A role for CRH and HPA activation in the regulation of plasticity signaling, neuroinflammation and emotional/mnesic behavior following global cerebral ischemia in rats

Patricia Barra de la Tremblaye

Thesis dissertation submitted to the

Faculty of Graduate and Postdoctoral Studies

in fulfillment of the requirements

for the Doctorate in Philosophy degree in Psychology

School of Psychology

Faculty of Social Sciences

University of Ottawa

ACKNOWLEDGMENTS

I would like to thank my committee members: Dr. Nafissa Ismail, Dr. William Staines, Dr. Zul Merali, Dr. Pamela Kent, and Dr. Claire-Dominique Walker, the external evaluator, who have manifested their distinguished skills and talents in their own fields by not only providing corrections and ideas to improve my thesis dissertation, but also for helping me develop research and professional skills that I hope to apply in my career. I would like to extend a sincere thank you to Dr. Charlie Thompson and Dr. Sarah Schock who have been mentors to me in helping me learn and perform neurobiochemical techniques used in the current thesis. Some professors also provided invaluable advice as I progressed through my PhD, including Dr. Cate Bielajew, Dr. Claude Messier, Dr. Alfonso Abizaid and Dr. Nathalie LeMarec, and I thank then sincerely. In the same line, I would like to thank, Jonathan James, Jacky Liang, and Sylvie Emond for their help and guidance in performing various neurobehavioral laboratory techniques and procedures. I would also like to thank the directors, faculty and administrative staff of the school of Psychology, from whom I have learned tremendously and who have facilitated my doctoral journey.

I would like to express my deepest appreciation to my doctoral supervisor, Dr. Hélène Plamondon. She has encouraged me to aim high and has helped me grow as a professional researcher. She has been a compassionate and most caring mentor, who conveyed a spirit of adventure in regards to research. Without her guidance and persistent help, this dissertation would have not been possible.

My doctorate has allowed me to meet amazing people that have become my most cherished friends, namely: Catrinel Girbovan, Idu Azogu, Julie Raymond, Nicolas Narvaez Linares, Simon Benoît, Lise Van de Beeck, Myriam Beaudry, Annie Girard, Laurence

Morissette, Emilie Gravel, Kristina Bradley, André Lauzon, Tina Yuan, and many more that I have met through the Graduate Student Association of the University of Ottawa.

Last but not least, I would also like to thank my mother, Léïla, whom I love with all my heart. She has always been there for me to encourage me in all my life's projects. I would like to express my deepest gratitude to the love of my life, Patrice Dumais, who has been so patient, kind, and helpful throughout the years, which have made this all possible. Without the support and love they both brought me, I would not have made it to the doctoral level.

Abstract

Depression occurs in about one third of patients with stroke and cardiac arrest. Hyperactivity of the stress system is the most commonly observed neuroendocrine change in major depressive disorder (MDD), which involves elevated levels in the cerebrospinal fluid of corticotropin-releasing hormone (CRH), a key stress neurohormone. Substantial evidence suggests that normalization of the stress system may be a requirement for successful treatment of MDD through region-specific changes in the mesocorticolimbic circuitry. Thus, alteration in the stress system may underlie the emotional and functional impairments observed following brain ischemic events. In addition, recent findings suggest that ischemic brain injury triggers a restorative process, creating a cerebral environment similar to that of early brain development, a period characterized by rapid neuronal growth and neuroplasticity, critical to optimize functional recovery of individuals post stroke. In particular brain-derived neurotrophic factor (BDNF), has been shown to play an important role in the pathophysiology of major depression and cerebral ischemia. However, whether CRH can mediate the expression of BDNF in the reparative process triggered by ischemic injury remains to be characterized. Therefore, the purpose of the current thesis is to characterize the effect of pharmacological blockade of CRH signaling at the onset of a global ischemic stroke, on emotional and cognitive behaviors, alteration in the neuroendocrine stress system, and markers of neuroplasticity including BDNF. To do this, an animal model of global cerebral ischemia with subsequent behavioral testing and postmortem brain analysis was used to determine underlying biochemical and behavioral changes modulated by CRH signaling following brain ischemia. This doctoral work will help elucidate the relationship between CRH and BDNF in the context of cerebral ischemia, and may provide insights for therapies targeting the stress system. These studies address considerations such as: the interplay

between stress, neuroplasticity and emotionality, and whether global ischemia can affect mood via changes in the HPA axis response.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

ABBREVIATIONS

4-VO	four-vessel occlusion model
AC	anterior cingulate cortex
ANOVA	analysis of variance
ANT	Antalarmin
BBB	blood brain barrier
Bcl-2	B-cell lymphoma-2
BDNF	Brain Derived Neurotrophic Factor
BLA	basolateral nucleus of the amygdala
BNST	bed nucleus of the stria terminalis
CA1	Cornu Ammonis area 1
CA3	Cornu Ammonis area 3
cAMP	cyclic AMP
CeA	central nucleus of the amygdala
CNS	central nervous system
CORT	corticosterone
COX2	cyclooxygenase 2
CPR	cardiopulmonary resuscitation
CREB	cAMP response element binding
CRH	corticotropin-releasing hormone
CRHR1	CRH type 1 receptors
CRHR2	CRH type 2 receptors
CVD	cardiovascular disease
DEX	dexamethasone
DG	dentate gyrus
EC	empty cage
EPM	Elevated plus Maze
FST	forced swim test
GDNF	glial cell-derived neurotrophic factor
GFAP	glial fibrillary acidic protein

GR	glucocorticoid receptors
HPA	hypothalamic-pituitary-adrenal
IBA1	ionized calcium binding adaptor
IL	infralimbic cortex
IL-1β, IL-1α, and IL-6	interleukins
iNOS	inducible nitric oxide synthase
ir	immunoreactive/immunoreactivity
LPS	lipopolysaccharide
LTD	long-term depression
LTP	long-term potentiation
MCAO	middle cerebral artery occlusion
MI	myocardial infarction
min	minute (s)
MMP	metalloproteinases
mPFC	medial prefrontal cortex
MR	mineralocorticoid receptors
mRNA	messenger ribonucleic acid
NAc	nucleus accumbens
NF-kB	nuclear factor kappa-light-chain-enhancer or activated b cells
NO	nitric oxide
NSFT	novelty suppressed feeding test
OFT	open field test
p75NTR	p75 neurotrophin receptor
pCREB	phosphorylated cAMP response element binding
PL	prelimbic cortex
PVN	paraventricular nucleus of the hypothalamus
ROS	reactive oxygen species
S1	Stranger rat 1
S2	Stranger rat 2
SIT	Three Chamber Social Approach Test
SPT	sucrose preference test
TH	tyrosine hydroxylase
TNFα	tumor necrosis factor α
TrkB	tropomyosin-related kinase B
UCN	urocortin
VEGF	vascular endothelial growth factor
VEH	vehicle
VTA	ventral tegmental area
YM-PAT	Y maze passive avoidance task

General Introduction

Cerebral ischemia refers to a deficiency in blood supply to the brain, reducing amounts of oxygen and essential nutrients to neurons and leading to a cascade of neurophysiological changes implicated in cellular degeneration (Lipton, 1999). In humans, this condition can result from a disruption in blood circulation within a focused area of the brain (i.e. obstruction or ruptures of a blood vessel in the brain: focal ischemia) or to the entire brain and periphery (global ischemia, often referred to as forebrain ischemia) such as experienced following cardiac and cardiopulmonary arrest, open heart surgeries or vascular accidents like an aorta aneurysm (Hadley, Papadakis, & Buchan, 2014). Ischemic strokes are more common than hemorrhagic strokes, accounting for approximately 83% of all cases (S. L. Paul, Srikanth, & Thrift, 2007). Although, ischemic stroke represents the most common severe neurological disorder (Barnett, 2010), it has recently declined from the third to the fourth leading cause of mortality in adults after heart disease, cancer, and chronic lower respiratory diseases (Towfighi & Saver, 2011). Rats are one of the most suitable species for experimental stroke animal models because of the pathophysiological similarities between rodents and humans (Yamori, Horie, Handa, Sato, & Fukase, 1976). Although rabbits, dogs, swine, and primates are more used in translational research, mice and rats are the most commonly used animal model as their encephalic vessels are similar to that of humans and they provide reliable and useful models of the pathology (Casals et al., 2011; Swindle, Makin, Herron, Clubb, & Frazier, 2012). The only clinically-validated treatment currently available post stroke is acute thrombolysis, the breakdown (lysis) of blood clots by pharmacological administration of tissue plasminogen activator (tPA). The utility of this approach is however constrained by the need to initiate treatment within 3–6 hours of symptoms and the risk of cerebral hemorrhage, which limit to less than 5% the percentage of stroke patients

apt to receive this treatment option (Y. Chen, Won, Xu, & Swanson, 2014). It therefore appears crucial to study new approaches able to treat a larger fraction of stroke patients. Hypothermia has been used safely as a neuroprotective treatment following ischemic stroke and cardiac arrest (Malek, Duszczyk, Zyszkowski, Ziembowicz, & Salinska, 2013; T. C. Wu & Grotta, 2013), but implementing this approach in the clinic is challenging.

Approximately 50% of patients who survive sudden cardiac arrest experience persistent motor and cognitive deficits, leaving them with complete or partial dependence on others (C. Lim, Alexander, LaFleche, Schnyer, & Verfaellie, 2004). The most common cognitive impairments following cerebral ischemia involve memory deficits and emotional impairments characterized by post-stroke depression (Bantsiele et al., 2009a; Hadidi, Treat-Jacobson, & Lindquist, 2009). Survivors of cerebral ischemia are 6 times more at risk of depression than healthy age-matched controls (M. Santos et al., 2009), depression affecting 33% of stroke patients (Maree L. Hackett, Yapa, Parag, & Anderson, 2005) and over 185,000 US stroke survivors annually (Hadidi et al., 2009). Both cognitive impairment and depression have relatively high prevalence in the acute stage (1st month) which gradually resorbs over a 6 month period following transient ischemic attack and minor stroke (Moran et al., 2014). These affective symptoms have been related to injury of the limbic system and hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis (Arborelius, Owens, Plotsky, & Nemeroff, 1999; Holsboer & Barden, 1996; Pariante & Lightman, 2008).

1. CRH and its Receptors

There is a general consensus that corticotropin-releasing hormone (CRH) is one of the most important modulators of the neuroendocrine, autonomic, immunological, and behavioral adaptation to stress (Becker, Abraham, Kindler, Helmeke, & Braun, 2007). The 41 amino acid

neuropeptide plays a neurotransmitter/neuromodulator role by acting on both the brain and periphery to coordinate the overall response of the body to stress (De Michele et al., 2005). CRH is synthesized as a larger inactive preprohormone (a precursor protein to a prohormone, being itself a precursor to a peptide/hormone) that undergoes proteolytic cleavage in the Golgi apparatus, mainly by prohormone convertase 2 (PC-2), and in some cases PC-1 to generate the active peptide (Laryea, Arnett, & Muglia, 2012). CRH containing neurons are widely distributed within the central nervous system (CNS) (Cummings, Elde, Ells, & Lindall, 1983), its major substrates being the parvocellular portion of the paraventricular nucleus of the hypothalamus (PVN, a key production site), cerebral cortex, locus coeruleus, the amygdala (especially the central nucleus, CeA) and hippocampus, all of which are important regions for stress adaptation, learning and memory (Bittencourt & Sawchenko, 2000). In the PVN, CRH is packaged into secretory vesicles and released in a circadian rhythm or in response to stress (Laryea et al., 2012).

CRH is a member of a peptide family including urocortin (UCN), UCN II and UCN III (Dautzenberg & Hauger, 2002; J. M. H. M. Reul & Holsboer, 2002). The effects of these peptides are mediated via two receptor types - CRHR1 and CRHR2 with almost 71% amino acid sequence similarity, but differential localization and affinity for CRH (Chalmers, Lovenberg, & De Souza, 1995). CRHR1 and CRHR2 are metabotropic receptors and thus, function slowly in activating signaling cascades to prime post-synaptic neurons for neurotransmitter or neuromodulator activity, unlike the fast activation of classical neurotransmitters (Gallagher, Orozco-Cabal, Liu, & Shinnick-Gallagher, 2008). Both CRH receptors signal by coupling to alpha subunits of stimulatory G (Gsα) proteins, leading to stimulation of adenylyl cyclase, generating the second messenger cyclic AMP (cAMP) (Lovenberg et al., 1995). Accumulation of

cAMP in the cytosol in turn stimulates the activation of protein kinase A (PKA) to phosphorylate the cAMP response element binding (CREB) protein by the cAMP response element (CRE) located in the CRH promoter in the cell nucleus, inducing transcription of stress related genes such as CRH and BDNF; see figure 1 in appendix A (Hauger, Risbrough, Oakley, Olivares-Reyes, & Dautzenberg, 2009).

Due to differences in N-terminal ligand-binding domains, CRH and UCN have high affinity for CRHR1, whereas UCN II and UCN III are the natural ligands for CRHR2 (Bonfiglio et al., 2011). CRHR1 is considered the primary receptor for CRH and mediates the behavioral stress response (Contarino, Heinrichs, & Gold, 1999; Müller & Wurst, 2004; K. Van Pett et al., 2000). CRHR1 is expressed throughout the CNS with highest concentrations in the cerebral cortex, hypothalamus, locus coeruleus, hippocampus, bed nucleus of the stria terminalis (BNST) and in the basolateral and medial nuclei of the amygdala, whereas CRHR2 displays abundant expression throughout the periphery and is present in more discrete brain locations, the densest concentrations being in the lateral septum, supraoptic nucleus, raphe nucleus, choroid plexus, nucleus tractus solitarii, cortical amygdala nucleus, and the ventromedial hypothalamus (Chalmers et al., 1995; Y. Chen, Brunson, Muller, Cariaga, & Baram, 2000; Kasia Van Pett et al., 2000). Regions of overlap between CRHR1 and CRHR2 distributions include the olfactory bulb, hippocampus, BNST, the nucleus accumbens (NAc) and medial septum (Lemos et al., 2012; J. M. H. M. Reul & Holsboer, 2002). Double immunohistochemistry staining revealed that CRHR1 is found in glutamatergic neurons of the cortex and hippocampus, in GABAergic neurons of the reticular thalamic nucleus, globus pallidus, and septum, and in dopaminergic neurons of the substantia nigra pars compacta and ventral tegmental area (Refojo et al., 2011).

Studies using animal models show opposite effects of the CRH receptors in modulating the central stress response (Heinrichs, Lapsansky, Lovenberg, De Souza, & Chalmers, 1997; Liebsch, Landgraf, Engelmann, Lorscher, & Holsboer, 1999; Preil et al., 2001). Behavioral and hormonal stimulatory effects of CRH can be ascribed to CRHR1-mediated actions (Muller et al., 2003; G. W. Smith et al., 1998; Timpl et al., 1998), whereas specific actions of the CRHR2-selective ligands UCN 2 or UCN 3 appear to play a role in dampening stress sensitivity (Greetfeld et al., 2009). Limbic CRHR1 is involved in mediating anxiety-related behaviors and feedback regulation of the HPA system. Studies using rodents to investigate efficient negative feedback show a rapid but transient increase in CRH and CRHR1 levels in the PVN after exposure to stressful conditions followed by an increase in cortical and hippocampal activation of CRHR2, causing a down-regulation of CRH and CRHR1 in these sites (Greetfeld et al., 2009; Skelton, Nemeroff, Knight, & Owens, 2000). Mice deficient in CRHR1 display decreased anxiety-like behavior and have an impaired stress response related to a severe glucocorticoid deficiency (G. W. Smith et al., 1998; Timpl et al., 1998). In contrast, CRHR2-mutant mice show increased anxiety-like behavior and heightened increases of CRH, ACTH and corticosterone in response to stress (Bale et al., 2000; Bale & Vale, 2003; Kishimoto et al., 2000). CRHR1 antagonists and antisense oligonucleotides are anxiolytic in rats while CRHR2 antagonists and antisense oligonucleotides increase despair-like behaviors and alter appetitive behaviors, such as suppressing appetite by urocortin, which is a high affinity CRHR2 ligand (Heinrichs et al., 1997; Liebsch et al., 1999). Together, these studies suggest that CRHR1 and CRHR2 act on neuronal systems inversely regulating stress and anxiety behaviors (Müller & Wurst, 2004).

2. Stress Response Following Stroke and Cardiovascular Disease (CVD)

In rodents, increased CRH concentrations range between 50 to 100% at the piriform cortex, the central nucleus of the amygdala, and the lateral, ventromedial and paraventricular nucleus of the hypothalamus within 4 h of global cerebral ischemia (Khan, Milot, Lecompte-Collin, & Plamondon, 2004). Similarly, elevated CRH mRNA levels in the amygdala and cerebral cortex are observed within hours of focal ischemia (Wong et al., 1995). Increased levels of CRH, norepinephrine and CRHR1 are observed in the rat PVN and pituitary after hypoxia, cold, or restraint stress (X. Q. Chen, Du, & Wang, 2004; T. Y. Wang et al., 2004). Hypoxia leads to increased CRH and CRHR1 mRNA expression in the hypothalamic PVN, as well as enhanced plasma ACTH and corticosterone (Xu, Chen, Du, & Wang, 2005). Serum and faecal corticosterone levels also increase after ischemia to reach back to basal levels in days post ischemia (Hwang et al., 2006; Kalliokoski et al., 2010). Perinatal hypoxia (prenatal day 19 to postnatal day 14) triggers a lasting dysregulation of basal and stress-induced corticosterone secretion up to six months postnatally, with CRH mRNA levels remaining elevated at the hypothalamic PVN even after corticosterone levels are back to baseline levels (Raff, Jacobson, & Cullinan, 2007). Notably, delayed alterations of CRH and its receptors have been demonstrated in response to focal and global cerebral ischemia (Khan et al., 2004; Neigh, Karelina, Zhang, et al., 2009). Increased activation of the HPA axis is associated with heightened sensitivity of the adrenal glands, maintaining elevated glucocorticoid levels (Fassbender, Schmidt, Mossner, Daffertshofer, & Hennerici, 1994; Radak, Resanovic, & Isenovic, 2014; Tu, Dong, Zhao, Yang, & Chen, 2013; Weidenfeld et al., 2011).

In humans, HPA axis activation has been reported to occur rapidly following cerebral ischemia (Fassbender et al., 1994; O'Neill, Davies, Fullerton, & Bennett, 1991; Wexler, 1970).

Dexamethasone treatment fails to suppress cortisol in acute stroke patients (Olsson, 1990; Olsson, Astrom, Eriksson, & Forssell, 1989) and hypercortisolism is associated with increased inflammatory response, high cytokine levels, fever and reduced white blood cell counts (A. Johansson, Olsson, Carlberg, Karlsson, & Fagerlund, 1997; Slowik et al., 2002), and is related to a high mortality rate and poor functional outcome following cerebral ischemia and cardiac arrest (de Jong, Beishuizen, de Jong, Girbes, & Groeneveld, 2008; Feibel, Hardy, Campbell, Goldstein, & Joynt, 1977; Marta-Moreno, Mostacero, Lopez del Val, & Morales-Asin, 1997; Murros, Fogelholm, Kettunen, & Vuorela, 1993; Tombaugh, Yang, Swanson, & Sapolsky, 1992). Early initiated and persisting release of cortisol is related to the severity of the brain injury, neurological deficits and clinical stroke syndrome (Anne et al., 2007; Christensen, Boysen, & Johannesen, 2004; Neidert et al., 2011). Patients showing elevated cortisol levels following cardiac surgery display higher cognitive and emotional impairments (Yin, Luo, Guo, Li, & Huang, 2007), suggesting that the stress response to cerebral ischemia is prognostically unfavourable.

3. Cascade of Physiological Events Following Ischemia

It has been well documented that abrupt deprivation of oxygen and glucose to neuronal tissues elicits a pathological cascade of physiological events, ultimately leading to neuronal death (Cheng, Al-Khoury, & Zivin, 2004). Normal cerebral blood flow ranges from 50 to 75 mL/100 g of brain tissue per minute. Upon an ischemic insult and the ensuing widespread depolarization blood flow can rapidly decrease to perfusion rates of ~18 mL/100 g of brain tissue per minute, neuronal cell death ensuing when perfusion reaches levels below 10 mL/100 g of brain tissue per minute (Harukuni & Bhardwaj, 2006). In cerebral ischemia, evolvement of tissue damage is characterized by three distinct phases (Heiss, 2012). During the *acute phase*, within a

few minutes of the ischemic event, energy failure is characterized by cell metabolism reaching threshold levels below those required to maintain basic housekeeping activities (~20% of pre-occlusion values). Such failure disrupts energy-dependent ionic pumps, leading to increased sodium and calcium influx and potassium efflux into the cells. Perturbation of the ionic equilibrium results in water transported into the cells causing osmotic swelling- i.e., edema. Neuronal damage results in the breakdown of energy-dependent Na+/K+-ATPases with subsequent cellular swelling and cytotoxic edema. During the *subacute phase,* the irreversible damage is expanded by spreading waves of depression-like depolarizations that emerge from the core ischemic region and propagate radially and spontaneously over large portions of the neighboring tissue - i.e., the penumbra that is functionally, and metabolically compromised, with blood flow ranging between 25% and 50% of pre-occlusion values, which are below the values required for axonal and synaptic activity, but not associated with irreversible damage. This phase of spreading depolarisations waves can last for approximately 6 h, although may persist over several days, in areas with or without impaired blood flow or energy metabolism. The unproportioned high metabolic workload and low oxygen supply lead to transient episodes of hypoxia, which increase lactate during the passage of each depolarization. Release of excitatory and inhibitory neurotransmitters, activation of receptors and receptor-operated ion channels, influx of calcium, free radicals formation, nitric oxide generation, dysfunction of endoplasmic reticulum, mitochondrial disturbances are some of the key factors involved in the propagation of ischemic damage. The last *phase of tissue injury* is delayed and may last for several days or even weeks, characterized by secondary phenomena such as blood brain barrier (BBB) disruption, inflammation, and programmed cell death (apoptosis). Such sequences of cellular events characterize global and focal ischemic damage.

Global cerebral ischemia is commonly induced in animal models by permanent occlusion of both vertebral arteries and temporary ligation of the two common carotid arteries (four-vessel occlusion model, 4-VO) (Krafft et al., 2012). In humans and animals, transient global cerebral ischemia leads to spatial learning and working memory deficits that are commonly associated with selective and delayed neuronal damage of the cornu ammonis 1 (CA1) hippocampal region (R. E. Hartman, J. M. Lee, G. J. Zipfel, & D. F. Wozniak, 2005; Lehotsky et al., 2009; Petito, Olarte, Roberts, Nowak, & Pulsinelli, 1998; von Euler, Bendel, Bueters, Sandin, & von Euler, 2006). Populations of hippocampal cells appear differentially vulnerable to the effects of ischemia, hippocampal interneurons being identified as more resistant than pyramidal neurons (Avignone, Frenguelli, & Irving, 2005). Moreover, there are two distinct hippocampal pyramidal output presenting distinct dendritic morphology and electrophysiological properties, that are both synergistically modulated by metabotropic glutamate and acetylcholine receptors, but with opposite outcomes on long-term neuronal excitability (Graves et al., 2012). Excess glutamate release in the CA1 pyramidal cells via stimulation of postsynaptic glutamatergic N-methyl-D-aspartate (NMDA) receptors creates an excitotoxic environment contributing to CA1 neuronal death (Bonde, Noraberg, Noer, & Zimmer, 2005; Lalonde & Mielke, 2014). As alluded, the extracellular glutamate efflux due to abnormal cellular depolarization and lack of reuptake under conditions in which energy stores are depleted, compromises normal ionic gradient across the neuronal membrane. This results in hyper-activation of the calcium-permeable ion channels on glutamatergic NMDA receptors, allowing excessive calcium influx (Dirnagl, Iadecola, & Moskowitz, 1999). Lack of cellular energy prevents the active calcium pumps from eliminating the elevated cytosolic calcium, resulting in hyperactivation of several calcium-dependent signaling pathways, which activate phospholipases and cyclooxygenases, leading to the

production of nitrogen and oxygen free-radicals. These functional alterations lead to peroxidation of the plasma, nuclear, and mitochondrial membranes and ensuing cell disintegration and DNA damage; they can also activate several pro-inflammatory genes, causing local and systemic inflammation (Lai & Todd, 2006). Interventions targeting glutamate excitotoxicity reduce acute ischemic cell death in animal models of focal and global ischemia, but these approaches are generally ineffective if not initiated prior to ischemia or within the first 6 hours thereafter (Ginsberg, 2008) and found not suitable for human therapy due to side effects including cytoplasmic vacuolization (Fix et al., 1993).

Pyramidal neurons in the hippocampal CA1 region are most susceptible to transient global cerebral ischemia, with neuronal death occurring 3-4 days post-ischemia due to apoptotic processes, a phenomenon referred to as “delayed neuronal death” (Choi, 1996; Kirino, 2000; Zheng, Zhao, Steinberg, & Yenari, 2003). Impairments related to hippocampal CA1 neuronal injury are observed within 48 to 72 hours after acute brain injury in humans (Bartsch et al., 2015). Hippocampal circuits are critically involved in processes mediating memory formation and consolidation (Kadar, Dachir, Shukitt-Hale, & Levy, 1998), and CA1 neuronal injury contributes to memory impairment and neurologic disability seen in humans with cognitive impairments (Bartsch et al., 2010). Metabolic changes in the CA1 region, assessed using MRI, appear to normalize to control values 7 days after reperfusion, a phenomenon that results in extensive neuronal death characterized by a ~75% loss in CA1 neuronal density, also related to neuronal shrinkage to which apoptosis and astrogliosis may contribute (Anderova et al., 2011). Delayed neuronal death is thus intermingled with cell death by apoptosis and necrosis, differently distributed within injured tissue (neurons in the core being predominantly necrotic while apoptosis is more abundant in the penumbral tissue) and temporally related to duration and

severity of the ischemic insult (Harukuni & Bhardwaj, 2006). Electron microscopy has detected necrotic and apoptotic markers in the hippocampal CA1 region after transient forebrain ischemia (Colbourne, Sutherland, & Auer, 1999; Dluzniewska, Beresewicz, Wojewodzka, Gajkowska, & Zablocka, 2005). Necrosis, a process that is not "regulated or programmed," is typically characterized by disruption of cellular homeostasis from energy failure due to severe mitochondrial injury, which leads to cellular swelling, membrane lysis, inflammation, vascular damage, and edema formation (Harukuni & Bhardwaj, 2006). Conversely, apoptosis, a process associated with DNA fragmentation, is characterized by cell shrinkage, chromatin aggregation, and preservation of cell membrane integrity and mitochondria without inflammation and injury to surrounding tissue (Harukuni & Bhardwaj, 2006). Neurons in the CA1 field of the hippocampus, show DNA fragmentation 3 days following ischemic insult, using TUNEL staining (J. N. Davis & Antonawich, 1997; Nitatori et al., 1995). Apoptotic cell death includes caspase-dependent and independent pathways (Love, 2003). Overexpression of Bcl-2, an anti-apoptotic protein, reduces neuronal apoptosis and/or prevents apoptosis of dentate granule cells delaying the loss of CA1 pyramidal neurons (H. D. Wang et al., 1999). Central infusion of a caspase inhibitor reducing pro-apoptotic caspase-3 expression, prevents delayed hippocampal CA1 cell loss, although fails to affect impairment of long-term potentiation in post-ischemic CA1 cells, suggesting that caspase inhibition alone cannot rescue functional plasticity (Gillardon, Kiprianova, Sandkuhler, Hossmann, & Spranger, 1999).

Importantly, the accumulation of unfolded proteins in the endoplasmic reticulum post ischemia triggers an unfolded protein response (UPR), which activates multiple signaling pathways contributing to neuronal death and apoptosis, through the production of reactive nitrogen species (RNS), increased inducible nitric oxide species (iNOS) and release of pro-

inflammatory cytokines (J. H. Chen, Kuo, Lee, & Tsai, 2014). Further, the production of reactive oxygen species (ROS) activates IRE1-α/TRAF2 complex formation, which regulates activity of TNF receptor-associated factor 2 (TRAF2), apoptosis signal-regulating kinase 1 (ASK1) and downstream kinase pathways including Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB) (J. H. Chen, Kuo, Lee, & Tsai, 2015; Rao & Bredesen, 2004). Administration of CRH confers proapoptotic effects on PC12 cell via activation of p38 MAPK (mitogen-activated protein kinase) and the Fas/Fas ligand system, which can be inhibited by application of CRHR1 antagonist Antalarmin (Dermitzaki, Tsatsanis, Gravanis, & Margioris, 2002). High CRH concentrations can induce cell apoptosis of primary cultured hippocampal neurons through UPR, increasing protein and mRNA levels of GRP78 (Endoplasmic reticulum stress response marker, glucose-regulated protein 78), induction of CHOP (proapoptotic transcription factor C/EBP homologous protein) and cleavage of caspase-12 protein, and activation of the IRE1 apoptotic pathway by recruiting the adaptor protein TRAF2 (TNF-receptor-associated factor 2), which recruits the apoptosis signaling kinase-1 (ASK1), which, in turn, activates c-jun N-terminal kinase (JNK) (Y. Zhang et al., 2012).

4. Neuroinflammatory Responses to Cerebral Ischemia

The brain's inflammatory response to focal and global ischemic insults is characterized by a rapid activation of microglia, followed by the infiltration of circulating inflammatory cells, including granulocytes (neutrophils), T cells, monocytes/macrophages, and other inflammatory components in the ischemic brain region (R. Jin, Yang, & Li, 2010). In the CNS, microglial cells act as resident immune cells that continuously scan the internal milieu with highly motile processes to defend against infections or toxic substances released from dying brain cells by scavenging and engulfing unwanted pathogens and cellular debris (Kettenmann, Hanisch, Noda,

& Verkhratsky, 2011; Kreutzberg, 1996). In the healthy brain, microglia are in a ramified resting state, characterized by a small cell body with fine, highly branched processes projecting out from the cell body. Following ischemia, microglia undergo phenotypic transformation to an activated state that includes an amoeboid and morphology in which they retract their processes, increase the size of cell body and become motile, migrating rapidly to the injury site (Lai & Todd, 2006). If the damage persists and the CNS cells die, microglia undergo further transformation and become phagocytic, removing cell debris, and engulfing polymorphonuclear neutrophils (Annunziato, Boscia, & Pignataro, 2013). Microglial proliferation peaks at 48 - 72 h, and lasts for several weeks after the initial ischemic injury (Denes et al., 2007). In the acute phase (minutes to hours) of ischemic stroke, microglia migrate to the damaged tissue and release neurotoxic factors, such as proinflammatory cytokines, including tumor necrosis factor alpha (TNFα), interleukin (IL-1β, IL-1α, and IL-6), and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), proteolytic enzymes, chemokines such as interferon γ-inducible protein 10 (IP10; CXCL10), and reactive oxygen species (ROS) (Amantea, Nappi, Bernardi, Bagetta, & Corasaniti, 2009; Kriz, 2006). These mediators induce the expression of adhesion molecules on cerebral endothelial cells, and on leukocytes, promoting the adhesion and trans-endothelial migration of circulating leukocytes (Yilmaz & Granger, 2008). In the subacute phase (hours to days), infiltrating leukocytes release cytokines and chemokines, especially excessive production of ROS and induction/activation of matrix metalloproteinases (MMP), mainly MMP-9, which amplify the brain-inflammatory responses through extensive activation of resident cells and infiltration of leukocytes, eventually leading to disruption of the BBB, brain edema, neuronal death, and hemorrhagic transformation (Amantea et al., 2009; Kriz, 2006).

Under quiescent condition, microglia facilitate neurogenesis (Ziv et al., 2006) while inflammation-induced microglial activation has been implicated in neurogenesis suppression (Ekdahl, Kokaia, & Lindvall, 2009; Mizumatsu et al., 2003). Microglia not only coordinate complex processes of neuroinflammation but may also be responsible for the activation of astrocytes in pathological conditions (Allan & Rothwell, 2003). Astrocytes are anatomically in juxtaposition to both neurons and blood vessels and contribute to the formation of the BBB, regulate cerebral blood flow in response to neuronal activity, provide metabolic substrates for neurons, and regulate oxidative balance in the brain (Laird, Vender, & Dhandapani, 2008). Astrocytes are preserved over the first 3 h of reperfusion (K. Yamashita et al., 1996), but later show extensive astrocyte death in the core of the lesion, while in the periphery astrocytes become reactive, and subsequently extend their processes in a single plane forming a glial scar in the peri-infarct area after cerebral ischemia (Sofroniew, 2009; Sofroniew & Vinters, 2010). This astrocytic scar is formed in a highly localized manner, eventually forming a physical barrier which limits immune cell infiltration and protects adjacent uninjured tissue (Schachtrup et al., 2010).

Reactive astrocytes have thicker main processes and soma and overexpress intermediate filaments glial fibrillary acidic protein (GFAP) and promote synthesis of IL-6, ciliary neurotrophic factor (CNTF), adhesion molecules, as well as other proteins generally associated with detrimental effects such as calcium-binding protein S100β, iNOS, and cyclooxygenase 2 (COX2) (Escartin & Bonvento, 2008; Pekny & Nilsson, 2005). Reactive astrocytosis is detected in the hippocampus between 4 and 7 days after an ischemic insult (Kielian & Esen, 2004). In the CA1 region, astrocytes and microglia have been shown to express mineralo- and gluco-corticoid receptor immunoreactivity 4 days after ischemia, with expression peaking 7 days after ischemia

(Hwang et al., 2006). In vitro and in vivo studies show that accumulation of activated microglial cells is linked to a secondary wave of tissue injury affecting neighboring neurons, raising the possibility that microglia play a role in neuronal death occurring in the secondary phase of ischemic injury (Y. Chen et al., 2014). Focal (B. Zhao et al., 2012), and global cerebral ischemia (W. Wang et al., 2014) potently activate microglia, and treatment with anti-inflammatory minocycline, a tetracycline that prevents microglial activation, confers neuroprotective action in global cerebral ischemic gerbils (Yrjanheikki, Keinanen, Pellikka, Hokfelt, & Koistinaho, 1998). Moreover, central injection of minocycline reduces anxiety-like behavior, microglial activation and hippocampal neuronal damage exacerbated by 3 weeks of 3 h daily restraint stress prior to 8 min cardiac arrest in mice (Neigh, Karelina, Glasper, et al., 2009).

Microglia are classically activated by exposure to T helper type 1 (TH1) cytokines such as interferon gamma (IFNγ) or bacterial lipopolysaccharide (LPS) (Goerdt et al., 1999). Stress exposure potentiates the CNS immune response by increasing plasma TNF-α and IL1β, and IL1β expression in the pituitary, hypothalamus, hippocampus, and cerebellum 24h after LPS injection (J. D. Johnson et al., 2002; Nguyen et al., 1998). Notably, stress exposure increases microglial activation and cytokine expression in the brain, and the vulnerability to stroke (Stuller, Jarrett, & DeVries, 2012). Thus, exposure to 6 h of immobilisation stress prior to permanent middle cerebral artery occlusion (MCAO) increases TNFα and IL-1β release in the cerebral cortex and worsens behavioral outcomes and infarct size, effects that can be reduced by pharmacological blockade of IL-1β (Caso, Moro, Lorenzo, Lizasoain, & Leza, 2007). In regards to stroke, central blockade of IL-1β attenuates exacerbated corticosterone levels, behavioral impairments, and infarct volume from a 6 h immobilisation stress exposure prior to focal ischemia (Caso et al., 2007). Of interest, clinical studies performed on women and men at risk of cardiovascular

disease showed that markers of inflammation including C-reactive protein and IL-6 are associated with depressive symptoms, although depression and inflammation remain independent predictors of cardiovascular disease (Empana et al., 2005; Vaccarino et al., 2007). Together, these findings suggest a bidirectional influence of the immune and stress systems, and potential involvement of the immune response in stress-induced affective disorder onset and progression following injury (Bale, 2009), as well as exacerbation of injury of neurons and other cell types following stroke (Y. Chen et al., 2014).

Notwithstanding these negative effects, microglial activation has been shown to exert neuroprotective actions in the injured CNS (Clarke et al., 2008). In contrast to TH1 and LPS, T-helper type 2 (TH2) increases microglial phagocytosis and triggers the secretion of anti-inflammatory cytokines such as IL-4 and IL-10, and the release of tissue remodeling molecules (Varin & Gordon, 2009). Increased IL-10 prior to ischemia is shown to reduce brain injury, and enhance recovery through its anti-inflammatory properties (Frenkel et al., 2003). IL-10 and glial cell-derived neurotrophic factor (GDNF) reduce neuronal apoptosis following hypoxic-ischemic brain damage in rats (S. J. Li et al., 2014). Activated microglia can also alleviate ischemic injury through secretion of brain derived neurotrophic factor (BDNF) (Elkabes, DiCicco-Bloom, & Black, 1996). BDNF promotes the proliferation and the phagocytic activity of microglia, and inhibits apoptosis in the brain (J. Zhang et al., 2003). Intranasal (Jiang et al., 2011) or intra-cerebral administration (Jiang et al., 2010) of BDNF prior and after MCAO upregulates IL10, increases activated phagocytotic microglia and reduces neurologic deficits, effects that can be abolished by the co-administration of anti-BDNF. Although stroke increases BDNF immunostaining in neurons and ependymal cells in the non-lesioned hemisphere (at 4 h and 24 h), highest increases in BDNF protein levels are found in the lesioned hemisphere in various cell

types including neurons, ependymal and microglial cells (at 24 h), endothelial cells of cerebral arterioles (at 4 h and 24 h) and astrocytes (at 8 days) (Bejot et al., 2011). In the hippocampus, the pattern of mature BDNF expression shows a more delayed increase (from 8 to 30d), which coincides with the evolution of synaptophysin expression (Madinier et al., 2013). A transient increase in BDNF-ir expression has been reported in CA1 neurons, as well as reactive astrocytes and microglia after global ischemia (T. H. Lee, Kato, Chen, Kogure, & Itoyama, 2002).

5. Neuronal Plasticity Following Cerebral Ischemia

In combination with other elements of the ischemic cascade, the inflammatory reaction triggers a rapid depletion in the number, length and turnover of dendritic spines, observed even a few minutes following cerebral ischemia (C. E. Brown, Wong, & Murphy, 2008; Liguz-Lecznar & Kossut, 2013). These changes have been proposed to underlie cognitive impairments following ischemic injury, memory deficits in the water maze test being associated with reduced dendritic length and arborization of hippocampal CA1 pyramidal neurons observed 16 weeks after forebrain ischemia (Jia, Zhang, Zhang, Liu, & Li, 2012; Titus et al., 2007). External factors that promote clinical recovery such as rehabilitation and pharmaceutical treatments are accompanied by dynamic restorative processes of maladaptive plasticity post stroke (Chollet, 2013; Jang, 2013). Even in the absence of treatment, the brain naturally undergoes moderate morphological and neuroanatomical reorganization quickly after injury, notably changes in dendritic branching, axonal sprouting and synaptogenesis (C. E. Brown & Murphy, 2008; S. Y. Kim & Jones, 2010). These structural changes are orchestrated by mechanisms of long-term potentiation (LTP) and depression (LTD), due to altered levels of excitatory and inhibitory neurotransmission (glutamate and GABA, respectively) and upregulation of growth factors, most notably BDNF (Nudo, 2011).

BDNF is a member of the neurotrophin family, which plays a crucial role in the survival, differentiation, connection and plasticity of neurons (Huang & Reichardt, 2001; B. Lu & Chow, 1999; B. Lu & Gottschalk, 2000; McAllister, Lo, & Katz, 1995; Schinder & Poo, 2000; Shao et al., 2010). It is the most abundantly expressed neurotrophin in the mature CNS (Hofer, Pagliusi, Hohn, Leibrock, & Barde, 1990), high concentrations being detected in the hippocampus, the cerebral cortex and the amygdala (Conner, Lauterborn, Yan, Gall, & Varon, 1997; Q. Yan et al., 1997). BDNF acts mainly via stimulation of the TrkB (tropomyosin-related kinase B receptor) receptor tyrosine kinase and the p75 neutrotrophin receptor (p75NTR) (Rodriguez-Tebar, Dechant, & Barde, 1990). In the adult hippocampus, it is essential for LTP (Figurov, Pozzo-Miller, Olafsson, Wang, & Lu, 1996; Korte et al., 1995; Korte, Staiger, Griesbeck, Thoenen, & Bonhoeffer, 1996; Minichiello et al., 2002), mediating learning and memory formation (Cunha, Brambilla, & Thomas, 2010; Korte et al., 1995). Memory-related synaptic plasticity involves enlargement of dendritic spines (Bourne & Harris, 2008; L. Y. Chen, Rex, Casale, Gall, & Lynch, 2007; Hering & Sheng, 2001; Holtmaat & Svoboda, 2009; Lynch, Rex, Chen, & Gall, 2008), whereas processes associated with memory loss involve shrinkage or loss of dendritic spines (Collingridge, Peineau, Howland, & Wang, 2010; Kasai, Fukuda, Watanabe, Hayashi-Takagi, & Noguchi, 2010). BDNF modulates activity-dependent synaptic plasticity by binding to its high-affinity TrkB receptor (Bramham & Messaoudi, 2005; Kang, Welcher, Shelton, & Schuman, 1997), mediating memory processing (Bekinschtein et al., 2007; Morris et al., 2003; Whitlock, Heynen, Shuler, & Bear, 2006) for a variety of learning tasks especially contextual fear conditioning (Bekinschtein et al., 2008; Hall, Thomas, & Everitt, 2000; I. Y. Liu, Lyons, Mamounas, & Thompson, 2004; Ma, Wang, Wu, Wei, & Lee, 1998; Monteggia et al., 2004; Ou & Gean, 2006; Rattiner, Davis, French, & Ressler, 2004), and spatial learning in the radial arm

maze (Mizuno, Yamada, Olariu, Nawa, & Nabeshima, 2000) and the Morris Water Maze (Kesslak, So, Choi, Cotman, & Gomez-Pinilla, 1998; Linnarsson, Bjorklund, & Ernfors, 1997; Mu, Li, Yao, & Zhou, 1999). BDNF deficiency results in retraction or atrophy of dendrites and impaired memory function (Horch, Kruttgen, Portbury, & Katz, 1999; McAllister, Katz, & Lo, 1997; McAllister et al., 1995; Tyler & Pozzo-Miller, 2003).

The phosphorylation of the cAMP response element binding (CREB) protein is considered to be essential for the synaptic plasticity underlying long-term memory formation and consolidation (Alberini, 2009). After contextual fear conditioning, phosphorylated CREB (pCREB) increases in the basolateral amygdala (BLA), central amygdala (CeA), through two independent phases of hippocampal ERK/CREB activation (C. Paul, Stratil, Hofmann, & Kleppisch, 2010; Stanciu, Radulovic, & Spiess, 2001; Trifilieff et al., 2006). pCREB is also elevated in the hippocampus when learning spatial tasks (Guzowski & McGaugh, 1997; Porte et al., 2011). Infusion of AP5, a NMDA receptor antagonist, in the dorsal hippocampus, just before a contextual fear conditioning session, decreases pCREB ratio in the BLA and CeA, supporting a role of the hippocampus in sending contextual information to the amygdala to serve as a conditioned stimulus (de Oliveira Coelho, Ferreira, Soares, & Oliveira, 2013). DNF/TrkB signaling in the amygdala is required for the acquisition of fear conditioning (Rattiner, Davis, French, et al., 2004; Rattiner, Davis, & Ressler, 2004).

BDNF has been implicated in brain disorders involving cognitive decline (Howells et al., 2000; Peng, Wuu, Mufson, & Fahnestock, 2005) and shown to be decreased in hippocampal and cortical regions of Alzheimer's disease patients (Hock, Heese, Hulette, Rosenberg, & Otten, 2000). Low BDNF levels also result in poor axonal outgrowth after brain injury (Naert, Ixart, Maurice, Tapia-Arancibia, & Givalois, 2011), while increased levels allow the remaining brain

tissue to reorganize and to recover function following injury (Dancause & Nudo, 2011). In addition, BDNF administration improves sensory-motor recovery after stroke in rats whereas chronic infusion of BDNF oligonucleotide antisense prevents rehabilitation-induced motor recovery (Maclellan et al., 2011). Both neurons (Comelli et al., 1992; Kokaia, Andsberg, Yan, & Lindvall, 1998; Rickhag, Teilum, & Wieloch, 2007; Sulejczak et al., 2007a; L. R. Zhao, Mattsson, & Johansson, 2000) and non-neuronal cells (Lai & Todd, 2008; Madinier et al., 2009; J. Neumann et al., 2006; Sato et al., 2009), located in and around the lesion site as well as in the contralateral hemisphere, contribute to increased BDNF production in the acute post-ischemic period creating a growth-positive environment. Interventions that upregulate BDNF expression increase neuroplastic changes and functional recovery (Chang et al., 2012; J. Chen, Qin, Su, Liu, & Yang, 2012; S. Guo, Som, Waeber, & Lo, 2012; Ploughman et al., 2009; Takeshima et al., 2011), whereas the opposite is observed upon BDNF blockade or depletion (Endres et al., 2000; Texel et al., 2011).

The protective role of BDNF has been demonstrated in various injury models including focal ischemia, hypoxia-ischemia and global brain ischemia and shown to involve up-regulation of anti-apoptotic B-cell lymphoma-2 (Bcl-2) and inhibition of intracellular calcium overload, attenuation of NMDA receptor-mediated toxicity, and promotion of neural regeneration through increased axonal sprouting, synaptogenesis and angiogenesis (A. Chen, Xiong, Tong, & Mao, 2013; Kiprianova et al., 1999). BDNF's beneficial effects on cell survival and synaptic transmission are thought to be mediated by TrkB receptors, while activation of the non-specific p75NTR receptors are speculated to play a role in mediation of cell death and functional impairment in a variety of neurodegenerative disorders (Hennigan, O'Callaghan, & Kelly, 2007). NMDA receptors and L-type voltage–sensitive calcium channels are activated by membrane

depolarization, which is induced by AMPA receptors, leading to an influx of intracellular calcium, resulting in activation of Ca2+ calmodulin-dependent (CaM) kinases II and IV, which activate CREB by phosphorylation at Ser-133, to encode the transcription of BDNF (Lipsky & Marini, 2007). BDNF-promoted increases in proximal dendritic arbors and spines occur via a CREB-dependent transcriptional regulation of a series of [Ca2+]-regulated enzymes and kinases pathways, which involve complex molecular pathways, which description is beyond the scope of the present thesis. However, it appears plausible that the functional recovery seen in our studies result from changes in neuronal plasticity and/or brain reorganization rather than neuronal protection per se.

6. Effects of Stress on Brain Plasticity

Over the last years, our research team has been examining pathophysiological changes affecting HPA axis activation post ischemia. This research has revealed similarities of the impact of potent stressors and that of an ischemic insult on the brain responses and functional changes. The following paragraphs review changes in brain plasticity in response to various stressors. We believe that this is instructive, considering that research in this area has been much more comprehensive than that more recently initiated using stroke models.

Numerous studies have demonstrated the effects of stress on BDNF expression and brain function via altered neurogenesis, dendrite length and spine density (Leuner & Shors, 2013). BDNF and TrkB receptors have been closely associated with structural and functional aspects of the adaptive response of hippocampal neurons to stress (Badowska-Szalewska, Spodnik, Klejbor, & Morys, 2010; Ewa, Beata, Ilona, Dariusz, & Janusz, 2012; Schaaf, De Kloet, & Vreugdenhil, 2000). Noteworthy studies have reported a striking contrast in dendritic remodeling patterns upon stress exposure between neurons of the hippocampus and the amygdala, involving atrophy

(shortening and debranching of apical dendrites) in pyramidal neurons of the hippocampus (J. J. Kim & Diamond, 2002; Lambert et al., 1998; Watanabe, Gould, & McEwen, 1992), and hypertrophy (enhanced dendritic arborization) in pyramidal and stellate neurons in the basolateral amygdala (Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005; Vyas, Jadhav, & Chattarji, 2006; Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002; Vyas, Pillai, & Chattarji, 2004), associated to memory impairments and anxiety-like behavior in the Elevated plus Maze (EPM), respectively. In support of these findings, various forms of chronic stress lead to reversible, but long-lasting, spatial memory impairments in rodents associated to retraction of apical dendrites of CA3 pyramidal neurons, suppression of hippocampal synaptic plasticity and altered expression of synaptic cell adhesion molecules in the hippocampus (Conrad, Galea, Kuroda, & McEwen, 1996; Luine, Villegas, Martinez, & McEwen, 1994; Magarinos, Orchinik, & McEwen, 1998; Watanabe et al., 1992; Woolley, Gould, & McEwen, 1990a) while also facilitating amygdaloidal related aversive learning (McGaugh, 2004; Poulos et al., 2009). Transgenic overexpression of BDNF increases spinogenesis in the BLA and anxiety-like behavior, (Govindarajan et al., 2006). Similarly, BDNF mRNA and protein levels, as well as TrkB phosphorylation increase in the amygdala hours following fear conditioning, and infusion of TrkB antagonists, before training impairs fear memory (Ou, Yeh, & Gean, 2010; Rattiner, Davis, French, et al., 2004). Interestingly, recent studies show BDNF to be increased in the dorsal and decreased in the ventral sub-region of the hippocampus following radial arm water maze training stress, suggesting that the dorsal portion is primarily associated with spatial navigation learning and the ventral portion regulating stress effects (Hawley, Morch, Christie, & Leasure, 2012; Zoladz et al., 2012). The duration of the stressor also appears determinant. For example, chronic immobilization stress (2h/day for 10 days) increases BDNF protein expression

in the BLA and decreases expression in the CA3 24 h following stress exposure, changes persisting up to 21 days in the BLA, while acute immobilization stress (a single 2 h episode) upregulates BDNF protein levels in the BLA although induces no change in the CA3, 24 h and 10 days later (Lakshminarasimhan & Chattarji, 2012). At the behavioral level, anxiogenic effects in the EPM have been observed following a 21 day stress-free recovery period and associated with dendritic hypertrophy in BLA neurons and atrophy in CA1 and CA3 pyramidal neurons of the hippocampus, although hippocampal changes are reversed upon an additional 21 day stress-free period (Vyas et al., 2006; Vyas et al., 2004). Of interest, while predator stress potentiates dendritic morphology changes in the amygdala in maladapted highly anxious rats (Adamec, Blundell, & Burton, 2005; Adamec, Blundell, & Collins, 2001), well adapted rats, which have low anxiety phenotypes, exhibit densely packed and shorter dendrites in BLA neurons (Mitra, Adamec, & Sapolsky, 2009). This resilience trait characterized by differential morphological effects in response to stress is further characterized by the upregulation of activity-regulated cytoskeletal (Arc*)* gene expression in the hippocampus CA1 neurons of well-adapted rats (Kozlovsky et al., 2008), while a down regulation of BDNF and up regulation of TrkB receptors is present in maladapted rats (Kozlovsky et al., 2007; Shi, Shao, Yuan, Pan, & Li, 2010).

The medial prefrontal cortex (mPFC) also undergoes extensive dendritic changes following various types of stressors (Amat et al., 2005; Radley et al., 2006). For instance, either daily 10 min restraint stress for one week (S. M. Brown, Henning, & Wellman, 2005), 21 days of daily 3-6 hour restraint stress (Cook & Wellman, 2004; Radley et al., 2006) or 21days of daily corticosterone injections (Wellman, 2001) result in typical changes restricted to apical dendrites of pyramidal neurons in the mPFC layers II-III, including the retraction of distal dendritic branches and spine loss. Moreover, the mPFC responds to stress in a lateralized fashion, in that

minor challenges stimulate the left hemisphere whereas severe stress activates the right hemisphere (Sullivan, 2004). In support of hemispheric asymmetry in dendritic alterations in the mPFC, 6 h-daily restraint over a week period leads to reduced length in basilar dendrites affecting the right hemisphere while a reduced number of spines on proximal dendrites is observed in both hemispheres. Postnatal separation stress also induces long lasting changes on pyramidal neurons, including altered dendritic complexity and spine density in the anterior cingulate cortex, the infralimbic cortex , the hippocampal formation and the amygdala (Bock, Gruss, Becker, & Braun, 2005; Helmeke, Ovtscharoff, Poeggel, & Braun, 2001; Helmeke, Poeggel, & Braun, 2001; Ovtscharoff & Braun, 2001; Poeggel et al., 2003). In adult rodents which were repeatedly stressed during the first postnatal weeks or raised in chronic social isolation after weaning, dendritic atrophy and spine loss are observed in the mPFC and in the hippocampal formation (Radley et al., 2006; Silva-Gomez, Rojas, Juarez, & Flores, 2003). In addition, prenatally stressed pubertal males show increased dendritic morphology in the CA1 and a decrease in the CA3 subfields of the hippocampus whereas, prenatally stressed adult rats show decreased spine density in both regions of the hippocampus (Martinez-Tellez, Hernandez-Torres, Gamboa, & Flores, 2009). Prenatal stress also leads to dendritic atrophy of neurons in the mPFC, more specifically, the anterior cingulate and orbitofrontal cortices (Murmu et al., 2006), especially when combined with postnatal chronic mild stress (Michelsen et al., 2007). In contrast, complex social housing for 12 weeks increases dendritic branching and length as well as spine density in the neocortical and parietal cortex (Sutherland, Gibb, & Kolb, 2010).

In recent years, BDNF expression in the ventral tegmental area (VTA)-nucleus accumbens (NAc) pathway has been suggested to contribute to the development of a depression-like phenotype in rodents. BDNF activity is upregulated by chronic social-defeat stress in the

NAc, and BDNF knockdown, in the mesolimbic dopamine pathway of adult mice, reduces susceptibility to stress and promotes antidepressant-like responses (Berton et al., 2006). In contrast, microinjections of BDNF into the NAc increases stress susceptibility (Krishnan et al., 2007) and anhedonic responses in the sucrose consumption test following chronic mild stress is associated with increased NAc-BDNF mRNA expression, which can be reversed by antidepressants (Bessa et al., 2013). Similarly, social defeat stress increases BDNF protein and mRNA expression in the VTA and NAc, associated with increased social avoidance behavior (Fanous, Hammer, & Nikulina, 2010; Johnston, Herschel, Lasek, Hammer, & Nikulina, 2015; Nikulina, Lacagnina, Fanous, Wang, & Hammer, 2012), effects that are prevented by BDNF depletion in the VTA (Fanous, Terwilliger, Hammer, & Nikulina, 2011). Intra-VTA BDNF infusion and virally-induced expression of the BDNF receptor in the NAc both accelerate the onset of immobility in the forced swim test (FST) (Eisch et al., 2003).

The integrative action of CRH is proposed to play an important role in the regulation of emotional tone within the neuroendocrine and the mesolimbic systems (Zorrilla, Logrip, & Koob, 2014), with CRH being proposed as a contextual signal required for phasic stimulation of BDNF secretion acting to regulate mood upon stress exposure (Walsh et al., 2014). For instance, anxiety (light/dark box test), anhedonia (sucrose preference test), and depressive-like behavior (FST), as well as increased ACTH and corticosterone plasma concentrations observed following chronic stress exposure coincides with increased CRH and BDNF mRNA expression in the hypothalamus and pituitary (Naert et al., 2011). Similarly, repeated stress exposure increases BDNF mRNA in CRH neurons of the PVN, lateral hypothalamus and pituitary, while decreased BDNF mRNA levels are observed in the hippocampus and cingulate cortex (M. A. Smith, Makino, Kim, & Kvetnansky, 1995). Notably, antidepressant treatment prevents stress-induced

increased CRH-ir in the PVN, TH-ir in the locus coeruleus and decreased BDNF mRNA levels in the hippocampus (B. Lee, Shim, Lee, & Hahm, 2013). Antidepressant treatment has also been shown to increase expression of BDNF phosphorylated CREB in the prefrontal cortex through activation of a MAPK p38 and ERK1/2 cascade (Alboni et al., 2010). CRH can also activate ERK1/2 pathway through a calcium-dependent early phase activation of cAMP and a second phase that internalizes CRHR1 in hippocampal neurons (Bonfiglio et al., 2013). These findings are important to consider, as adaptive changes, affecting regulation of stress signals, would likely alter structural plasticity in the hippocampus, prefrontal cortex and amygdala, inducing associated memory and emotional behaviors (B. S. McEwen, 2010). Such interactions within discrete brain pathways are susceptible to play a role in regulating mood. Akin to stressors, the cAMP-CREB cascade, involving modulation of BDNF expression, represents a crucial signaling pathway in brain plasticity induced following focal and global cerebral ischemia (M. W. Kim et al., 2005; J. H. Lee et al., 2009). At present, its contribution to recovery of cognitive and emotional processes remain largely unknown.

7. Can CRH play a role in regulating neuroplastic changes post ischemia?

Considering that global ischemia mimics different effects associated with stressor exposure, and the acknowledged contribution of CRH in regulating excitatory glutamatergic synaptic transmission in limbic circuits (Gallagher, Orozco-Cabal, Liu, & Shinnick-Gallagher, 2008), we believe that the neuropeptide could play a significant role in neuroplasticity following global ischemia. For example, electrophysiology recordings from neurons in the CeA, show that CRH enhances the frequency of spontaneous excitatory postsynaptic currents, suggesting that CRH can increase presynaptic glutamate release (Silberman & Winder, 2013). The role of CRH in regulating memory processes and emotional tone is also pertinent to ischemia-induced

cognitive impairments and post-stroke depression (Deak et al., 1999; H. L. Wang, Wayner, Chai, & Lee, 1998). Notably, recent research indicates bidirectional influence of CRH and BDNF signaling in the brain. Thus, BDNF through TrkB-CREB signaling induces the expression of CRH in the PVN, without increasing corticosterone levels (Jeanneteau et al., 2012). BDNF has been considered a stress-responsive intercellular messenger since central administration of BDNF induces a gradual increase in the CRH mRNA signal in the parvocellular portion of the PVN, accompanied by increases in ACTH and corticosterone plasma concentrations (Givalois et al., 2004). CRH injected into the dentate gyrus produces a dose-dependent (0.1µg, 1.0 µg and 10 µg) increase in BDNF mRNA levels, and consistently and markedly improves retention performance of rats in an inhibitory avoidance learning task, an effect that could be blocked by BDNF antisense oligonucleotide treatment (Ma, Chen, Wei, & Lee, 1999). Upregulation of BDNF also occurs via a CRHR1 activated cyclic AMP-Protein kinase A (PKA)-CREB signaling pathway in cerebellar granular cultures (see figure 2 in appendix A) (Bayatti, Hermann, Lutz, & Behl, 2005). Importantly, CRHR1 activation has been identified as a key regulator of the extracellular signal-regulated kinase–mitogen-activated protein kinase (ERK-MAPK) cascade, which potentiates BDNF-stimulated TrkB signaling in locus coeruleus neurons (Traver, Marien, Martin, Hirsch, & Michel, 2006). In vitro, a 1 h CRH exposure has been shown to impair synaptic plasticity in hippocampal neurons and expression of the postsynaptic density protein 95 (PSD-95) (Y. Chen, E. A. Kramar, et al., 2012; Gounko et al., 2012). CRH overexpression also impairs CREB phosphorylation in the hippocampus (Kasahara, Groenink, Olivier, & Sarnyai, 2011), and reduces BDNF transcription in the hippocampal CA1 region (Flandreau, Ressler, Owens, & Nemeroff, 2012).

Via its ability to regulate numerous brain functions and processes affected post ischemia, CRH could play a determinant role in post ischemic outcomes. CRH regulates learning and memory processes by binding to its receptors in the hippocampus and amygdala (E. H. Lee, Hung, Lu, Chen, & Chen, 1992; E. H. Lee, Lee, Wang, & Lin, 1993; J. Radulovic, Ruhmann, Liepold, & Spiess, 1999a; Zorrilla, Schulteis, et al., 2002), where it modulates synaptic plasticity by promoting neuronal excitability through increased LTP (Bayatti, Zschocke, & Behl, 2003). Transient increase facilitates learning and memory (E. H. Lee et al., 1993; J. Radulovic et al., 1999a; Row & Dohanich, 2008) while chronically elevated CRH levels leads to progressive loss of hippocampal pyramidal neurons and impairs spatial memory (Brunson, Eghbal-Ahmadi, Bender, Chen, & Baram, 2001; Fenoglio, Brunson, & Baram, 2006; Heinrichs et al., 1996). Transgenic mice overexpressing CRH exhibit poor learning performance in the Morris Water Maze (Heinrichs et al., 1996; Koob, Cole, Swerdlow, Le Moal, & Britton, 1990; Stenzel-Poore, Duncan, Rittenberg, Bakke, & Heinrichs, 1996). Previous studies show that CRH increases neuronal excitability via a lasting enhancing effect on synaptic efficacy in the hippocampus (H. L. Wang et al., 1998), CA1 population spikes, and firing frequency of rat hippocampal neurons (Aldenhoff, Gruol, Rivier, Vale, & Siggins, 1983; Blank, Nijholt, Eckart, & Spiess, 2002; Hollrigel, Chen, Baram, & Soltesz, 1998). CRH signaling within the amygdala and the hippocampus has been shown to affect acquisition and consolidation of recent (Kolber et al., 2008; Pitts & Takahashi, 2011; Pitts, Todorovic, Blank, & Takahashi, 2009; Roozendaal, Brunson, Holloway, McGaugh, & Baram, 2002) and remote (Thoeringer et al., 2012) memories. Enhancement of synaptic hippocampal function following acute stress has been shown to involve CRH release within the hippocampus and excitatory neuromodulating action of CRH released by

cortical and hippocampal GABAergic interneurons (Becker et al., 2007; Y. Chen, Bender, Frotscher, & Baram, 2001; Joels & Baram, 2009; Swanson, Sawchenko, Rivier, & Vale, 1983).

In the hippocampus CA1 and CA3 pyramidal layers, CRH is released from GABAergic interneurons and binds on CRHR1 receptors located on the dendritic spines of glutamatergic neurons (Y. Chen, Andres, Frotscher, & Baram, 2012; Y. Chen, E. A. Kramar, et al., 2013). Thus, CRH increases neuronal excitability of CA1 pyramidal neurons by modulating somatic voltage-gated ionic currents important for the generation of action potentials, an effect that is mediated through activation of CRHR1 expressed on glutamatergic neurons (Kratzer et al., 2013). Acute stress activates CRHR1 on CA1 and CA3 pyramidal cells, enhancing neuron excitability and promoting synaptic efficacy and LTP (Blank et al., 2002; Y. Chen et al., 2004; Y. Chen, Fenoglio, Dube, Grigoriadis, & Baram, 2006; Cibelli, Corsi, Diana, Vitiello, & Thiel, 2001; X. X. Yan, Baram, Gerth, Schultz, & Ribak, 1998). However, CRH overexpression impairs LTP and enhances LTD leading to spatial learning and memory deficits (Ivy et al., 2010; J. J. Kim & Diamond, 2002).

Multiple studies support CRH effects on hippocampal plasticity to be preferentially mediated by CRHR1 activation. CRH-CRHR1 signaling has been shown to play a role in promoting discrete plastic changes, such as nectin-3–mediated axodendritic adhesion in hippocampal neurons, which mediates learning and memory function under basal conditions (X. D. Wang et al., 2013). Increased dendritic branch length of primary hippocampal culture neurons upon exposure to CRH can be reversed when cells are treated with CRHR1 antagonist, Antalarmin, but not with CRHR2 antagonist Astressin 2B (Sheng et al., 2012). Under conditions of stress, dendritic atrophy of CA1 pyramidal cells and memory impairments observed in rats have been prevented by CRHR1 blockade (Y. Chen et al., 2010; X. D. Wang, Y. Chen, et al.,

2011b). CRH-induced dendritic spine loss through CRHR1 signaling requires the activation NMDA receptors and downstream recruitment of calcium-dependent enzyme, calpain, resulting in the breakdown of spine actin-interacting proteins including spectrin (Andres et al., 2013). Forebrain CRHR1 deficiency blocks spatial memory impairment, and promotes CA1 LTP and spine density in the CA3 hippocampal areas in mice exposed to 21 days social-defeat stress (X. D. Wang, G. Rammes, et al., 2011). Central administration of CRHR1 antagonist, NBI 30775, 30 min prior restraint stress prevents hippocampal spine loss and dendritic atrophy via regulation of actin filaments located in the cytoskeleton of synaptic spines (Y. Chen, Dube, Rice, & Baram, 2008) while chronic treatment with the CRHR1 antagonist, SSR125543, prevents the decrease of hippocampal neuronal synaptic transmission associated with object recognition deficits in mice exposed to unavoidable foot-shock stress (Philbert, Belzung, & Griebel, 2013). Bilateral infusion of CRHR1 antisense oligodeoxynucleotide in the dorsal hippocampus, 48 h following 10 min predator odor exposure stress, prevents depletion of BDNF protein levels and anxiety (Kozlovsky, Zohar, Kaplan, & Cohen, 2012). In sum, although mechanisms remain to be elucidated, these studies support bidirectional interplay between CRH and BDNF in various brain circuits.

8. HPA Activation and Emotional Impairment Following Stroke and Cardiovascular Accidents

Depression is the most commonly observed mood disorder following stroke, expressed in the early acute phase with depressive symptoms persisting after 1 year, and associated with stroke severity, neurological impairment, poor recovery in activities of daily life, and higher risk of mortality (Kouwenhoven, Kirkevold, Engedal, & Kim, 2011; Robinson & Spalletta, 2010; Vataja et al., 2001). A recent meta-analysis found that approximately one third of stroke patients

experience depression in the first weeks to months following stroke (Ayerbe, Ayis, Wolfe, & Rudd, 2013). Stroke survivors are 6 times more at risk of developing depression compared to normal aging controls (M. Santos et al., 2009). Conversely, depression increases the risk of developing stroke, independently of hypertension and diabetes (Dong, Zhang, Tong, & Qin, 2012) and is associated with a 2-4 fold increased risk of developing cardiovascular disease and mortality after myocardial infarction (Joynt, Whellan, & O'Connor, 2003; Liebetrau, Steen, & Skoog, 2008). Suicidal thoughts can develop shortly after the onset of acute stroke, especially in patients with recurrent strokes, and who have depression prior or after having a stroke (Kishi, Kosier, & Robinson, 1996; Kishi, Robinson, & Kosier, 1996; C. O. Santos, Caeiro, Ferro, & Figueira, 2012). Noteworthy, post stroke depression (PSD) is consistently associated with cognitive impairments such as impaired learning, executive dysfunction, and memory, orientation, language and attention deficits (Chatterjee, Fall, & Barer, 2010; Fultz, Ofstedal, Herzog, & Wallace, 2003; Melkas et al., 2010; Spalletta, Guida, De Angelis, & Caltagirone, 2002). Cardiopulmonary arrest survivors similarly develop a broad range of neurological and behavioral impairments affecting daily functioning, participation in society and quality of life (Lundgren-Nilsson, Rosen, Hofgren, & Sunnerhagen, 2005; Pearn, 2000; Wachelder et al., 2009). Impaired attention and executive function (Mangus et al., 2014; Moulaert, Verbunt, van Heugten, & Wade, 2009), along with emotional disorders including anxiety and depression (Larsson, Wallin, Rubertsson, & Kristofferzon, 2014; Miranda, 1994; Moulaert, Wachelder, Verbunt, Wade, & van Heugten, 2010; Reich, Regestein, Murawski, DeSilva, & Lown, 1983) are common sequelae. These impairments in cardiac-arrest-survivors have largely been attributed to neuronal damage affecting hippocampal neurons and are linked to cognitive impairments in spatial and episodic/declarative memory, which are also evident in animal models of global

cerebral ischemia (Blum et al., 2012; Gainotti et al., 2004; Petito, Feldmann, Pulsinelli, & Plum, 1987; Taraszewska, Zelman, Ogonowska, & Chrzanowska, 2002).

Clinical studies revealed that 40 % of acute ischemic stroke survivors display both anxiety and depressive symptoms (Vuletic, Sapina, Lozert, Lezaic, & Morovic, 2012). Anxiety is more frequently reported than depressive symptoms in the acute stage of ischemic stroke and although there is comorbidity between the two, they often occur independently in stroke patients (Barker-Collo, 2007; Fure, Wyller, Engedal, & Thommessen, 2006), supporting multifactorial changes related to cerebrovascular disease, and the possibility that depression occurs as an expression of altered brain function and not merely a psychological response to an adverse situation (Vuletic et al., 2012). Neuroimaging studies suggest that PSD is aggravated by the proximity of macrovascular lesions to anterior and posterior areas of the prefrontal cortex (Barker-Collo, 2007; Hama et al., 2007; Naarding et al., 2007; A. Singh et al., 2000; Tateno, Kimura, & Robinson, 2002; Vataja et al., 2004). Extensive research, captured by the vascular depression hypothesis (M. K. Sun & Alkon, 2013) supports effects of ischemic insults on brain structures and neural circuits involved in mood regulation. These include the medial prefrontal cortex, amygdala, hippocampus, and ventromedial parts of the basal ganglia (Drevets, Price, & Furey, 2008; Robinson, Kubos, Starr, Rao, & Price, 1984), which are important sites for glucocorticoid negative feedback (S. M. Smith & Vale, 2006). Cumulative microvascular lesions in these brain regions are also associated with PSD (Brodaty, Withall, Altendorf, & Sachdev, 2007; Nishiyama et al., 2010; M. Santos et al., 2009).

The vulnerability of the hippocampus to neuronal damage following cerebral ischemia and its key role as negative feedback regulator of HPA axis activity has led to suggest that disruption of HPA axis functioning likely contributes to functional impairments (E. S. Brown,

Rush, & McEwen, 1999). Dysregulation of the HPA axis is characterized by diminished feedback inhibition by glucocorticoid accompanied by increased CRH secretion from the hypothalamic PVN and extra-hypothalamic neurons (Gadek-Michalska, Tadeusz, Rachwalska, & Bugajski, 2013), see figure 3 in appendix A. Glucocorticoid hormones terminate stress response via a negative feedback action at the levels of the pituitary, hypothalamus and limbic brain areas, the latter of which includes the hippocampus, amygdala and septum (Kellendonk, Gass, Kretz, Schutz, & Tronche, 2002). For example, daily injection of corticosterone for 21 consecutive days decreases body weight and increases immobility behavior in the FST (Gregus, Wintink, Davis, & Kalynchuk, 2005; S. A. Johnson, Fournier, & Kalynchuk, 2006). In addition, chronic corticosterone exposure reduces open-arm exploration in the EPM, and duration of head dipping activity in the hole-board test, as well as increases serum levels of corticosterone, CRH expression in the hypothalamus, and TH expression in the locus coeruleus, while also decreasing BDNF and TrkB mRNA expression in the hippocampus (B. Lee et al., 2014; H. Lim et al., 2012). Reduced hippocampal BDNF secretion may limit the ability of the hippocampus to provide HPA axis inhibition under stressful conditions (Murakami, Imbe, Morikawa, Kubo, & Senba, 2005), while increased BDNF expression in the PVN under such conditions may promote hyperactivation of the HPA axis (Givalois et al., 2004).

In recent years, BDNF has also received attention as a critical physiological regulator of depression and anxiety, with antidepressant drug treatments exerting significant effects to regulate levels in the prefrontal cortex and the hippocampus (Castren, 2005; Castren, Voikar, & Rantamaki, 2007). Depression is known to lower BDNF expression in the hippocampus, in part through modification of the chromatin structure (R. S. Duman, 2005; R. S. Duman & Monteggia, 2006). A meta-analysis examining serum BDNF levels in depression confirms that serum BDNF

levels are lower in patients with depression when compared to healthy control subjects (Sen, Duman, & Sanacora, 2008). BDNF expression in the hippocampus is increased in post-mortem brains of subjects treated with antidepressants at the time of death (B. Chen, Dowlatshahi, MacQueen, Wang, & Young, 2001). BDNF expression is upregulated by antidepressant treatment (Dias, Banerjee, Duman, & Vaidya, 2003) and BDNF induces direct antidepressant-like effects in rats (Siuciak, Lewis, Wiegand, & Lindsay, 1997). Postmortem analysis of depressed patients' brains revealed reduced BDNF levels, which appeared restored by antidepressant treatment (Karege, Bondolfi, et al., 2005; Karege, Vaudan, Schwald, Perroud, & La Harpe, 2005). Notably, chronic peripheral diffusion of BDNF prevents reduced neurogenesis and depression and anxiety behaviors induced following chronic unpredictable stress in mice (Schmidt & Duman, 2010). Reduced hippocampal volume is amongst the most frequently reported finding in neuroimaging studies of major depression patients (Cole, Costafreda, McGuffin, & Fu, 2011) and stress is a common risk factor for stroke in humans (Guiraud, Amor, Mas, & Touze, 2010). Thus, ischemia puts individuals at high risk of developing depression, as common underlying mechanisms resemble those of nonvascular depression such as sensitivity to stress and altered neurotrophic activity.

9. Objectives of the Study

The relationship between stress intensity and cognitive function follows an inverted U shaped curve response whereby memory performance is improved in conditions of optimal stressor intensity, while impaired under conditions above or below optimal stress (Martin et al., 2009; Salehi, Cordero, & Sandi, 2010). Recent experiments have demonstrated the beneficial effects of reducing corticosterone and norepinephrine levels, through pharmacological treatment, on memory performance of ischemic animals in spite of hippocampal degeneration at time of

testing (M. R. Milot & Plamondon, 2011b), suggesting that changes in stress response and/or emotional reactivity play a significant role in ischemic impairments. Sunstained CRH elevations contribute to region-specific dendritic remodeling via CRHR1 activation, leading to cognitive and emotional behavioral changes. Despite the increasing evidence supporting the bidirectional relationship between CRH and BDNF in depression and chronic stress, it has yet to be examined after global cerebral ischemia.

The overall goal of this thesis aims to verify whether this neurochemical stress pathway, involving BDNF and CRH, is the subcellular basis of emotional and cognitive impairments observed following global cerebral ischemia. Together with immunohistochemical staining of BDNF, TrkB, CRH and CRHR1 in limbic structures of the brain, this behavioral data will serve to foster a better understanding of the relationship between endogenous regulators of brain plasticity and of the neuroendocrine stress system. Considering ischemic stroke as a severe systemic stressor, the current thesis project aims to answer the question as to whether CRH through CRHR1 signaling may regulate region specific alterations in neuronal plasticity as evidenced through BDNF/TrkB expression following cerebral ischemia. Ischemia-induced changes in BDNF/TrkB mRNA or protein expression are expected to reflect the neuroplastic changes observed in animals models of chronic stress and depression, where BDNF is down-regulated by stress in the hippocampus and frontal cortex but is increased by stress in the nucleus acumens and amygdale (see figure 4 in appendix A).

Furthermore, the current thesis will verify emotional and cognitive dysfunction in ischemic animals that may be explained by neurotrophic changes orchestrated through CRHR1 signaling. More precisely, this research project characterizes: (1) the possibility of persistent dysregulation of the neuroendocrine system post ischemia by observing changes in

immunoreactivity of stress markers in discrete brain regions of the limbic system; (2) the effects of global cerebral ischemia, and selective CRHR1 blockade on HPA function and corticosterone secretion at multiple baseline intervals and following an acute restraint stress; (3) the effect of cerebral ischemia and selective CRHR1 blockade on site-specific changes in mRNA, protein and immunoreactivity of BDNF and associated markers of neural plasticity examined 30 days post ischemia; (4) the impact that global ischemia, alone and combined with prior blockade of CRHR1 receptors, exerts on social, depressive-like, anxiety-like and cognitive behavior; (5) the impact of cerebral ischemia and CRHR1 antagonist on hippocampal neuronal degeneration and inflammation. We predict that intracerebroventricular administration of CRHR1 antagonist prior to ischemia will attenuate ischemia-induced site-specific changes in BDNF expression, leading to improved cognitive and emotional responses post ischemia. In the hopes of discovering potential therapeutic strategies, this thesis project will contribute to uncovering the molecular and cellular mechanisms underlying emotional and cognitive vulnerability observed following forebrain ischemia starting in animals and later being applied in humans.

Appendix A

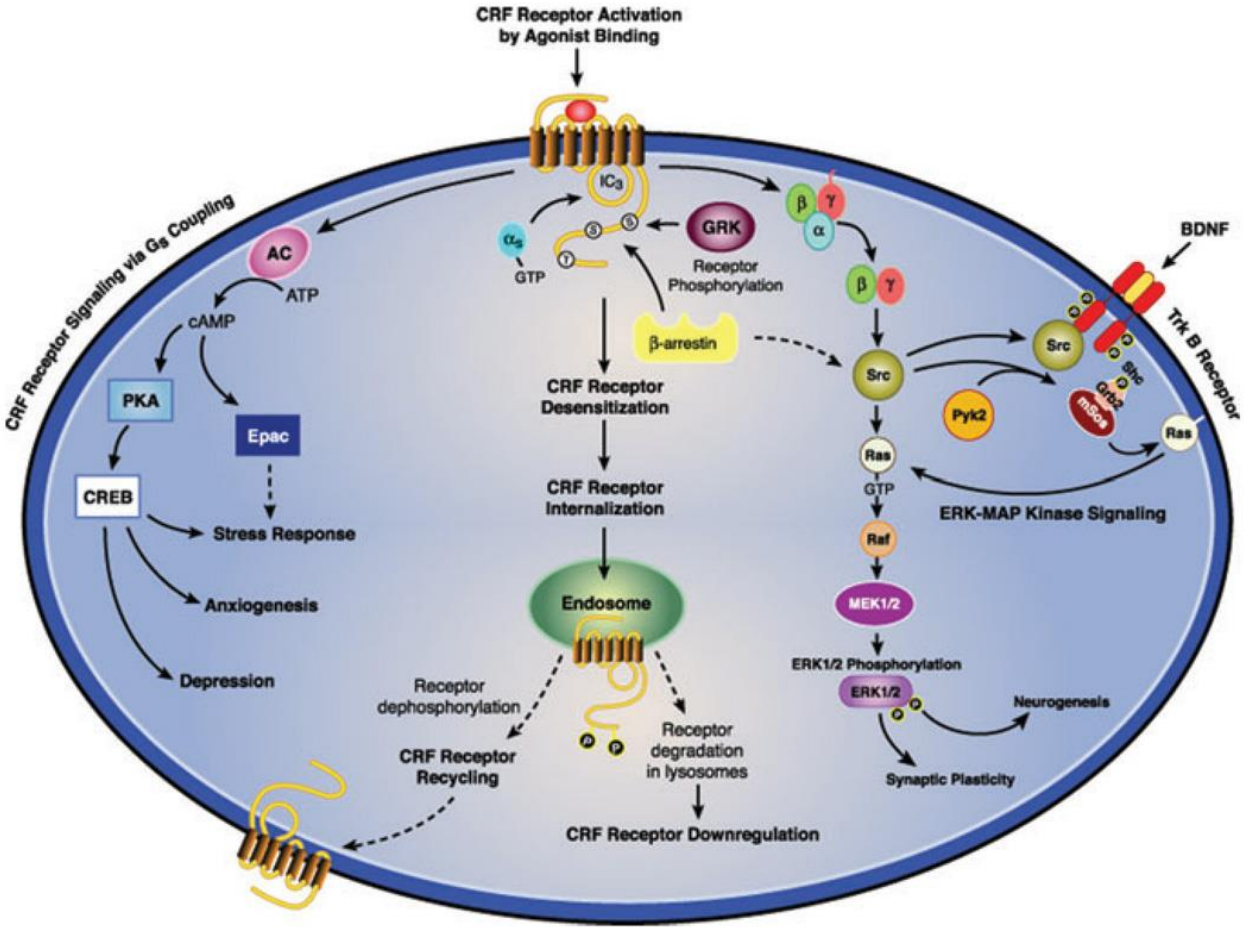

Figure 1. Major intracellular signal transduction pathways for CRF_1 and CRF_2 receptors. While the dominant mode of signaling for both CRF receptors signaling is Gs-coupled AC-PKA cascade, they may also signal via the PLC-PKC and ERK-MAPK cascades. CRF receptor AC-PKA signaling is stringently regulated by GRK- and arrestin-mediated homologous desensitization. Although the mechanisms regulating CRF receptor ERK-MAPK signaling are not yet fully understood, this pathway may potentiate BDNF-stimulated TrkB receptor function. Adapted from "Role of CRF receptor signaling in stress vulnerability, anxiety, and depression" by Hauger, Risbrough, Oakley, Olivares-Reyes, & Dautzenberg, 2009, Ann N Y Acad Sci, 1179, 120-143.

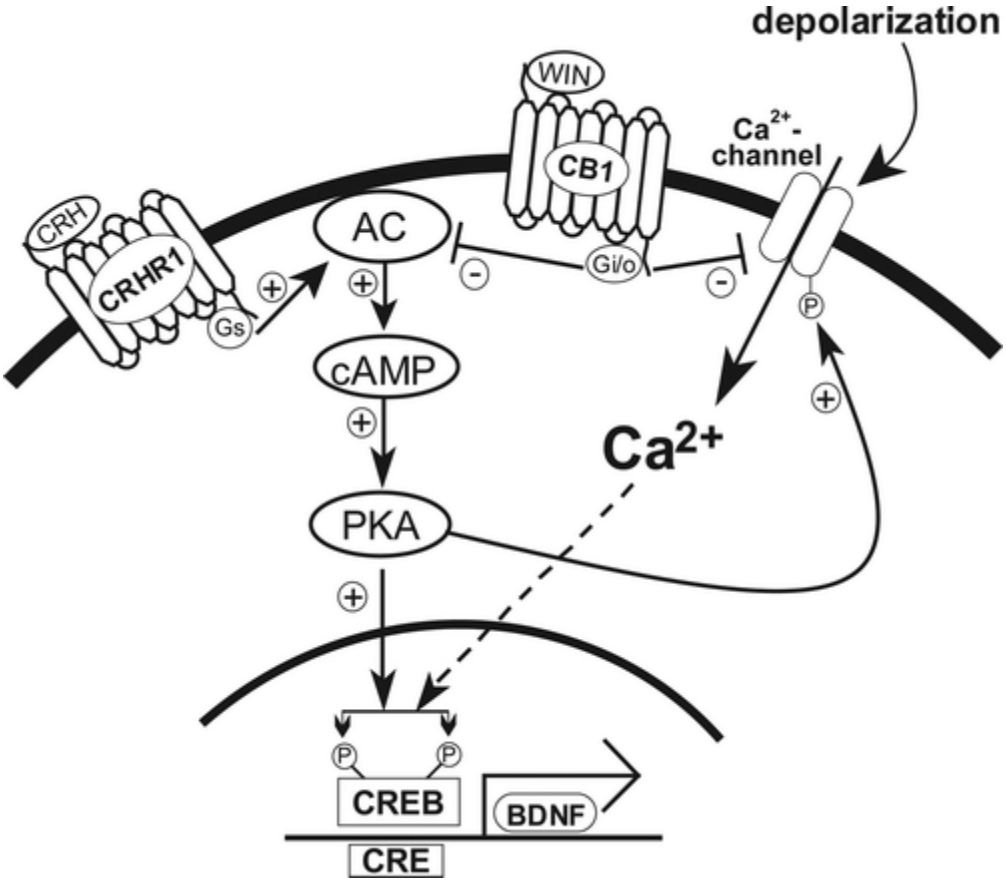

Figure 2. A schematic representation showing the intracellular pathways that may mediate the interaction between CRHR1 and CB1, leading to the regulation of BDNF expression. Stimulation of CRHR1 with CRH activates AC, and subsequent cAMP production leads to the activation of PKA. Activated PKA phosphorylates CREB, which initiates the expression of the BDNF. Moreover, PKA is known to phosphorylate Ca2+ channels, which would lead to an increased influx of Ca2+ and thus activation of BDNF expression. Depolarization of the cells with KCl (35 mm) also leads to increased BDNF expression. Adapted from "Corticotropin-releasing hormone-mediated induction of intracellular signaling pathways and brain-derived neurotrophic factor expression is inhibited by the activation of the endocannabinoid system" by Bayatti, N., Hermann, H., Lutz, B., & Behl, C. (2005), Ann N Y Acad Sci, 1179, 120-143. Endocrinology, 146(3), 1205-1213.

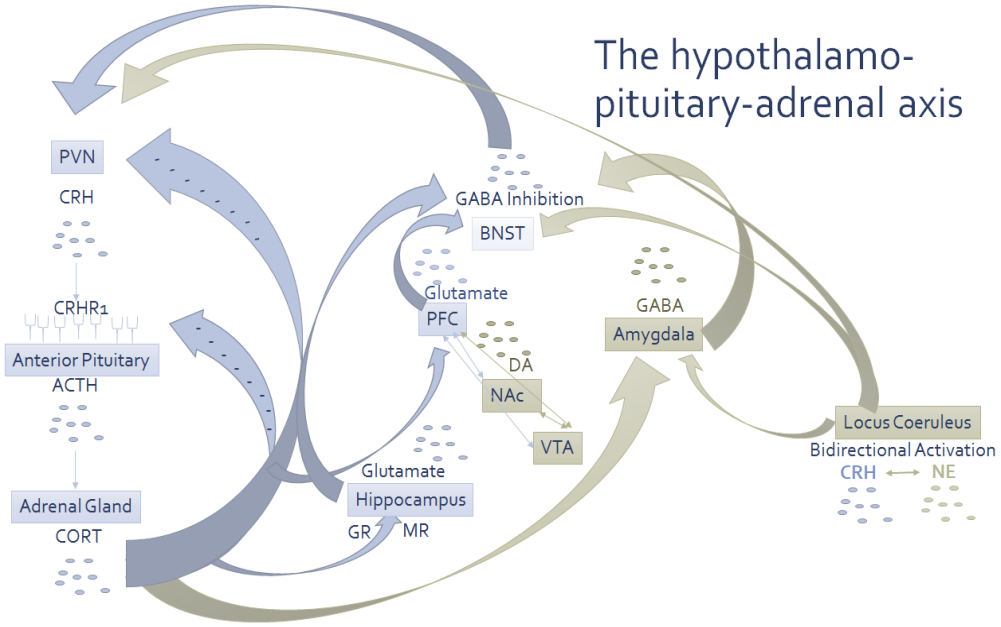

Figure 3. A schematic representation showing the feedback control of the HPA axis. The HPA axis is mediated by activation of specific neurons in the medial parvocellular subdivision of the paraventricular nucleus that synthesize and release CRH into the portal system of blood vessels of the median eminence to stimulate release of ACTH into the systemic circulation, through CRHR1-induced cleavage of POMC by stimulating cAMP generation, from the anterior pituitary gland, which in turn stimulates glucocorticoid synthesis and secretion from the adrenal cortex. The HPA axis is finely tuned via negative feedback from circulating glucocorticoids that bind to two receptor subtypes, the high-affinity mineralocorticoid receptor (MR) and lower affinity glucocorticoid receptor (GR). Direct inhibition of the HPA occurs through GR effects on corticotropic cells and on the CRH neurons. Glucocorticoids also act in the hippocampus through MR and GR, to increase CA1 firing. Hippocampal glutamatergic excitation may inhibit PVN neurons through an inhibitory circuit involving GABAergic projection from the bed nucleus of the stria terminalis (BNST) to CRH neurons. Other potential inhibitory sites for GR action are in the prefrontal cortex and in the basolateral amygdala (BLA); projections from these nuclei are also relayed to PVN through the BNST (shown by gray arrows). In contrast, GR in the central nucleus of the amygdala (CeA), and in nucleus accumbens (NAc)**,** inducing an increase in activity of dopaminergic neurons of the ventral tegmental area (VTA), have a stimulatory effect on HPA activity (shown by gold arrows). CRH activates norepinephrine (NE), and norepinephrine in turn activates CRH in the locus coeruleus in response to stressors. NE also stimulates CRH release in the PVN, BNST, and CeA. CRH is localized within a subpopulation of GABAergic neurons in CeA and BNST. CRH itself enhances GABA inhibitory postsynaptic potentials (IPSCs) in the CeA and BNST through CRHR1 activation. Pathophysiologies, including cerebral ischemia, which alter the ability of these brain regions to maintain tightly regulated HPA activity result in disturbances in CORT levels or excessive CORT secretion in response to stimuli.

Brain Region	Change
Hippocampus: CA1, CA3	↓ spine density in apical dendrites ↓ BDNF protein/mRNA ↓ PSD95 protein
PFC	↓ spine density ↓ BDNF
Amygdala: BLA	↑ spine density in apical and basal dendrites ↑ BDNF
Nucleus Accumbens	↑ spine density in Nac ↑ BDNF

Table 1. Dendritic spine density, and BDNF mRNA or protein expression in the depression neurocircuitry. Dendritic spine density and BDNF is down-regulated by stress in the hippocampus and cortex but increased by stress in the nucleus accumbens and amygdala.

Article 1

Published as: de la Tremblaye, P. B., Raymond, J., Milot, M. R., Merali, Z., & Plamondon, H. (2014). Evidence of lasting dysregulation of neuroendocrine and HPA axis function following global cerebral ischemia in male rats and the effect of Antalarmin on plasma corticosterone level. Horm Behav, 65(3), 273-284.

Authorial contributions

Experiment 1

Patricia Barra de la Tremblaye contributed to study design, performed and interpreted the study and statistical analysis, and drafted and revised the manuscripts. She also prepared all brain sections and performed the immunohistochemical analyses for all proteins and brain regions analyzed. Dr. Marc Milot performed ischemic surgeries, and behavioral testing in the Radial Arm Maze.

Experiment 2

Patricia Barra de la Tremblaye performed the surgeries for cannulae implantation, and four-vessel occlusions for all experimental groups. She also collected over a thousand blood samples with the help of Julie Raymond, an honor student co-supervised by Patricia. Via a collaboration with Dr. Zul Merali, Jon James assisted in running the radioimmunoassays to analyze CORT concentrations.

Dr. Hélène Plamondon supervised the research project, interpreted the statistical analysis, and revised the manuscript.

Abstract

Abnormal function of the neuroendocrine stress system has been implicated in the behavioral impairments observed following brain ischemia. The current study examined long-term changes in stress signals regulation 30 days following global cerebral ischemia. Experiment 1 investigated changes in the expression of corticotropin releasing hormone (CRH) and its subtype 1 receptor (CRHR1), glucocorticoid receptors (GR) in the paraventricular nucleus of the hypothalamus (PVN), the central nucleus of the amygdala (CeA), and the CA1 subfield of the hippocampus. Tyrosine hydroxylase (TH) was determined at the locus coeruleus (LC). Experiment 2 investigated the role of central CRHR1 activation on corticosterone (CORT) secretion at multiple time intervals following global ischemia after exposure to an acute stressor. Findings from Experiment 1 demonstrated a persistent increase in GR, CRH and CRHR1 immunoreactivity (ir) at the PVN, reduced GR and CRHR1 expression in pyramidal CA1 neurons, and increased LC TH expression in ischemic rats displaying working memory errors in the radial arm Maze. Findings from Experiment 2 revealed increased CORT secretion up to 7 days, but no longer present 14 and 21 days post ischemia. However upon an acute restraint stress induced 27 days following reperfusion, ischemic rats had increased plasma CORT secretions compared to sham-operated animals, suggesting HPA axis hypersensitivity. Antalarmin (2μg/2μl) pretreatment significantly attenuated post ischemic elevation of basal and stress-induced CORT secretion. These findings support persistent neuroendocrine dysfunctions following brain ischemia likely to contribute to emotional and cognitive impairments observed in survivors of cardiac arrest and stroke.

Keywords: HPA dysregulation, corticotropin releasing hormone, glucocorticoid receptors, CRHR1, immunohistochemistry, global cerebral ischemia, rat.

1. Introduction

The effects of transient global cerebral ischemia on rat behavior such as long-lasting spatial learning and memory deficits are commonly associated with selective neuronal damage of the CA1 hippocampal region (R. Hartman, J. Lee, G. Zipfel, & D. Wozniak, 2005; Lehotsky et al., 2009). However, increasing evidence supports the involvement of stress regulators in the pathophysiology of cerebral ischemia, which could contribute to behavioral impairments. For example, corticotropin releasing hormone (CRH) release, protein and mRNA expression as well as corticosterone (CORT) levels remain elevated for days in discrete brain regions and in the periphery following cerebral ischemia (Hwang et al., 2006; Khan et al., 2004; Wong et al., 1995). Past studies have also shown that plasma levels of norepinephrine (NE) are up-regulated during (Gustafson, Westerberg, & Wieloch, 1991), in the hours (Globus et al., 1989), and even weeks (Pich et al., 1993) after brain ischemia. These findings suggest that global ischemia represents a potent physiological stressor. If the effects of such a stressor persist over time, the allostatic load may result in maladaptive physiological and behavioral responses (Beauchaine, Neuhaus, Zalewski, Crowell, & Potapova, 2011).

Upon exposure to physiological or psychogenic stressors, the HPA axis initially responds via increased synthesis and release of CRH from the paraventricular nucleus of the hypothalamus (PVN). PVN CRH neurons project to the median eminence where CRH is released and binds to CRHR1 receptors in the anterior pituitary gland inducing the release of adrenocorticotropic hormone (ACTH) into the blood stream. ACTH subsequently triggers secretion of glucocorticoids from the adrenal cortex. A negative feedback mechanism by glucocorticoids then acts to inhibit HPA axis activation via mineralocorticoid (MR) and glucocorticoid (GR) receptors present at the pituitary, hypothalamus and hippocampus (J. M. Reul & de Kloet, 1985), which

regulate HPA activity in a complementary fashion; MRs regulate CORT secretion under basal conditions and are activated in the initial phase of the stress reaction, followed by GR activation to terminate the stress response (Joels, Karst, DeRijk, & de Kloet, 2008). Both MRs and GRs are highly abundant in CA1 pyramidal neurons of the hippocampus, a brain structure known to inhibit HPA activity as well as for its importance in learning and memory (Sarabdjitsingh, Meijer, Schaaf, & de Kloet, 2009). In response to stress, CRH also activates locus coeruleus (LC) neurons to stimulate the release of NE in forebrain terminal projections, which in turn stimulates the release of CRH at the PVN and central nucleus of the amygdala (CeA), indicating a strong relationship between NE and CRH in modulating HPA activation (Koob, 1999; Reyes, Valentino, & Van Bockstaele, 2008; Roozendaal, Schelling, & McGaugh, 2008). Among other brain regions, the amygdala has been shown to facilitate adrenocortical responses through interactions with the PVN (Feldman, Conforti, & Weidenfeld, 1995; Van de Kar & Blair, 1999). The lateral part of the CeA contains the highest density of CRH cell bodies within the amygdaloid complex while a more modest amount is found within the basolateral nucleus of the amygdala (BLA) (Hsu, Chen, Takahashi, & Kalin, 1998; Pitts et al., 2009). It is worthy of note that exposure to various stressors as well as ischemia increase extracellular CRH release and/or mRNA expression at the CeA (Hsu et al., 1998; Khan et al., 2004; Merali, McIntosh, Kent, Michaud, & Anisman, 1998; Merlo Pich et al., 1995).

Recently, findings from our laboratory (M. R. Milot & Plamondon, 2011a) demonstrated elevated CORT secretion in ischemic animals in response to Barnes Maze testing simultaneously with spatial memory deficits. A single dose of the glucocorticoid synthesis inhibitor metyrapone prior to vessel occlusion concomitantly attenuated post testing CORT secretion and spatial memory deficits in ischemic animals despite not providing neuroprotection in the hippocampal

CA1 area. In addition, pre-testing administration of the alpha2-adrenoceptor agonist clonidine (which inhibits NE release) attenuated ischemia-induced working memory impairments while opposite effects were obtained with the antagonist yohimbine. These findings support the notion that global ischemia sensitizes systems regulating stress responses and emotionality even at delayed intervals post-ischemia, effects which, if prevented, can reduce post ischemic memory impairments even in the presence of hippocampal damage.

Given the role of CRH and glucocorticoid in stress-induced emotional and cognitive impairments (Muravieva & Alberini, 2010; Ryan, Vollmayr, Klyubin, Gass, & Rowan, 2010; Vouimba, Yaniv, & Richter-Levin, 2007), the current study sought to characterize the impact of 10 minute global ischemia on long-term changes in the expression of key neuroregulators involved in the neuroendocrine stress response. Experiment 1 determined post ischemia immunochemical expression of GR, CRH and its type 1 receptor (CRHR1) in brain regions known to participate in HPA axis regulation and emotional and memory functions, namely the hypothalamic PVN, the amygdalar CeA, the CA1 of the hippocampus. Further, tyrosine hydroxylase (TH) immunoreactivity in the LC at a delayed 30 day-interval following global ischemia was also determined. Spatial learning ability was tested using Radial Arm Maze task and hippocampal cell death was determined by quantification of CA1 neurons of the CA1 subfield. We then investigated whether a CRHR1 antagonist (Antalarmin) administered prior to ischemia would regulate plasma CORT levels across 30 days after reperfusion and affect hyper-arousal to stressful conditions in ischemic rats. To do so, Experiment 2 explored time-dependent changes in basal and stress-induced CORT secretion and the role of Antalarmin in adrenocortical glucocorticoid secretion. This study is the first to concomitantly characterize alterations of CRH, adrenocortical and the noradrenergic system at a delayed interval following global cerebral

ischemia and to assess the role of CRHR1 receptors in post ischemic glucocorticoid regulation under basal and stressful conditions. Together with prior evidence of high CORT secretion observed in response to behavioral testing, persistent changes in the expression of distinct stress signals would support a long-term impact of cerebral ischemia on HPA reactivity.

2. Materials and methods

2.1 General methodology

2.1.1 Animals

Male Wistar rats (N =16 and N = 40 in Experiments 1 and 2, respectively) weighing between 250–320g at time of surgery were obtained from Charles River Laboratories (Rochefort, Québec, Canada) and habituated to the housing facility for a minimum of two weeks before surgery. Rats were individually housed and maintained on a 12 h light/dark cycle (lights on at 7:00 AM) with free access to water and standard rat chow. Room temperature was maintained at 21–23 °C with 60% relative humidity. The experimenter handled all the rats daily for 2-3 min in the four days preceding the first day of surgery, and two days prior to initiation of spatial memory testing performed in Experiment 1. All experiments and procedures were in accordance with the guidelines set by the Canadian Council of Animal Care and approved by the University of Ottawa Animal Care Committee. Efforts were made to minimize the number of animals used and their suffering.

2.1.2 Four-vessel occlusion surgery

Forebrain ischemia was induced using the four-vessel occlusion (4VO) model as previously described (Pulsinelli & Brierley, 1979). Briefly, rats were anesthetized using isoflurane (2-3%) mixed with oxygen. The core temperature was maintained at 37±0.5 °C

throughout surgery and during ischemia by means of a feedback-regulated heating blanket connected to a rectal thermometer. The vertebral arteries were irreversibly occluded by electrocoagulation and a small-diameter silk thread looped around the carotid arteries to facilitate subsequent occlusion. Sham-operated animals underwent anesthesia and received the same dorsal and ventral surgical incisions as the ischemic group without electrocoagulation of the vertebral arteries. Twenty-four hours later, rats were briefly anesthetized and carotid arteries re-exposed for clamping. Cerebral ischemia occurred between 7:30-9:30 AM. After discontinuing anesthesia and at first sign of wakefulness (sniffing and limb movements) the pair of carotid arteries was occluded with microvascular clamps for a 10 min period, the rats freely ventilating.

2.2 Experiment 1

2.2.1 Spatial memory: Radial Arm Maze

Groups of sham (n=7) and ischemic (n=8) subjects were transported from their vivarium to the Radial Maze testing room and allowed to rest at least 30 minutes prior to testing (See Fig. 1 for experimental timeline). In order to ensure motivation to complete the Radial Maze tasks, rats were maintained at 90% of their body weight from day 7 post-reperfusion when pre-surgery weights were surpassed. Animals were weighed daily and received 15-20 grams of standard rat chow following behavioral testing. Every seven days 5 grams was added to their target weight to maintain a normal growth curve. The Radial Maze was evenly illuminated at 300 lux, surrounded by distinct extra-maze visuo-spatial cues, and elevated 50 cm above the floor. The maze consisted of eight arms (60x12 cm with a 5 cm lip around each arm) extending radially from a central octagonal area (32 cm in diameter with a 30 cm high clear Plexiglas wall). Plexiglas sliding doors allowed entry into each arm. The experimenter sat behind a panel where he could

observe and record behavior unobtrusively and manipulate the overhead strings to open and close the maze doors. During all testing the experimenter was blind to the groups.

Pre-training in the Radial Maze began on day 8 post ischemia and lasted four days. During the pre-training sessions, Froot Loop (FL) pieces were scattered in the arms and center of the Radial Maze, gradually reduced to be placed only in the small wells located at the end of the arms (and not visible to the rats from the center of the maze) on the fourth pre-training session. During this pre-training phase, rats were placed in the center of the maze facing a random direction, all 8 arms closed. All the arms were opened after 10 s and rats allowed exploring the maze and eating the food for 15 min. After returning to the central area following an arm entry, the Radial Maze arms were all closed and re-opened after a 10 s interval. After the last pre-training day, the training phase was initiated and conducted for 12 consecutive days (one trial per day) using the same procedure and with one FL piece placed in the well at the end of each of the eight arms. A working memory error was recorded when a rat re-entered a previously visited arm. A trial ended when a total of 9 arm entries had been made. The maze was cleaned between each trial with a 15% ethanol solution.

2.2.2 Brain tissue preparation

Thirty days following reperfusion, rats were deeply anesthetized using sodium pentobarbital and transcardially perfused with a solution of 0.9% saline, followed by 4% paraformaldehyde containing 0.2% picric acid in 0.1M phosphate buffer (pH= 7.4). The brains were removed from the skull, post fixed in the same fixative for 1h and then immersed overnight in a 20% sucrose solution and in a 10% sucrose solution for the subsequent 24 h, and then frozen and stored at -80 ◦C. Brains were sectioned into 14μm-thick coronal sections using a cryostat and mounted onto Superfrost Plus slides (Fisher Scientific, Canada). The localization of collected

brain tissue was determined for the different regions of interest according to coordinates from the Paxinos and Watson atlas (1997) (see fig.2): Paraventricular nucleus of the hypothalamus (PVN, -1.30 – -2.12 mm posterior to Bregma), the CA1 pyramidal layer of the hippocampus (CA1, -2.80 – -4.16 mm), the central nucleus of the amygdala (CeA, -2.30 – -3.30 mm), and the locus coeruleus (LC, Bregma -9.80 – -10.52 mm).

2.2.3 Immunohistochemical detection

Brain sections were washed in 0.01M phosphate-buffered saline (PBS) five times for three minutes and exposed to a pretreatment solution (97% PBS, 1% BSA, 2% Triton X-100) for 30 minutes at room temperature. Sections were then incubated overnight at room temperature using rabbit polyclonal antibody for CRH (1:200, Millipore Corporation), goat polyclonal antibody for CRHR1 (1:200, Santa Cruz Biotechnology), rabbit polyclonal antibody for GR (H-300) (1:200, SantaCruz Biotechnology), and mouse monoclonal antibody for TH (1:1000, Millipore Corporation). Following incubation, slides were rinsed and Alexa 594-conjugated donkey anti-rabbit (1:500, Invitrogen Canada Inc), Alexa 488-conjugated donkey anti-goat (1:500, Invitrogen Canada Inc) or donkey anti-mouse (1:500, Invitrogen Canada Inc) secondary antibodies applied for 2 h at room temperature to reveal immunopositive cells. All the primary and Alexa fluor-conjugated secondary antibodies were diluted in the blocking solution previously described. Special controls were also performed in order to test the specificity of the antibody being used. Following three rinses, slides were incubated with 1µg/ml (Hoechst 33342, Invitrogen Canada Inc) for 10 min at room temperature to label cell nuclei. An anti-fade medium containing 0.1% *p*-phenylenediamine in phosphate buffered glycerol was then applied and the slides coverslipped and sealed with nail polish. The immunofluorescent signal detection was accomplished using an Olympus DX51microscope (Center Valley, PA, USA). Digital images of

immunofluorescence were obtained using the Progress Pro 2.7.6 software under 20 × magnification. For all regions of interest, immunoreactive cell bodies or processes were quantified using Image J software (Image J, National Institutes of Health) and the method described by Hayes and colleagues (Hayes, Knapp, Breese, & Thiele, 2005). Percentages of optical densities (Mean Grey Values: estimates of the staining intensity) from a selected brain region relative to a subthreshold background were obtained. This technique required to initially subtract the background and then measure intensely labeled area. Four anatomically matched pictures of the left and right hemispheres of the brain were used to produce an average immunoreactivity score for each brain region in each animal. Data are presented as background corrected standardized image densities for each brain.

2.2.4 Determination of CA1 Neuronal Cell Death

Coronal brain sections (14μm) at the level of the dorsal hippocampus (-2.80– -4.16 mm posterior to Bregma) were stained with thionin for quantification of CA1 neuronal damage in sham and ischemic rats. The number of surviving neurons was counted using a LEICA DAS light microscope attached to a SONY digital camera, and image analysis software Norton Eclipse (v 6.0). Only pyramidal neurons showing a rounded cell body, a distinct cytoplasm, a clear nuclear outline and a visible nucleolus were counted within 1 mm length area in the CA1 region. A mean value was obtained from six bilateral measurements per animal in each experimental group. The neuronal density for a given animal represents the mean of both the right and left neuronal cell densities.

2.2.5 Statistical Analysis

All analyses were performed using SPSS Statistics 19. All behavioral and histological assessments were conducted by experimenters blind to group identity. Homogeneity of variance

was tested for all variables using the Levene's statistic and when homogeneity was not assumed, the Welch Correction was applied for heterogeneity of variance. For the Radial Maze, the 12 day testing period was grouped into 2-day test blocks for the analysis. A one-way repeated measures ANOVA, with group as the independent factor and test block (six test blocks of 2 trials) as the repeated factor was performed to assess the number of working memory errors in ischemic and sham-operated animals. The Mauchly's test was significant, thus the Huynh-Feldt correction was applied to adjust degrees of freedom. Following omnibus analysis, planned pairwise comparisons (separate one-way ANOVAs) were conducted. Immunohistochemical data (image J IR scores) were individually analyzed for each brain region using one-way ANOVAs to determine between group differences. A one-way ANOVA also compared neuronal density values of the CA1 layer of the hippocampus in sham-operated and ischemic animals 30 days after ischemia. Results are expressed as mean $\pm$ Standard Error of the Mean (SEM). Statistical significance is obtained when $p < 0.05$. A univariate GLM was used to calculate partial Eta squared ($\eta p2$) and a hand calculation was performed to generate Cohen's d (*d*) values to give an indication of effect sizes. $\eta p2$ (ranging from 0.0 to 1.0) describes the proportion of variance in a particular dependent variable (e.g., working memory errors) that is accounted for by the effect of an independent variable, and Cohen's d represents the magnitude of the difference between groups in standard units.

2.3 Experiment 2

2.3.1 Cannulae implantation

One week after acclimatization to the animal facility (see Fig. 1 for experimental timeline), rats (n =40) underwent surgery for stereotaxic implantation of a guide cannula into the third ventricle for drug treatment administration as previously described (Charron, Frechette,

Proulx, & Plamondon, 2008). Rats were anesthetized using isoflurane (2-3 %) mixed with oxygen and positioned in a stereotaxic instrument. Stainless steel guide cannulae (22 gauge) were implanted using the following coordinates: 4.3 mm posterior to Bregma, 0.0 mm lateral to the midline and 4.3 mm ventral to the skull surface according to the Paxinos and Watson atlas (1997). Guide cannulae were secured to the skull with dental cement and four anchor screws. A dummy cannula was inserted into the guide cannula to prevent occlusion.

2.3.2 Drug treatment

Antalarmin hydrochloride ($C_{24}H_{34}N_4$●HCl; MW= 415.0; Sigma–Aldrich Inc., St. Louis, MO), a potent nonpeptide CRHR1-selective receptor antagonist was dissolved in 0.9% saline containing 10% Cremophor (Sigma–Aldrich Inc.) as suggested in studies using similar dosage of Antalarmin infused directly in discrete brain nuclei (Bledsoe, Oliver, Scholl, & Forster, 2011; X. Liu et al., 2011). Ischemic and sham rats were intracerebroventricularly (icv) injected with either Antalarmin (2ug/2ul) or vehicle 30 minutes prior to 10-min global ischemia or sham occlusion, creating 4 groups: Ant-Sham (n=10), Ant-Ischemia (n=10), Veh-Sham (n=10), and Veh-Ischemia (n=10). The selection of icv drug dosage and time of drug administration were based on the previous literature and a previous study from our lab using CP-154,526, an analog of Antalarmin (Khan et al., 2004; Telegdy & Adamik, 2008). Antalarmin was selected in this study as it exhibits higher affinity for the CRH type 1 receptor than CP-154,526 (K_1=0.8 for Antalarmin vs. 2.7 nM for CP-154,526) (Zorrilla, Valdez, Nozulak, Koob, & Markou, 2002).

2.3.3 Restraint Stress

On the 27th day following ischemia or sham operation, animals were subjected to restraint stress by placing them in a plastic restrainer for 15 minutes during the light phase (between 9 AM and 11:00 AM).

2.3.4 Blood collection

Blood samples were collected from all animals prior to each surgical procedure (cannula implantation, ischemic surgery and vessel occlusion) and 30, 60, and 120 minutes and 24 h following sham occlusion or global ischemia. To monitor the long-term effects of CRHR1 antagonist on CORT secretion, blood samples were also collected 3, 7, 14 and 21 days after induction of 10 minute ischemia. To collect blood, the tail vein nick procedure was used as previously described (M. R. Milot, James, Merali, & Plamondon, 2012). Two blood drops per sample were collected at each stated time interval on Schleicher and Schuell specimen collection paper (Whatman International Ltd., Maidstone, UK). Two days after restraint stress, thus thirty days following occlusion, rats were decapitated.

2.3.5 Corticosterone radioimmunoassay

Blood corticosterone levels were assessed using a commercial rat CORT radioimmunoassay kit according to the manufacturer's instructions as previously described (M. R. Milot et al., 2012). Briefly, 3.0 mm diameter circles were punched from the Schleicher and Schuell specimen collection papers containing the blood drop samples, using a Gem Hole Punch (McGill Inc., Marengo, IL), and placed in a tube containing 200 µl of Dulbecco's phosphate-buffered saline (DPBS; Sigma-Aldrich, St. Louis, MO) having 0.1% gelatin (Avantor Performance Materials, Phillipsburg, NJ). The tubes were shaken in an orbital shaker at 90 rpm for one hour at 24 °C and then refrigerated for 48 h at 4 °C before radioimmunoassay procedure. Followed the manufacturer's protocol to prepare drop samples and standards, we placed 25 µl of each sample and 25 µl of steroid diluent in each of two tubes for duplicate analysis. We analyzed 25 µl of each provided concentration standards diluted with 25 µl of DPBS, in triplicate, to generate a standard curve of corticosterone concentration. Standard and sample tubes were

analyzed in a HP Cobra II gamma counter (Canberra-Packard, Meriden, CT). Corticosterone concentrations in drop blood samples were quantified in units of pg corticosterone per punch.

2.3.6 Statistical Analysis

CORT levels data were analyzed using a mixed ANOVA design with two independent factors Surgery and Drug and one repeated factor time to explore the change in CORT levels prior to (Baseline = before surgeries and prior to occlusion) and following 10 minute ischemia exposure (30, 60, 120 minutes; 24 hours; 3, 7, 14 and 21 days), and for pre and post (30, 60 and 120 min) restraint time intervals (on post ischemic day 27). The Mauchly's test being significant, the Huynh-Feldt correction was applied to adjust degrees of freedom to more conservative values. Significant main effects or interactions were further explored and corrected Bonferroni post hoc analysis used for comparison between the four groups. All results are shown as means ± SEM. and p value <0.05 are considered significant for all analyses.

3. Results

3.1 Experiment 1

3.1.1 Working memory in the Radial Maze

Fig. 3 shows the effect of 10 minutes forebrain ischemia on working memory errors during within-trial entries 2–5 over the course of the 6 test blocks. The repeated measure ANOVA indicated a main effect of Group ($F_{(1,12)} = 14.47$; $p<0.003$, $\eta p2 = 0.547$), Time (test blocks) ($F_{(5,60)} = 11.87$; $p<0.001$, $\eta p2 = 0.497$) but no Group X Time interaction ($F_{(5,60)} = 2.21$; $p=0.064$, $\eta p2 = 0.156$). Global ischemia significantly increased working memory errors at test blocks 1 ($p = 0.046$), 2 ($p =0.032$) 3 ($p = 0.04$), and 4 ($p = 0.024$).

3.1.2 GR expression in the PVN of the hypothalamus and in the CA1 subfield of the hippocampus

Fig. 4 shows effects of 10 minutes global ischemia on GR immunoreactivity in the PVN of the hypothalamus and in the CA1 subfield of the hippocampus. Global ischemia resulted in a significant reduction of GR ($_{\text{Welch statistic}}$ =6.93, p 0.28, ηp2 = 0.37, *d*= 5.92) in the CA1 hippocampal subregion and in a significant increase of GR in the PVN (Welch statistic = 20.92, p = 0.01, ηp2 = 0.59, d= 9.32) of the ischemic as compared to the sham group.

CRHR1 expression in the PVN of the hypothalamus and in the CA1 subfield of the hippocampus

Fig. 5 shows effects of 10 minutes global ischemia on CRHR1 immunoreactivity in the PVN of the hypothalamus and in the CA1 of the hippocampus. Global ischemia resulted in a significant reduction of CRHR1 expression in the CA1 hippocampal subregion (Welch statistic = 24.10, p = 0.01, ηp2 = 0.67, d= 11.11) and in a significant increase in CRHR1 immunoreactive expression in the PVN ($F_{(1, 13)}$ = 41.78, p =.000, ηp2 = 0.76, d= 13.92) of ischemic compared to sham rats.

3.1.3 CRH expression in the hypothalamic PVN, amygdalar CeA and hippocampal CA1

Fig. 6 shows the effects of 10 minutes global ischemia CRH expression in the PVN, the CeA, and the CA1. Ischemic animals showed higher CRH levels in the PVN ($F_{(1, 13)}$ = 37.52, p < 0.05, , ηp2 = 0.74, d= 13.19) while a significant decrease in the density of CRH neurons was found in the CeA ($F_{(1, 13)}$ = 4.74, *p* = .048, ηp2 = 0.27, d= 4.69) in rats submitted to global cerebral ischemia as compared to sham operated animals. There were no significant differences in CRH immunoreactivity in the CA1 of the hippocampus for ischemic compared to sham animals ($F_{(1, 13)}$ = 3.28, p = .093, ηp2 = 0.23, d= 4.29).

3.1.4 TH expression in the LC

Fig. 7 shows effects of 10 minutes global ischemia TH expression in the LC. Thirty days following global cerebral ischemia higher TH expression was present in the LC of ischemic ($F_{(1, 13)} = 14.29$, p =.002, ηp2 = 0.52, d= 8.14) compared to sham animals.

3.1.5 CA1 neuronal injury

Fig. 8 shows the impact of 10 min forebrain ischemia on CA1 survival 30 days post-reperfusion. One-way ANOVA revealed a between group effect on neuronal density. Ischemic rats displayed a significant increase in CA1 neuronal injury 30 days after reperfusion (Mean = 122.71, SEM = 5.76, $F_{(1, 13)} = 41.84$, p = 000, ηp2 = 0.76, d= 13.93) as compared to sham operated animals (Mean = 193.74, SEM = 9.75).

3.2 Experiment 2

3.2.1 Plasma CORT levels following occlusion

The pattern of changes in corticosterone levels induced by global cerebral ischemia decreased with time but with a lower magnitude to those of Antalarmin treated ischemic animals and of both sham groups (Fig. 9, top panel). A two-way repeated measures ANOVA of CORT levels revealed significant main effects of time ($F_{(10,350)} = 205.840$, $p<0.001$), surgery ($F_{(1,35)}= 59.647$, $p<0.001$) and drug ($F_{(1,35)}= 57.515$, $p<0.001$), with significant surgery X drug ($F_{(1,35)}= 12.451$, p = 0.001) and time X surgery X drug ($F_{(10,350)} = 3.32$, $p<0.001$) interactions. Bonferroni-corrected post hoc pairwise comparisons revealed significant differences between ischemic-saline and sham animals at different intervals, persisting up to day 7 post occlusion ($p<0.001$). At 14 and 21 days, CORT levels were no longer different between the ischemic-saline animals and all other rat groups ($p > 0.05$). Antalarmin-treated ischemic animals showed a

suppressive effect of CORT secretion apparent 30 and 60 min following occlusion with CORT levels distinct to those of ischemic-saline animals ($p<0.01$) and similar to saline ($p>0.05$) and Antalarmin-treated sham rats ($p = 0.27$ at the 60 min time interval). There was no difference between ischemic and sham animals at baseline. Animal groups that underwent the ischemic surgery showed higher elevations in CORT levels measured 24 h following Day 1 of the two-day 4VO surgical procedures than sham-operated groups ($p<0.01$).

3.2.2 Effects of 15 min Restraint Stress on CORT Secretion

To test the hypothesis that ischemic animals display a hyperactive neuroendocrine stress response, we assessed CORT levels prior to and 30, 60 and 120 minutes following restraint stress (Fig. 9, bottom panel). A two-way repeated measures ANOVA of CORT levels revealed significant main effects of time ($F_{(4,105)} = 99.515$, $p<0.001$), surgery ($F_{(1,35)}= 4.289$, $p = .046$) and drug ($F_{(1,35)} = 21.878$, $p<0.001$), with a significant surgery X drug interaction ($F_{(1,35)} = 4.357$, $p = 0.044$) but no significant time X surgery X drug interaction ($F_{(4,105)} = .534$, $p = 0.660$). Overall, ischemic-saline rats displayed relatively higher CORT levels across time points (30: $p<0.005$; at 60: $p<0.05$ & 120:$p <0.05$ minutes) following restraint stress compared to ischemic and sham Antalarmin treated groups but only for the 30 minute interval for the sham-saline group ($p= 0.048$), despite a general decrease over time for all groups. CORT levels prior to restraint, on Day 27 post occlusion, were similar across all groups ($p>0.05$).

4. Discussion

Although memory impairments are commonly associated to neuronal damage following global ischemia (H. P. Davis, Baranowski, Pulsinelli, & Volpe, 1987; Gionet et al., 1991; Hagan & Beaughard, 1990), recent studies have demonstrated that factors such as physical exercise (van Praag, Christie, Sejnowski, & Gage, 1999), enriched environment (Janssen et al., 2010;

Ploughman, Attwood, White, Dore, & Corbett, 2007) and diets (Correia Bacarin et al., 2013; Girbovan, Morin, & Plamondon, 2011; Roberge, Hotte-Bernard, Messier, & Plamondon, 2008) led to functional preservation and rapid functional recovery post ischemia despite unaffected CA1 neuronal injury. Moreover, pharmacological treatments reducing CORT secretion and NE levels enhanced hippocampal-dependent memory performance in ischemic animals in spite of CA1 neuronal degeneration at time of testing (M. R. Milot & Plamondon, 2011a). These findings support increased sensitization and responsiveness of systems regulating stress hormones and a role for such changes in memory function following global ischemia.

The current study aimed to characterize specific changes in neurochemical/endocrine function present at a remote 30 day time interval following brain ischemia. Our findings indicate that neuronal death in this model is accompanied by decreased expression of glucocorticoid and CRH type 1 receptors located on hippocampal pyramidal neurons (Y. Chen et al., 2000; Takeda & Tamano, 2012). These changes may be interpreted as evidence of long-lasting impact of global ischemia on negative feedback regulation of the HPA axis. This proposition is supported by heightened HPA reactivity and alterations in circulating CORT reported following exposure to potent physiological stressors, including global ischemia (Ladd, Huot, Thrivikraman, Nemeroff, & Plotsky, 2004; M. R. Milot & Plamondon, 2011a; Uys et al., 2006). In addition, our findings indicate significant elevations of CRH- and CRHR1-ir at the PVN. CRH and CRHR1 have been shown to substantially and rapidly increase in this region following exposure to various stressors (Imaki et al., 2001; Makino et al., 1995; Rivest, Laflamme, & Nappi, 1995). Furthermore, CRH-producing neurons of the PVN directly trigger an increase in CRHR1 mRNA expression after stress onset (Konishi, Kasagi, Katsumata, Minami, & Imaki, 2003), and increased CRH-ir at the PVN induced by chronic hypoxia can be blocked by administration of

the CRHR1 antagonist CP-154,526, suggesting mutual regulation following a stress episode (T. Y. Wang et al., 2004; Xu et al., 2005). Noteworthy, GR are highly expressed in PVN CRH neurons (Cintra et al., 1987; Uht, McKelvy, Harrison, & Bohn, 1988). Under normal conditions, glucocorticoids suppress CRH activation by binding to GR receptors at the PVN and hippocampus (Erkut, Pool, & Swaab, 1998; Itoi et al., 1987). Reduced GR-positive neurons at the CA1 of the hippocampus in ischemic rats support the notion of an underlying reduction in the negative feedback effects of glucocorticoids on the HPA axis, resulting in elevated CRH- and CRHR1-ir in ischemic compared to sham animals. Concomitant to such changes, we observed increased PVN GR-ir. Although the exact mechanism remains to be clarified, changes in PVN sensitivity to glucocorticoid feedback action may result in adaptation of these receptors to a new setpoint of enhanced HPA activity after the ischemia. In a similar fashion, fornix lesion has been shown to also impair the outcome of corticosterone action in the hippocampus through efferent pathways towards the PVN, leading to increase expression of CRH and GR mRNA expression in this region (Han et al., 2007). Changes in PVN expression of key stress signals in the current study likely reflect differences in immunoreactivity rather than cell density considering a recent study showing high resistance of hypothalamic neurons to acute ischemic injury induced by oxygen and glucose deprivation in rat brain slices (Brisson & Andrew, 2012). Consistent with our finding, a recent study showed increased PVN and decreased dorsal hippocampal CRHR1 expression 21 days following cardiac arrest in mice, suggesting persistent sensitization of the HPA axis (Neigh, Karelina, Zhang, et al., 2009).

Unlike CRH type 1 receptors, which reside mainly on dendritic spines of CA1 pyramidal cells, we observed no changes in CA1 CRH-ir in ischemic compared to sham animals. This is likely attributable to the CRH peptide being located exclusively in a subset of GABAergic

interneurons less prone to ischemic damage (Y. Chen et al., 2004). Indeed, counts of hippocampal cells immunoreactive for glutamic acid decarboxylase showed that the GABA interneurons survive ischemia (Johansen, 1993). This finding is consistent with data collected at shorter term intervals and showing no alterations in *in vivo* CRH release from the CA1 in the hours following global ischemia or post mortem CRH assessment performed 4, 24 and 72 h following global ischemia (Khan et al., 2004).

Concomitant with HPA axis changes, ischemic animals showed heightened TH expression in adrenergic neurons of the LC compared to sham rats 30 days following reperfusion. Past studies have shown that brain infarction triggers a hyper-activation of the sympathetic nervous system, triggering elevation of plasma catecholamine concentrations and systemic release of cytokines (Oto et al., 2008). Post ischemic locomotor hyperactivity has also been associated with increased norepinephrine levels (Robinson & Bloom, 1977). Given that the LC contains the major group of noradrenergic perikarya (A6 cell group) of the brain (Dahlstrom & Fuxe, 1964; Debure et al., 1992), the higher levels of TH observed in the LC after ischemia are likely indicative of an upregulation of this neurotransmitter. Moreover, the fact that increased TH expression was observed 30 days post ischemia suggests that such changes may not represent a residual consequence of LC activity during the induction of the ischemic insult but rather an indirect effect of the heightened sensitivity of the HPA axis in ischemic rats (McDevitt et al., 2009), as observed in our study (e.g., PVN).

At the CeA, our findings revealed decreased CRH-ir relative to expression in sham animals. This observation is consistent with a recent study showing significant losses of CRH-positive neurons at the CeA six weeks following hypoxia–ischemia induced in neonatal rats, a phenomenon associated with hyperactivity in response to novel open field exposure in ischemic

rats (Carty et al., 2010). Alterations at longer survival intervals contrast with rapid elevations of CRH release, protein and mRNA levels in the amygdala observed at short time intervals following traumatic brain injury and global or focal ischemia in rats (Khan et al., 2004; Roe, McGowan, & Rothwell, 1998; Wong et al., 1995). Although the exact contribution of such changes remains unknown, increase in CRH expression at shorter term intervals likely contributes, above regulatory actions of the stress response, to plastic remodeling affecting amygdalar and hippocampal neurons following various psychogenic and physiological stressors, including cerebral ischemia (Kovalenko et al., 2006; Martone, Hu, & Ellisman, 2000; Ruan, Han, Shi, Lei, & Xu, 2012). Elevated CORT levels have been reported to significantly increase CRH in the CeA (Myers & Greenwood-Van Meerveld, 2010; Shepard, Barron, & Myers, 2000), a phenomenon that could lead to neuronal damage in this brain region post ischemia. The role of CeA CRH-ir in HPA axis regulation after cerebral ischemia requires further investigation considering that CeA brain lesion has been reported to exert minimal effects on CORT or ACTH secretion following acute stress (Prewitt & Herman, 1997).

Recent findings have supported a role for CRH type 1 receptors in the regulation of the HPA axis upon stress exposure (X. D. Wang, Y. Chen, et al., 2011a). In Experiment 2, we used Antalarmin to assess the role of central CRHR1 activation on basal and stress-induced glucocorticoid secretion following global ischemia. Our findings indicate significantly enhanced CORT secretion up to 7 days post occlusion, after which secretion profile on days 14, 21 and 27 was comparable as that observed in sham-operated animals. Similar CORT activation has been reported within the initial post ischemic week (Craft & Devries, 2009; M. R. Milot & Plamondon, 2011a). However, this study is the first to have measured basal CORT concentrations at longer post ischemic intervals. Notably, although CORT secretion eventually

resumed to normal values, ischemic animals showed CORT hyper secretion in response to a 15 min restraint stress imposed 27 days post ischemia, indicating persistent sensitization of the HPA axis to stressors. These findings are concordant with recent observations from our group showing heightened neuroendocrine reactivity in ischemic compared to sham rats upon exposure to the stressor of behavioral testing in a Barnes Maze (M. R. Milot & Plamondon, 2011a). Of note, both sham and 4-vessel occlusion led to increased CORT secretion at the early 30 and 60 min time intervals following vessel exposure, although the magnitude of such changes was dependent on stressor intensity and significantly more elevated in ischemic compared to sham operated rats. Pretreatment with Antalarmin significantly reduced CORT secretion in both groups. Importantly, although CORT inhibition was only apparent during the first hour in the case of Antalarmin-treated sham rats, suggesting that effects might be contingent to direct blockade of the receptors, CORT secretion was significantly reduced for many days in pretreated ischemic animals. This suggests that pre-ischemic modulation of CRH action appears to condition the brain's response to global ischemia, a phenomenon that has lasting effects on post ischemic HPA axis regulation. Of interest, the exposure of all animals to a 15 min restraint stress 27 days following reperfusion led to the observation that Antalarmin had similar effects even at this remote time interval, reducing stress-induced CORT secretion in both sham and ischemic rats, with comparable magnitude when accounting for initial differences of HPA reactivity in sham and ischemic animals. It thus appears that a single Antalarmin administration may act to precondition the brain's response to remote stressor exposure. These findings are consistent with studies showing that CRHR1 antagonist administration markedly inhibited plasma CORT secretions observed upon repeated periods of hypoxia (X. Q. Chen, Xu, Du, Wang, & Duan, 2005). These observations may also be relevant to the reported prevention of ischemia-induced hyperactivity

in a novel open field following global cerebral ischemia by the CRHR1 antagonist CP 154 526 in the absence of altered CA1 neuronal protection (Plamondon & Khan, 2006). One possible explanation may be that Antalarmin administration impacted the psychological appraisal of stress/arousal associated with ischemia or sham-operation such that any future manipulations (such as restraint) are appraised according to past experiences. In this context, decreasing glucocorticoid levels via metyrapone administration is effective in reducing the strength of an emotional memory in a long-lasting manner in humans (Marin, Hupbach, Maheu, Nader, & Lupien, 2011).

Conclusion

The current study represents the first demonstration of brain-region specific alterations in important stress regulators following brain ischemia and of a role of central CRH type 1 receptor activation to changes in CORT secretion and later physiological responses to acute stressor exposure. Dysregulation of the HPA axis and hippocampal/amygdalar circuitries are acknowledged as important regulators of memory and emotional impairments observed in several neurological and affective disorders including schizophrenia, multiple sclerosis, Alzheimer's disease and epilepsy (A. Zobel et al., 2004). Rats exposed to global ischemia or myocardiac infarction show depressive-like behaviors, a phenomenon associated with injury of the limbic system and that can be prevented by antidepressant treatments (Bah, Kaloustian, Rousseau, & Godbout, 2011; Bantsiele et al., 2009a; Wann et al., 2006). In this context, our findings support the need to perform long-term studies to uncover the role of neuroendocrine dysfunction in post ischemic emotional impairments. Depression affecting 33% of ischemic survivors (Maree L. Hackett et al., 2005), such investigations could shed light on alternative therapeutic treatments to improve cognitive and/or emotional impairments.

5. Acknowledgments

The authors thank Sylvie Emond and Jon James for excellent technical assistance in the animal facility and in conducting the CORT RIA assessments, respectively. We are also grateful to Dr. Alfonso Abizaid for his assistance in troubleshooting the CRH antibody for immunohistochemical analysis and Dr. Pam Kent for comments on the manuscript. This work was supported by a grant from the National Science and Engineering Research Council of Canada to Dr. Hélène Plamondon and internal funding from the University of Ottawa. The authors declare having no conflict of interest regarding the work described here.

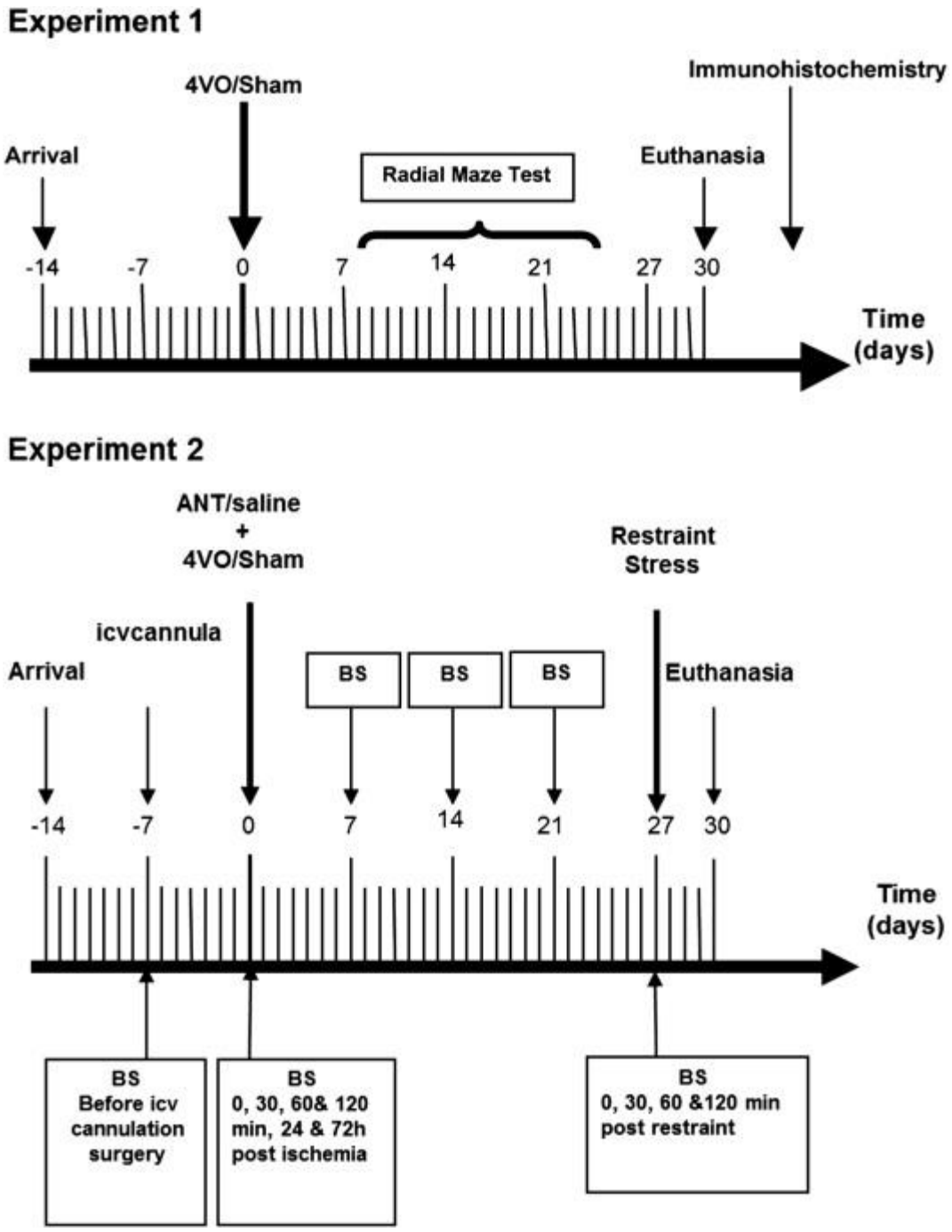

Fig.1. Timeline for Experiment 1 (top panel) and Experiment 2 (lower panel). Day 0 refers to the day of induction of 4 vessel occlusion (4VO), day 8 is the start of Radial Maze testing. In Experiment 2, Antalarmin (2µg/2µl) or vehicle was icv administered 30 minutes before sham or carotid occlusion. Blood Samples (BS) were collected at various intervals during the experiment. On day 27 post ischemia or sham occlusion, animals were exposed to a 15 min restraint stress.

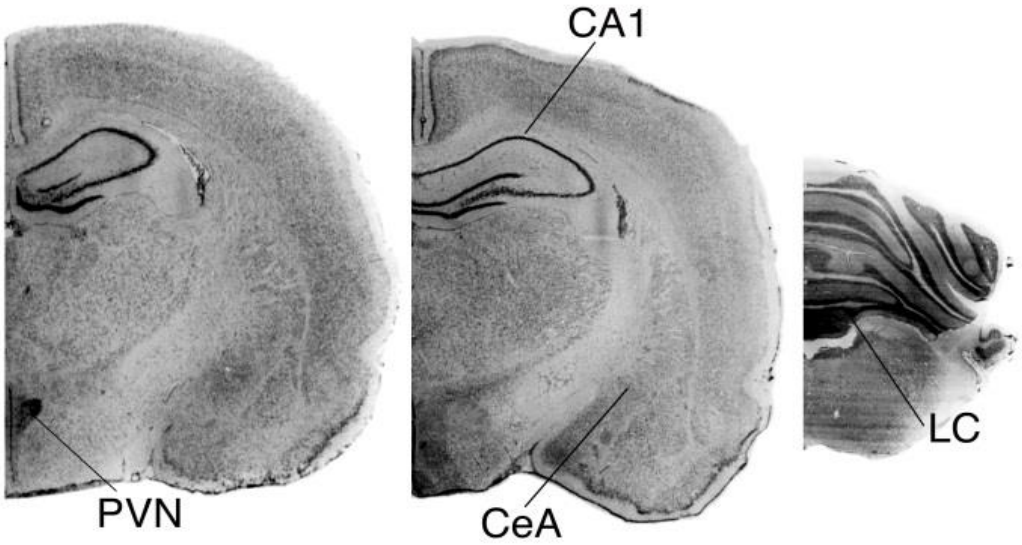

Fig. 2. Photomicrographs of rat coronal brain sections depicting selected brain regions for immunohistochemical detection.

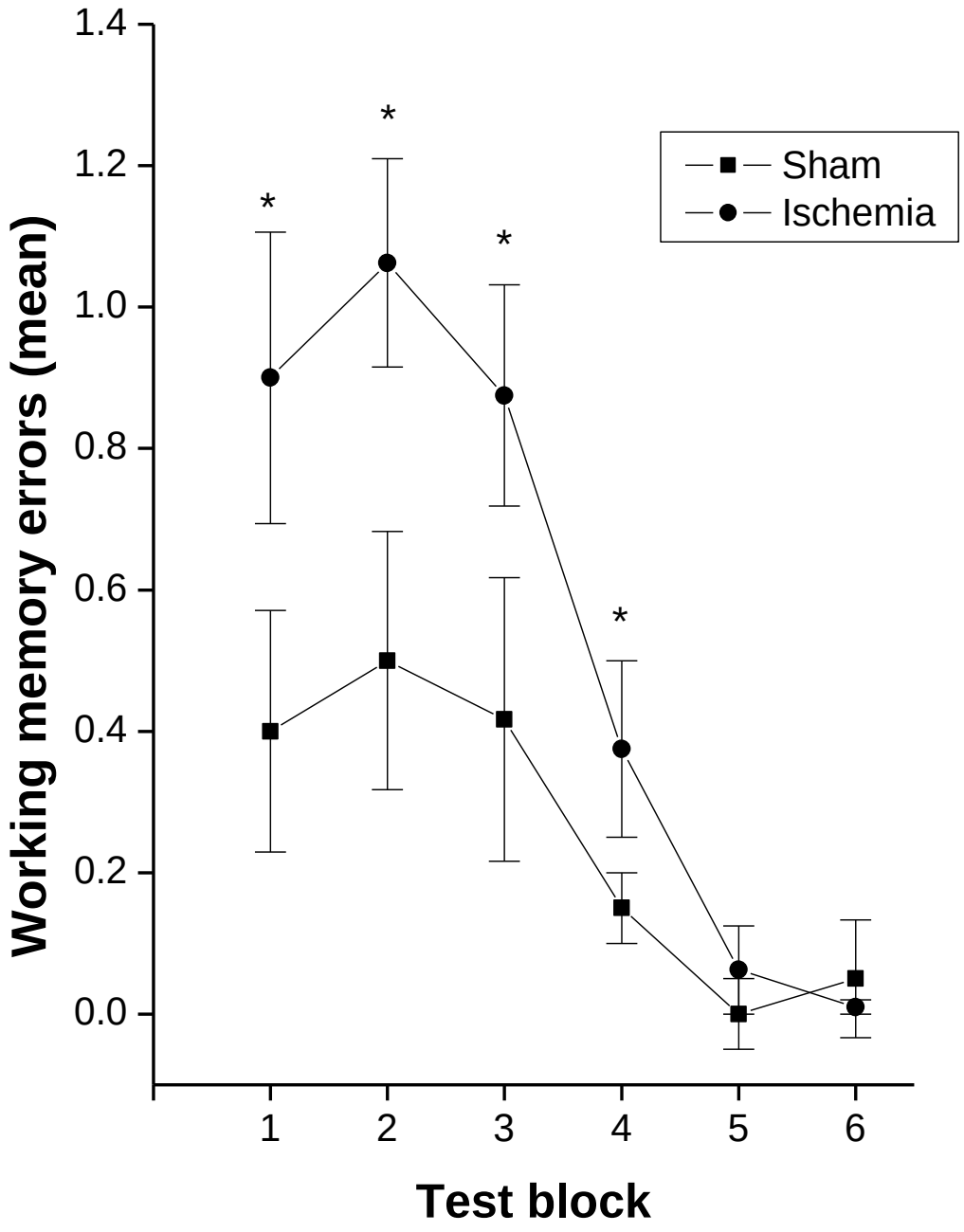

Fig.3. Memory/learning performance as measured in the Radial Arm Maze. Ischemic animals in Experiment 1 showed spatial memory impairments as demonstrated by increased number of working memory errors in the first four testing blocks. Values represent means ± SEM. *P< 0.05.

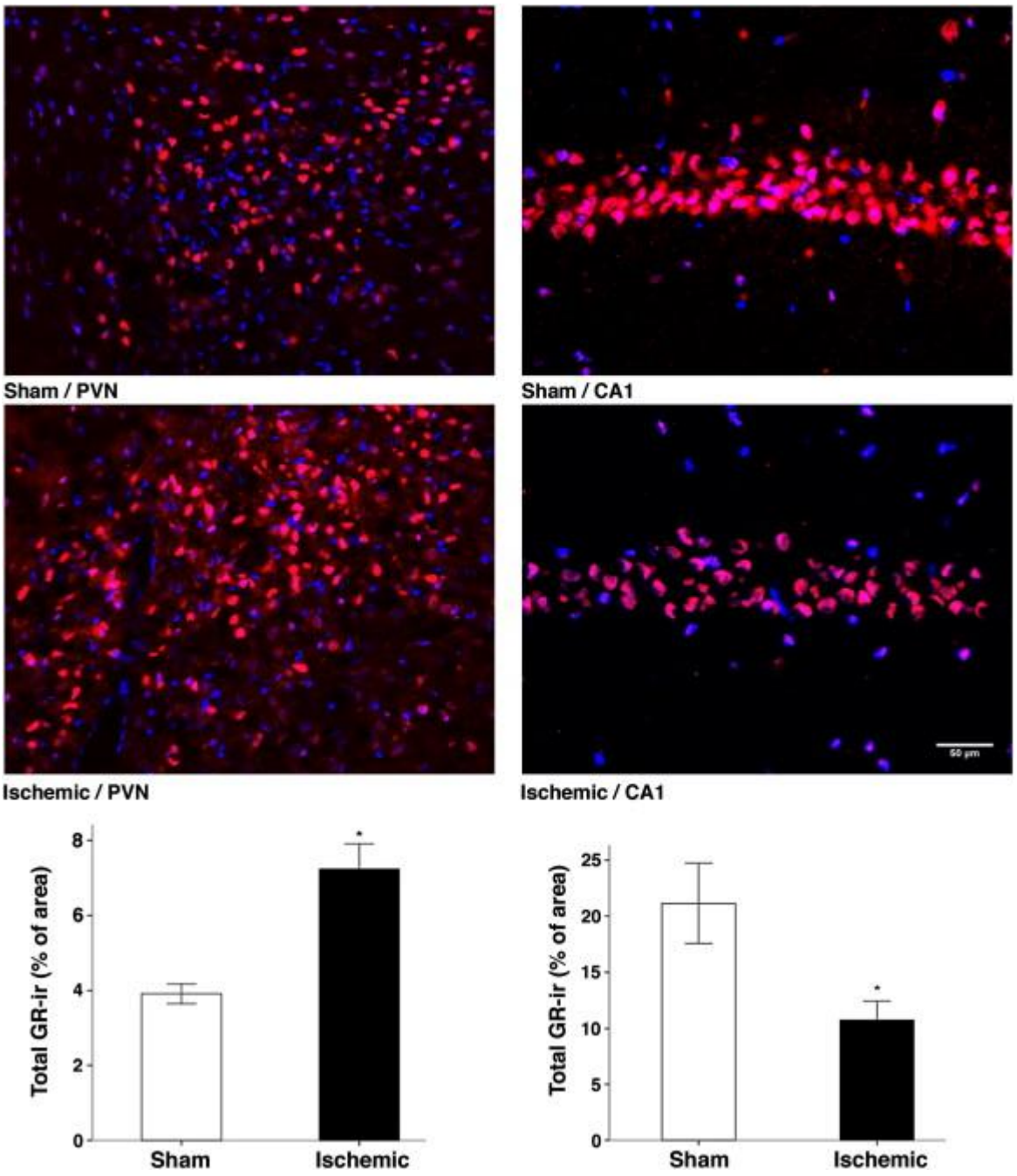

Fig. 4. Representative photomicrographs of GR-ir at the paraventricular nucleus of the hypothalamus (PVN) and hippocampal CA1 layer 30 days post ischemia. Increased versus decreased GR-ir was detected in ischemic compared to sham rat at the PVN and CA1 layer, respectively. Results are expressed as mean ± SEM. * $p < 0.05$.

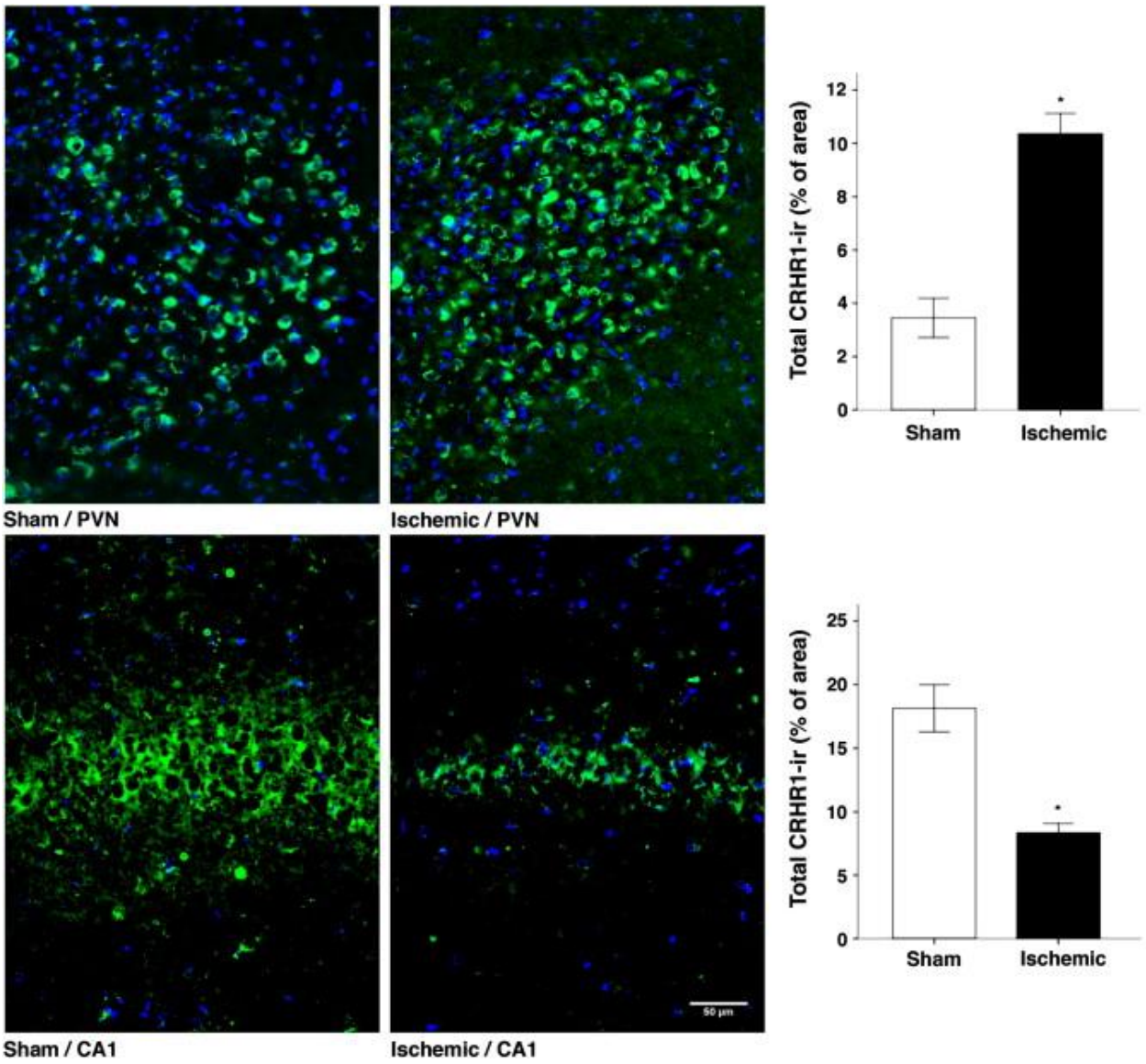

Fig. 5. Representative photomicrographs of CRHR1-ir at the PVN and CA1 layer 30 days post ischemia. Increased versus decreased CRHR1-ir expression was detected in ischemic compared to sham rats at the PVN and CA1 pyramidal layer, respectively. Results are expressed as mean ± SEM. * $p < 0.05$.

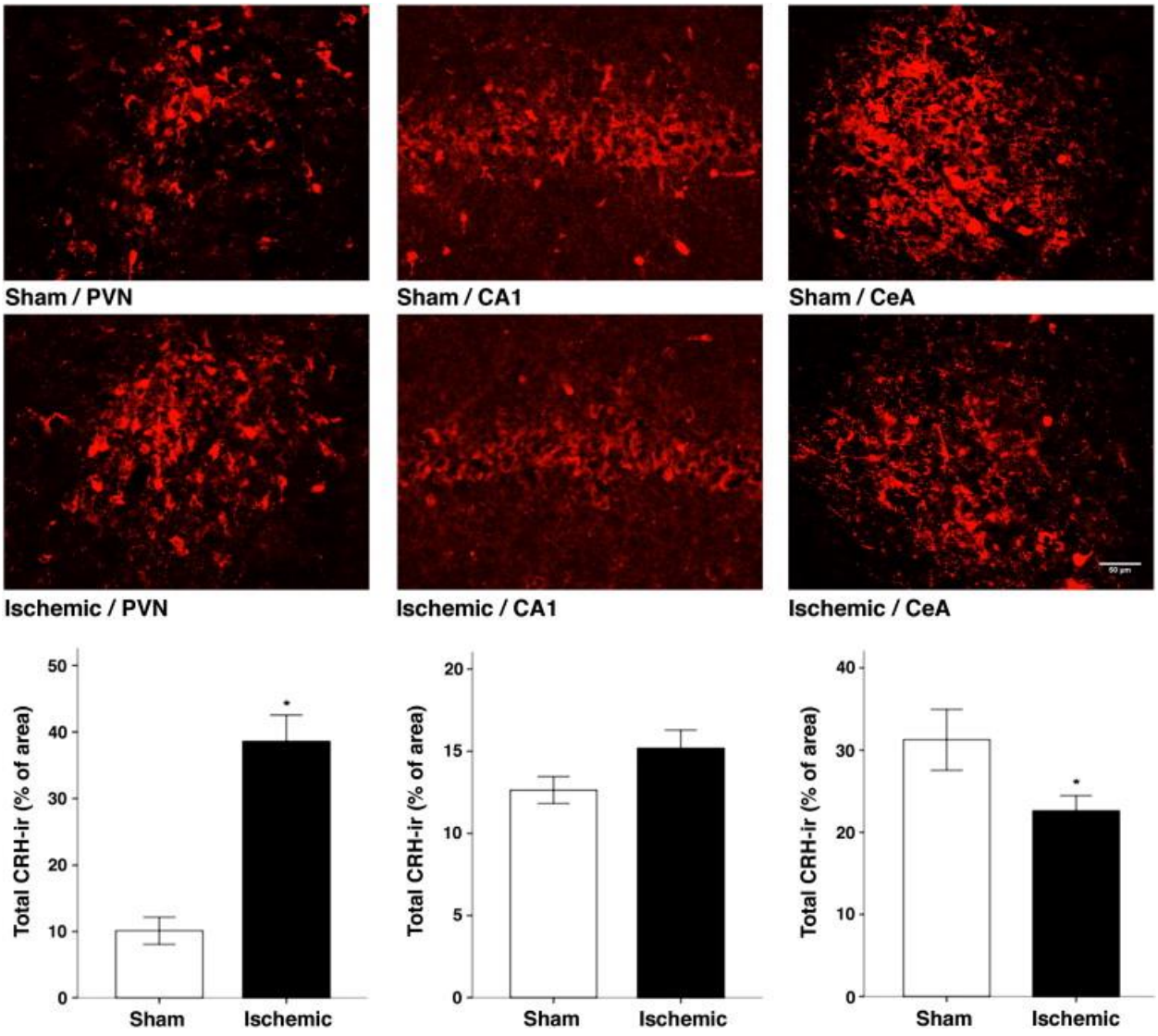

Fig. 6. Representative photomicrographs of CRH-ir at the PVN, hippocampal CA1 layer and central nucleus of the amygdala (CeA) in sham and ischemic rats 30 days following global ischemia. Significant increase and decrease in the density of CRH immunoreactive neurons in ischemic compared to sham rats in the PVN and in the CeA, respectively, with no significant differences observed at the CA1 layer. Results are expressed as the mean ± SEM. * $p < 0.05$.

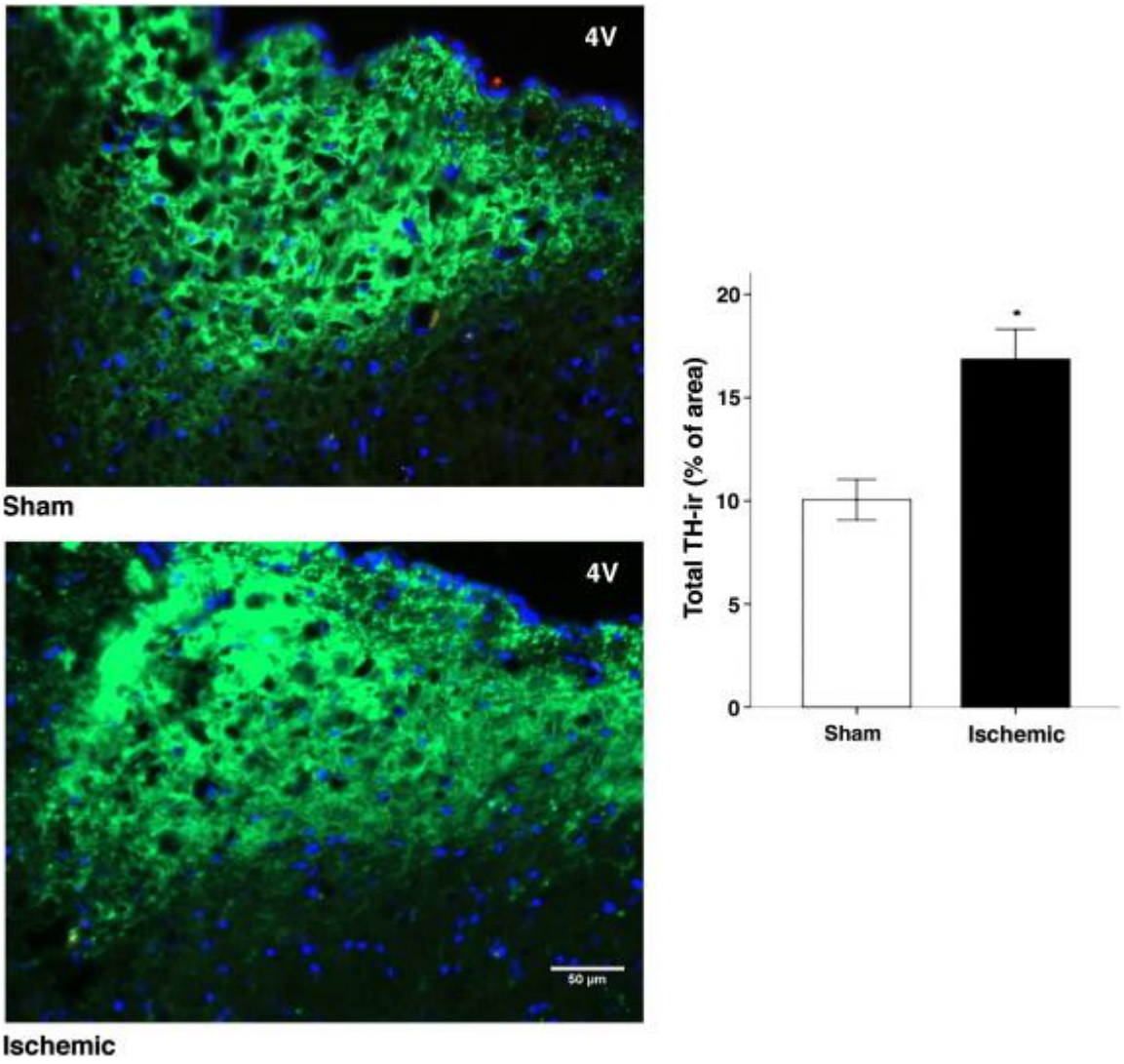

Fig. 7. Representative photomicrographs of TH-ir expression at the locus coeruleus (LC) in sham and ischemic rats 30 days following global ischemia. Increased TH expression was detected in ischemic compared to sham rats. Results are expressed as the mean ± SEM. * $p < 0.05$.

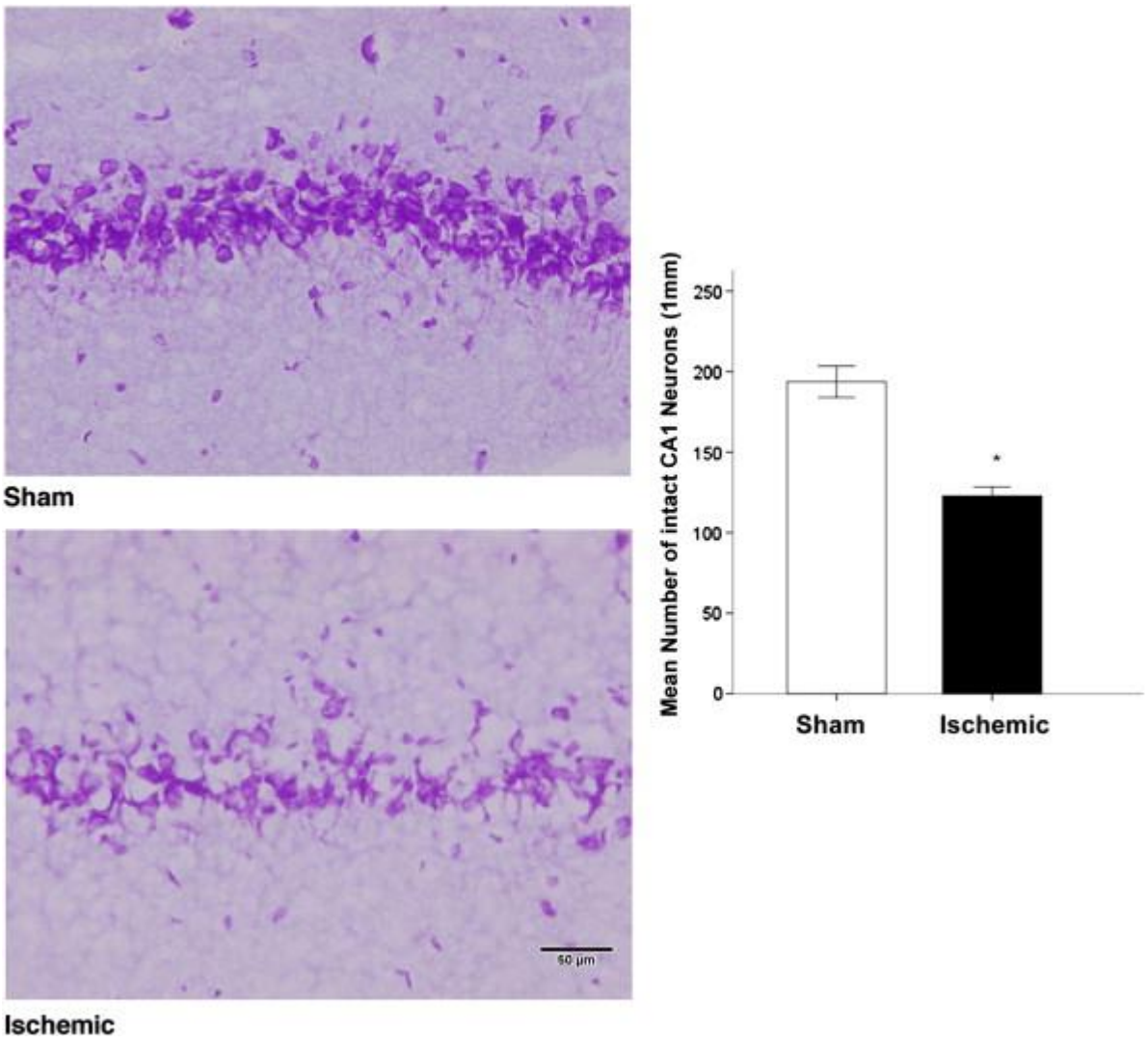

Fig. 8. Representative photomicrographs of thionin-stained CA1 neurons in sham and ischemic animals. CA1 neuronal density was significantly reduced in ischemic compared to sham animals 30 days following reperfusion. Results are expressed as mean ± SEM. * $p < 0.05$.

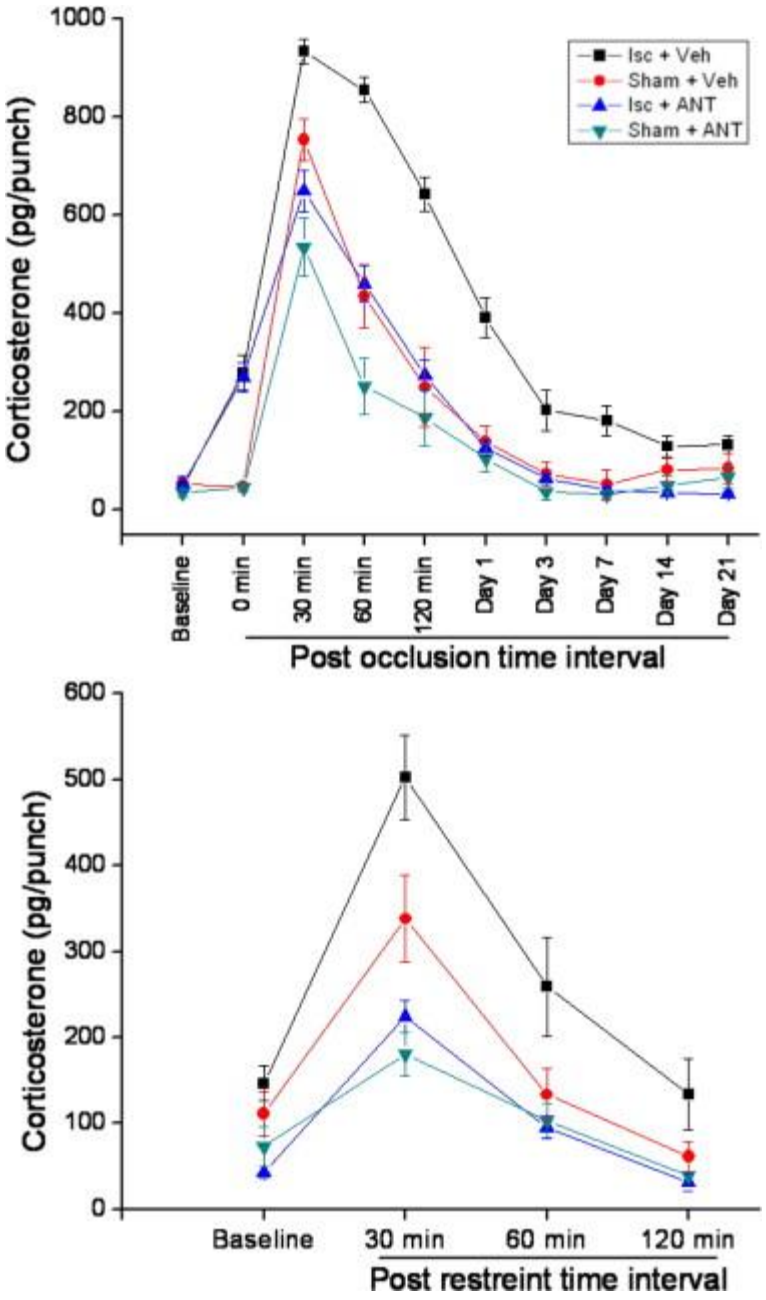

Fig. 9. Top panel shows the effect of 10 min global ischemia or sham occlusion on blood CORT secretions measured up to 21 days following reperfusion. Increased CORT secretion remained elevated up to day 7 in ischemic animals. Antalarmin pretreatment significantly inhibited the immediate surge in CORT secretion in sham and ischemic rats, an effect persisting up to 7 days in ischemic animals. Bottom panel shows the effect of 10 min restraint stress induced on day 27 post reperfusion on blood CORT concentrations (pg/punch). Acute restraint stress significantly elevated CORT secretion in saline- compared to Antalarmin-treated rat groups. Antalarmin was effective in reducing the immediate surge in CORT in both sham and ischemic rats, and in suppressing ischemia-induced increases in CORT secretion over the entire post restraint period. Values are expressed as mean ± SEM. * $p < 0.05$.

Article 2

Submitted as: de la Tremblaye, P. B., Narvaez Linares, N., Shock, S., & Plamondon, H. (2016). Blockade of CRHR1 receptors regulates social and depressive-like behaviors and expression of BDNF and TrkB in mesocorticolimbic regions following global cerebral ischemia.

Authorial contributions

Patricia Barra de la Tremblaye contributed to the study design, performed and interpreted the statistical analysis, and drafted and revised the manuscripts. She performed surgeries for cannulae implantations, and 4 vessels occlusions, behavioral testing, immunohistochemical analyses for all experimental groups. Nicolas Narvaez Linares an honor students that Patricia co-supervised blindly analyzed the social interaction test behaviors and helped in conducting immunohistochemistry. Patricia performed Western Blotting and RT-PCR procedures under the supervision of Dr. Sarah Shock. Dr. Hélène Plamondon supervised the research project, interpreted the statistical analysis, and revised the manuscripts.

Abstract

Increased HPA axis activation and CRH release characterize the brain's response to global cerebral ischemia. Recently, CRH via activation of CRH type 1 receptors (CRHR1) has been shown to regulate Brain Derived Neurotrophic Factor (BDNF) secretion and emotional behavior. The current study investigates the impact of CRHR1 blockade on BDNF/TrkB signaling expression in the mesolimbic circuitry, and social and depressive-like behavior following global ischemia. Adult male Wistar rats (N = 50) were injected with Antalarmin (2µg/µl) or a saline vehicle 30 min prior to 10 minute global cerebral ischemia (4VO model) or sham occlusion. Levels of BDNF and TrkB receptors were determined by immunohistochemistry, Western blot and Rt-PCR in subregions of the medial prefrontal cortex (mPFC), nucleus accumbens (NAc) and ventral tegmental area (VTA) 30 days post ischemia. The Three Chamber Social Approach Test (SIT) assessed sociability and preference for social novelty, and the forced swim test (FST), sucrose preference test and novelty suppressed feeding test (NSFT) characterized anxiety and depression. Corticosterone levels and organ (thymus, seminal and adrenal glands) weights were determined as additional physiological indices of stress. Global ischemia led to reduced BDNF and TrkB protein and mRNA expression in the mPFC, while heightened levels were found in the NAc. This was accompanied by increased immobility time in the FST and reduced sucrose preference in ischemic rats. However, ischemic rats showed heightened sociability and preference for social novelty accompanied by reduced latency to feed in the NSFT. Antalarmin treatment normalized ischemic biochemical and behavioral changes to levels of saline-treated sham and home cage rats. Together, these findings support that CRHR1 activation upon brain ischemia exerts lasting effects on plasticity markers in the mesocorticolimbic circuitry, likely playing a role in socio-emotional impairments following cardio- or cerebro-vascular accidents.

Keywords: Global Cerebral Ischemia; Antalarmin; Depressive-like Behaviour; Social Interaction; TrkB; BDNF; Corticosterone.

1. Introduction

Adverse social consequences post stroke include emotional impairments and depression, manifested by a low self-esteem, an altered self-image, and a redefinition of one's social role depending on cognitive or physical changes (Barrett, 2010). Dysregulation of HPA axis activation in major depression includes impaired inhibition of cortisol release, higher baseline cortisol values, and an overactive response to psychological stressors, which have been related to abnormalities in activation of corticotropin-releasing hormone (CRH) (Sher, Oquendo, Burke, Cooper, & Mann, 2013). This is interesting considering that CRHR1 blockade has beneficial effect in the treatment of anxiety and depression in humans (Holsboer & Ising, 2008). Similarly, although basal corticosterone (CORT) secretion normalizes 7 days after global cerebral ischemia, exposure to an acute restraint stress 27 days post reperfusion induces hypersecretion of plasma CORT in rats, which is prevented by pretreatment with Antalarmin (ANT), a specific CRH type 1 receptors (CRHR1) antagonist (de la Tremblaye, Raymond, Milot, Merali, & Plamondon, 2014). CRHR1-ir expression remains elevated in the PVN at remote intervals post-ischemia, while reduced receptor expression is observed in the CA1 subfield of the hippocampus, supporting its contribution to ischemia-induced HPA axis dysregulation (de la Tremblaye et al., 2014).

Recently, CRHR1 activation has been shown to modulate the expression of brain-derived neurotrophic factor (BDNF) and its high-affinity tropomyosin-related kinase B (TrkB) receptor (Bayatti et al., 2005), which are critical for neuronal survival, synaptic plasticity and memory function, also participating in the pathophysiology of depressive disorders and stroke (Bennett & Lagopoulos, 2014; Platenik et al., 2013). Severe stress exposure reduces BDNF expression in the hippocampus resulting in depressive behavior, which can be restored by antidepressant

treatment, along with improved behavior (R. S. Duman & Monteggia, 2006; Koponen et al., 2005). BDNF gene methylation is also associated with depression in coronary artery disease (Bozzini et al., 2009) and stroke (J. M. Kim et al., 2012; J. M. Kim et al., 2008) survivors, persistence of this phenomenon being associated with worsening depressive symptoms over one year follow-up (J. M. Kim et al., 2013). Accordingly, BDNF serum concentrations are decreased in post-stroke depression patients (Zhou et al., 2011) while lower TrkB mRNA and protein levels have been reported in the prefrontal cortex of suicide subjects (Dwivedi et al., 2003). Similarly, chronic exposure to unpredictable stress in rats reduces dendritic branching and spine density in the mPFC (Goldwater et al., 2009; Radley et al., 2004) and BDNF and TrkB protein and mRNA levels in the hippocampus and the frontal cortex while impairing sucrose consumption (Banerjee, Hazra, Ghosh, & Mondal, 2014). Noteworthy, reduced BDNF at the PFC has been associated with increased immobility time in the forced swim test (FST) in non-treated rats (Sequeira-Cordero, Mora-Gallegos, Cuenca-Berger, & Fornaguera-Trias, 2014). In humans, decreased CRH binding in the frontal cortex has also been reported in suicide victims (Nemeroff, Owens, Bissette, Andorn, & Stanley, 1988) while the gene–gene interaction between CRHR1/BDNF has been identified as an important contributing factor to the pathophysiology of recurrent major depressive disorder in the Chinese population (Xiao et al., 2011).

Contrary to observations at the hippocampus, stress increases BDNF expression in the nucleus accumbens (NAc) and the ventral tegmental area (VTA) (Cleck, Ecke, & Blendy, 2008; Fanous et al., 2010; Nikulina et al., 2012), and infusion of BDNF in the VTA or the NAc have been shown to produce depressive-like effects (Berton et al., 2006; Eisch et al., 2003). Interestingly, stress induces activation of the mesocorticolimbic dopamine system (Tidey & Miczek, 1996), and BDNF co-localizes with tyrosine hydroxylase-ir neurons of this system

(Seroogy et al., 1994). Stimulation of dopamine synthesis also promotes the expression of BDNF (Okazawa, Murata, Watanabe, Kamei, & Kanazawa, 1992), while BDNF infusion into the VTA and NAc increases dopamine utilization (Horger et al., 1999; Pierce & Bari, 2001). In this context, a 30-min middle cerebral artery occlusion (MCAo) in mice elicited depression-like symptoms associated with degeneration of dopaminergic neurons, reduced dopamine levels and dopamine transporter density along with increased BDNF protein levels in ischemic striatum, which could be reversed by chronic antidepressant treatment initiated 7 days after stroke (Kronenberg et al., 2012). These findings suggest that alterations of BDNF signaling can regulate the reward circuitry and highlight the importance of BDNF-dopamine interplay in the development of PSD (Ghitza et al., 2010).

At present, the role of CRHR1 blockade on depressive-like behavior and expression of neuroplasticity in the mesocorticolimbic system after global cerebral ischemia has yet to be characterized. The goals of the current study therefore aim to determine the effects of pre-ischemic CRHR1 blockade on motivation, social interaction, anhedonia and depressive-like behaviors, as well as characterize changes in TrkB and BDNF protein and mRNA expression within the mPFC and NAc, using western blot and PCR techniques. Site-specific changes in BDNF and TrkB distribution in the subregions of the mPFC: the anterior cingulate cortex (CG1), prelimbic cortex (PL) and infralimbic cortex (IL), as well as the subregions of the NAc the core (NAcC) and shell (NAcS), and in the VTA were determined by immunohistochemistry.

2. Methods

2.1 Animals

Male Wistar rats (N = 96, n = 50 underwent behavioral testing, and n = 46 were used for the mRNA and protein analysis) weighing between 250 and 320 g at time of surgery were

obtained from Charles River Laboratories (Rochefort, Québec, Canada) and habituated to the housing facility for a minimum seven days before surgery. Rats were individually housed and maintained on a 12 h light/dark cycle (lights on at 7:00 AM) with free access to water and standard rat chow. Room temperature was maintained at 21-23 °C with 60% relative humidity. The experimenter handled all rats daily for 2-3 min for four days preceding surgery, and two days before behavioral testing. All experiments and procedures were conducted in accordance with the guidelines set by the Canadian Council of Animal Care and approved by the University of Ottawa Animal Care Committee. Efforts were made to minimize the number of animals used and their suffering.

2.2 ICV Cannulation and Drug Preparation

One week after arrival to the animal facility (see Fig. 1 for experimental timeline), rats underwent surgery for stereotaxic implantation of a guide cannula into the third ventricle for drug treatment administration, as previously described (de la Tremblaye et al., 2014). Briefly, rats were anesthetized using isoflurane mixed with oxygen (2-3%) and positioned in a stereotaxic instrument. Stainless steel 22-gauge guide cannula (Plastics One Inc.) were stereotaxically implanted using the following coordinates: 4.3 mm posterior to Bregma, 0.0 mm lateral to the midline and 4.3 mm ventral to the skull surface according to Paxinos and Watson atlas (1997) and secured to the skull using four anchor screws and dental cement. A dummy cannula was inserted into the guide cannula to prevent occlusion. The drug was administered via a 28-gauge stainless steel injector (Plastics One Inc.) projecting 0.5 mm below the tip of the guide cannula. Cannula placement into the 3rd ventricle was confirmed in thionin-stained slices.

Antalarmin hydrochloride (Sigma-Aldrich Inc.), a selective CRHR1 antagonist, was dissolved in 0.9% saline containing 10% Cremophor (Sigma-Aldrich Inc.). Ischemic and sham

rats were injected with Antalarmin (2µg/2µl; icv) or the vehicle solution 30 min prior to 10-min global ischemia or sham occlusion, creating 4 experimental groups: Ischemia-Antalarmin (IA; n = 12), Ischemia-Vehicle (IS; n = 12), Sham-Antalarmin (SA; n = 10), and Sham-Vehicle (SS; n = 10). A home cage (HC; n = 6) group, which did not undergo surgery or drug treatment, participated in behavioral testing.

2.3 Four-vessel occlusion surgery

The four-vessel occlusion (4VO) model was used to induce forebrain ischemia as previously described (Pulsinelli & Brierley, 1979). Briefly, rats were anesthetized using isoflurane (2-3%) mixed with oxygen. The core temperature was maintained at 37 ± 0.5 °C throughout surgery and during ischemia using a feedback-regulated heating blanket connected to a rectal thermometer. The vertebral arteries were irreversibly occluded by electrocoagulation and a small-diameter silk thread looped around the carotid arteries to facilitate subsequent occlusion. Sham-operated animals underwent anesthesia and received the same surgical incisions as the ischemic group without electrocoagulation of the vertebral arteries. Twenty-four hours later, rats were briefly anesthetized and carotid arteries re-exposed for clamping. Cerebral ischemia occurred between 7:30-9:30 AM. After discontinuing anesthesia and at first sign of wakefulness, the carotid arteries were occluded with microvascular clamps for 10 min in freely ventilating rats.

2.4 Behavioral Testing

2.4.1 Three Chamber Social Approach Test

On the 8th day following reperfusion, sociability and preference for social novelty were assessed using the three chamber social approach test (Toth & Neumann, 2013). Rats acclimated

to the room 30 min prior testing, which took place in the early afternoon to minimize testing-related anxiety and circadian variations in activity. The test was carried out using a slightly modified open field arena separated into three compartments (center compartment: 20×50×50 cm; left and right compartments: 40×50×50 cm) by clear Plexiglas walls that have arched doorways to allow free access among chambers via the middle chamber. During the acclimation trial, each rat was placed in the center compartment and allowed to freely explore for 5 min. During the 10 min sociability trial, a Stranger rat (S1), an unfamiliar but gender and weight matched Wistar rat, was placed inside a meshed cage located in one of the two side compartments, while an identical but empty meshed cage (EC) was placed in the opposite side compartment. The side location of S1 was counterbalanced between animals. During the 10 min social novelty trial, S1 remained in one of the meshed cages and a novel Stranger rat (S2) was placed into the empty meshed cage. The arena was cleaned with 70% ethanol between each session. The stranger rats were placed in meshed cages to prevent direct physical contacts and ensure that all social behaviors were initiated or terminated by the experimental rat. The animals serving as strangers were habituated to placement in the mesh cages during 5 min sessions for the three days preceding the test. Rat behavior was recorded with an overhead camera, connected to a PC computer. Time of direct (sniffing and stretching in an active, exploratory manner around the cage) and indirect (exploration in specific chamber but without contact to the cage) interactions with S1 & EC and S1 & S2, and time spent in the two chambers were determined by an experimenter unaware of the rats' treatment conditions, using OD log software (ODlog 2.0, USA). The duration of walking, rearing, grooming, and freezing was also recorded.

2.4.2 The Forced Swim Test (FST)

The FST originally developed by Porsolt et al. (Porsolt, Anton, Blavet, & Jalfre, 1978) has proven highly valuable for assessing the antidepressant properties of the majority of currently available antidepressants (Petit-Demouliere, Chenu, & Bourin, 2005). We used the modified rat FST in which case, depth of water and number of behaviors scored are increased to improve the sensitivity of the test (Masana, Castane, Santana, Bortolozzi, & Artigas, 2012). Rats are placed in a clear Plexiglas cylinder (20 cm wide and 46 cm high) filled with a 30 cm depth of water (temperature between 22-25ºC) for 15 min. Following this period, they are removed from the tank, dried off with paper towel and placed into a cage warmed by a heating pad before being returned to their home cages. Twenty-four hours later, rats are re-exposed to the test for 5 min and their behavior assessed. The sessions are recorded using a video camera mounted from the side allowing visualization of the four animal's paws. Escape-directed behaviors and immobility are scored every 5 s of the 300 s testing period using a data logging software (ODlog 2.0, USA). Escape-directed behaviors include (a) climbing, with vertical movement of the forepaws; and (b) swimming, with horizontal movement throughout the swim chamber; (c) Immobility is defined as the lack of movement in three of the four paws, with movement in the fourth paw being only necessary to keep the rat afloat (Overstreet, 2012). The time-sampling technique enables a convenient method for quantifying and distinguishing the active behaviors from immobility. Increased immobility is indicative of heightened despair (Slattery & Cryan, 2012).

2.4.3 Sucrose Preference Test

The sucrose preference test is a measure of the hedonic state or ability to experience pleasure of an animal. Reduced sucrose preference indicates decreased sensitivity to reward, a fundamental feature in clinical depression (Overstreet, 2012). Anhedonia was determined by

measuring preference for a sucrose solution over water, using a two-bottle free-choice method (Filho et al., 2015). The basal sucrose preference was evaluated two days after arrival to the animal facility and four days prior to surgery. Rats were provided with a free choice from two 100 ml glass bottles containing 1 % sucrose or water for four consecutive days, the position of the bottles being switched every 12 h. Liquid intake (consumption of water and sucrose solution) was measured by weighing the bottles each morning between 7:00 and 9:00 AM. Then, starting 14 days following global ischemia, rats were re-exposed to pre-weighted bottles of 1% sucrose solution or water. At this time interval, rats had free access to the bottles for four consecutive days, the bottle position being switched every 12 h. The amount of consumed liquids was determined by weighing the bottles prior and every 24 h post exposure. Sucrose preference was evaluated via calculating the ratio between the volume of sucrose solution consumed over the total liquid intake (sucrose preference = sucrose consumption/(sucrose solution consumption + tap water consumption) × 100%). Anhedonia is inferred from reduced sucrose preference in rats.

2.4.4 Novelty Suppressed Feeding Test (NSFT)

The novelty-suppressed feeding test was performed on day 27 following reperfusion. Similar to the open field test, the NSF test is based on rodents' innate fear of novel spaces and the ability of the rat to resolve a conflict between a context that accentuates anxiety and a drive to approach an appetitive stimulus (W. Liu & Zhou, 2012). On the testing day, the rat is transferred from its home cage to a novel larger plastic cage (17×17×12 inches) with the floor covered with 2 cm of wooden chip bedding and a light beaming to brightly illuminate (450 lux) the center zone. Food but not water is removed from the home cage for a fasting period of 24 h before the NSF test. Animals are brought to the testing room at least 30 min before the test. At the time of testing, a single weighed food pellet (regular rat chow) is placed on a white filter paper

positioned in the center of the box. The rat is placed in a corner of the box and its movements are videotaped for a maximum of 10 min. The latency taken to eat the pellet (defined as the rat sitting on its haunches and biting the pellet with the use of his forepaws) was noted using a stopwatch. Immediately afterwards, the animal is transferred to its home cage, and the amount of food consumed by the rat in the subsequent 5 min is measured.

2.5 Physiological Measurements

2.5.1 Body Weight

Animal weights were recorded before each of the surgical procedures, and at 7, 14, 21 and 30 days following sham occlusion or 10 min global ischemia.

2.5.2 Food Consumption

Rat chow consumption over a 24 h period was measured from day 7 to day 14 following reperfusion for all animals. During this period, rats were provided with a pre-weighed food ration every morning at 9:00 AM. Daily food consumption was determined by weighing the amount of food remaining in the cages (including food pieces that might have fallen inside the cage) 24 h later.

2.5.3 Trunk Blood and Organ Collection

Thirty days following occlusion, rats were removed one by one from their housing room and rapidly decapitated with a sharp guillotine, and trunk blood collected in 1.5 ml EDTA coated tubes. The blood was centrifuged at 4000 rpm for 10 min at 4°C, and the serum was decanted and stored at −80°C until determination of CORT levels using commercially available radioimmunoassay kits (MP Biomedicals, USA) (M. R. Milot et al., 2012). Following decapitation, the adrenal glands, thymus, and seminal vesicles were rapidly extracted and

weighed according to previously described methods (Tortora & Amitrano, 2013). The organs wet weight values were normalized using each rat's body weight before statistical analysis.

2.6 Biochemical Assays

2.6.1 Immunohistochemistry

Rat brains were quickly removed following decapitation, frozen in dry ice before storing at −80°C. Cryostat cut 14 µm coronal sections were collected through the prefrontal cortex (Bregma ∼−3.2 to −2.2 mm), nucleus accumbens (NAc, Bregma ∼ −2.70 to −1.70 mm), and ventral tegmental area (VTA, Bregma ∼−5.60 to −6.30 mm), using the rat brain atlas (Paxinos & Watson, 1997), and mounted onto Superfrost Plus slides (Fisher Scientific, Canada). Brain sections were postfixed using 4% PFA for 5 min at RT, rinsed (3 × 5 min) with PBS, and blocked in 1% BSA in 0.02% Triton/PBS for 30 min. They were then incubated overnight at 4°C in blocking buffer containing a polyclonal rabbit anti-BDNF- (1:500, Santa Cruz Biotechnology Inc., USA) or a polyclonal rabbit anti-TrkB (1:500, Santa Cruz Biotechnology Inc., USA) primary antibodies. After 3 × 5 min PBS rinses, slides were incubated for 2 h at RT with a fluorescent-conjugated donkey anti-rabbit secondary antibody (1:400, Invitrogen Canada Inc.) and rinsed. A last incubation for 10 min at RT with a Hoestch nuclear stain solution (1:20000 Hoechst 33342, Invitrogen Canada Inc.) was performed before mounting the slides.

Immunofluorescence labeling was visualized using an Olympus DX51microscope (Center Valley, PA, USA) and 6 bilateral digital images of BDNF and TrkB-ir expression from the mPFC, NAcC (core), NAcS (shell), and VTA analyzed by a blinded investigator, using Image J software (Image J, National Institutes of Health), as previously described (de la Tremblaye et al., 2014). Optical densities (Mean grey values) determined the staining intensity using the threshold technique. Data are presented as background corrected standardized image

densities for each brain region. The boundaries of the mPFC and its subregions were determined using the corpus callosum and the midline (Paxinos & Watson, 1997). Immunolabeled cells in the anterior cingulate cortex (AC), the prelimbic cortex (PL), and the infralimbic cortex (IL) were determined by centering the field within each subregion at 100 × using the corpus callosum and the midline as a guide, then shifting to a 200 × magnification. The anterior commissure and lateral ventricle were used as landmarks for the NAcS, NAcC, ensuring that the image was within the subregion of interest.

2.6.2 Tissue preparation for RT-PCR and mRNA determination

An additional subset of rats (N = 46, 4 experimental groups; n = 10 per group; and 1 group of naïve/unoperated animals; n = 6) from the same experimental design were decapitated 30 days after ischemia. Rat brains were carefully removed and placed in a stainless-steel mold to obtain 2 mm thick coronal sections from the fresh brain tissue. The PFC and NAc were rapidly dissected on an ice-cold aluminium plate covered with a filter paper dampened with PBS. Dissected tissue was placed in 1.5 ml Eppendorf tubes, flash-frozen in liquid nitrogen and rapidly stored at −80°C.

2.6.3 RNA Extraction, Reverse Transcription and PCR

According to the manufacturer's protocol, frozen tissue was homogenized in 1 ml TRIzol reagent (Invitrogen Canada Inc.) using a Sonicator (Fisher Scientific, Misonix XL2000 Ultrasonic Homogenizer). Homogenates were incubated at RT for 5 min in order to completely dissociate nucleoprotein complexes. Subsequently, 0.2 ml of chloroform was added and the homogenate was shaken vigorously for 15 s and incubated for 5 min at RT. Samples were subsequently centrifuged at 12 000 g for 15 min at 4 °C. The aqueous phase, containing RNA, was mixed with 0.5 ml of isopropanol, incubated at RT for 10 min, and centrifuged at 12 000 g

for 10 min at 4°C. The resulting RNA pellet was washed in 75% ethanol, centrifuged at 7500 g for 5 min at 4°C, air dried and dissolved in 20 µl 0.1% DEPC water, and stored at −80°C until analysis. The RNA concentration was determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer. The ratio between the absorbance values at 260 and 280 nm was used to estimate RNA purity. Total RNA (2µg) from each sample was reverse transcribed for 60 min at 42°C using SuperScriptTM II (200 units, Invitrogen Canada Inc.) and First Strand Buffer (Invitrogen Canada Inc.), in the presence of 0.5 µg/µl oligo dT, 10 mM DL-dithiothreitol and 1mM deoxynucleotide triphosphate solution (dNTP mix, Invitrogen Canada Inc.). The cDNA was stored at −20°C.

PCR was performed, using specific primers (Invitrogen Canada Inc.) designed to selectively amplify mRNA for BDNF, TrkB, and GAPDH (housekeeping gene). The sequences of primers were as follows: TrkB (For-5'GGCCAAGAATGAATATGGTAA 3'; Rev-5'TTGAGCTGGCTGTTGGTGAT 3'), BDNF (For-5′-ATGGGACTCTGGAGAGCGTGAA-3′; Rev-5′-CGC CAGCCA ATTCTC TTT TTGC-3′), GAPDH was used as loading control (For-5′-CATGGCCTTCCGTGTTCCTACCC-3′; Rev-5′-CCTCGGCCGCCTGCTTAC-3′). Amplifications of cDNA (1µl) obtained from RT were performed in PCR buffer containing 10mM dNTPs, 2 mM MgCl2, 10µM of each specific primer, and 2µl of Taq polymerase in a total volume of 25µl. Negative control samples lacking cDNA were processed in parallel with the same experimental protocol. cDNA amplifications were performed in an Eppendorf thermocycler (Brinkmann) after 30-32 cycles, using the following conditions: denaturation at 94°C/1 min, annealing temperature 60°C/1 min (BDNF), 53°C/1 min (TrkB), or 60°C/1 min (GAPDH), extension 72°C for 1 min, and final extension 72°C for 8 min. For each set of primers, control experiments, involving varying the number of cycles, were performed to define the linear

range for PCR amplification. PCR products were electrophoresed on 2% agarose gels together with a 100 bp DNA Ladder (FroggaBio Inc.), and visualized under u.v. light using ethidium bromide. The intensity of PCR products was measured with an image analysis system Image Lab 4.1 (Bio-Rad Laboratories Canada Ltd.) and normalized using GAPDH amplification as control.

2.6.4 Western Blotting

Western blotting was used to determine TrkB, presynaptic marker synapsin and post-synaptic marker PSD-95 protein levels. Micropunched tissue from the mPFC and NAc was homogenized in a lysis buffer, containing 10 µl of inhibitor cocktail in 500 ml of radioimmunoprecipitation assay (RIPA) buffer (Sigma-Aldrich Inc.). The homogenates were centrifuged, the supernatants collected and total protein concentration determined according to the Bradford method (Bio-Rad Laboratories Canada Ltd.). After boiling with sodium dodecyl sulfate (SDS) for 5 min, samples were electrophoresed on an 8% polyacrylamide gel under constant 100 mV current. Separated proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane using transfer stacks/iBlot gel transfer device (Invitrogen Canada Inc.). Nonspecific binding sites were blocked using 10% milk in Tris-Buffered Saline and Tween 20 (TBST, 20 mM Tris–HCl pH 7.6, 137 mM NaCl, 0.05% Tween-20), and membrane was incubated overnight at 4°C with anti-TrkB (1:2000, Santa Cruz Biotechnology Inc., USA), anti-synapsin (1:500, PhosphoSolutions, LLC) or anti-PSD95 (1:1000, Zymed Laboratories Inc.). Following TBS washes, a 2 h incubation with an anti-rabbit or anti-mouse IgG horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology, Inc. USA) was performed at room temperature. Immunoblots were then rinsed three times with TBST, and then visualized on X-ray films (Fisher Scientific), after exposure to an enhanced chemiluminescence, ECL, kit (Millipore Canada Ltd.). The molecular size of each protein was determined by running

a protein ladder (FroggaBio Inc.) in an adjacent lane. Membranes were stripped with TBST containing Reblot Plus solution (Millipore Canada Ltd.) for 20 min at room temperature in between antibodies. The film signals were digitally scanned and quantified using Image J software. Anti-actin (1:50000, Abcam Inc.) was used as an internal control such that data were standardized according to actin values.

2.7 Statistical Analysis

All statistical analyses were conducted using SPSS Statistical Software (version 20.0). The assumption of homogeneity of variance was verified. Separate two-way ANOVAs were performed to analyze the social behavior data as well as immunoreactive, protein and mRNA levels for the specific markers. When main effects or interactions were detected, one-way ANOVAs including the HC group were conducted to assess between group differences followed by Bonferroni-corrected post-hoc comparisons. For all analyses, $p < .05$ was considered statistically significant. Data are presented as means ± SEM, with $p < 0.05$ to represent significant analyses.

3. Results

3.1 Three Chamber Social Approach Test

No side preference (right versus left chamber) was found during the habituation period.

3.1.1 Sociability

Fig. 2 respectively illustrates the time of direct (A) and indirect (B) interaction with the stranger rat (S1) versus the time spent exploring the empty meshed cage (EC) (upper panel), or the time interacting with a new stranger rat (S2) versus a familiar stranger S1 (lower panel).

Preferences ratios for time of direct (C) and indirect (D) interaction are presented in both upper and lower panels.

3.1.1.1 *Direct Interaction Time (DIT) with S1, EC and S1/EC Preference Ratio*

Two-way ANOVA on DIT with S1 indicated no main effects but a surgery x drug interaction ($F(1, 47) = 7.578$, $p = 0.008$), which ANOVA showed attributable to IS rats spending increased DIT with S1 compared to SS ($p = 0.004$), IA ($p = 0.016$), and HC ($p = 0.037$). Two-way ANOVA on the preference ratios for S1 versus EC indicated a surgery x drug interaction ($F(1, 47) = 14.893$, $p = 0.001$). Between group differences ($F(4, 54) = 5.370$, $p < .0001$) were related to increased time spent with S1 in IS rats compared to SS ($p \leq 0.05$). SA also showed a preference for S1 compared to SS ($p = 0.003$) and HC ($p = 0.016$) groups (Sociability, Fig. 2 A & C).

3.1.1.2 *Indirect Interaction Time (ITT) with S1 and S1/EC Preference Ratio*

Two-way ANOVA on S1/EC preference ratio revealed a main effect of drug ($F(1, 47) = 4.024$, $p = 0.051$) and a surgery x drug interaction ($F(1, 47) = 5.570$, $p = .022$). Simple effects analyses indicated that SS had lower ratios than IS ($F(1, 47) = 4.087$, $p = 0.049$) and SA ($F(1, 47) = 9.008$, $p = 0.004$) rats (Sociability, Fig. 2 B & D). There were no other significant effects.

3.1.1.3 *Behavioral Measures during the Social Approach Test*

There were no main effects or interaction for time or frequency of rearing, grooming and freezing behaviors ($p > 0.05$). For locomotion, a main effect of drug ($F(1, 47) = 12.002$, $p = 0.001$) and a surgery x drug interaction ($F(1, 47) = 5.709$, $p = 0.021$) were observed attributable to reduced locomotion time in SA compared to IA ($p = 0.013$), SS ($p = 0.001$) and HC ($p = 0.001$) rats (data not shown).

3.1.2 Social Novelty

3.1.2.1 *Direct Interaction Time (DIT) with S2, S1, and S2/S1 Preference Ratio*

Two-way ANOVA on DIT with S2 revealed a surgery x drug interaction ($F(1, 47) = 14.989$, $p = 0.001$) due to IS rats spending increased DIT with S2 compared to SS ($p = 0.001$), IA ($p = 0.001$) and HC ($p = 0.004$) groups. ANT treatment normalized behavior in ischemic rats bringing S1 versus S2 interaction to similar levels as that observed in SS rats, while ANT led to increased DIT with S2 in sham rats ($F(1, 47) = 4.348$, $p = 0.043$). Two-way ANOVA on S2/S1 preference ratio indicated a surgery x drug interaction ($F(1, 47) = 20.239$, $p = 0.001$) due increased preference ratio in IS compared to IA rats and SS ($p \leq 0.007$) groups. SA also showed higher preference ratio than SS and HC ($p \leq 0.005$) groups (Social Novelty; Fig. 2 A & C).

3.1.2.2 *Indirect Interaction time (IIT) with S2, S1, and S2/S1 Preference Ratio*

Two-way ANOVA on IIT with S2 indicated no main effects but a surgery x drug interaction ($F(1, 47) = 7.797$, $p = 0.008$). Between group differences ($F(4, 51) = 3.884$, $p = 0.008$) are due to IS ($p = 0.037$) and SA ($p= 0.028$) groups showing increased ITT with S2 compared to HC rats. For ITT with S1, a main effect of drug ($F(1, 47) = 4.917$, $p = 0.032$) and a surgery x drug interaction ($F(1, 47) = 7.572$, $p = 0.009$) were found. One-way ANOVA ($F(4, 51) = 4.440$, $p = 0.004$) revealed increased IIT with S1 by IA rats compared to all other groups, except the HC group ($p < 0.05$). For the preference ratio of IIT, a surgery x drug interaction ($F(1, 47) = 18.856$, $p = 0.001$) was observed due to IS and SA rats spending more IIT with S2 than S1 compared to SS ($p = 0.008$), IA ($p = 0.005$) and HC ($p = 0.03$) groups (Social Novelty; Fig. 2 B & D).

3.1.2.3 *Behavioral Measure during the Social Novelty Test*

No significant effects were obtained for time and frequency of locomotion, rearing and grooming ($p > 0.05$). A drug effect ($F (1, 47) = 35.47$, $p = 0.01$) was found for immobility time attributable to elevated immobility in vehicle compared to ANT-treated rats.

3.2 Forced Swim Test (FST)

Fig. 3 A & B illustrates the time spent and frequency of swimming, climbing, and immobility. For swimming time, a main effect of surgery ($F (1, 47) = 14.891$, $p = 0.001$) and a surgery x drug interaction ($F (1, 47) = 4.332$, $p = 0.043$) were found. One-way ANOVA ($F (4, 54) = 7.924$, $p = 0.001$) revealed reduced swimming time in IS rats compared to all groups ($p \leq 0.003$) except IA rats ($p = 0.086$). For swimming frequency, only a surgery effect ($F (1, 47) = 11.488$, $p = 0.001$) was found attributable to elevated swimming frequencies in ischemic compared to sham rats. For climbing time and frequency, there were no main effects or interaction ($p > 0.05$). For immobility time, main effects of surgery ($F (1, 47) = 24.589$, $p = 0.000$) and drug ($F (1, 47) = 4.597$, $p = 0.037$) were found. One-way ANOVA ($F (4, 54) = 11.255$, $p = 001$) revealed increased immobility time in IS compared to all other groups ($p < 0.005$). For the frequency of immobility only an effect of surgery ($F (1, 47) = 20.761$, $p = 0.000$) was found related to increased immobility in ischemic compared to sham rats.

3.3 Sucrose Preference Test (SPT)

Fig 3 C shows sucrose preference ratio for the six time points examined, 3 baseline ratios collected pre-ischemia, and 3 collected from Day 14 post ischemia. Two-way repeated measures ANOVA revealed a main effect of time ($F (2.7, 123) = 16.246$, $p < 0.001$), but no surgery and drug effects or interaction ($p > 0.05$). One-Way ANOVA failed to show group differences for the baseline measures ($p > 0.05$), but revealed decreased sucrose preference in IS compared to sham

and HC rats ($p < 0.05$) on post ischemic days 14 ($F (4, 51) = 3.136$, $p = 0.022$), 15 ($F (4, 51) = 3.164$, $p = 0.021$) and 16 ($F (4, 51) = 7.889$, $p = 0.000$), which was prevented by ANT treatment ($p < 0.05$).

3.4 Novelty Suppressed Feeding (NSF)

Two-way ANOVA revealed a surgery effect ($\boldsymbol{F}$ (1, 45) = 7.985, $\boldsymbol{p}$ = 0.007) and a surgery x drug interaction ($\boldsymbol{F}$ (1, 45) = 25.433, $\boldsymbol{p}$ = 0.001), attributable to reduced feeding latencies in IS compared to SS and IA groups ($p \leq 0.005$). ANT significantly reduced feeding latencies in sham rats ($p \leq 0.01$). The surgery effect is manifest by comparing HC and SS groups (Fig. 3 D).

3.5 Physiological Measures

3.5.1 Body Weight

Two-way repeated measures ANOVA on body weight revealed a main effect of time ($F (3, 158.5) = 644.375$, $p = 0.001$), surgery ($F (1, 52) = 11.91$, $p < 0.001$) and a time x surgery interaction ($F (3, 158.5) = 19.026$, $p = 0.001$). Ischemic rats showed reduced body weight gain compared to sham rats, which reached significance on the day following surgery and 24h, 72h and 7, 14, and 21 days following occlusion ($p \leq 0.009$)(Fig. 4, upper graph).

3.5.2 Food Intake

Two-way repeated measures ANOVA revealed an effect of time (days) ($F (6, 312) = 3.697$, $p = 0.001$), surgery ($F (1, 52) = 39.210$, $p = 0.000$), and a time x surgery interaction ($F (6, 312) = 2.204$, $p = 0.042$). Ischemic groups showed reduced food intake compared to sham rats from post ischemic day 7 to 13 ($p < 0.001$). HC did not differ from sham rats (Fig. 4, bottom graph).

3.5.3 CORT Serum Levels

Fig. 5 A shows trunk blood CORT levels measured 30 days post ischemia. Two-way ANOVA revealed a surgery effect ($F(1, 41) = 5.267$, $p = 0.027$) and a surgery x drug interaction ($F(1, 41) = 5.503$, $p = 0.024$) due to higher CORT levels in IS compared to SS and IA groups ($p \leq 0.01$).

3.5.4 Organ Weights

Two-way ANOVA on the adrenal gland to total body weight ratios revealed main effects of surgery ($F(1, 51) = 62.665$, $p = 0.001$) and drug ($F(1, 51) = 23.018$, $p = 0.001$). One-way ANOVA ($F(4, 56) = 24.382$, $p < 0.001$) revealed higher ratios in IS compared to all other groups ($p < 0.001$). IA had higher ratios than SA rats ($p < 0.001$) (Fig. 5 B). For the thymus, surgery ($F(1, 51) = 63.709$, $p = 0.000$) and drug ($F(1, 51) = 5.205$, $p = 0.027$) effects were found. IS rats had a smaller thymus than all other groups ($p < 0.001$), while IA remained different from sham groups ($p < 0.001$) (Fig. 5 C). For the seminal vesicles, surgery ($F(1, 51) = 26.306$, $p = 0.000$) and drug ($F(1, 51) = 6.315$, $p = 0.015$) effects were found. Between group differences ($F(4, 56) = 22.11$, $p < 0.001$) revealed smaller ratios in IS compared to sham and HC groups ($p <0.001$). ANT treatment mitigated the suppressive effect of ischemia on the seminal vesicles weight ($p = 0.05$) (Fig. 5 D).

3.6 Biochemical Measures

3.6.1 BDNF-Immunoreactivity (ir) in the mPFC

Fig. 6 shows diminished BNDF-ir expression across all mPFC sub-regions (CG1, PrL and IL) post ischemia, which was prevented by ANT-treatment. Within the CG1 zone, main effects of surgery ($F(1, 40) = 8.699$, $p = 0.005$) and drug ($F(1, 40) = 14.115$, $p < 0.001$) were

found. BDNF-ir was reduced in IS compared to all groups, except HC rats ($p < .0005$). Within the PrL, a surgery ($F (1, 40) = 23.340$, $p < 0.001$) and a surgery x drug interaction ($F (1, 40) = 8.489$, $p = 0.006$) were found. PrL-BDNF-ir was reduced in IS compared to all groups ($p < .05$). Similarly, a surgery ($F (1, 40) = 6.026$, $p= 0.019$) and surgery x drug interaction ($F (1, 40) = 14.716$, $p < .000$) were found at the IL due to reduced BDNF-ir in IS rats.

3.6.2 TrkB-Immunoreactivity in the mPFC

Fig. 7 shows a post ischemic diminution in TrkB-ir in CG1, PrL and IL sub-regions. Two-way ANOVAs performed on these subregions revealed main effects of surgery ($F (1, 40) = 65.889$, $p < 0.001$; ($F (1, 40) = 21.159$, $p < 0.001$; ($F (1, 40) = 6.771$, $p = 0.013$, respectively), which were related to reduced TrkB-ir in ischemic compared to sham groups.

3.6.3 TH-, BDNF- and TrkB-Immunoreactivity in the NAc

Within the core zone (Fig. 8), analysis of TH-ir showed main effects of surgery ($F (1, 36) = 19.083$, $p < 0.001$), drug ($F (1, 36) = 8.616$, $p = 0.007$), and surgery x drug interaction ($F (1, 36) = 4.831$, $p = 0.036$). IS showed the highest TH-ir of all groups ($p < 0.05$), which was attenuated by ANT treatment. For BDNF-ir, main effects of surgery ($F (1, 36) = 29.217$, $p< 0.001$), drug ($F (1, 36) = 41.771$, $p = < .001$), and surgery x drug interaction ($F (1, 36) = 24.045$, $p < 0.001$) were found. Between group differences ($F (4, 40) = 25.726$, $p < .001$) were due to significantly elevated BDNF-ir in IS compared to all other groups ($p < 0.001$). Two-way ANOVA on TrkB-ir revealed a surgery ($F (1, 36) = 39.869$, $p < 0.001$) and a surgery x drug interaction ($F (1, 36) = 6.937$, $p = 0.012$). One-way ANOVA ($F (4, 40) = 17.707$, $p < .001$) indicated decreased TrkB-ir in in IS and IA compared to all other groups ($p \leq 0.01$).

Within the shell zone (Fig. 9), analysis of TH-ir showed main effects of surgery ($F (1, 36) = 22.455$, $p < 0.001$) and drug ($F (1, 36) = 6.593$, $p = 0.016$). One-way ANOVA ($F (4, 40) =$

12.969, $p < .001$) revealed elevated TH-ir in IS and IA compared to HC and SA groups ($p < 0.01$). HC differed from SS rats ($p= 0.022$). For BDNF-ir, a surgery ($F (1, 36) = 16.977$, $p< 0.001$) and a marginal effect of drug ($F (1, 36) = 3.857$, $p = 0.057$) were observed, associated to increased BDNF in ischemic compared to sham and HC groups. Analysis of TrkB-ir showed main effects of surgery ($F (1, 36) = 7.026$, $p = 0.012$) and drug ($F (1, 36) = 4.966$, $p = 0.032$). Reduced expression was present in ischemic ($M = 19.466$, $SEM = 0.938$) compared to sham ($M = 22.981$, $SEM = 0.938$) groups, and ANT elevated TrkB-ir expression ($M = 22.701$, $SEM = 0.938$) compared to Veh ($M = 19.746$, $SEM = 0.938$) treatment.

3.6.4 TH-, BDNF- and TrkB-Immunoreactivity in the VTA

For TH-ir, only a drug effect ($F (1, 36) = 11.243$, $p = 0.002$) was found related to increased TH-ir in Veh- compared to ANT-treated rats. For BDNF-ir, a surgery ($F (1, 36) = 34.217$, $p < 0.001$) and surgery x drug interaction ($F (1, 36) = 21.604$, $p < 0.001$) were found. One-way ANOVA ($F (4, 40) = 16.561$, $p < .001$) revealed significantly lower BDNF-ir in IS compared to all groups ($p < 0.05$), except the HC ($p = 0.910$). SS showed elevated expression compared to HC. For TrkB-ir, a drug effect ($F (1, 36) = 22.851$, $p = 0.001$) and a surgery x drug interaction ($F (1, 36) = 12.43$, $p = 0.001$) were found. One-way ANOVA ($F (4, 41) = 10.417$, $p < 0.001$) showed reduced TrkB-ir in IS compared to all groups ($p \leq 0.02$), except the HC (Fig. 10).

3.7 Western Blot and Reverse Transcription Polymerase Chain Reaction (RT-PCR) Analyses

3.7.1 Protein and mRNA Expression at the Nucleus Accumbens

Fig. 11 (E-G) shows TrkB, Synapsin, and PSD95 protein expression at the NAc. For TrkB, a surgery x drug interaction ($F (1, 12) = 6.362$, $p = 0.027$) was found, which ANOVA ($F (4, 15) = 5.046$, $p = 0.009$) revealed related to elevated TrkB expression in SS compared to IS ($p = 0.05$)

and HC (p = 0.01) groups. For synapsin, main effects of surgery (F (1, 12) = 67.782, p = 0.001) and drug (F (1, 12) = 6.316, p = 0.027) and a surgery x drug interaction (F (1, 12) = 40.757, p = 0.000) were found. One-way ANOVA (F (4, 15) = 51.384, p =0.001) revealed elevated synapsin protein levels in SA and HC compared to SS, IS and IA groups (p < 0.001). For PSD-95, main effects of surgery (F (1, 12) = 49.340, p = 0.000) and drug (F (1, 12) = 21.851, p = 0.001) were found. Ischemic groups showed reduced PSD95 levels compared to sham rats and ANT-treatment also reduced expression. For BDNF and TrkB mRNA expression (Fig.11, E-G), main effects of drug were found (F (1, 12) = 19.614, p = 0.001 and F (1, 12) = 7.642, p = 0.014, respectively) both attributable to reduced mRNA levels in ANT- compared to VEH-treated animals.

3.7.2 Protein and mRNA Expression at the Prefrontal Cortex

Fig. 12 (E-G) shows TrkB, Synapsin, and PSD95 protein expression at the PFC. For TrkB, a surgery effect (F (1, 12) = 21.225, p = 0.001) was detected associated with reduced expression in ischemic compared to sham rats. For synapsin, main effects of surgery (F (1, 12) = 49.228, p = 0.000) and drug (F (1, 12) = 6.365, p = 0.027) were found. One-way ANOVA (F (4, 15) = 18.241, p =0.000) indicated increased synapsin expression in sham groups compared to ischemic and HC groups (p < 0.005). For PSD-95, a surgery effect (F (1, 12) = 41.678, p = 0.001) was observed related to reduced PSD95 expression in ischemic (M = 0.523, SEM = 0.071) compared to sham (M = 1.170, SEM = 0.071) animals. For mRNA expression (Fig.12, E-G), main effects of surgery (F (1, 12) = 4.566, p = 0.054) and drug (F (1, 12) = 81.594, p = 0.001), and a surgery x drug interaction (F (1, 12) = 21.736, p = 0.001) were found for BDNF. One-way ANOVA (F (4, 15) = 47.162, p =0.001) indicated that this was due to increased expression in ANT-treated and HC groups (p < 0.05). For TrkB, a surgery x drug interaction (F

(1, 12) = 9.614, p = 0.009) was found related to reduced mRNA expression in IS compared SS and IA rats ($p \leq 0.03$). In contrast, ANT reduced mRNA expression in sham rats (p = 0.037).

4. Discussion

To our knowledge, the current study is the first to characterize the role of CRHR1 activation on the regulation of plasticity signaling molecules within the mesocorticolimbic circuitry following global ischemia, and determine associated changes in socioemotional behavior in an animal model mimicking cardiac arrest in humans.

Behaviorally, ischemic rats showed heightened sociability and preference for social novelty in the SIT. ANT treatment normalized social behavior in ischemic animals, and enhanced sociability in the sham animals. These observations contrast impaired social interaction observed in rodent models of focal ischemia, traumatic brain injury and diffuse microembolic infarcts (Nemeth, Shurte, McTigue, Nemeroff, & Neigh, 2012; D. K. Pandey, Yadav, Mahesh, & Rajkumar, 2009; Verma, Friedler, Harris, & McCullough, 2014). An unfamiliar testing situation is traditionally viewed as an anxiogenic condition, and decreased social interactions used as an index of anxiety (File, 1980). Although mechanisms remain to be determined, reduced cell density in the basolateral nucleus of the amygdala and CRH-ir in the central nucleus of the amygdala have been observed following global ischemic and hypoxia in rats (de la Tremblaye & Plamondon, 2011b) and global ischemic rats have been shown to spend more time in open zones of the EPM and OFT (Dunbar, de la Tremblaye, Azogu, & Plamondon, 2015), possibly indicative of decreased anxiety and/or increased impulsivity. Notably, the PFC has been implicated in the top-down regulation of emotion and social behavior in humans and rodents (Avale et al., 2011; Hartley & Phelps, 2010; V. L. Wall, Fischer, & Bland, 2012). PFC lesions increase impulsivity (Hehar, Yeates, Kolb, Esser, & Mychasiuk, 2015) and impair social

behaviors in rodents (Spikman, Timmerman, Milders, Veenstra, & van der Naalt, 2012) and humans (Adolphs, 2009; Meyer-Lindenberg & Tost, 2012). Reduced PFC activation during a working memory task has also been associated with impaired social function in depressed patients (S. Pu et al., 2012).

In the last years, socio-emotional responses in humans and animals have been closely associated with changes in neurotrophic factors. In the current study, diminished BDNF expression levels in the PFC and hippocampus is similar to that induced following chronic unpredictable mild stress (Luo et al., 2015; Yi et al., 2014). Reduced BDNF and TrkB immunoreactivity and mRNA expression at the PFC in ischemic rats was accompanied by reduced protein levels of PSD-95 and synapsin. In this context, atrophy of dendrites and reduced BDNF levels in the mPFC in stressed animals have been proposed to underlay altered synaptic efficacy of efferent and afferent connections of the mPFC with limbic, striatal and basal forebrain structures, contributing to depression-like behavior (C. H. Duman & Duman, 2015). It is also interesting to note that inactivation of the mPFC leads to increased social interaction time in adolescent rats (van Kerkhof, Damsteegt, Trezza, Voorn, & Vanderschuren, 2013). PFC dopamine receptors appears involved with antagonism of D3 receptor improving social novelty discrimination in juvenile rats, while D2 receptor blockade impairs such behavior (Loiseau & Milian, 2009; Watson et al., 2012).

Heightened impulsivity could also mediate ‘reduced anxiety’ in global ischemic animals. Namely, top-down control of impulsive behavior proposed through serotonergic and dopaminergic connections between regions such as the hippocampus, amygdala and PFC, sending afferents to the NAc and VTA is intriguing to explore (Dalley, Everitt, & Robbins, 2011; Dalley & Roiser, 2012). For instance, impulsive deficits can be ameliorated by repeated

treatment with a serotonin/noradrenaline reuptake inhibitor through enhanced BDNF and PSD-95 protein levels, increased dendritic spine density and excitatory currents in surviving neurons of the lesioned ventromedial PFC of rats (Tsutsui-Kimura, Yoshida, Ohmura, Izumi, & Yoshioka, 2015). Motor impulsivity and increased novelty-seeking has also been associated with decreased dopamine levels at the PFC (Freund et al., 2014) and D1 receptors and BDNF mRNA levels within the dorsal hippocampus (Ferland et al., 2014). The hippocampus being the most vulnerable region to global ischemic insult (Schmidt-Kastner & Freund, 1991), it is interesting to contemplate that increased sociability and preference for social novelty, as well as a reduced latency to feed in the NSF test may in part be related to altered dopamine levels in the mesolimbic circuitry feeding into the PFC (Dalley, Mar, Economidou, & Robbins, 2008).

Biochemically, our findings support reduced TrkB, but heightened BDNF and TH immunoreactivity in the NAc core and shell in ischemic rats, which were reversed by ANT treatment. Notably, inputs from the PFC to NAc core originate dorsally in the cingulate and prelimbic cortices whereas the NAc shell is innervated primarily by neurons located more ventrally in the infralimbic cortex (Berendse, Galis-de Graaf, & Groenewegen, 1992; Sesack, Deutch, Roth, & Bunney, 1989). Thus, consistent patterns of ir-expression in the NAc core and shell portions may be related to ischemia-induced inhibition of BDNF and TrkB observed across the mPFC subfields. While BDNF mRNA expression is relatively low in the NAc (Seroogy et al., 1994), TrkB is widely expressed at the brain site (Altar et al., 1994). Our findings demonstrate increased BDNF and TrkB mRNA levels in the NAc of SS and IS, which were significantly attenuated by ANT-treatment, although changes at the protein levels were not correlated. Reduced PSD-95 and synapsin protein level expression was also apparent in ischemic rats compared to naïve and sham control groups, a phenomenon not affected by CRHR1

antagonism. Such changes could support dysfunctional neurotransmitter release in the ischemic brain (Chi, Greengard, & Ryan, 2001). In addition, BDNF synthesis in NAc neurons likely influences TrkB receptor activation on dopamine afferents and retrograde transport to dopamine cell bodies in the VTA (Graham et al., 2009). Conversely, BDNF synthesized in VTA dopamine neurons can be released locally and act reciprocally on TrkB receptors to augment dopamine cell excitability (L. Pu, Liu, & Poo, 2006) or undergo anterograde transport and release in the NAc to activate TrkB receptors on NAc neurons (Altar et al., 1997). Interestingly, our findings indicate that BDNF and TrkB receptors at the NAc appear more sensitive to forebrain ischemia than at the VTA. Increased TH expression in the NAc and VTA 30 days post ischemia may be time-dependent as a recent study from our lab showed reduced TH-ir in the same regions 9 days post ischemia, which was accompanied by reduced anxiety in the EPM and OFT (Dunbar et al., 2015).

In terms of functional relationships, VTA and NAc receive strong glutamatergic inputs from the PFC, hippocampus, and amygdala, which in turn are innervated by VTA dopamine neurons, forming a critical neurocircuit in the regulation of motivation and mood (Nestler, 2015). The intra-VTA infusion of BDNF produces depression-like behavior, while the blockade of BDNF activity in the NAc produced antidepressant-like effects (Eisch et al., 2003; Nestler & Carlezon, 2006). A marked reduction of BDNF protein and pTrkB expression in the mPFC is found in rats developing learned helplessness following inescapable foot-shocks, while opposite changes are reported in the NAc (Shirayama et al., 2015; C. Yang, Shirayama, Zhang, Ren, & Hashimoto, 2015). Heterozygous BDNF+/Met mice, carrying the human BDNF Val66Met polymorphism, exhibit heightened depression-like behaviors and decreased BDNF levels and apical dendritic spine density in the PFC following stress (H. Yu et al., 2012). Bilateral infusion

of the TrkB agonist 7,8-DHF into the infralimbic area of the PFC exerts antidepressant effects, while bilateral infusion of the TrkB antagonist ANA-12 into the shell and core of the NAc induces antidepressant effect following repeated inescapable foot-shocks (Shirayama et al., 2015) and LPS injection (J. C. Zhang, Wu, et al., 2015). Furthermore, LPS-induced depressive-like behaviors are associated with reduced spine density and BDNF protein levels at the PFC, while opposite changes were present at the NAc. NAc injection of the TrkB antagonist ANA-12 reduced depressive-like behaviors in LPS-treated mice (J. C. Zhang, Wu, et al., 2015) as well as blocked the ability of phasic stimulation to induce social avoidance (Walsh et al., 2014).

To date, few studies have examined the effects of global cerebral ischemia on emotional behavior in rodents (Bantsiele et al., 2004; Bantsiele et al., 2009b) despite the high prevalence of stroke patients living with depression (Almeida & Xiao, 2007). In the current study, global cerebral ischemia led to increased immobility in FST and decreased sucrose preference, indicative of a depressive phenotype. Prolonged immobility and reduced swimming time were attenuated by ANT treatment. Immobility and swimming frequency were higher in ischemic animals due to rats going in and out of immobility and swimming behaviors while the sham groups were more consistent in their swimming pattern. Increased immobility and decreased swimming time in the forced swim test have demonstrated depressive-like behaviors in rodent models of myocardial infarction (MI) (Bah et al., 2011) and middle cerebral artery occlusion in mice (MCAO) (Deplanque, Venna, & Bordet, 2011; Kronenberg et al., 2012). MI rats also showed reduced preference for a sucrose solution, indicative of anhedonia (Bah et al., 2011). Pre-treatment with pentoxifiline, a pro-inflammatory cytokine inhibitor or post-treatment with the SSRI fluvoxamine effectively reduced depressive-like behaviors but failed to improve brain infarct size or change in spontaneous motor activity (Bah et al., 2011; Deplanque et al., 2011).

Importantly, increasing evidence supports the dysregulation of the neuroendocrine system in depression and its relationship with increased proinflammatory cytokines and diminished neurotrophic support leading to neuronal loss, reduced neurogenesis and dendritic atrophy in human and animal studies of depression (Colla et al., 2007; Maletic et al., 2007).

The anhedonic and depressive-like responses observed in ischemic rats is interesting considering that chronic CORT injections similarly reduce sucrose preference and prolong immobility time in the FST in mice (Y. C. Li et al., 2015). In this study, elevated HPA reactivity post ischemia is suggested through increased post mortem CORT levels and adrenal gland weight ratios while thymus and seminal vesicle ratios were reduced, all being prevented by pre-ischemic ANT treatment concomitant to normalized emotional responses in treated animals. Notably, PFC lesions can alter plasma CORT levels (Diorio, Viau, & Meaney, 1993; Feldman & Conforti, 1985; Feldman & Weidenfeld, 1999; Kovacs & Makara, 1988; Magarinos, Somoza, & De Nicola, 1987; Mizoguchi, Ishige, Aburada, & Tabira, 2003) and reduced number of glucocorticoid-receptors in the mPFC, hippocampus or PVN in aged rats can block the suppressive effect of the synthetic glucocorticoid dexamethasone (DEX) into these brain loci (Mizoguchi et al., 2009). Increased serum levels of CRH, CORT, and ACTH are also associated with downregulation of hippocampal BDNF and TrkB protein expression following 21 days of chronic mild stress in mice (Y. Chen, Liu, Wu, & Nice, 2013). Patients with depressive and anxiety disorders often show a hyperactive HPA axis characterised by increased levels of cortisol, hypertrophy of pituitary and adrenal cortex and over-expressed hypothalamic CRH (S. S. Wang, Kamphuis, Huitinga, Zhou, & Swaab, 2008), mostly ascribed to impaired feedback regulation of the HPA axis, possibly caused by altered function of the glucocorticoid receptors (Anacker, Zunszain, Carvalho, & Pariante, 2011).

Neuroendocrine studies provide strong indications that hyperactivity of CRH-related brain circuits, resulting in a dysregulation of the HPA axis system, plays a causal role in the symptomatology of affective and anxiety disorders (Keck & Holsboer, 2001). Prior stress can sensitize the HPA axis by reducing efficacy of glucocorticoid negative feedback, characterized by intact or enhanced ACTH response to a subsequent stressor (De Souza & Van Loon, 1982; Le Mevel, Abitbol, Beraud, & Maniey, 1979; O'Connor et al., 2004; O'Connor et al., 2003; Weiser, Osterlund, & Spencer, 2011). A depression-like phenotype in GR heterozygous (GR+/-) mice by the DEX/CRH test is evident by much higher blood CORT levels than wild-type mice (Ridder et al., 2005). Adverse consequences of severe or chronic stress also include adrenal gland hypertrophy (Nemeroff et al., 1992; Rubin, Phillips, McCracken, & Sadow, 1996), thymic involution (Tarcic, Ovadia, Weiss, & Weidenfeld, 1998), and reduced male fertility as characterized by smaller seminal vesicles (Blanchard et al., 1995; Weathington, Arnold, & Cooke, 2012). Stress also reduces food intake and body weight, effects dependent on the intensity of a stressor (Maniam & Morris, 2012; Marti, Marti, & Armario, 1994). Interestingly, adult male rats treated twice daily over 11 days with ANT (20 mg/kg; i.p.) displayed decreased plasma ACTH and CORT concentrations compared with vehicle-treated rats (Bornstein et al., 1998), indicating that CRHR1 is important in initiating the stress response, and appears involved in cortisol signaling associated with mood disturbances (Spijker & van Rossum, 2012).

The CRH system plays a key role in social behavior (Hostetler & Ryabinin, 2013). Social interaction (SI) is inversely associated with anxiety, such that decreased levels of SI are indicative of an anxiogenic state (File & Hyde, 1978). CRH and stressors reduce social interaction via CRHR1 activation (B. M. Campbell, Morrison, Walker, & Merchant, 2004; Dunn & File, 1987; Zorrilla & Koob, 2004), and a single injection of NBI3b1996 (10 and 30 mg/kg), a

CRHR1 antagonist can prevent reduced SI in repeatedly stressed animals (Gehlert et al., 2005). In contrast, central administration of the CRHR1 agonist, stressin1-A, increases anxiety-like behavior in the social interaction and shock-probe tests, while infusion of urocortin 3, a CRHR2 agonist, failed to affect anxiety and social interaction (Y. Zhao et al., 2007). Notably, CRH appears necessary for successful short-term social recognition as non-selective CRH receptor antagonist (D-Phe CRF12–41) and CRH binding protein inhibitor (r/h CRH(6–33)) dose-dependently impair social memory (Heinrichs, 2003). Mice over-expressing CRH also show heightened social recognition and memory upon a 24 h later retest (Kasahara, Groenink, Kas, et al., 2011).

In this study, ischemic rats showed reduced body weight gain and food intake, elevated serum CORT levels, increased adrenal gland and reduced thymus and seminal glands weight ratios. These features were significantly attenuated by ANT treatment. CRHR1 blockade also had a robust effect normalizing social behavior in ischemic rats and increasing interactions in sham groups, indicating that hyper arousal state may mediate social responses to novel environments (M. R. Milot & Plamondon, 2011a). As the encounter with a novel conspecific can be mildly stressful, the differences between sham and ischemic rats could be explained by an increased sensitivity of ischemic rats to stressors (de la Tremblaye et al., 2014) or a failure to adapt to environmental changes (Tonissaar et al., 2008). Notably, isolation rearing has been associated with robust increases in social interaction with a novel conspecific (Ferdman, Murmu, Bock, Braun, & Leshem, 2007; Hermes, Li, Duman, & Duman, 2011), an effect accompanied with decreased c-fos expression in the PFC (V. L. Wall et al., 2012). Social interaction has been shown to improve recovery to a higher extent than physical activity following focal cerebral ischemia and combined with an enriched environment allowing free physical activity to provide

the best therapeutic strategy (B. B. Johansson & Ohlsson, 1996). Despite the benefits of social interactions, it is unlikely that ischemic animals in the current study be attracted to social encounters as a therapeutic measure (Venna, Xu, Doran, Patrizz, & McCullough, 2014). Previous experiments rather suggest that increased social interaction following global ischemia may be a consequence of dysfunction affecting emotional brain circuitry, namely the mesocorticolimbic circuitry and HPA axis activation.

Conclusion

In conclusion, our study shows that global ischemia induced depression-like behavior, as well as alterations in BDNF/TrkB protein and mRNA density within the mesoccorticolimbic circuitry. More specifically, this study is the first to support that altered expression of BDNF, TrkB and TH expression in the NAc and PFC could underlay depressive-like behaviors observed in ischemic rats. Furthermore, our findings support that inhibition of CRHR1 receptors alters the magnitude of the stress cascade upon ischemia leading to normalization of BDNF/TrkB expression in PFC and NAc, which has important benefits on normalizing HPA axis activation and post stroke depression.

5. Acknowledgments

The authors thank Dr. Sarah Schock, Dr. William Staines, and Dr. Charlie Thompson for their help in performing western blotting and RT-PCR techniques in the current study. This work was supported by a grant from the National Science and Engineering Research Council of Canada to Dr. Hélène Plamondon and internal funding from the University of Ottawa. The authors declare having no conflict of interest regarding the work described here.

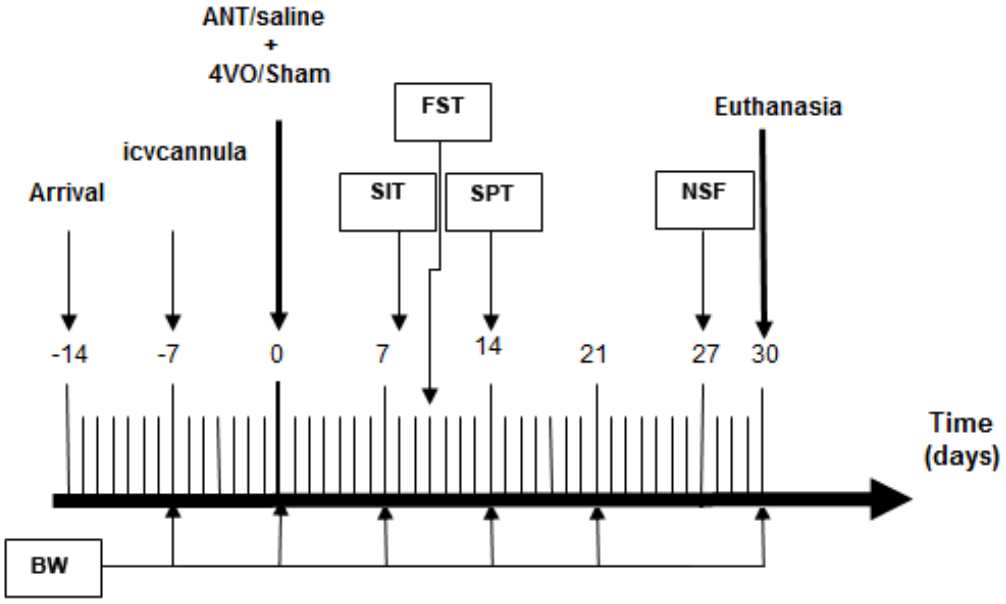

Fig. 1. Experimental timeline. Arrows indicate the days the various experimental procedures were conducted. After a 7-day acclimation period, rats underwent cannula implantation followed 7 days later by the ischemic/sham surgeries. Antalarmin (2 μg/2 μl) or vehicle was icv injected 30 min prior sham or carotid occlusion. The different testing procedures were conducted on the indicated days. SIT (day 8) - social interaction test; FST (day 9) - forced swim test; SPT (day 14) - sucrose preference test and on NSFT (day 27) - novelty suppressed feeding test. Body weight (BW) was assessed after each surgical procedure and every week until 30 days post reperfusion.

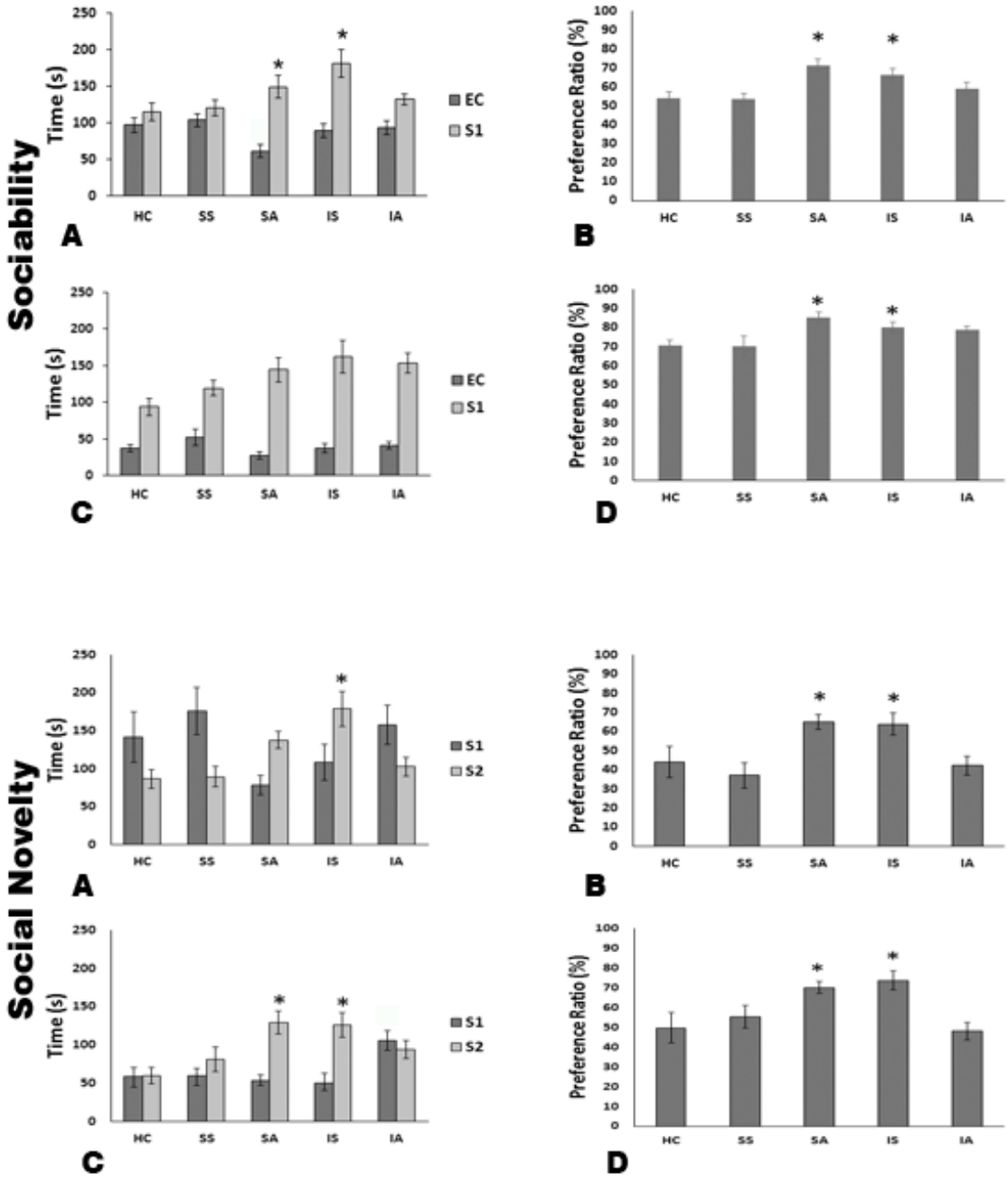

Fig. 2. **Sociability -** *Top figure*. Graphs A & C respectively show direct (DIT) and indirect (IIT) interaction time with stranger rat 1 (S1) versus the empty cage (EC), and B & D show preference ratios for the direct and indirect interaction. Increased direct interaction time with S1 and preference ratio support enhanced sociability by IS and SA groups. **Social Novelty Preference -** *Bottom figure*. Graphs A & C respectively show DIT and IIT with the novel stranger rat (S2) versus the familiar rat (S1), and B & D show preference ratios for the direct and indirect interaction. The IS and SA groups spent significantly more time directly and indirectly interacting with the novel stranger rat (S2) than the familiar rat (S1). In contrast, SS, IA, and HC groups showed more DIT with the familiar S1rat. Data are expressed as mean ± SEM. *$p < 0.05$.

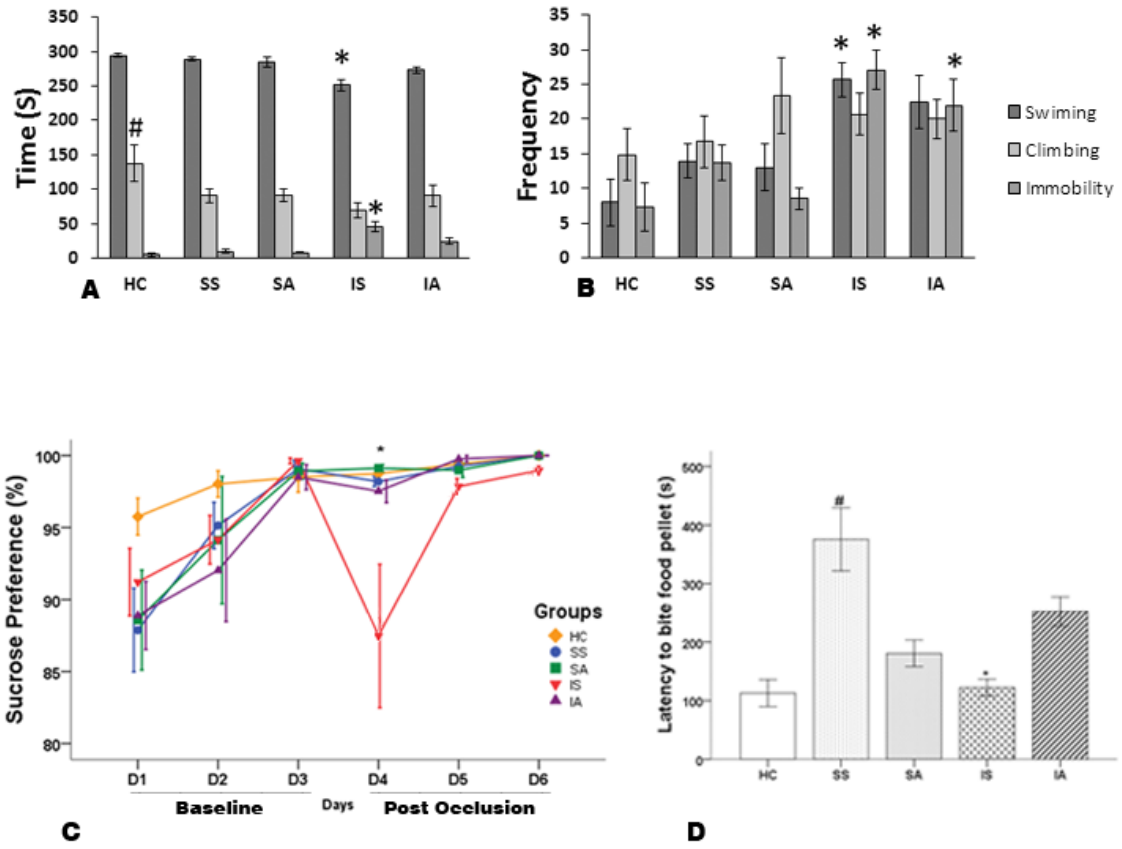

Fig. 3. Ischemia-induced depressive- and anhedonic behaviors are prevented by Antalarmin treatment. **Forced Swim Test** (Top panel graphs) - Time spent (**A**) and frequency (**B**) of swimming, climbing, and immobility. IS showed increased immobility and reduced swimming time compared to all groups ($p \leq 0.005$). Antalarmin prevented these changes. Interestingly, ischemic rats more frequently switched behavior (giving up much quicker) than control groups, increasing their swimming and immobility frequencies (*$p \leq 0.05$). #, indicates increased climbing time in HC compared to all groups ($p \leq 0.05$). **Sucrose preference** (**C)** assessed 14 days after occlusion (D4 on graph) was transiently reduced in IS rats compared to all other groups (*$p \leq 0.05$). **Novelty suppressed feeding** (**D)** IS showed reduced feeding latencies compared to SS and IA groups (*$p \leq 0.005$). #, indicates increased latency in SS compared to all groups ($p \leq 0.05$). Data are expressed as mean ± SEM.

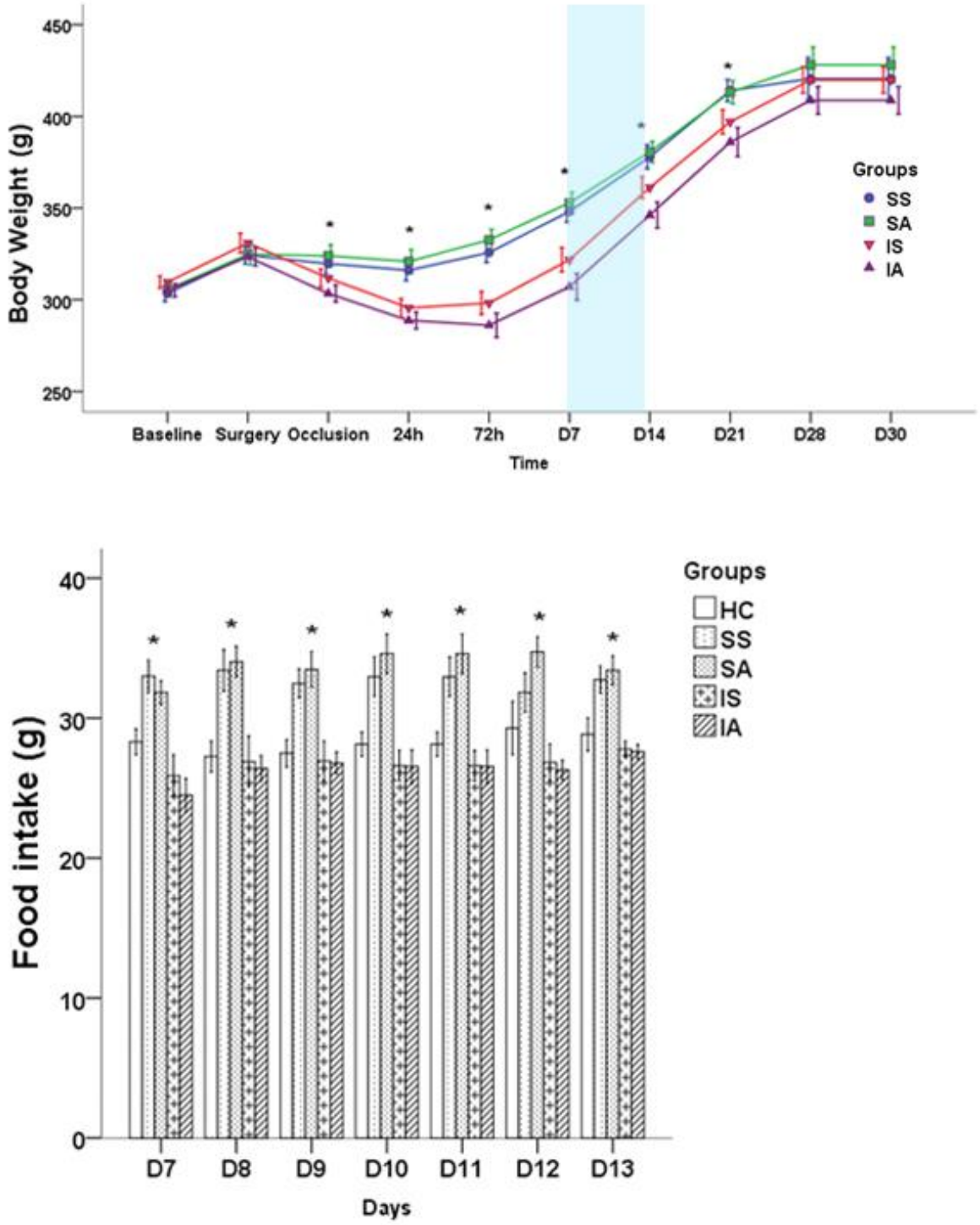

Fig. 4. **Body weight** (upper graph) assessed at baseline, after each surgery and weekly over a 30 day experimental time course. Ischemic rats show lower body weight than sham rats from the time of occlusion to the 21st day post ischemia. **Food intake** (lower graph) measurements initiated 7days post occlusion and for a 1-week period, which is indicated by the shaded area in the upper graph. Food intake was reduced in ischemic compared to sham operated rats. Antalarmin had no effect on these measures. Values represent means ± SEM. *$p < 0.05$.

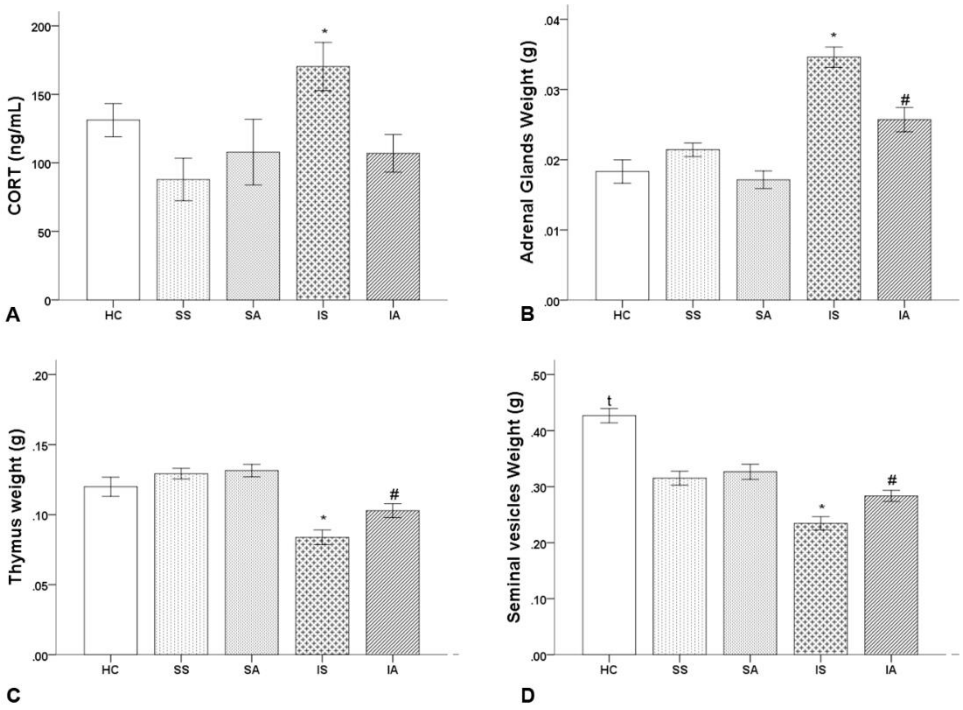

Fig. 5. CORT measurements and organ weights 30 days post ischemia. Ischemia increased CORT serum levels (**A**), and adrenal weight (**B**), but reduced the weight of the thymus (**C**) and seminal vesicles (**D**) (* $p < 0.05$). Antalarmin reversed these effects ($^{\#}$ $p < 0.05$). Values are expressed as means ± SEM.

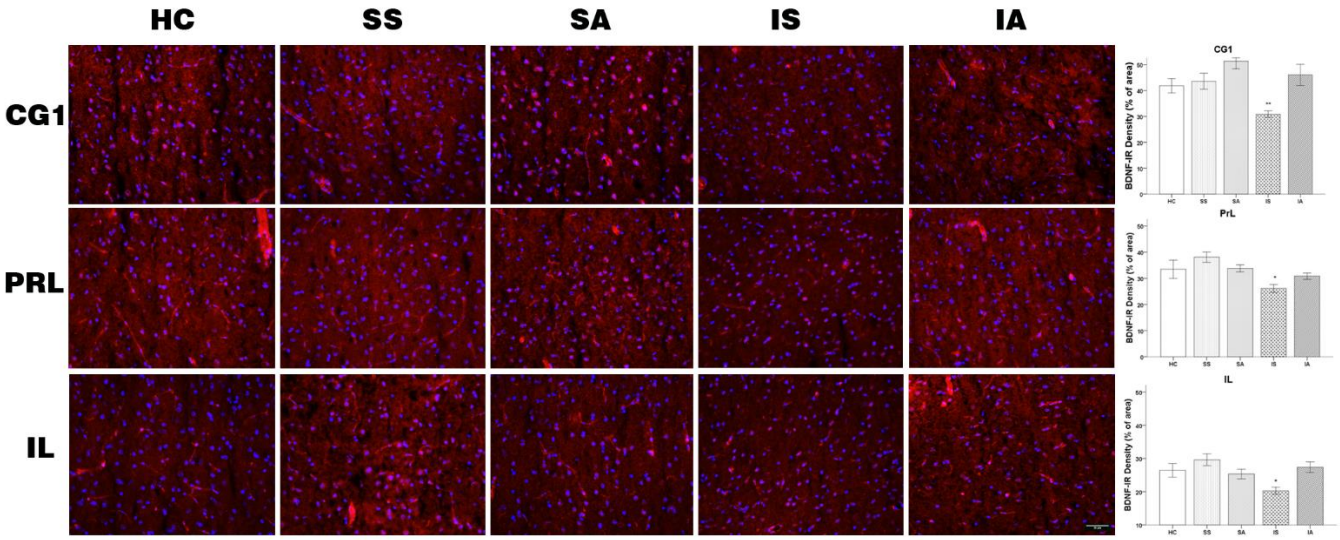

Fig. 6. BDNF-ir expression in the mPFC 30 days post ischemia. Representative photomicrographs of BDNF (red) and Hoescht (blue) immunopositive labeling within the cingulate, pre- and infra-limbic cortices [CG1, PrL, and IL, respectively] at 200X magnification. Histograms show mean percent optical densities for each of the groups. Ischemia reduced BDNF-ir in all mPFC subregions, and Antalarmin prevented such BDNF. Data are expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$.

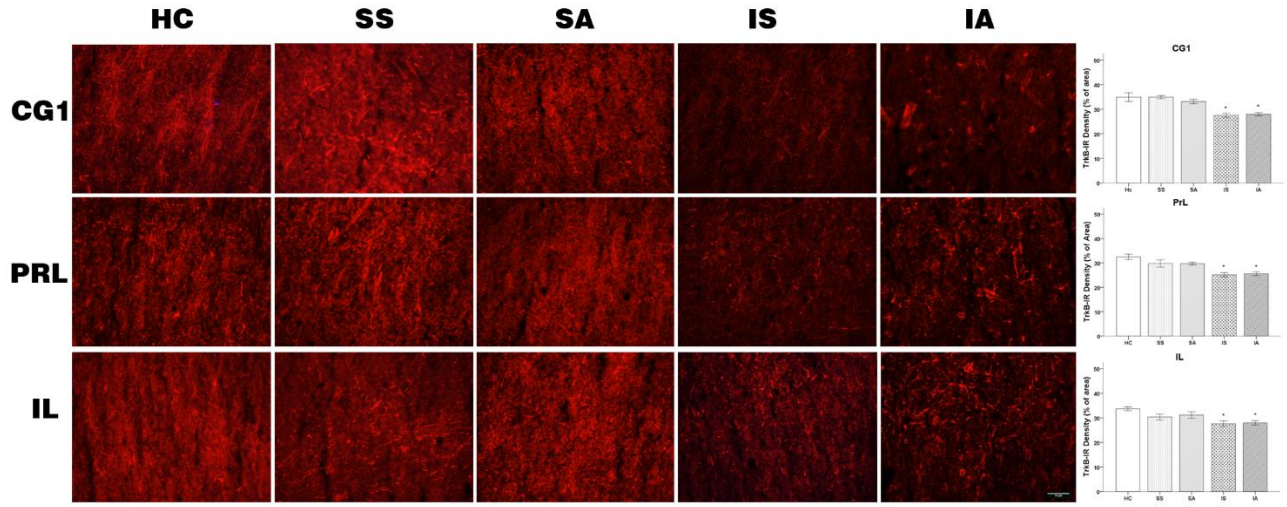

Fig. 7. TrkB-ir expression in the mPFC 30 days post ischemia. Representative photomicrographs of TrkB (red) immunopositive labeling within the cingulate, pre- and infra-limbic cortices [CG1, PrL, and IL, respectively] at 200X magnification. Histograms show mean percent optical densities for each of the groups. Ischemia reduced TrkB in all subregions, although Antalarmin showed no regulatory effects. Data are expressed as mean ± SEM. *$p < 0.05$.

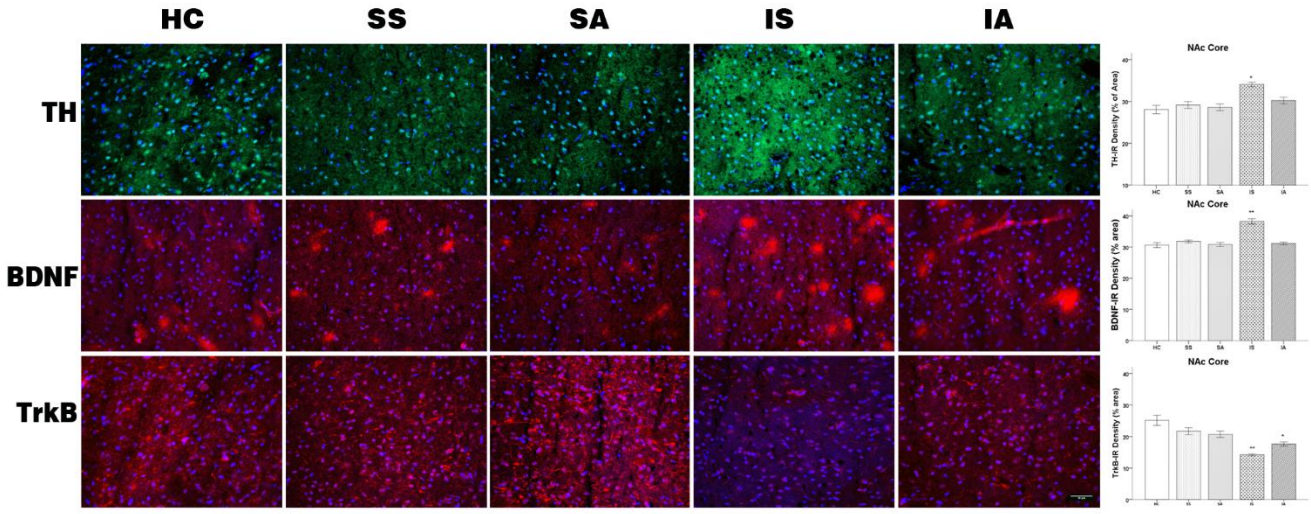

Fig. 8. TH, BDNF and TrkB expression in the NAc core 30 days post ischemia. Representative photomicrographs of BDNF or TrkB (red), TH (green), and Hoescht (blue) immunopositive labeling within the NAc core at 200X magnification. Histograms show the mean percent optical densities for each of the groups. Ischemia increased BDNF and TH, but reduced TrkB expression. Antalarmin fully (BDNF and TH) and partially (TrkB) reversed these effects. Data are expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$.

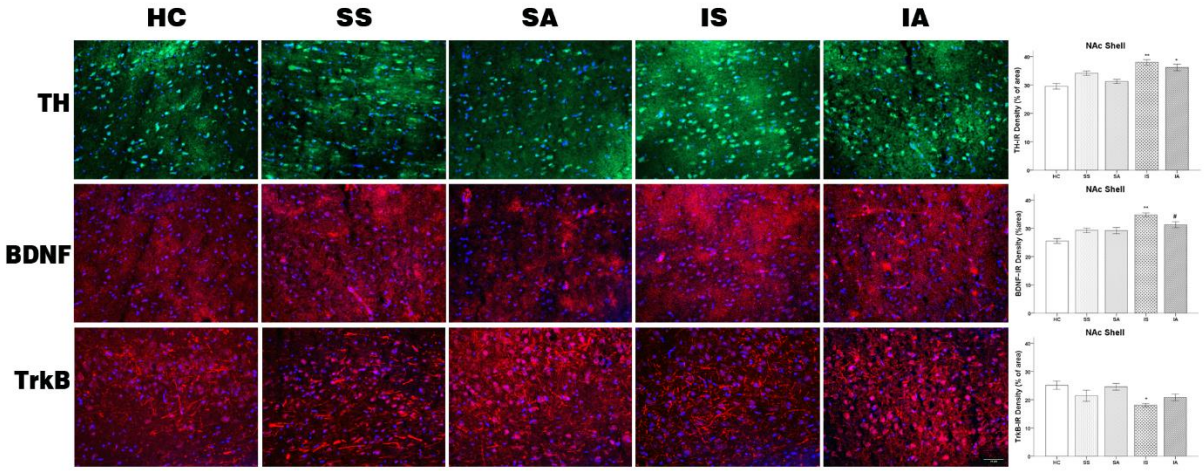

Fig. 9. TH, BDNF and TrkB expression in the NAc shell 30 days post ischemia. Representative photomicrographs of BDNF & TrkB (red), TH (green), and Hoescht (blue) immunopositive labeling at 200X magnification. Histograms show mean percent optical densities for each group. IS increased TH- and BDNF-ir compared to HC and SA, and to SS, SA and HC, respectively. Antalarmin had a marginal effect to attenuate IS-increased BDNF. Both ischemic groups showed reduced TrkB-ir expression. Data are expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$.

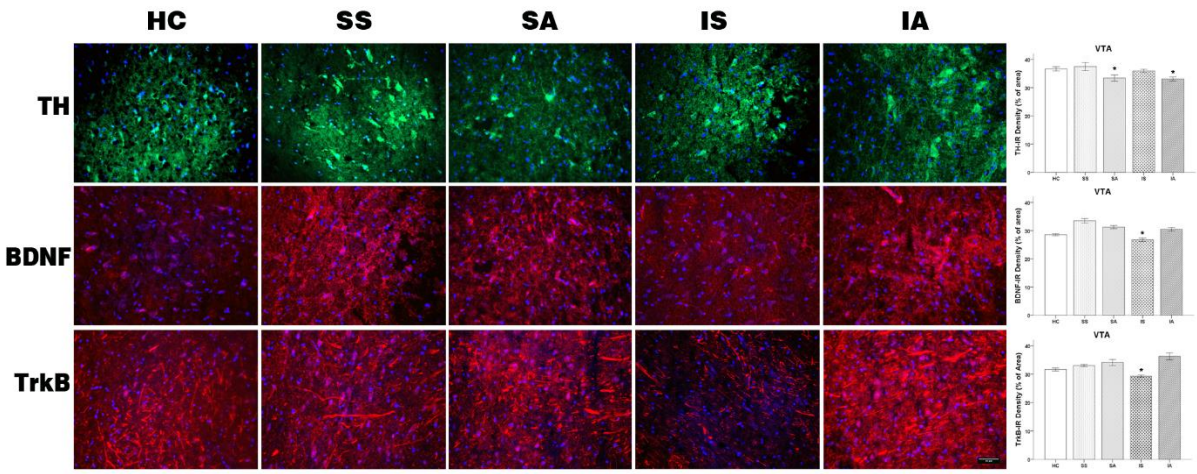

Fig. 10. TH, BDNF and TrkB expression in the VTA 30 days post ischemia. Representative photomicrographs of BDNF & TrkB (red), TH (green), and Hoescht (blue) immunopositive labeling at 200X magnification. Histograms show the mean percent optical densities for each group. For TH-ir, only a main effect of drug was found, ANT reducing expression. Ischemia reduced BDNF and TrkB-ir compared to all groups (*$p < 0.05$), except the HC. Data are expressed as mean ± SEM.

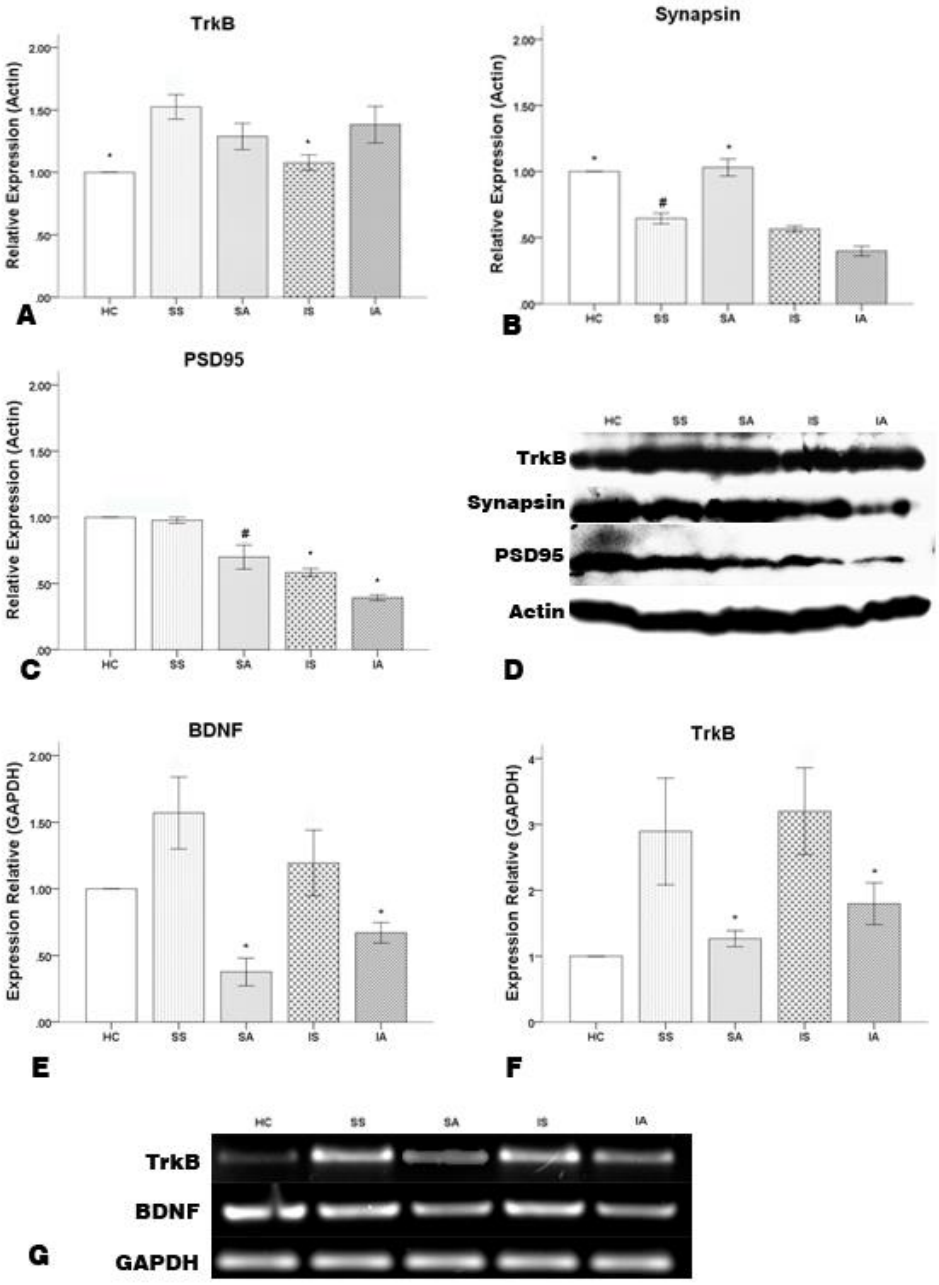

Fig. 11. Protein and mRNA expression in the NAc 30 days after global cerebral ischemia. Average values for the optical density of immunoreactive bands, normalized relative to β-actin following Western blot for TrkB (A), Synapsin (B), and PSD-95 (C). Average values of the optical density of bands normalized relative to GAPDH following RT-PCR for BDNF (E) and TrkB (F). D & G shows representative protein and mRNA bands expressed as a ratio of β-actin or GAPDH, respectively, and normalized to the value of HC controls. Analyses revealed elevated TrkB protein in SS compared to IS ($p = 0.05$) and HC ($p = 0.01$). Synapsin and PSD95 were elevated in control compared to ischemic groups, and ANT reduced sham PSD95 expression. Effects on BDNF and TrkB mRNA levels are due to ANT reducing mRNA levels in sham and ischemic rats (n = 4-5 /group). Values represent means ± SEM, $^{*,\#}$ $p \leq 0.05$.

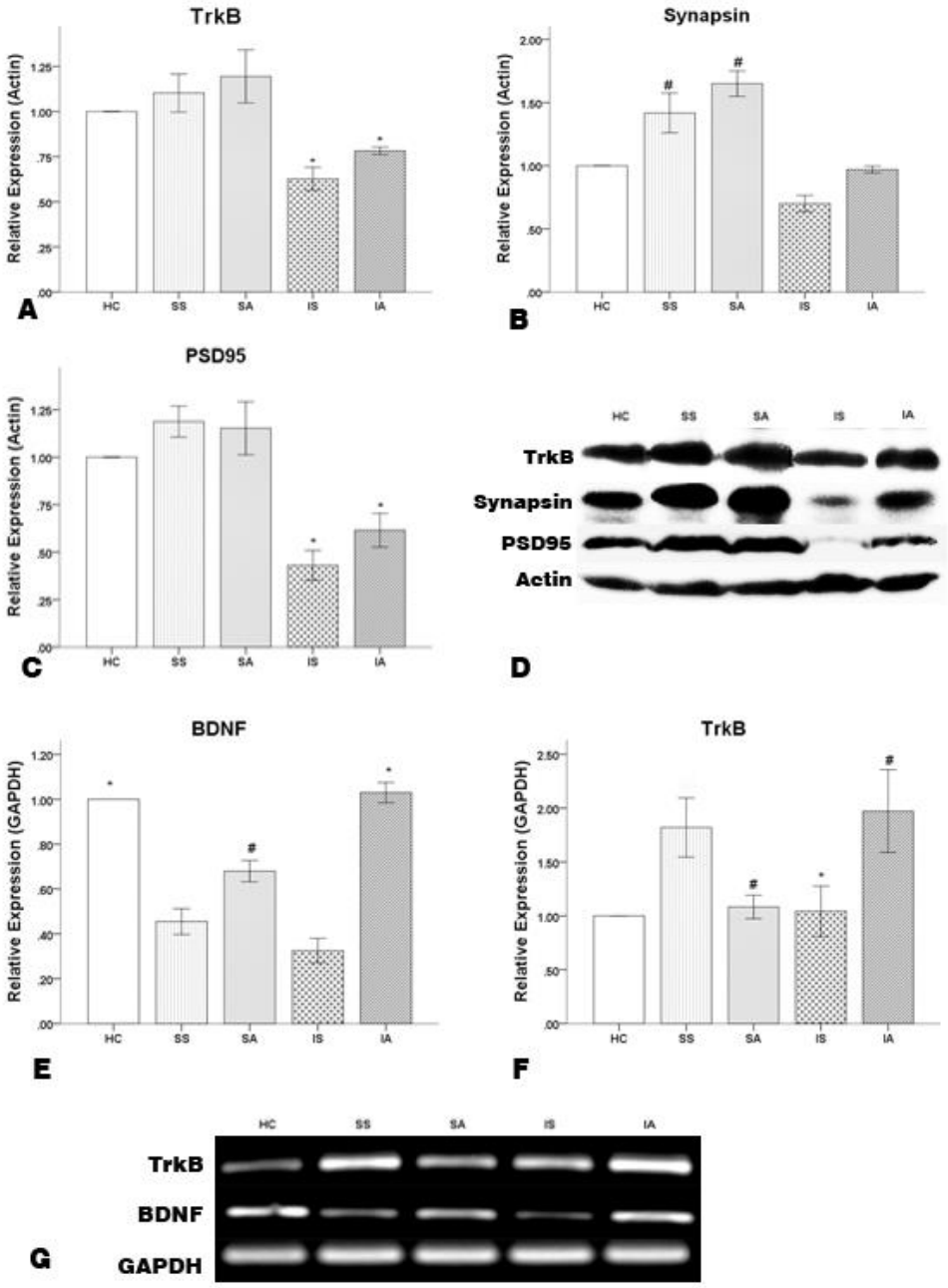

Fig. 12. Protein and mRNA expression in the PFC 30 days after global cerebral ischemia. Average values for the optical density of immunoreactive bands, normalized relative to β-actin following Western blot for TrkB (A), Synapsin (B), and PSD-95 (C). Average values of the optical density of bands normalized relative to GAPDH following RT-PCR for BDNF (E) and TrkB (F). D & G shows representative protein an0d mRNA bands expressed as a ratio of β-actin or GAPDH, respectively, and normalized to the value of HC controls. Findings showed a significant reduction in TrkB, synapsin, and PSD95 levels in ischemic rats (n = 4-5 /group), accompanied by reduced BDNF mRNA levels in IS compared to all groups except SS. TrkB mRNA levels were reduced by ischemia although ANT affected expression in an opposite manner in sham and ischemic animals. Values represent means ± SEM, $^{*,\#}$ $p \leq 0.05$.

Article 3

Submitted as: de la Tremblaye, P. B., Schock, S., & Plamondon, H. (2016). Beneficial effect of CRHR1 Antagonism using Antalarmin on hippocampal, amygdalar and hypothalamic BDNF/TrkB/pCREB plasticity signaling, microglial activation and neuronal damage, and CORT secretion: Relationship to anxiety and spatial and aversive learning following global ischemia.

Authorial contributions

Patricia Barra de la Tremblaye contributed to the study design, performed and interpreted the statistical analysis, and drafted and revised the manuscripts. She also performed surgeries for cannulae implantations, and 4 vessels occlusions, behavioral testing, immunohistochemical analyses for all experimental groups. She performed Western Blotting and RT-PCR procedures under the supervision of Dr. Sarah Shock. Dr. Hélène Plamondon supervised the research project, interpreted the statistical analysis, and revised the manuscripts.

Abstract

The current study examined effects of corticotropin-releasing hormone type 1 receptor (CRHR1) blockade using Antalarmin (ANT) prior to global cerebral ischemia on markers of neuroplasticity and inflammatory, as well as cognitive/behavioral impairments. Male Wistar rats (N = 50) were subjected to sham surgery or global cerebral ischemia using the four vessel occlusion (4VO) model. ICV injection of ANT (2μg/2μl) or a vehicle was administered 30 min prior to ischemia. Behavioral testing was initiated 7 days post ischemia and included assessment of anxiety and locomotor behavior in the Elevated plus Maze and Open Field, and fear and spatial learning in a Y-Maze Passive Avoidance Task and in the Barnes Maze, respectively. Immunofluorescence served to detect brain derived neurotrophic factor (BDNF) and TrkB expression in the hippocampus, basal lateral amygdala (BLA) and the paraventricular nucleus of the hypothalamus (PVN) 30 days post reperfusion, and expression of markers association with neuronal injury and inflammation, including NeuN, glial fibrillary acidic protein (GFAP), ionized calcium binding adaptor (IBA1) and tumor necrosis factor α (TNFα). Findings revealed improved spatial memory and fear retention in ANT-treated ischemic rats. ANT also attenuated ischemia-induced increased and decreased BDNF and TrkB mRNA and protein levels at the amygdala and hippocampus, respectively. The prolonged heightened BDNF and TrKB expression at the PVN also appeared influenced by CRHR1 signaling. ANT blunted post-ischemic IBA1, GFAP and TNFα-immunoreactivity (ir) in all hippocampal sub-regions and conferred neuronal protection in the CA1 and BLA. Together, these findings suggest that CRHR1 activation upon cardiovascular insults significantly contributes to ensuing neuronal and functional changes influencing post ischemic recovery.

Key words: Global cerebral ischemia; Antalarmin; Neurodegeneration; Memory; BDNF; Inflammation, Rats.

1. Introduction

Akin to different stressors, activation of the HPA axis is markedly increased following focal (Weidenfeld et al., 2011) and global (de la Tremblaye et al., 2014) cerebral ischemia, as well as after cardiac arrest (Neigh, Karelina, Zhang, et al., 2009). At the PVN, heightened CRH activation has been described at early 24 h (Khan et al., 2004), and delayed (9 and 30 days) intervals following global cerebral ischemia (de la Tremblaye et al., 2014; Dunbar et al., 2015). Notably, CRH administration upon an ischemic insult also shows dose-related effects, with low doses injected before or as late as 8 h after global cerebral ischemia enhancing neuronal survival and improving spatial memory deficits in rats, while higher dosages have no effect (Charron, Fréchette, Proulx, & Plamondon, 2008). In vitro, low CRH doses administered 8 h after KCN insult also enhances survival in primary cortical neurons from 30–50 % (Charron et al., 2009), which is similar to dose-related protection conferred by CRH following glutamate-induced excitotoxic damage in hippocampal neurons (Elliott-Hunt, Kazlauskaite, Wilde, Grammatopoulos, & Hillhouse, 2002). Of interest, intraventricular administration of α-helical CRH, a non-selective CRH receptor antagonist, induces dose-related protection of hippocampal CA1 neurons following 10 min forebrain ischemia (Lyons, Anderson, & Meyer, 1991) or excitotoxic brain damage (Strijbos, Relton, & Rothwell, 1994). More recently, central administration of α-helical CRH or systemic administration of the CRHR1 antagonist (CP154,526) enhanced functional recovery without affecting neuronal injury (Plamondon & Khan, 2006). These findings raise the possibility that improved functional recovery conferred by CRH blockade can be in part mediated by altered brain plasticity.

Indeed, complex and site-dependent interactions between CRH and BDNF have been described, a phenomenon possibly involving glial and neuronal sources of secretion, as BDNF is

produced in neurons and microglial cells (Ferrini & De Koninck, 2013), expressing CRHR1 receptors (Stevens et al., 2003). In vitro, nanomolar CRH concentrations lead to a rapid CRHR1- and Gβγ-dependent increase in cAMP response element binding (CREB) phosphorylation in rat hippocampal pyramidal neurons (Stern, Meitzen, & Mermelstein, 2011), which contributes to the regulation of BDNF production, and indirectly to neuronal survival, differentiation and synaptic function (Cortes-Mendoza, Diaz de Leon-Guerrero, Pedraza-Alva, & Perez-Martinez, 2013; Tanaka, 2001; Tardito et al., 2006). Activation of microglial cells by CRH similarly stimulates BDNF release (M. J. Wang et al., 2007; W. Wang, Ji, & Dow, 2003). Activated microglia have been shown to secrete BDNF and to alleviate ischemic injury (Elkabes, DiCiccoBloom, & Black, 1996). A recent in vitro study on spinal cord injury demonstrated that CRH facilitates axonal outgrowth by stimulating BDNF release from microglial cells (Yuan et al., 2010). Similarly, findings from cerebellar granular cell cultures also support enhancing effects of CRH on BDNF signaling through CRHR1 activation (Bayatti et al., 2005). BDNF also acts as a downstream target of CRH signaling, mediating the neuroprotective effects of CRH against oxidative stress (Behl & Clement, 2011).

Following brain ischemia, there are increased levels in growth factors and excitatory neurotransmitters allowing brain tissue to reorganize and recover function (Dancause & Nudo, 2011). Thus, recovery of forelimb function after focal ischemia has been shown dependent on BDNF-mediated structural plasticity (Ploughman et al., 2009; Schabitz et al., 2004; Schabitz et al., 2007). From 2-24 h after forebrain ischemia, BDNF mRNA increases in the granule cells of the dentate gyrus, a phenomenon partially blocked by an AMPA receptor antagonist (Lindvall et al., 1992). At early time points following stroke, BDNF immunolabeling is robustly expressed in microglial cells, endothelial cells of cerebral arterioles, astrocytes as well as neurons and

ependymal cells of the lesioned hemisphere (Bejot et al., 2011). Thirty days following focal ischemia, increased BDNF secretion is associated with elevated synaptophysin and GAP-43 (a marker of neuritogenesis) expression (Madinier et al., 2009). BDNF promotes the phagocytic activity, and inhibits microglial apoptosis in the injured brain (J. Zhang et al., 2003). Intranasal or intracerebral BDNF delivery prior and after MCAO results in reduced cell loss, apoptosis and neurological deficits via upregulation of activated and phagocytotic microglia along with increased anti-inflammatory IL10 and NF-κB and reduced pro-inflammatory TNF-α protein and mRNA expression signaling molecules, an effect abolished by co-administration of anti-BDNF (Jiang et al., 2010).

Multiple studies have added to our understanding of how BDNF operates in neural circuits as a stress-responsive intercellular messenger. These studies have revealed opposing effects of BDNF in particular brain regions (Autry & Monteggia, 2012). Chronic and acute stress exposure lead to increased BDNF mRNA and protein levels in the PVN (Arunrut, Alejandre, Chen, Cha, & Russo-Neustadt, 2009; Murakami et al., 2005; Nibuya, Takahashi, Russell, & Duman, 1999; Pizarro et al., 2004; M. A. Smith & Cizza, 1996). Acute and chronic central infusion of BDNF increase PVN-CRH levels, a phenomenon accompanied by increased ACTH and corticosterone levels (Givalois et al., 2004; Naert, Ixart, Tapia-Arancibia, & Givalois, 2006; Toriya et al., 2010). Moreover, genetic disruption of glucocorticoid receptors, resulting in disinhibition of the HPA axis, leads to an upregulation of CRH and BDNF expression in the hypothalamus (Jeanneteau et al., 2012). Likewise, immobilization stress increases BDNF expression at the BLA, a duration-dependent effect lasting up to 21 days (Lakshminarasimhan & Chattarji, 2012). BDNF mRNA levels are elevated in the BLA 1-12 h after fear conditioning training (Ou et al., 2010). During fear consolidation, there is a rise in BDNF mRNA levels

peaking 2 h after conditioning and increasing TrkB phosphorylation in the BLA, a phenomenon thought initiated to replenish BDNF stores post stress (Marmigere, Givalois, Rage, Arancibia, & Tapia-Arancibia, 2003).

In contrast, BDNF levels are downregulated in the hippocampus following single (Fuchikami, Morinobu, Kurata, Yamamoto, & Yamawaki, 2009; T. Lee, Saruta, Sasaguri, Sato, & Tsukinoki, 2008; M. A. Smith & Cizza, 1996; Ueyama et al., 1997a) and repeated immobilization stress (Nibuya, Morinobu, & Duman, 1995; M. A. Smith, Makino, Kvetnansky, & Post, 1995). Chronic unpredictable mild stress reduces BDNF protein levels and dendritic spine densities in CA1 and CA3 pyramidal neurons, impairing LTP and *in vivo* transmission at CA3-CA1 synapses (Qiao, An, Ren, & Ma, 2014). Five weeks of chronic mild stress induces cognitive deficits, associated with reduced cell proliferation, synaptogenesis and BDNF expression in the hippocampus and enhanced levels of CRH, ACTH and CORT (S. Li et al., 2008). In contrast, infusion of BDNF into the hippocampus protects against restraint stress-induced learning deficits in the water maze (Radecki, Brown, Martinez, & Teyler, 2005). Intra-hippocampal injection of the glucocorticoid receptor antagonist RU38486 prior training impairs avoidance memory and reduce pTrkB, pERK2, pPLCγ and pCREB levels, effects prevented by BDNF administration immediately after training (D. Y. Chen, Bambah-Mukku, Pollonini, & Alberini, 2012).

Considering earlier findings, the current study has two main goals: to verify the effect of pre-ischemic blockade of CRHR1 signaling on the spatial memory and fear-response, as well as investigate the genetic and proteomic levels of BDNF/TrkB expression in discrete brain regions involved in mood and memory at 30 days post global cerebral ischemia. To do this, a single injection of a highly selective CRHR1 antagonist, Antalarmin, was icv administered 30 min

before global ischemia. Emotional and spatial memory was evaluated using the Y Maze passive avoidance task (YM-PAT) and the Barnes Maze tests. Immunohistochemistry detected alteration in post ischemic distribution and density of BDNF, TrkB, pCREB, NeuN, GFAP, IBA1 and TNFα in the CA1, CA3 and dentate gyrus (DG), sub-regions of the hippocampus, the BLA and PVN. Western blot and RT-PCR analysis of TrkB, BDNF and Synapsin complemented these findings. Neuronal death in the hippocampal CA1 and amygdalar BLA was determined, and CORT levels used to assess altered HPA reactivity.

2. Methodology

2.1 Animals

Male Wistar rats were obtained from Charles River Laboratories (Rochefort, Québec, Canada). Two distinct sets of animals were used in this study. For in vivo assessment, rats (N = 50) were divided into 4 experimental groups: (n = 10-12 per group; and 1group of naïve/unoperated animals; n= 6). For post mortem analysis (Western blot and PCR), a second set of animals (N = 46) was divided into 4 experimental groups; n = 10 per group; and 1group of naïve/unoperated animals; n= 6. They were individually housed and maintained on a 12 h light/dark cycle (lights on at 7:00 AM) with free access to water and standard rat chow. Room temperature was maintained at 21–23 °C with 60% relative humidity. Rats were habituated to the housing facility for a minimum of one week before surgery and weighed 250-320g at the time of surgery. The experiments were carried out during the light phase between 9:00 AM and 4:00 PM. All rats were handled daily for 2-3 minutes in the four days preceding the day of surgery to minimize stress. All experiments were carried out in accordance with the guidelines set by the Canadian Council of Animal Care and received ethical approval from the University of Ottawa Animal Care Committee.

2.2 Intracerebroventricular (ICV) Cannulation and Drug Infusion

One week after acclimatization to the animal facility (see Fig. 1 for experimental timeline), rats (N = 44; N = 40) underwent surgery for stereotaxic implantation of a guide cannula into the third ventricle as previously described (de la Tremblaye et al., 2014). Rats were anesthetized using isoflurane (2–3%) mixed with oxygen and positioned in a stereotaxic instrument. Permanent 22-gauge stainless steel guide cannula (Plastics One Inc.) were stereotactically placed using the following coordinates: 4.3 mm posterior to Bregma, 0.0 mm lateral to the midline and 4.3 mm ventral to the skull surface according to the Paxinos and Watson atlas (1997). Guide cannulae were secured to the skull with dental cement (Jet Liquid acrylic resin, Lang Dental Manufacturing Co., Inc.), and four anchor screws (Plastics One Inc.). A dummy cannula (Plastics One Inc.) was inserted into the guide cannula to prevent occlusion. The drug was administered via a 28-gauge stainless steel injector (Plastics One Inc.) placed in, and projecting 0.5 mm below, the tip of the guide cannula. Antalarmin hydrochloride (Sigma-Aldrich Inc.) (2µg/2µl) or vehicle containing 0.9% saline with 10% Cremophor (Sigma-Aldrich Inc.) were intracerebroventricularly (icv) injected 30 min prior to 10-min global ischemia or sham occlusion, creating 4 groups: Ischemia-Antalarmin (**IA**; n = 12), Ischemia-Vehicle (**IS**; n = 12), Sham-Antalarmin (**SA**; n = 10), and Sham-Vehicle (**SS**; n = 10). A home cage (**HC**; n = 6). For PCR and Western blot analysis, groups were divided as follows: Ischemia-Antalarmin (**IA**; n = 12), Ischemia-Vehicle (**IS**; n = 12), Sham-Antalarmin (**SA**; n = 10), and Sham-Vehicle (**SS**; n = 10), home cage controls (**HC**; n = 6). The selection of drug dosage and administration were based on the previous literature (Briscoe, Cabrera, Baird, Rice, & Woods, 2000; Jaszberenyi et al., 2009; Telegdy & Adamik, 2008; H. Wang, Spiess, Wong, & Zhu, 2011; Zorrilla, Schulteis, et al., 2002) and earlier work from our lab using CP-154,526, an analog of Antalarmin (Khan et

al., 2004). Antalarmin was selected for its high affinity for CRH type 1 receptors (K_D=0.8 nM) (Zorrilla, Valdez, et al., 2002).

2.3 Forebrain Ischemia

One week following cannulation, rats were subjected to the four-vessel occlusion model as previously described (Pulsinelli & Brierley, 1979; Pulsinelli, Brierley, & Plum, 1982). Briefly, rats were anesthetized by inhalation of 2-2.5% isoflurane dissolved in 1.0 L/min O_2. The vertebral arteries were irreversibly occluded by electrocauterization and a small-diameter silk thread was looped around the carotid arteries to facilitate subsequent occlusion. Sham-operated animals (n =14) underwent anesthesia and received the same dorsal and ventral surgical incisions as the ischemic group without electrocauterization of the vertebral arteries. Twenty-four hours later, rats were briefly anesthetized and carotid arteries re-exposed for clamping. Cerebral ischemia was induced after discontinuing anesthesia and at first signs of wakefulness (sniffing and limb movements) by occluding the pair of carotid arteries with microvascular clamps for a 10 minute period in freely ventilating rats. The core temperature was regulated throughout the surgery by means of a feedback regulated heating blanket connected to a rectal thermometer (Homeothermic Control Unit, Harvard Instruments, Natick, MA), and supported with a heating pad during vessel occlusion and in the hours following surgery and reperfusion.

2.4 Behavioral Testing

Rats were handled once a day for 2-3 days before behavioral testing. Animal handling and behavioral coding were performed by an experimenter blind to the animal groups.

2.4.1 Elevated plus Maze (EPM)

The EPM consisted of two opposing open arms (50 cm × 10 cm with a 5 mm clear Plexiglas lip), an open 10 × 10 cm center area and two opposing closed arms (50 cm × 10 cm with 40 cm high walls). The maze stood 60 cm above the floor. White curtains surround the maze and behavior was monitored using an overhead camera. On day 7 following reperfusion, groups of sham and ischemic rats were transported from the vivarium to the testing room and allowed to habituate at least 30 min before testing. Upon testing, the rat was placed in the center of the EPM facing one of the open arms of the maze. Each rat was allowed to explore the maze freely for 5 min and the time spent and the number of entries in the open and closed arms, risk assessment and crossing behaviors recorded using data logging software (ODlog 2.0, USA). A closed or open-arm entry was operationally defined when the rat places all four paws into one arm. Risk assessment was defined as a stretch-attend response, where the rat stretches its body forward and sniffs or visually scans the open arm. Head entry into the open arm was required while paws and body remained in the center area or closed arm. Crossing behavior was recorded each time the animal crosses the center zone going from one arm to its opposite arm. The EPM was cleaned with 70% ethanol after each test.

2.4.2 Novel Open Field Test (OFT)

The open field arena was made of gray Plexiglas (LWH: 75 cm×75 cm× 30 cm) with a grid dividing the floor into 36 identical squares. The arena stood on a table 90 cm above the floor and white curtains separated the arena and the recording zones. Following the EPM test, animals had at least 30 min of acclimation prior to testing. They were then placed in a random corner of the OFT facing the wall, and permitted to explore the environment for 5 min. The rats' behavior was monitored using an overhead camera and data logging software (ODlog 2.0, USA) used to

record the number of squares crossed in the center and the periphery, and the number of rearing (defined as standing upright on hind legs) and grooming behaviors. The total frequency of square entries determined locomotor activity and rearing behaviors was quantified as an index of exploratory behavior. Reduced number of crossings in the center versus the peripheral zones is a valid index of anxiety in rodents, the center zone being a more anxiogenic zone (Prut & Belzung, 2003). The arena was thoroughly cleaned with 70% ethanol before testing the next rat.

2.4.3 Y-Maze Inhibitory Avoidance Task (YM-PAT)

The YM-PAT measures the retention of fear or the learning ability to avoid a previously punished stimulus (Azogu et al., 2015; Vitale, 2011). Aversive learning was assessed 17 days following reperfusion. It consisted of a Plexiglas structure with three arms (LWH: 35.5 X 15 X 30 cm), placed on a table (90 cm high) and surrounded by curtains with a video camera placed overhead. First, the rats were placed in the maze facing the wall of one of the 3 maze arms and the latency (L1) for the rat to enter one of the other two arms was recorded. Once the animal entered an arm (when all four paws are enclosed), a sliding divider was placed to trap the rat in the selected arm. The rat then received four jets of condensed air (air puff) in its face with 15 sec intervals before the rat was placed back in its cage. After a 5 min delay, inhibitory avoidance was assessed by placing the rat back in the original starting arm with a unique access to the aversive arm (the other arm being blocked). The latency (L2) to enter the aversive arm (max 300 sec) was recorded. The difference between L1 and L2 determined retention of the aversive stimuli.

2.4.4 Barnes Maze (BM)

The BM is a spatial memory test that requires the rat to learn the position of a hiding hole in order to escape a brightly lit open maze surface (Harrison, Reiserer, Tomarken, & McDonald, 2006). The test took place 20 days post reperfusion and was conducted as previously described

(de la Tremblaye & Plamondon, 2011a; M. R. Milot & Plamondon, 2011a). The maze consisted of a circular structure (d = 122 cm; C = 376.8 cm) placed 100 cm above the floor with 18 circular holes at equal distance from each other (d = 10 cm). One of the holes led to an escape box held in place by a drawer-style system. A black drape was placed around the edge of the maze and hung to the floor reducing visibility of the box via other holes. Black curtains surrounding the maze displayed spatial cues (poster, calendar, and geometrical shapes). Two floodlights (150W each) placed over the maze which provided uniform and bright illumination motivated the rat to escape. A camera was placed over the maze to monitor behavior. Using the spatial cues, the rat could reach the escape box on every trial. Each rat was randomly assigned one of the four escape box locations, which remained the same for the rest of the trials. Training sessions were conducted over the course of 5 days (day 1-5), one session a day and each session consisted of 2 trials of maximum 5 min each. Exceptionally, on the first day each rat went through 3 trials. The initial trial allowed the rats to become comfortable with the maze and the location of the escape box. The test began by placing the rat under a starting box in the middle of the maze (an opaque plastic container). After 30 sec, the start box was lifted via a pulley system starting the testing period. Once the rat entered the escape box, the hole was covered and the rat remained in the box for 90 sec (only for the first trial of the initial training day). Immediately after the first trial, a second trial repeated these conditions. This procedure was repeated for the following 4 training days, each daily session including two trials separated by a 5 min interval. If the rat did not enter the escape box within 5 min, it was gently guided toward and into it. After each trial, the rats returned to their cage and the Barnes Maze and escape box cleaned (70% ethanol) before testing the rat a second time after a 5 min interval. Trials were videotaped and videos digitally converted in MPEG format using a convertor system. Dependent measures including escape latency (sec),

total path length (cm) and speed (cm/sec) were analyzed with the EthoVision XT 7.1 tracking system (Noldus, Leesburg, Va). The number of working and reference errors were recorded by a blind experimenter. A working error was defined when a rat sniffed or looked at a hole not linked to the escape box. A reference error occurred when a rat sniffed or looked at a hole that did not contain the escape box more than once.

2.5 Collection of Trunk Blood

Thirty days following occlusion, rats were removed one by one from the vivarium, lightly anesthetized with isoflurane and quickly decapitated with a guillotine. Trunk blood was collected and centrifuged at 4000 rpm for 10 min at 4^0C, and the serum decanted and stored at −80°C until determination of CORT levels by a radioimmunoassay as previously described (M. R. Milot et al., 2012).

2.6 Brain Tissue Preparation

Following decapitation, brains were removed from the skulls and immediately frozen in dry ice before storing at −80ºC. Using a Leica cryostat, 14 μm-thick coronal sections were mounted onto polarized Superfrost Plus slides (Fisher Scientific, Canada). The regions of interest were localized on the collected brain tissue according to coordinates from the Paxinos and Watson atlas (1997): the dorsal hippocampus (Bregma, ~ -2.80 to - 4.16 mm), the basolateral nucleus of the amygdala (BLA, Bregma, ~ -2.12 to -3.30 mm) and the paraventricular nucleus of the hypothalamus (PVN, Bregma, ~ -1.30 to -2.12 mm).

An additional subset of rats (N=46) from the same experimental design were decapitated according to the same procedure, but the brains quickly removed from the skull were placed in a stainless-steel mold to obtain fresh tissue for protein and mRNA analysis. The dorsal and ventral hippocampi, as well as the amygdala were rapidly dissected from 2 mm thick coronal sections on

an ice-cold aluminium plate covered with a PBS dampened filter paper. Dissected tissue was placed in 1.5 ml Eppendorf tubes, flash-frozen in liquid nitrogen and stored at -80°C.

2.7 Immunohistochemistry

Cryostat cut 14 µm sections were postfixed for 5 min at room temperature using a 4% paraformaldehyde solution containing 20% picric acid, washed (3 × 5 min) with PBS (1 mM, phosphate buffered saline), and blocked in PBS with 0.02 % Triton X-100 and 0.01 g/ml bovine serum albumin (BSA) for 30 min. Sections were then incubated at 4°C overnight in blocking buffer containing either a polyclonal rabbit BDNF (1:500, Santa Cruz Biotechnology, Inc.) or TrkB (1:500, Santa Cruz Biotech, Inc.) antibodies. Sections were rinsed in PBS (3 × 5 min), and subsequently incubated for 2h at RT with a fluorescent-conjugated donkey anti-rabbit secondary antibody (1:400, Invitrogen Canada Inc.), followed by three rinses. Slides were incubated with Hoechst nuclear stain solution for 10 min at RT (1:20000 Hoechst 33342, Invitrogen Canada Inc.), and rinsed three times before mounting. To assess the presence of neuroinflammation, the same procedure was performed on hippocampal sections using the following primary antibodies: mouse anti-NeuN (1:500, Millipore, Ltd.), mouse anti-GFAP (1:1000, ab7260, Abcam Inc.), rabbit anti-IBA1 (1:1000, 019-19741, Wako Chemicals, Inc.) or mouse anti-TNFα (1:200, sc-1351, Santa Cruz Biotechnology, Inc.), and incubated for 24 h at 4°C. Slices were then incubated at room temperature for 2 h with either Alexa 488-conjugated donkey anti-mouse IgG (1:500, Invitrogen Canada Inc.) or Alexa 594-conjugated donkey anti-rabbit IgG (1:500, Invitrogen Canada Inc.) secondary antibodies. Immunofluorescence labeling was visualized on six bilateral digital images using an Olympus DX51microscope (Center Valley, PA, USA) using the Progress Pro 2.7.6 software under 200X magnification as previously described (de la Tremblaye et al., 2014). Immunolabeling in the hippocampus (CA1, CA3, and DG), the BLA, and PVN was

analyzed by a blinded investigator, using Image J software (Image J, National institutes of health). Optical densities (mean grey values), estimates of the staining intensity, were obtained by using the threshold technique. Data are presented as background corrected standardized image densities for each brain region.

2.8 RNA extraction, Reverse transcription and PCR.

According to the manufacturer's protocol, frozen tissue was homogenized in 1 ml TRIzol Reagent (Invitrogen Canada Inc.) using a Sonicator (Fisher Scientific, Misonix XL2000 Ultrasonic Homogenizer). Homogenates were then incubated at RT for 5 min in order to completely dissociate nucleoprotein complexes. Subsequently, 0.2 ml of chloroform was added and the homogenate was vigorously shaken for 15 sec and incubated for 5 min at RT. Samples were subsequently centrifuged at 12 000 g for 15 min at 4 °C. The aqueous phase, containing RNA, was mixed with 0.5 ml of isopropanol, incubated at RT for 10 min, and centrifuged at 12 000 g for 10 min at 4 °C. The resulting RNA pellet was re-suspended in 75% ethanol, centrifuged at 7500 g for 5 min at 4 °C, air dried and dissolved in 20 µl 0.1% DEPC water, and stored at −80 °C until analysis. The RNA concentration was determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer. The ratio between the absorbance values at 260 and 280 nm was used to estimate RNA purity. Total RNA (2µg) from each sample was reverse transcribed for 60 min at 42°C using SuperScriptTM II (200 units, Invitrogen Canada Inc.) and First Strand Buffer (Invitrogen Canada Inc.), in the presence of 0.5 µg/µl oligo dT, 10 mM DL-dithiothreitol and 1 mM deoxynucleotide triphosphate solution (dNTP mix, Invitrogen Canada Inc.). The cDNA was stored at −20°C.

For PCR, specific primers were designed to selectively amplify **TrkB** mRNA expression (For-5'GGCCAAGAATGAATATGGTAA 3'; Rev-5'TTGAGCTGGCTGTTGGTGAT 3'),

BDNF mRNA expression (For-5′-ATGGGACTCTGGAGAGCGTGAA-3′; Rev-5′-CGC CAGCCA ATTCTC TTT TTGC-3′), **GAPDH** mRNA expression was used as a housekeeping gene (For-5′-CATGGCCTTCCGTGTTCCTACCC-3′; Rev-5′-CCTCGGCCGCCTGCTTAC-3′). Amplifications of cDNA (1 µl) obtained from RT were then performed in a mixture consisting of PCR buffer containing 10mM dNTPs, 2mM MgCl2, 10 µM of each specific primer, and 2µl of Taq polymerase in a total volume of 25µl. Control samples lacking reverse transcriptase were processed in parallel with the same experimental protocol. cDNA Amplifications were performed in an Eppendorf thermocycler (Brinkmann) after 30-32 cycles, using the following conditions: denaturation at 94°C for 1 min, annealing temperature 60 °C/1 min (BDNF), 53 °C/1 min (TrkB), or 60 °C/1 min (GAPDH), extension 72 °C for 1 min, and final extension 72 °C for 8 min. For each set of primers, control experiments, involving varying the number of cycles, were performed to define the linear range for PCR amplification. PCR products were electrophoresed on 2% agarose gels together with a 100 bp DNA Ladder (FroggaBio), and visualized under u.v. light using ethidium bromide. The intensity of PCR products was measured with an image analysis system Image Lab 4.1 (Bio-Rad) and normalized using GAPDGH amplification as control.

2.9 Western Blotting

Protein levels of TrkB and the presynaptic marker synapsin were determined by Western blotting. Tissues from the dorsal and ventral hippocampi and the amygdala were homogenized in a lysis buffer, containing 10 µl of inhibitor cocktail in 500 ml of RIPA buffer. The homogenates were centrifuged and the supernatants collected. Total protein concentration was determined according to the Bradford method (BioRad). After boiling with SDS for 5 min, samples were electrophoresed on an 8% polyacrylamide gel under constant current (100 mV). Separated

proteins were transferred onto a PVDF membrane using transfer stacks/iBlot gel transfer device (Invitrogen Canada Inc.). Nonspecific binding sites were blocked with 10% milk in TBST (20 mM Tris–HCl pH 7.6, 137 mM NaCl, 0.05% Tween-20) overnight at 4^0C, and incubated with either anti-TrkB (1:2000, Santa Cruz Biotechnology, Inc.) or anti-synapsin (1:500, PhosphoSolutions, LLC), followed by three washes with TBST, and a subsequent 2 h incubation at room temperature with an anti-rabbit or anti-mouse IgG horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology, Inc.). Immunoblots were washed three times with TBST and later visualized on X-ray films (Fisher Scientific) following exposure to an enhanced chemiluminescence kit (Millipore, Ltd.). The molecular size of the protein was determined by running a protein ladder (FroggaBio, Inc.) in an adjacent lane. Membranes were stripped with TBST containing Reblot Plus solution (Millipore, Ltd.) for 20 min at room temperature in between antibodies. The film signals were digitally scanned and quantified using Image J software. Anti-actin (1:50000, Abcam, Inc.) was used as an internal control and data standardized according to actin values.

2.10 Neuronal Injury Assessment

Coronal brain slices (14μm) were stained with thionin for quantification of CA1 and CA3 and BLA neurons in both sham and ischemic rats. Slices were examined at 200X magnification using a LEICA DAS microscope attached to a SONY digital camera, and image analysis software Norton Eclipse (v 6.0). Cells with clear cytoplasmic outlines, a circular shape and a clear nucleus outline were counted as viable and functional cells. Counting was done on three brain slices for each rat on both the right side and the left side. Neuronal density for a given animal represented the average of these six measures. Values are expressed as mean ± SEM.

2.11 Statistical Analysis

All analyses were performed using IBM SPSS Statistics 20. Behavioral and histological assessments were conducted by experimenters blind to group identity. Behavioral data was analyzed using a mixed ANOVA design with two independent factors surgery and drug and one repeated factor time for the Barnes Maze to explore the change in latency, distance to reach the target box, distance to zone, reference and working memory errors and speed (m/s), and for the Y Maze to explore the change in latency to enter the aversive zone and the ratio between trials. Homogeneity of variance was assumed for all variables using the Levenes statistic. When the Mauchly's test was significant, the Huynh-Feldt correction was applied to adjust degrees of freedom to more conservative values. Significant main effects or interactions were further explored and corrected using the Bonferroni post hoc analysis for comparison between the four groups. Separate two-way ANOVAs analyzed all other data. Bonferroni corrected simple effect tests were applied when significant interactions were detected. The home cage control group was not included in the initial two-way ANOVA analyses as it created an unbalanced design. When main effects or interactions were detected, one-way ANOVAs including the HC group were conducted followed by Tukey's post hoc comparisons. Results are expressed as mean ± Standard Error of the Mean (SEM). Statistical significance is obtained when $p < 0.05$.

3. Results

3.1 Elevated plus Maze

For time spent in the open arms, a main effect of drug ($F(1, 50) = 12.061$, $p = 0.001$) was found, attributable to increased time spent in the anxiogenic zones by ANT-treated groups. Bonferroni post hoc comparisons following one-way ANOVA ($F(4, 57) = 13.636$, $p = 0.001$), including the 5 groups, indicated increased time spent by SA compared to IS groups and by the

HC group compared to all other groups ($p < 0.01$) (Fig.1a). For the frequency of entries into the open arms, a drug effect ($F (1, 50) = 9.239$, $p = 0.004$) was also found, with post hoc comparisons showing the same between group differences [ANOVA ($F (4, 57) = 10.951$, $p = 0.001$)] (Fig.1b). For time spent and frequencies of entries in the closed arms, only main effects of drug ($F (1, 50) = 12.317$, $p = 0.001$; $F (1, 50) = 4.193$, $p = 0.046$, respectively) were found. Consistent with observations in the open zones, post hoc comparisons [ANOVA ($F (4, 57) = 14.154$, $p = 0.001$] revealed that the IS group spent more time in the closed arms than the SA ($p = 0.029$) and HC rats, these two latter groups spending less time than all other groups ($p < 0.01$). For the frequency of entries in the closed arms, post hoc comparisons upon one-way ANOVA ($F (4, 57) = 11.343$, $p = 0.001$) revealed increased number of entries in the closed arms by the HC group compared to all other groups ($p < 0.01$), suggesting increased locomotion in this animal group (data not shown).

3.2 Open Field Test

For locomotion time in the centre zone a significant surgery x drug interaction ($F (1, 42) = 6.723$, $p = 0.013$) but no main effects were observed. Post hoc comparisons following one-way ANOVA ($F (4, 53) = 7.043$, $p = 0.001$) revealed heightened centre locomotion time in HC rats compared to all groups ($p < 0.05$) except SA rats ($p = 0.070$) (Fig. 1c). A significant surgery x drug interaction ($F (1, 42) = 7.242$, $p = 0.010$) was also present for squared crossed in the center zone. One-way ANOVA revealed significant differences between groups ($F (4, 53) = 4.274$, $p = 0.005$), showing that the SS group made less entries in the centre zone than SA ($p = 0.025$) and HC control groups ($p = 0.05$) (Fig.1d). In time spent in the periphery of the OFT, an effect of drug ($F (1, 42) = 10.11$, $p = 0.003$) was found, with one-way ANOVA ($F (4, 53) = 3.446$, $p = 0.015$) showing reduced locomotion time in the periphery for the SS group compared to IA

animals ($p = 0.024$). For squares crossed in the periphery, a drug effect ($F (1, 42) = 8.225$, $p = 0.006$) was also found due to increased locomotion in ANT-treated groups (data not shown).

3.3 Y-Maze Inhibitory Passive Avoidance Task

Fig. 4 displays the mean latency to enter one of the arms (trial 1) and the mean latency to enter the aversive arm (trial 2) (Upper graph) and the ratio (% difference) between the two trials (Bottom graph). Two-way repeated-measures ANOVA showed main effects of time ($F (1,47)= 38.621$, $p = .001$) and surgery ($F (1,47)= 31.556$, $p =.001$), and time x surgery ($F (1,47) = 24.981$, $p = .001$), and surgery x drug ($F (1,47) = 5.079$, $p = .029$) interactions. All groups had higher latencies to enter the arm of the Y Maze after exposure to the aversive stimulus, except the ischemic animals that had comparable latencies for both trials ($p = .370$). Pairwise comparisons indicated that the IS group had shorter latencies to enter the aversive arm than SS animals ($p = 0.008$), but not in comparison to IA animals ($p = 0.203$). The IA group also had shorter latencies than the SA group ($p = 0.025$), and no differences were apparent between the sham groups ($p = .068$). One-way ANOVA revealed group differences for the percent ratio between latency 1 and 2 ($p \leq 0.01$). The IS rats were significantly different from all groups ($p < 0.005$), while IA rats took more time to enter the aversive arm than IS rats ($p = 0.004$), and only significantly differed from SS rats ($p = 0.008$).

3.4 Barnes Maze Test

For the latency to find the escape box, two-way repeated measures ANOVA showed main effects of drug ($F (1,47) = 10.909$, $p = .002$), surgery ($F (1,47) = 27.530$, $p = .001$), and time ($F (3.882, 182.434) = 195.361$, $p = .001$), as well as a surgery x drug ($F (1,47) = 5.252$, $p = 0.026$) and time x surgery ($F (3.882, 182.434) = 8.310$, $p = .001$) interactions. All groups had reduced latencies as the number of trials increased. Pairwise comparisons for the interaction

between time and surgery indicated that the ischemic group had higher latencies to reach the escape box than sham animals on all trials ($p < 0.05$) except on Day 1 trials 1 & 2 ($p = 0.24$ and 0.13). For the surgery x drug interaction, pairwise comparisons revealed higher latencies in IS compared to SS ($p = 0.001$) and IA ($p = 0.001$) groups (Fig.3a). For traveled distance, main effects of drug ($F (1,47)= 9.840$, $p = .002$), surgery ($F (1,47) = 10.692$, $p =.001$), and time ($F (4.091, 192.279) = 149.359$, $p = .001$), as well as surgery x drug ($F (1,47)= 14.253$, $p =.001$), time x drug ($F (4.091, 192.279)= 2.934$, $p = 0.021$), time x surgery ($F (4.091, 192.279) = 4.753$, $p = 0.001$), and time x drug x surgery ($F (4.091, 192.279) = 3.661$, $p = 0.006$) interactions were found. Ischemic animals traveled more distance to reach the escape box compared to sham groups and ANT treatment reduced distance traveled. All groups traveled shorter distance as the number of trials increased. Pairwise comparisons indicated that IS traveled longer distances to reach the escape box than SS and IA ($p \leq 0.001$) groups (Fig.3b). For the number of working memory (WM) errors, main effects of surgery ($F (1,47) = 15.826$, $p = .001$), drug ($F (1,47) = 11.743$, $p = .001$), and time ($F (4.299, 202.058) = 209.910$, $p = 0.001$), as well as a surgery x drug interaction ($F (1,47)= 7.388$, $p =.009$) were found. Ischemic animals made more WM errors compared to sham groups, and ANT treatment reduced WM errors. All groups made fewer errors as the number of trials increased. Pairwise comparisons indicated that IS animals made more WM errors in reaching the escape box than SS and IA ($p \leq 0.001$) groups (Fig.3c). For the number of reference memory (RM) errors, mains effect of surgery ($F (1,47) = 10.105$, $p =.003$), drug ($F (1,47)= 6.211$, $p = .016$), and time ($F (3.153, 148.174) = 107.239$, $p =.001$), as well as surgery x drug ($F (1,47)= 6.455$, $p = .014$), time x treatment ($F (3.153, 148.174) = 2.708$, $p = .045$), and time x surgery ($F (3.153, 148.174) =4.129$, $p = .007$) interactions were found. Ischemic animals made more RM errors compared to sham groups, and ANT-treatment reduced

the number of RM errors. All groups made fewer RM errors as the number of trials increased. Pairwise comparisons indicated that IS animals made more RM errors to reach the escape box than SS and IA groups ($p \leq 0.001$). One-way ANOVA on individual days showed that the IS group made more RM errors than all groups on all trials ($p < .05$) preceding trial 2 on day 3 (Fig.3d). For the average speed (velocity), main effects of drug ($F(1,47)= 4.823$, $p = 0.033$) and time ($F(3.005, 141.223) = 105.208$, $p = .001$) were found. ANT-treated groups had an overall slower speed in comparison to VEH-treated groups. Velocity however increased for all groups as they progressed through the test trials ($p <.05$) (data not shown).

3.5 CORT Serum Levels

Fig. 5 shows CORT levels, measured in extracted serum from trunk blood samples collected 30 days post ischemia. Two-way ANOVA revealed a main effect of surgery ($F(1, 36) = 16.041$, $p = 0.001$), attributable to higher CORT levels in ischemic (Mean = 72.919, SEM = 6.121) compared to sham (Mean = 38.247, SEM = 6.121) animals. Bonferroni post hoc comparisons following one-way ANOVA ($F(4, 38) = 6.404$, $p = 0.001$) revealed higher CORT levels in IS compared to control groups ($p < 0.005$).

3.6 Assessment of neuronal density in the hippocampal CA1 and CA3

Fig. 6 shows the impact of ischemia and ANT treatment on CA1 and CA3 cell survival 30 days post-reperfusion using NeuN-ir staining and the Nissl stain thionin. For NeuN-ir staining (Fig. 6 a, b & d) in the CA1, two way ANOVA revealed main effects of drug ($F(1, 36) = 17.084$, $p = .001$) and surgery ($F(1, 36) = 184.962$, $p = .001$) and a surgery x drug interaction ($F(1, 36) = 16.717$, $p = 0.001$). Simple effects tests indicated that both ischemic groups had lower NeuN expression compared to sham groups ($p \leq 0.001$), and ANT significantly increased NeuN expression post ischemia ($p = 0.001$). For the CA3, a main effect of surgery ($F(1, 36) = 60.839$,

p = .001) and a surgery x drug interaction (F (1, 36) = 7.732, p = .009) were found. Simple effects tests indicated that the ischemic group had lower NeuN expression than sham groups (p < 0.001), although the IA had increased NeuN expression compared to the IS group (p = 0.005). For the DG, two-way ANOVA failed to show main effects of surgery and drug or a surgery x drug interaction (p > 0.05) (data not shown). For thionin staining (Fig. 6 a, c & e) in the CA1, main effects of surgery (F (1, 36) = 276.315, p = 0.001), drug (F (1, 36) = 22.501, p = 0.0001), and a surgery x drug interaction (F (1, 36) = 17.762, p = 0.001) were found. Ischemic animals had significantly higher CA1 injury compared to sham groups (p = 0.001), which was attenuated by ANT treatment (p = 0.001). At the CA3, main effects of surgery (F (1, 36) = 91.064, p = 0.001) and drug (F (1, 36) = 8.292, p = 0.007), and surgery x drug interaction (F (1, 36) = 9.254, p = 0.004) were found. Ischemia increased CA3 neuronal injury compared to both sham groups (p < 0.001), and cell death was attenuated by ANT treatment (p = 0.001). As for the BLA, main effects of surgery (F (1, 36) = 138.287, p = 0.001) and drug (F (1, 36) = 7.998, p = .008) were found. Post hoc tests following one-way ANOVA (F (4, 41) = 42.391, p = .001) revealed reduced cell density in ischemic compared to control groups (p < 0.001). ANT reduced ischemic injury (p = 0.026), conferring partial protection (data not shown).

3.7 GFAP- and IBA1-ir in Hippocampal CA1 and CA3 Regions

Fig. 8 shows the impact of ischemia and ANT treatment on GFAP and IBA1 expression in the CA1, CA3 thirty days post-reperfusion. For *GFAP-ir* (Fig. 7 a, b & d) in the CA1, main effects of drug (F (1, 36) = 8.707, p = .006) and surgery (F (1, 36) = 13.254, p = .001) were found. The ischemic groups had higher GFAP expression than sham animals, which was reduced by ANT treatment. One-way ANOVA revealed between group differences (F (4, 42) = 6.512, p = 0.001) due to higher levels of GFAP levels in IS rats compared to all groups (p < 0.05) except

for IA animals ($p > 0.05$). For the CA3, main effects of drug ($F (1, 36) = 15.395$, $p = .001$) and surgery ($F (1, 36) = 40.029$, $p = .001$) were found. The ischemic groups had elevated GFAP-ir compared to sham animals, which was reduced by ANT treatment. One-way ANOVA revealed between group differences ($F (4, 42) = 16.943$, $p = 0.001$) due to higher GFAP in the IS group compared to all groups ($p < 0.01$). The IA group had significantly higher GFAP-ir compared to the SA group ($p = 0.015$). For IBA1-ir (Fig 7. a, c & e) in the CA1, main effects of drug ($F (1, 36) = 5.529$, $p = .024$), and surgery ($F (1, 36) = 114.294$, $p = .001$) were found, due to higher IBA1-ir expression in ischemic compared to all groups ($p < 0.01$) [ANOVA ($F (4, 42) = 35.529$, $p = 0.001$)]. For the CA3, a main effect of surgery ($F (1, 36) = 109.371$, $p = 0.001$) was found, related to higher IBA1-ir levels in ischemic rats compared to all groups [ANOVA ($F (4, 42) = 36.422$, $p < 0.01$)].

3.8 TNFα-ir in the hippocampal CA1 and CA3 regions

Fig. 8 shows the impact of ischemia and ANT treatment on TNFα expression in the CA1, and CA3 thirty days post-reperfusion. For the CA1, main effects of drug ($F (1, 36) = 4.550$, $p = .040$) and surgery ($F (1, 36) = 17.165$, $p = .001$) were found, due to elevated TNFα-ir expression in ischemic compared to control animals. Post hoc comparisons following one-way ANOVA ($F (4, 42) = 7.674$, $p = 0.001$) identified higher TNFα in IS rats compared to all other groups ($p < 0.05$). The IA group only differed significantly from the SA group ($p = 0.031$). For the CA3, main effects of drug ($F (1, 36) = 5.912$, $p = .020$), and surgery ($F (1, 36) = 32.821$, $p = .001$) were found, higher TNFα expression being observed in ischemic rats compared to sham animals, which was reduced by ANT treatment in sham rats. The IA group only significantly differed from SA animals ($p = 0.001$).

3.9 BDNF- TrkB- and pCREB-immunoreactivity at the hippocampal CA1 and CA3 regions

Fig. 9 and 10 show the impact of ischemia and ANT treatment at the CA1 and CA3 hippocampal layers 30 days post-ischemia, respectively, for BDNF, TrkB and pCREB-ir. For BDNF at the CA1, main effects of drug ($F(1, 36) = 19.055$, $p = .001$) and surgery ($F(1, 36) = 73.288$, $p = .001$) were found. One-way ANOVA revealed differences between groups ($F(4, 40) = 35.173$, $p = 0.000$), due to higher BDNF levels in IS rats compared to all other groups ($p < 0.01$). The ischemic-ANT group had lower expression than the ischemic-VEH group ($p = 0.001$), although it remained higher than that of sham and HC groups ($p < 0.05$). For BDNF-ir at the CA3, main effects of drug ($F(1, 36) = 13.176$, $p = .001$), surgery ($F(1, 36) = 11.797$, $p = .002$) and a significant surgery x drug interaction ($F(1, 36) = 7.277$, $p = 0.011$) were found. One-way ANOVA revealed significant differences between groups ($F(4, 40) = 9.949$, $p = 0.001$), due to higher BDNF-ir in IS rats compared to all other groups ($p < 0.001$).

For TrkB at the CA1, main effects of drug ($F(1, 36) = 4.899$, $p = 0.033$), and surgery ($F(1, 36) = 18.738$, $p = 0.001$) were found. One-way ANOVA revealed differences between groups ($F(4, 41) = 8.347$, $p = 0.001$), due to higher TrkB in the the IS group compared to all other groups ($p < 0.01$), except the IA group, which did not significantly differ from any of the groups ($p > 0.05$). For the CA3, a main effect of surgery ($F(1, 36) = 17.222$, $p = .001$) was found. One-way ANOVA revealed differences between groups ($F(4, 41) = 6.680$, $p = 0.001$), due to higher TrkB in IS rats compared to all other groups ($p < 0.01$), except the IA group which did not significantly differ from any of the groups ($p > 0.05$).

For pCREB at the CA1, a main effect of surgery ($F(1, 36) = 13.432$, $p = 0.001$) and a surgery x drug interaction ($F(1, 36) = 45.287$, $p = 0.001$) were found. One-way ANOVA revealed differences between groups ($F(4, 40) = 10.185$, $p = 0.001$), due to higher pCREB levels

in IS animals compared to all other groups ($p < 0.05$), except the HC group, which also had higher pCREB-ir than the SS group ($p = 0.008$). For the CA3, a surgery x drug interaction ($F (1, 36) = 14.426$, $p = 0.001$) was found. Simple effects tests indicated that the IS group had significantly more CA3-pCREB-ir than SS and IA animals ($p \leq 0.01$).

3.10 BDNF- and TrkB-immunoreactivity at the BLA and PVN

Fig. 11 (a, b & c) shows the impact of ischemia and ANT treatment on BDNF and TrkB expression in the BLA and PVN, 30 days post-reperfusion. For the BLA and BDNF-ir, a surgery x drug interaction ($F (1, 36) = 8.922$, $p = 0.005$) was found. One-way ANOVA revealed differences between groups ($F (4, 41) = 4.193$, $p = 0.006$), due to higher BDNF levels for the SA group compared to the IA group ($p = 0.017$). For TrkB-ir at the BLA, a main effect of surgery ($F (1, 36) = 36.509$, $p = 0.001$) was found. One-way ANOVA ($F (4, 41) = 12.537$, $p = 0.001$) showed that this was due to higher TrkB-ir in ischemic compared to sham and HC groups ($p < 0.01$).

At the PVN [Fig. 11 (a, d & e)] and for BDNF-ir main effects of drug ($F (1, 36) = 16.373$, $p = 0.001$), surgery ($F (1, 36) = 17.822$, $p = 0.001$) and a surgery x drug interaction ($F (1, 36) = 26.110$, $p = 0.001$) were found. One-way ANOVA revealed significant differences between groups ($F (4, 41) = 17.541$, $p = 0.001$), due to higher BDNF-ir in IS animals compared to all other groups ($p < 0.001$). For TrkB-ir, a main effect of surgery ($F (1, 36) = 7.380$, $p = .010$) was found, which ANOVA ($F (4, 41) = 3.776$, $p = 0.011$) showed attributable to higher TrkB-ir in the IS rats compared to SA and HC groups ($p \leq 0.05$).

Protein (Western Blot) and mRNA (RT-PCR) Expression in the Dorsal Hippocampus

Fig. 12 shows the impact of ischemia and ANT treatment on TrkB and synapsin protein expression (Fig. 12 a, b & c) in the dissected dorsal hippocampus, 30 days post-reperfusion. For

TrkB protein expression, a main effect of surgery ($F (1, 12) = 11.209$, $p = 0.006$) was found, which one-way ANOVA ($F (4, 15) = 4.489$, $p = 0.014$), attributed to higher TrkB level in the SS compared to IA group ($p = 0.044$). For synapsin expression, a main effect of surgery ($F (1, 12) = 30.707$, $p = 0.001$) and a surgery x drug interaction ($F (1, 12) = 7.110$, $p = 0.021$) were found. One-way ANOVA revealed differences between groups ($F (4, 15) = 12.431$, $p = 0.001$) due to lower synapsin in IS animals compared to all other groups ($p < 0.005$), except the IA group, which differed from the SS group ($p = 0.003$).

For BDNF mRNA (Fig. 12 d & f), we found a main effect of surgery ($F (1, 16) = 30.375$, $p = 0.001$), which ANOVA ($F (4, 20) = 10.405$, $p = 0.001$) revealed attributable to higher BDNF mRNA levels in both sham groups compared to the ischemic and HC groups ($p < 0.05$). For TrkB mRNA (Fig. 12, e & f), main effects of surgery ($F (1, 16) = 28.690$, $p = 0.001$) and drug ($F (1, 16) = 7.482$, $p = 0.015$) were found. One-way ANOVA revealed differences between groups ($F (4, 20) = 16.553$, $p = 0.001$) due to elevated TrkB mRNA expression in the SS group compared to all other groups ($p < 0.005$). The SA group showed higher levels than the HC group ($p = 0.001$).

3.11 Protein (Western Blot) and mRNA (RT-PCR) Expression in the Dorsal Hippocampus

Fig. 13 (a, b & c) shows the impact of ischemia and ANT treatment on TrkB and synapsin protein expression in the dissected amygdala, 30 days post-ischemia. For TrkB, a main effect of surgery ($F (1, 12) = 7.883$, $p = 0.016$) was found, which ANOVA ($F (4, 15) = 4.135$, $p = 0.019$) showed related to higher TrkB protein in IA rats compared to the HC group ($p = 0.033$). For synapsin, a main effect of surgery ($F (1, 12) = 14.499$, $p = 0.002$) was found, indicating higher synapsin protein expression in ischemic compared to sham animals. One-way ANOVA revealed differences between groups ($F (4, 15) = 5.716$, $p = 0.005$), due to higher protein levels

of synapsin in the amygdala of IA rats compared to all other groups ($p < 0.005$), except the IS group ($p > 0.05$).

For BDNF mRNA (Fig. 13 d & f), main effects of surgery ($F (1, 12) = 43.646$, $p = 0.001$) and drug ($F (1, 12) = 7.606$, $p = 0.017$) were found. One-way ANOVA ($F (4, 15) = 17.197$, $p = 0.001$) revealed differences between groups due to reduced BDNF mRNA levels in SS animals compared to all other groups ($p < 0.05$), except the SA group which only differed from the IA group ($p = 0.004$). For TrkB mRNA, a main effect of surgery ($F (1, 12) = 5.591$, $p = 0.036$) and a surgery x drug interaction ($F (1, 12) = 22.061$, $p = 0.001$) were found. Differences between groups ($F (4, 15) = 13.386$, $p = 0.001$) were due to higher TrkB mRNA levels in the SA and HC groups compared to SS and IA animals ($p < 0.005$).

4. Discussion

The current study showed that blockade of CRHR1 receptors has a significant impact on functional and biochemical responses induced post ischemia. ANT treatment reduced spatial memory impairment in the Barnes Maze, improved aversive learning in the YM-PAT and regulated anxiety-like behaviors in the EPM and OFT. Pre-ischemic CRHR1 blockade partially reversed ischemia-induced neuronal damage and attenuated immunohistochemical expression of neuroinflammation (GFAP, IBA1, TNFα) although not reaching significance. Notably, ischemia increased BDNF and TrkB-ir at the CA1 and CA3 subfields of the hippocampus, and similar to what was observed with glial immunostaining, ANT reduced expression although not to a significant level. Interestingly however, ANT treatment led to apparent differences in the immunostaining distribution in ischemic rats especially apparent for IBA1- and TrkB-ir at the CA3, and BDNF- and TrkB-ir at the CA1. Using Western blotting and RT-PCR analyses, our findings highlight alterations in BDNF, TrkB and synapsin levels predominantly affecting the

vulnerable dorsal, rather than ventral, portion of the hippocampus where both protein and mRNA expression were diminished 30 days post ischemia. At the PVN, heightened post ischemic BDNF- and TrkB-ir, together with elevated trunk blood serum CORT concentrations, were reduced by ANT treatment. Increased TrkB-ir was observed in ischemic groups at the BLA, independently of CRH-R1 blockade.

4.1 Beneficial effects of Antalarmin treatment on post-ischemic memory deficits

Behaviorally, CRHR1 blockade attenuated ischemia-induced spatial memory impairments in the Barnes Maze. This positive outcome resembles memory enhancing effects on Barnes Maze performance observed in ischemic rats pre-treated with the glucocorticoid inhibitor metyrapone (M. R. Milot & Plamondon, 2011a). This may suggest that akin to metyrapone-treated rats, reduced CORT secretion also observed in ANT-treated ischemic animals may contribute to improved memory function. Despite reduced CA1 pyramidal neurons being commonly associated with impaired spatial memory performance, diminished glucocorticoid secretion has been shown to alleviate memory impairment in the absence of reduced ischemic damage (Y. D. Zhao et al., 2013). Notably, notwithstanding the groups, all rats showed learning as they progressed through trials, indicating that spatial learning is possible despite ischemic damage, which supports compensatory mechanisms. In the YM-PAT, ischemic rats selected more rapidly an arm during the initial trial and showed reduced latency to enter the aversive arm, where they received repeated jets of air, during the second trial. Such responses are consistent with reduced freezing behavior in the contextual fear conditioning test observed following cardiac arrest in rodents, and associated with CA1 injury and synaptic dysfunction in the dorsal hippocampus (Cohan et al., 2015), likely contributing to memory processing of reactive fear responses (F. C. Yang & Liang, 2014). Recent studies also identify the BLA as a sensitive brain

region to hypoxic-ischemic damage (Carty et al., 2010; de la Tremblaye & Plamondon, 2011a), which is consistent with reduced amygdalar density observed in VEH-ischemic compared to sham-operated and HC rats in the current study. Our observations are also consistent with ~25% neuronal attrition at the BLA and reduced fear conditioning reported following 10 minute global cerebral ischemia (de la Tremblaye & Plamondon, 2011a). Notably, ANT-treated ischemic rats showed increased latency to re-enter the aversive arm compared to VEH-treated ischemic rats, concomitant with increased neuronal survival at the BLA.

4.2 Increased expression of neuroinflammation signaling molecules in the ischemic brain appears partly affected by CRHR1 activation and staining distribution related to injury severity.

Our findings support inhibitory effects of pre-ischemic blockade of CRHR1 on ischemia-induced expression of distinct markers of neuroinflammation. ANT partially attenuated expression of reactive glial cells and TNFα levels in all subregions of the hippocampus 30 days post ischemia. This is consistent with a pro-inflammatory role for CRH upon ischemia, which appears mediated via CRHR1 in a cAMP dependent manner and thought to contribute to reduced cell survival (Baigent, 2001). Cerebral ischemia triggers inflammation within hours, persisting for days following brain insult (B. Z. Sun et al., 2014). The damaged brain tissue activates astrocytes and microglia which release pro-inflammatory cytokines such as TNF-α (R. Jin et al., 2010; Kriz, 2006), which ultimately results in increased cell death (Sharma & Kumar, 1998; Vila, Castillo, Davalos, & Chamorro, 2000; Watters & O'Connor, 2011). Among suggested roles, reactive astrocytes are thought to contribute to glial scarring post ischemia, produced by extracellular matrix substrates that become a barrier to dendritic and axonal regeneration after injury (Cregg et al., 2014).

In the current study, predominant and circumscribed expression of IBA1-positive cells was observed within the injured pyramidal CA1 and CA3 subregions in ischemic VEH-treated animals 30 days post ischemia, labeled cells presenting shorter processes and plump cell bodies, phenotypically associated with an activated state. This is consistent with previously reported post-ischemic morphological changes in reactive microglia (Gehrmann, Banati, Wiessner, Hossmann, & Kreutzberg, 1995; Girbovan & Plamondon, 2015; F. Lu et al., 2014; Morioka, Kalehua, & Streit, 1991; Yrjanheikki et al., 1998). These IBA1-positive cells may be blood-derived macrophages having a primary phagocytic role (R. Jin et al., 2010). Notably, ANT-treated ischemic rats showed a clearly distinctive hippocampal IBA1-ir expression characterized by sparsely distributed IBA-1-positive cells showing highly ramified processes affecting brain tissue adjacent to the pyramidal cell layers, which distribution pattern appear similar to that found in sham-operated animals. These observations are interesting as reactive microglia have been shown to secrete BDNF (Coull et al., 2005; Nakajima, Tohyama, Kohsaka, & Kurihara, 2002), which levels are significantly increased in infarcted brain regions following stroke (Sulejczak et al., 2007b). BDNF mRNA levels increase in both degenerating and surviving neurons in the acute post-stroke period (Comelli et al., 1992; Rickhag et al., 2007; L. R. Zhao et al., 2000). Subsequently however, BDNF production shifts from neurons to non-neuronal/non-astrocytic cell phenotypes, which co-label with the microglia marker IBA-1 (Livingston-Thomas, McGuire, Doucette, & Tasker, 2014). Such observations are pertinent to the response observed 30 days post ischemia in our study, a time interval where neuronal damage is thought completed. Thus, the increased BDNF- and TrkB-ir expression (and to a lower extent pCREB) in the CA1 and CA3 subfields of the hippocampus, with modest changes at the DG, could in part be regulated by heightened secretion of plasticity signals by glial cells. In addition, increased CORT

levels observed in the ischemic rats suggest that activation of glucocorticoid signaling persisting at delayed post ischemic interval may contribute to changes in plasticity signaling expression in affected brain regions. Indeed, increased plasticity signaling at the amygdala post ischemia is consistent with heightened sensitivity of the hippocampus in various stress models and involving increased dendritic and synaptogenesis. Current observations observed at remote post-ischemic intervals need to be further explored. They strengthen recent findings using selective markers to study reactive microglia, which have proposed active roles for microglia in recovery of the injured brain, including beneficial outcomes. Thus, transplantation of human microglial cells following focal ischemia reduces ischemic impairments and cellular apoptosis (Narantuya et al., 2010) while microglial depletion enhances excitotoxic injury and cytokine and chemokine production following ischemic reperfusion (Faustino et al., 2011). In this context, the differential distribution of IBA1-ir observed at the CA3 layer in vehicle- and ANT-treated ischemic groups is intriguing, and could support differential recruitment dependent on degree of injury. Cell damage being alleviated by ANT, IBA1 recruitment in the CA3 resembles that observed in sham animals, where expression is heightened in the penumbra zone of the CA3 pyramidal layer.

4.3 A dual role for BDNF secretion in the ischemic brain?

Transient forebrain ischemia is known to rapidly upregulate BDNF mRNA expression in the hippocampus, a phenomenon thought to be mediated through glutamate release (Tsukahara et al., 1994). Increased BDNF mRNA expression in the hippocampus has been proposed to occur via glutamate activation of AMPA, rather than NMDA receptors (Zafra, Hengerer, Leibrock, Thoenen, & Lindholm, 1990) and to promote neuroprotective actions (Lindvall et al., 1992; Tsukahara et al., 1994). The CA1 pyramidal neurons also show significant increase in pCREB in the hours following ischemia (K. Jin, Mao, Simon, & Greenberg, 2001; Kuramoto et al., 1998),

which rapidly decreases along with reduced BDNF expression (Z. J. Zhang, Peng, Zhu, & Wang, 2012). Similarly, full-length TrkB protein expression in the affected cortex and striatum is decreased 24 h after focal cerebral ischemia in male rats (Tian et al., 2013). Decreased pCREB protein levels in the hippocampus is associated with impaired LTP in the CA1 region following chronic intermittent hypoxia (A. M. Wall, Corcoran, O'Halloran, & O'Connor, 2014).

Our observations 30 days post ischemia contrast with the reported decreased BDNF protein levels at the vulnerable CA1 layer observed at earlier time intervals following 10 min of forebrain ischemia associated with elevated BDNF gene and protein expression in the more resistant DG and CA3 regions (Kokaia et al., 1996). In these studies, aggravation of brain damage in CA3 and other brain regions has been associated with suppressed BDNF gene expression, as brain regions become more vulnerable to ischemic damage (Uchino, Lindvall, Siesjo, & Kokaia, 1997). Consistent with reduced plasticity signaling, an 18% decrease in CA1 spine density has been observed two weeks following cardiac arrest, correlated with impaired post-ischemic water maze learning (Neigh et al., 2004).

Our findings reveal heightened BDNF immunostaining in the CA1 and at the CA3 thirty days post ischemia, corresponding to the degree of cellular injury, which was reduced by ANT in ischemic rats. TrkB expression followed a similar trend as BDNF, but changes were not as strong in pCREB expression due to higher levels in HC and sham rats. Of interest, Western blotting performed on dissected dorsal hippocampal tissue showed reduced TrkB expression. Physiologically, reduced TrkB receptor expression appears relevant considering elevated BDNF-ir post ischemia. The same is true for synapsin expression, which supports synaptic efficacy, being affected by ischemic damage in a degree dependant manner. At the mRNA level, RT-PCR data suggest depleted BDNF mRNA at the dorsal hippocampus in ischemic compared to sham

groups. Having assessed both BDNF protein and mRNA levels, it seems reasonable that depleted BDNF mRNA stores are due to increased release of the protein at this delayed post ischemic interval. Conversely, sham-operated groups show elevated BDNF mRNA at the dorsal hippocampus and reduced BDNF-ir expression. It is interesting to note that TrkB protein levels assessed in the dissected dorsal hippocampus show a differential expression profile, as levels determined more precisely in the CA1 and CA3 layers using immunohistochemistry, raising an important point as to the specificity of the tissue expression being detected.

At the amygdala, we observed a trend towards increased TrkB protein levels in ischemic animals which reached significance when looking more specifically at TrkB-ir in the BLA. This was paralleled by reduced TrkB mRNA expression in ischemic groups compared to SA and HC rats while BDNF mRNA was increased in ischemic compared to sham groups. Concordant increases in BDNF mRNA and TrkB protein may indicate dynamic proteins to receptors interactions. Similarly, the profile of BDNF-ir at the BLA is concordant with TrkB mRNA expression, supporting mutual influence of these signaling molecules. In the current study, interpretation is facilitated by measurements of both mRNA and protein levels, supporting dynamic fluctuations between BDNF and TrkB expression in the context of ischemic pathophysiology. Synapsin protein expression is also interesting, being reduced at the dorsal hippocampus in the ischemic brain, and increased at the amygdala. These findings are consistent with opposite structural changes leading to reduced versus increased synaptogenesis at the hippocampus and amygdala, respectively, following exposure to various stressors in rodents (Serafini, 2012; J. C. Zhang, Yao, et al., 2015).

The role of BDNF may be especially important in the degenerative change that occurs in hippocampal neurons after forebrain ischemia as the hippocampus has the highest BDNF

mRNA content relative to other brain regions (Hofer et al., 1990). However, studies are not clear as to its role under normal and pathological states. Central BDNF infusion has been shown to reduce necrosis in the hippocampal CA1 region after transient forebrain ischemia in rats (Beck, Lindholm, Castren, & Wree, 1994), and to promote neural regeneration, angiogenesis, and functional recovery of MCAO rats along with reduction of cell damage (Guan et al., 2012). Physical exercise performed before and after stroke as a form of rehabilitation increases BDNF levels, coincident with improved recovery (Ke, Yip, Li, Zheng, & Tong, 2011; G. Kim & Kim, 2013). Of interest, enriched environment enhances memory performance and BDNF protein expression in the dentate gyrus, although it failed to affect CA1 cell death following global ischemia (Gobbo & O'Mara, 2004), while others have shown enriched environment to prevent loss of spine density in the hippocampus and improved object recognition in hypoxic-ischemic rats (J. J. Rojas et al., 2013).

Nonetheless, BDNF upregulation after cerebral traumatic events is not always sufficient to promote recovery (B. Li et al., 2010). For instance, contrasting above mentioned effects, BDNF or proBDNF activation of p75 neurotrophin receptors can induce axon degeneration in cultured sympathetic neurons (K. K. Singh et al., 2008), and also following ischemic reperfusion in mice (Lorentz et al., 2013). Intra-amygdala kainic acid-mediated status epilepticus enhances phosphorylated TrkB immunoreactivity in apical dendritic shafts and spines of CA1 pyramidal cells, and within mossy fiber axons, as well as in giant synaptic boutons of dentate granule cells (Helgager, Liu, & McNamara, 2013), while inhibition of TrkB initiated after status epilepticus and maintained for 2 weeks prevents recurrent seizures, ameliorates anxiety-like behavior, and limits loss of CA3 neurons when tested weeks to months later (G. Liu et al., 2013). Together these findings render difficult a clear interpretation of the contribution of

pCREB/BDNF/TrkB to cellular preservation and functional recovery. In our study, BDNF signaling is persistent in the most injured brain regions 30 days post ischemia and associated with diminished functional recovery.

4.4 CRH and the Ischemic Cascade

Following cerebral ischemia, increased CRH release, mRNA and protein expression (Khan et al., 2004), likely enhance excitation in key limbic circuits, possibly increasing the risk of excitotoxicity (T. Z. Baram & Ribak, 1995; Ribak & Baram, 1996), in a concentration dependent manner (Charron et al., 2009). The vast majority of hippocampal CRH-expressing neurons are GABAergic interneurons (Y. Chen, R. A. Bender, et al., 2001), terminals of these CRH-expressing interneurons being strategically positioned to influence the excitability of the principal hippocampal neurons via alternated release of CRH and GABA.

Transient ischemic brain injury initiates a complex sequence of events which is known to modify the function of the HPA axis (Radak et al., 2014). Assessment of basal CORT secretion has been shown to be increased in the hours and days following global cerebral ischemia as compared to sham animals with heightened stress-induced elevations in CORT levels of ischemic rats induced by mild (spatial memory testing observed 11-14 days after ischemic injury) (M. R. Milot & Plamondon, 2011a) and more severe (15 minute restraint stress, 2 months after ischemic injury) stressors (Roberge, Messier, Staines, & Plamondon, 2008). Interestingly, a single systemic injection of metyrapone, a glucocorticoid suppressor, administered prior to ischemia onset lowers plasma CORT levels in the hours and days after reperfusion and concurrently reduces Barnes Maze post-testing CORT elevations and spatial memory impairments despite similar CA1 damage to that of vehicle-treated rats at time of testing (M. R. Milot & Plamondon, 2011a). These data indicate that suppression of the adrenocortical

activation at the onset of an ischemic episode, through glucocorticoid synthesis inhibition, appears sufficient to attenuate sensitization and increased responsiveness of the neuroendocrine system. In the current study, a similar effect was observed with ANT inducing partial reversal of trunk blood elevated CORT concentrations in ischemic animals. Altered HPA axis functioning during basal and challenging conditions after ischemic brain injury, supports dysfunctional negative feedback regulation, resulting in heightened CORT secretion, a phenomenon characterizing various pathological states, including depression which affects over a third of stroke patients (Gothe et al., 2012).

At the hypothalamic PVN, BDNF and TrkB immunoreactivity was heightened post ischemia and reduced by ANT treatment. This increase is consistent with elevated BDNF mRNA levels in PVN-CRH neurons following acute or repeated immobilization stress (M. A. Smith, Makino, Kim, et al., 1995). Notably, central BDNF infusion, both acute (Givalois et al., 2004) and continuous (Naert et al., 2006), has been shown to be associated with increased plasma corticosterone concentrations. Chronically infused BDNF increases CRH-ir at the PVN reducing food intake and body weight, an effect that can be attenuated by central blockade of CRH receptors using α-helical-CRH9-41 (Toriya et al., 2010). As for TrkB, PVN CRH-ir neurons have been shown to strongly express TrkB proteins following administration of the neurotoxin colchicine in rodents (Toriya et al., 2010). Thus, considering that CRH- and CRHR1-ir increased in the PVN following global ischemia, it appears likely that ANT treatment reduced BDNF and TRKB expression through CRHR1 blockade within the PVN (de la Tremblaye et al., 2014).

4.5 Conclusion

In the current study, Antalarmin conferred a neuroprotective effect on the ischemia-induced brain injury and reduced spatial memory deficits in the Barnes Maze and aversive

learning in the YM-PAT. These changes were accompanied by ANT-mediated anti-inflammatory effects as well as improved HPA axis regulation post ischemia. We also demonstrated a relationship of activation of CRHR1 receptors upon ischemia and the cascade of plasticity signaling in affected brain regions. Enhanced neuronal survival associated with delayed changes in plasticity signaling at the BLA and CA1 may suggest intermingled roles of CRHR1 in the regulation of emotional/cognitive impairment following global ischemia. Together, these findings provide novel insights on effects of an ischemic insult on multiple pathways regulating a variety of brain processes, certain mimicking, while others contrasting physiological responses associated with potent stressors. These findings also support therapeutic tools aimed at minimizing stress pathways activation in the hours of an ischemic insult.

5. Acknowledgments

The authors thank Simon Benoit and Marika Bonneville for their help in performing histological analyses and behavioral scoring. This work was supported by a grant from the National Science and Engineering Research Council of Canada to Dr. Hélène Plamondon and internal funding from the University of Ottawa. The authors declare having no conflict of interest regarding the work described here.

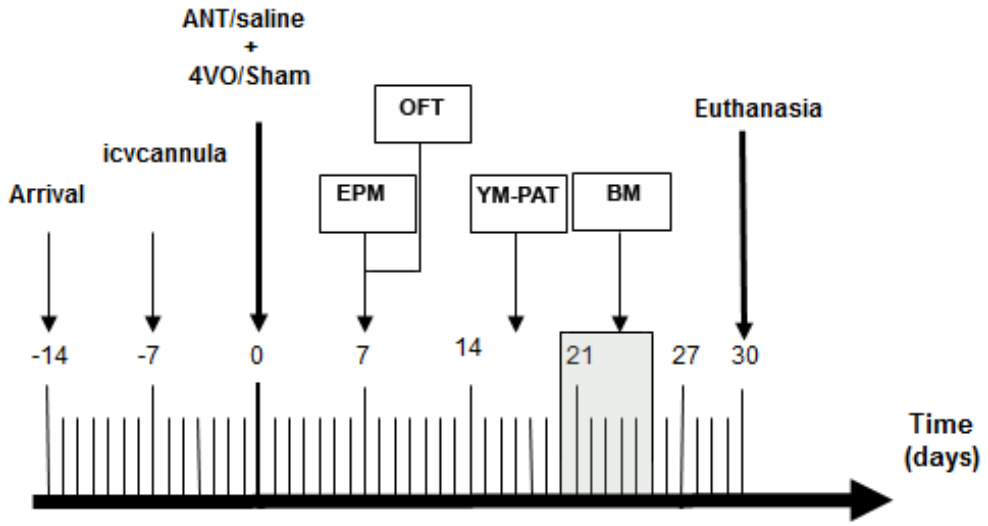

Fig. 1. Experimental timeline. Rats arrived to the animal facility one week prior to cannulae implantation surgery. On day 0, after 7 days of recuperation, rats underwent 4 vessel occlusion (4VO). Antalarmin (2 μg/2 μl) or vehicle was icv administered 30 min before sham or carotid occlusion. Animals were tested on day 7 in the Elevated plus Maze (EPM) and Open Field Test (OFT), on day 17 in the Y Maze Passive Avoidance task (YM-PAT), and starting on day 20 in the Barnes Maze (BM) for 5 days. Rats were euthanized 30 days post reperfusion.

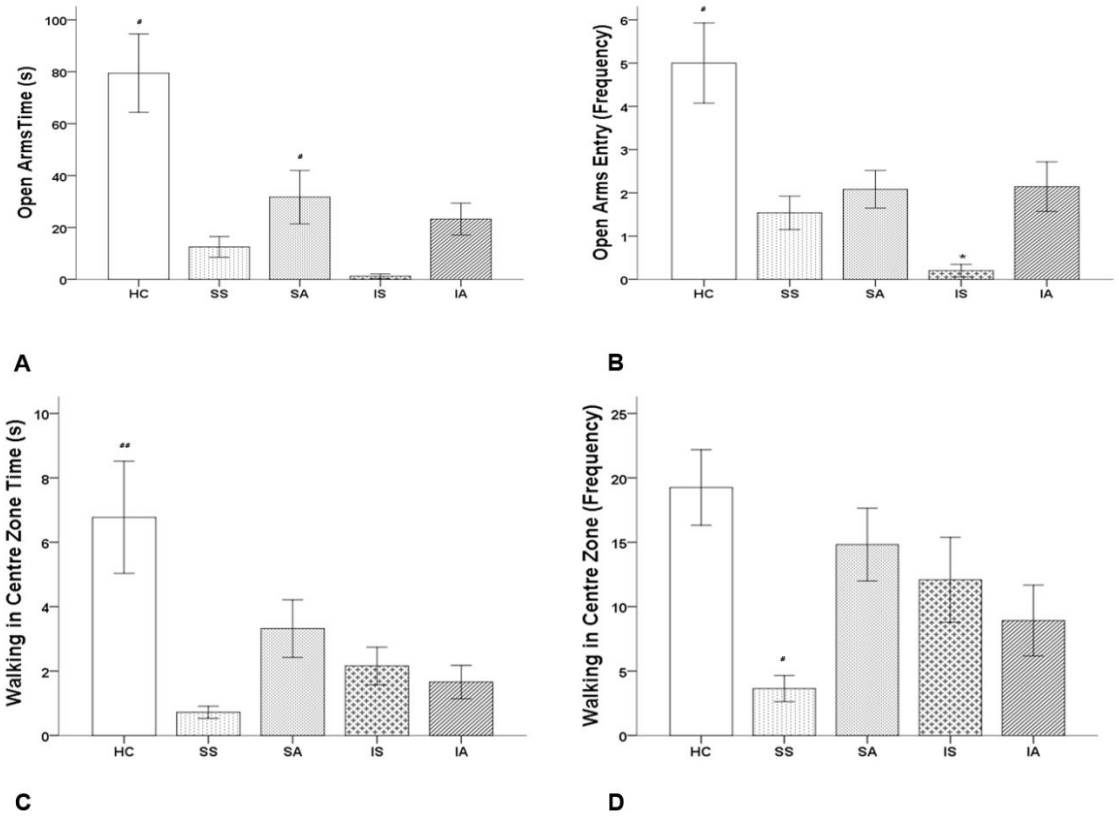

Fig. 2. (A) Time spent and (B) frequency of entry in the open arm of the EPM for each experimental group. All surgical groups spent less time in the open arm in comparison to the HC group, with Antalarmin attenuating anxiety-like behaviors in sham and ischemic groups. (C) Time and (D) frequency of walking in the OFT center zone. All surgical groups spent reduced time in the center zone compared to HC rats, ANT attenuated this behavior. IS ventured more in the center zone than the SS group. Data are expressed as mean ± SEM. **$p < 0.01$, #$p < 0.05$

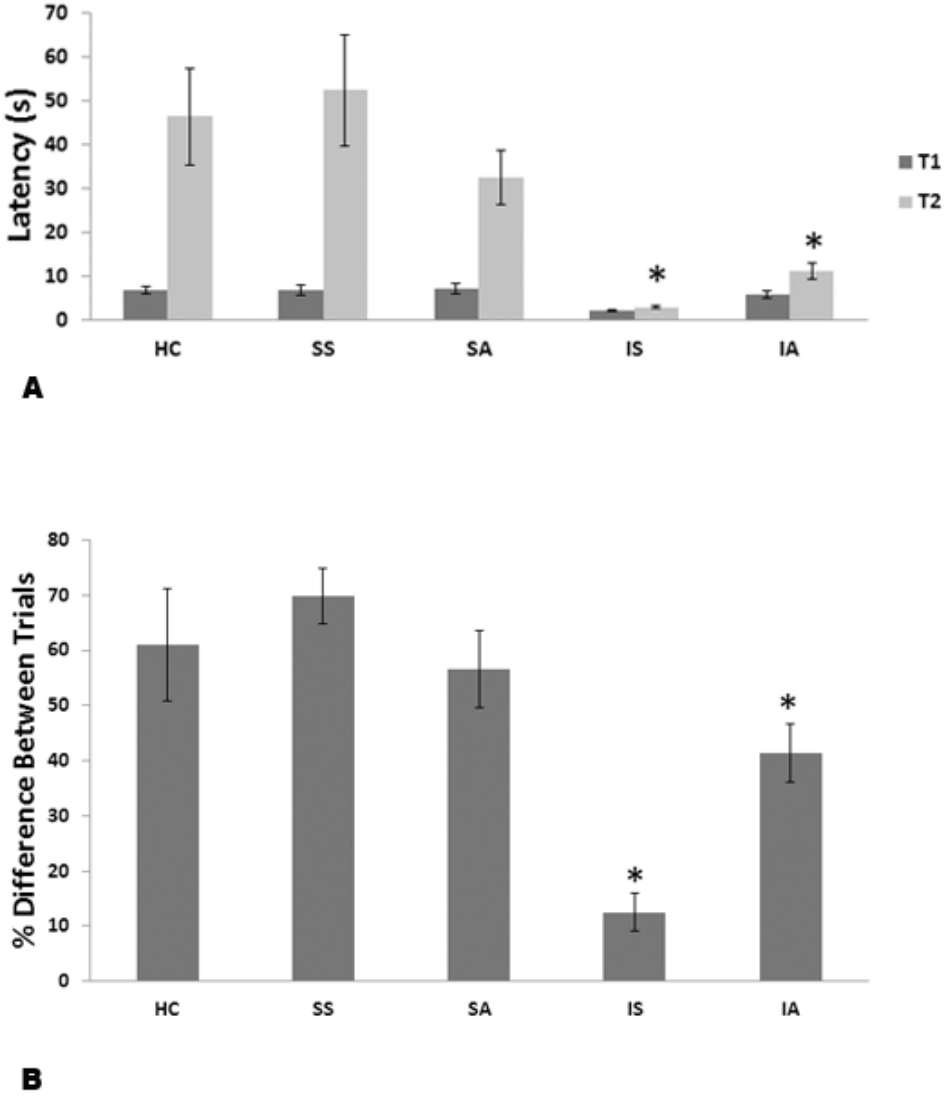

Fig. 3. Global ischemia led to reduced latency to re-enter the aversive arm in the Y Maze Passive Avoidance task, and ANT partially reversed this response. (A) shows the latency to enter the aversive arm on Trials 1 and 2 (T1 and T2). (B) indicates the percent difference between both trials. Results are presented as mean latencies ± SEM. *$p < 0.05$.

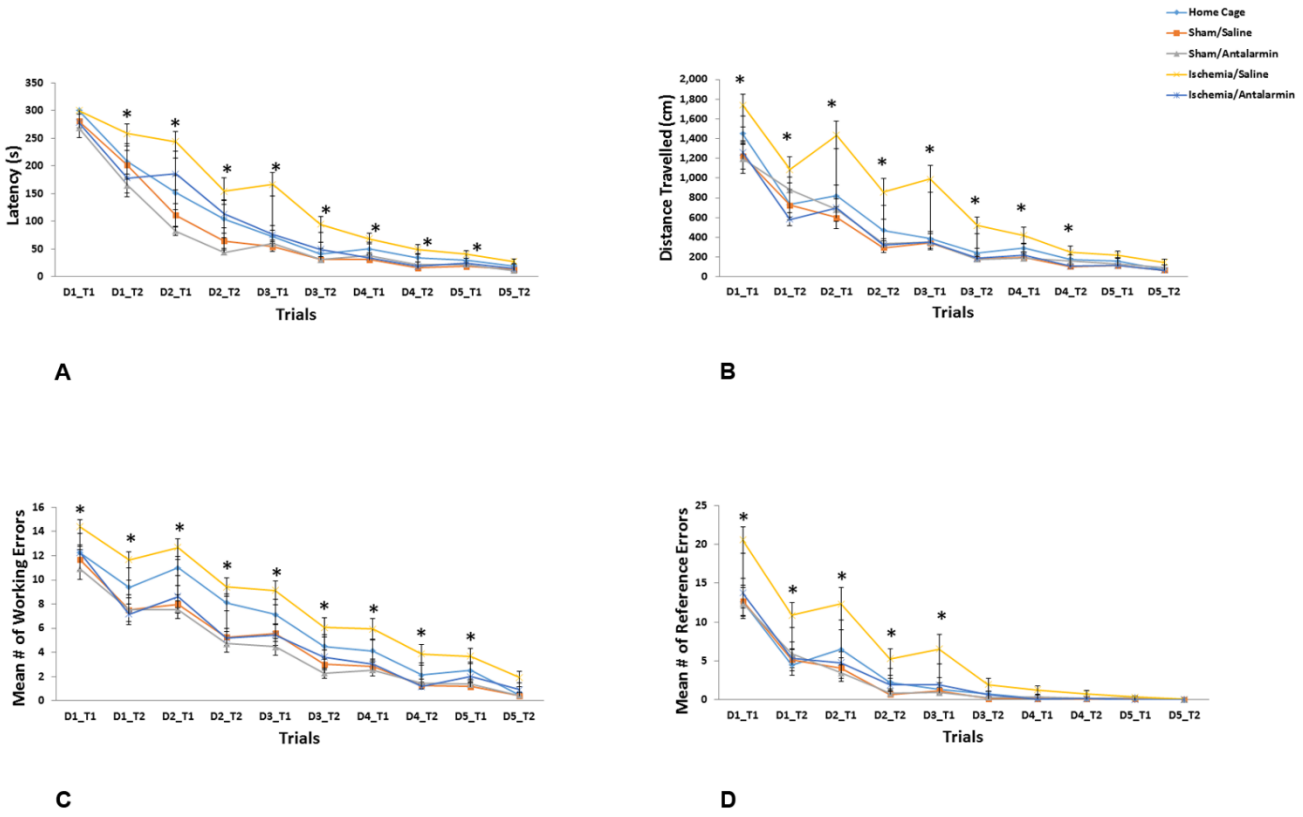

Fig. 4. Ischemia increased (A) latency, (B) distance traveled, (C) working and (D) reference errors, to find the escape box in the Barnes Maze. Antalarmin prevented ischemia-induced memory impairments. Animals received two trials per day for 5 consecutive days. Results are presented as mean ± SEM. * $p < 0.05$.

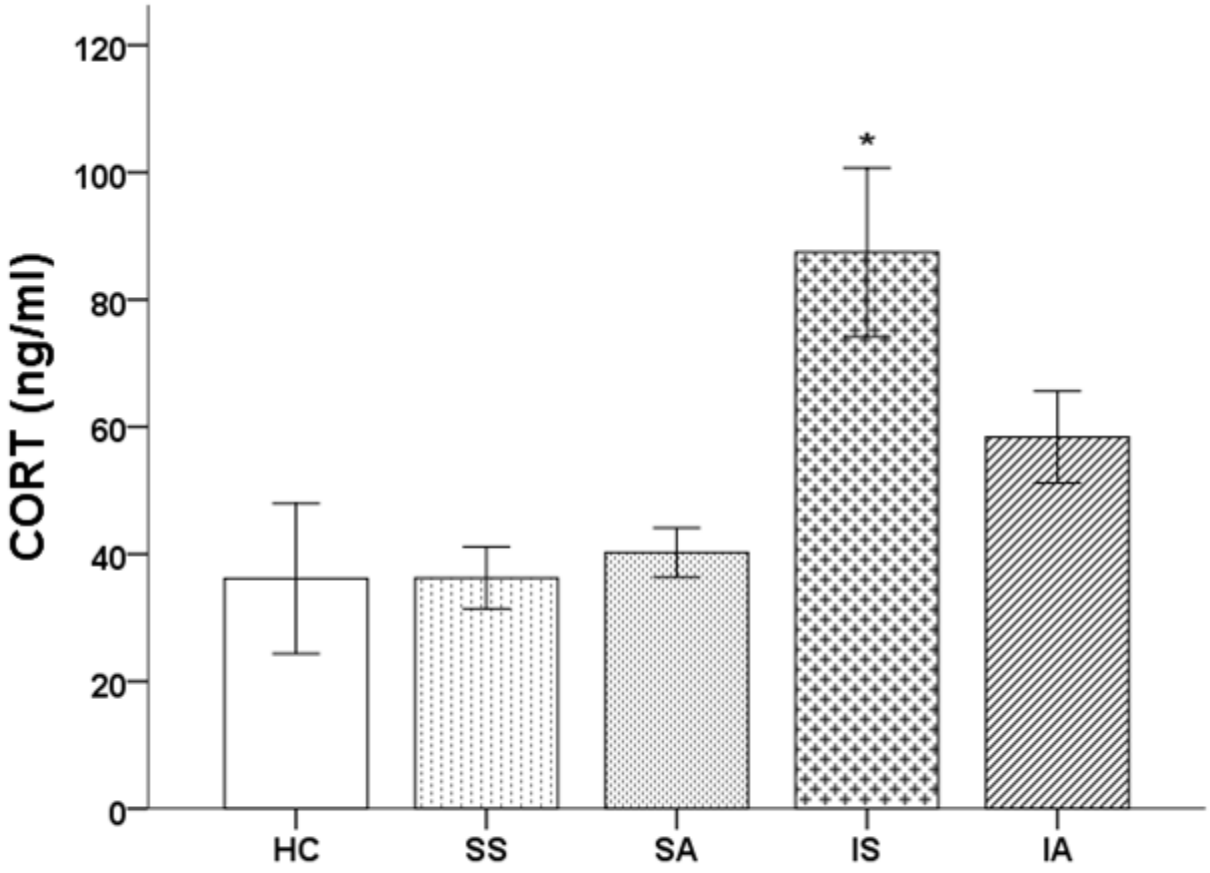

Fig. 5. Global ischemia led to increased serum corticosterone levels 30 days following reperfusion, an effect prevented by Antalarmin treatment. Values are means ± SEM. *$p < 0.05$.

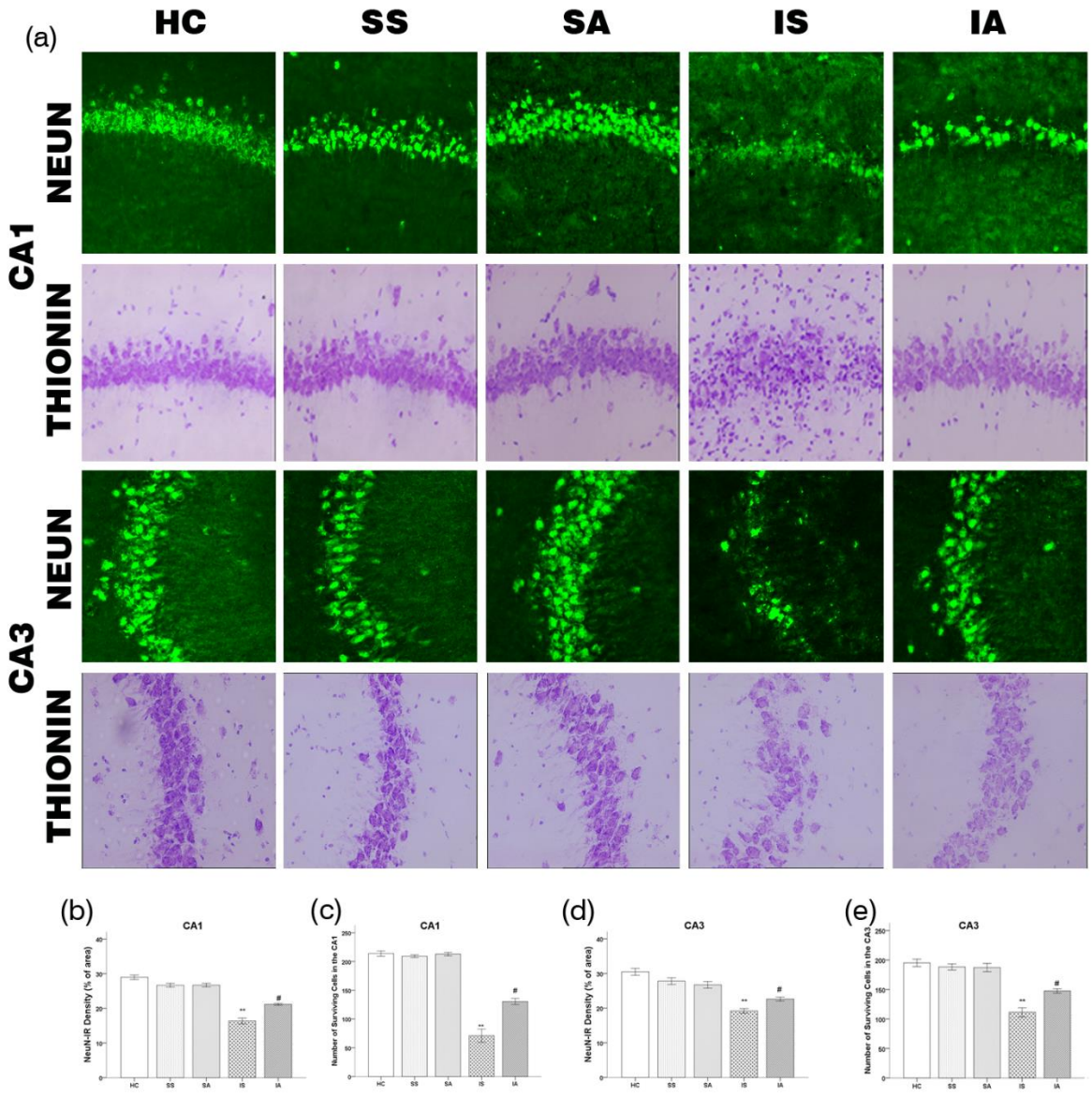

Fig. 6. Global ischemia led to reduced neuronal density in the hippocampus (*), which was partially reversed by Antalarmin 30 days post ischemia (#). (a) Representative photomicrographs of NeuN immunostaining (green) and thionin Nissl staining (purple) in the CA1 (top) and CA3 (bottom) hippocampal subfields at 200X magnification. Histogram representation of CA1 (b-c) and CA3 (d-e) mean percent optical densities for NeuN and cell counts for thionin staining. Data are expressed as mean ± SEM. **p < 0.01,

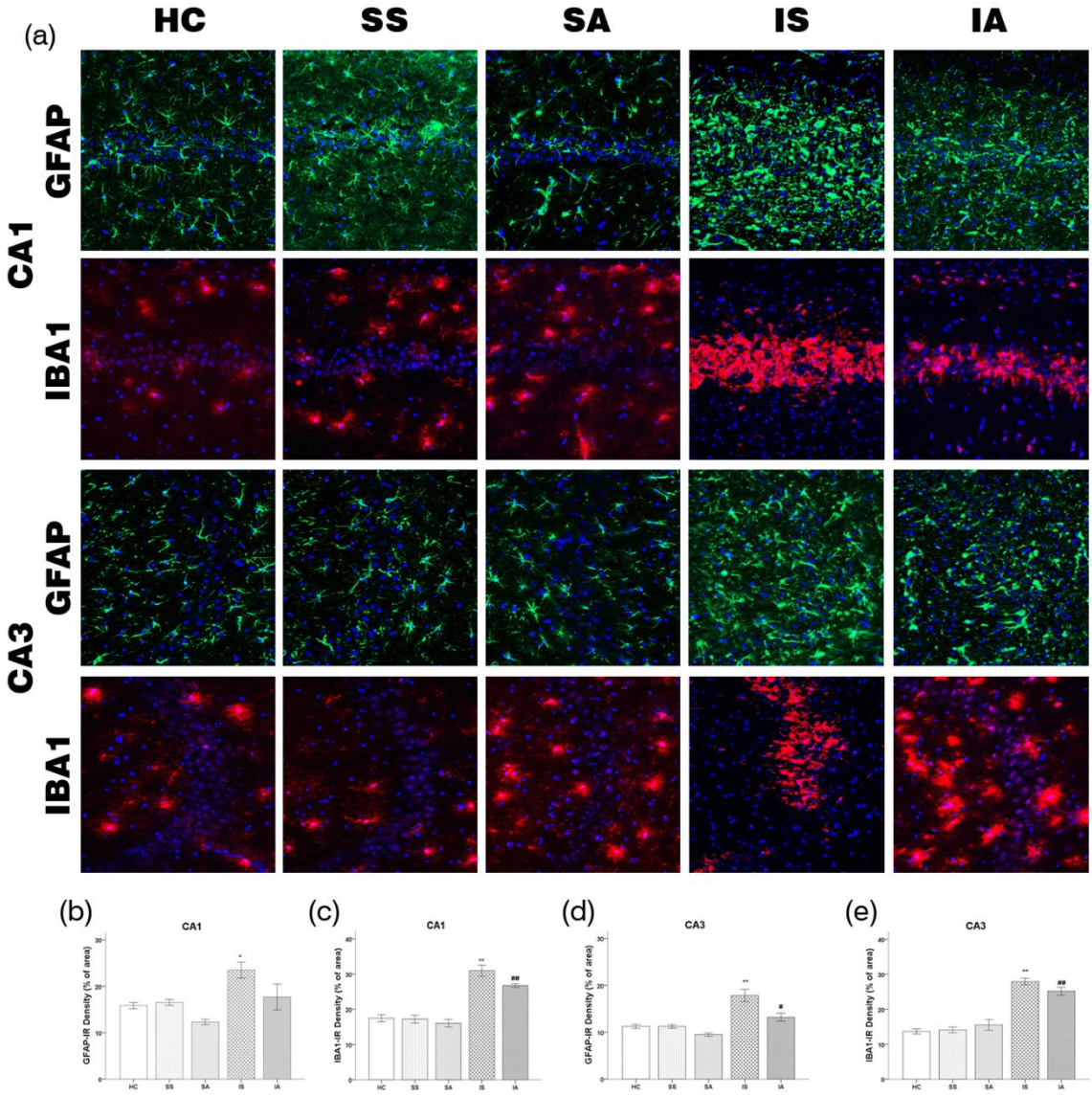

Fig. 7. Global ischemia led to a significant increase in IBA1 and GFAP hippocampal staining, which was partially reduced by Antalarmin treatment. (a) Representative photomicrographs of GFAP (green), IBA1 (red), and Hoescht (blue) immunopositive labeling within the CA1 (top) and CA3 (bottom) subfields of the hippocampus at 200X magnification. Histograms show the mean percent optical densities for GFAP and IBA1 in the CA1 (b-c) and CA3 (d-e), for each of the groups. Data are expressed as mean ± SEM. *$p < 0.05$, #$p < 0.05$, **$p < 0.01$.

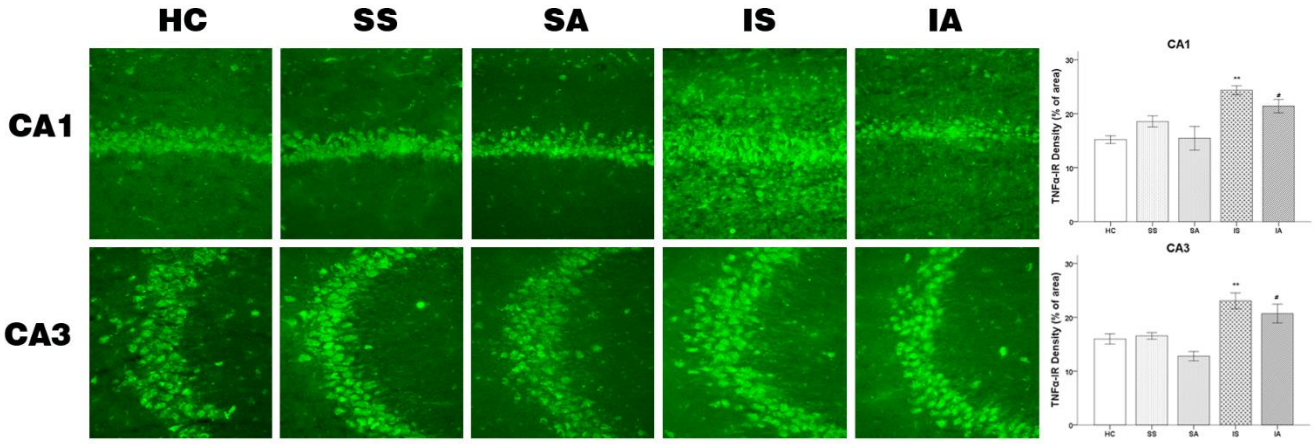

Fig. 8. Global ischemia led to a significant increase in TNF-α hippocampal staining, which was partially reduced by Antalarmin treatment. Representative photomicrographs (left) of TNFα (green) and Hoescht (blue) immunopositive labeling within the CA1 and CA3 subfields of the hippocampus at 200X magnification. Histograms (right) show the mean percent area optical densities in the CA1 and CA3 for each of the groups. Data are expressed as mean ± SEM. *$p < 0.05$, #$p < 0.05$, **$p < 0.01$.

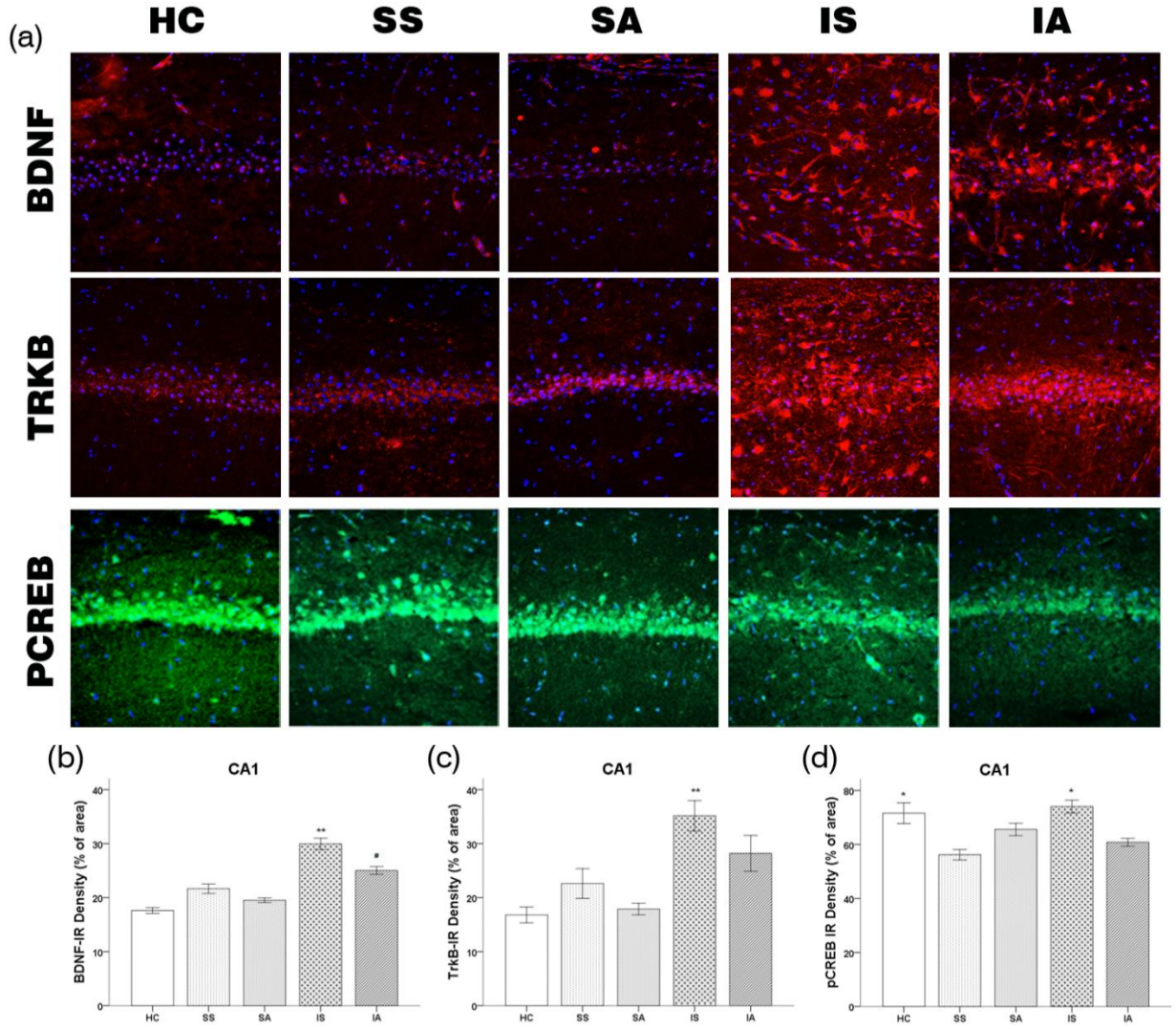

Fig. 9. Global ischemia led to a significant increase in BDNF, TrkB, and pCREB-ir expression in the CA1, which was reduced by Antalarmin treatment. For pCREB, levels were also elevated in the HC rats (a) Representative photomicrographs of BDNF (red), TrkB (red), pCREB (green), and Hoescht (blue) immunopositive labeling within the CA1 subfield of the hippocampus at 200X magnification. Histograms show the mean percent area optical densities for (b) BDNF, (c) TrkB, and (d) pCREB in the CA1 for each of the groups. Data are expressed as mean ± SEM. *p < 0.05, #p < 0.05, **P < 0.01.

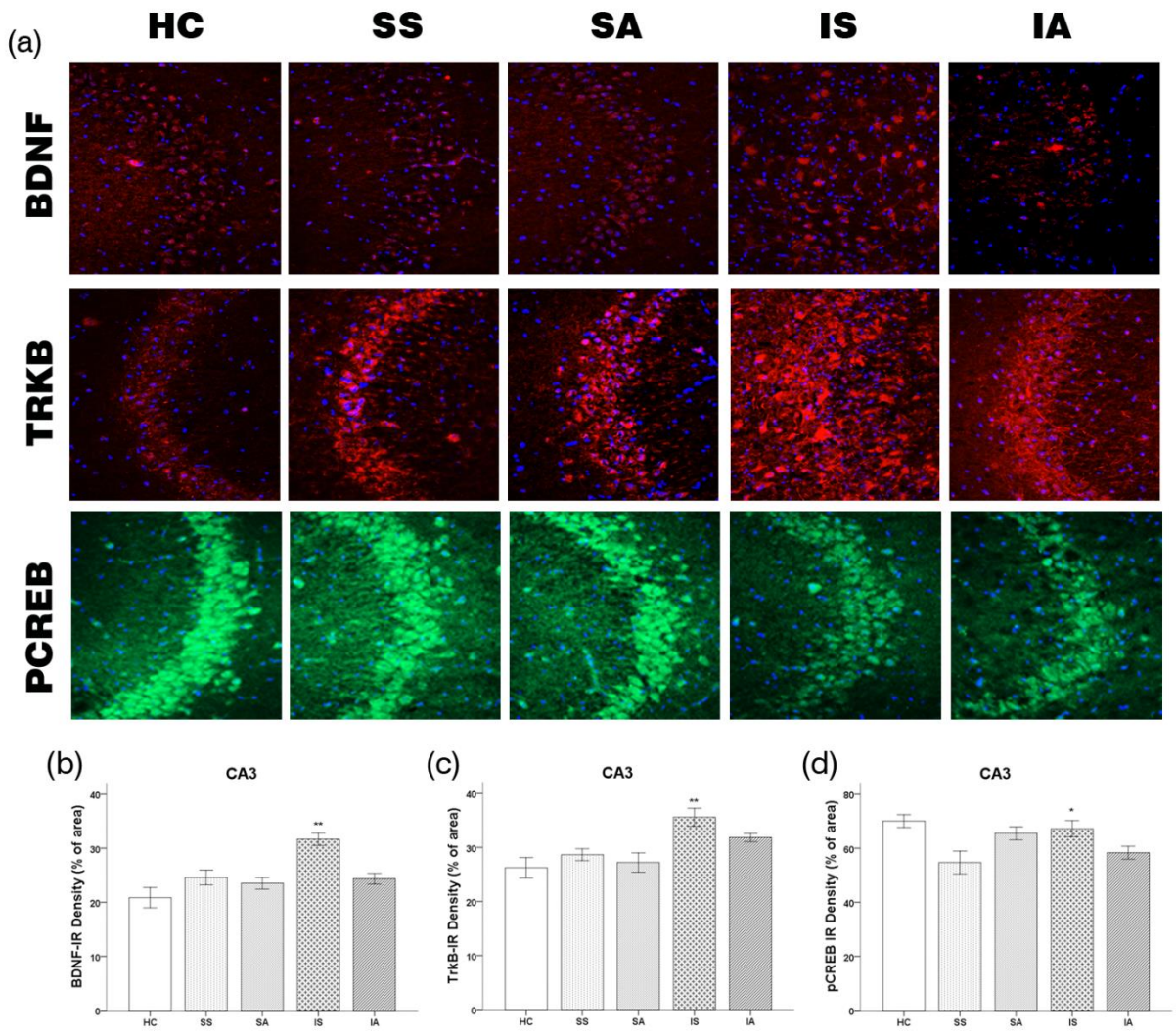

Fig. 10. Global ischemia led to a significant increase in BDNF, TrkB, and pCREB expression in the CA3, which was reduced by Antalarmin treatment. (a) Representative photomicrographs of BDNF (red), TrkB (red), pCREB (green), and Hoescht (blue) immunopositive labeling within the CA3 subfield of the hippocampus at 200X magnification. Histograms show the mean percent area optical densities for (b) BDNF, (c) TrkB, and (d) pCREB in the CA3 for each of the groups. Data are expressed as mean ± SEM. *$p < 0.05$, #$p < 0.05$, **$p < 0.01$.

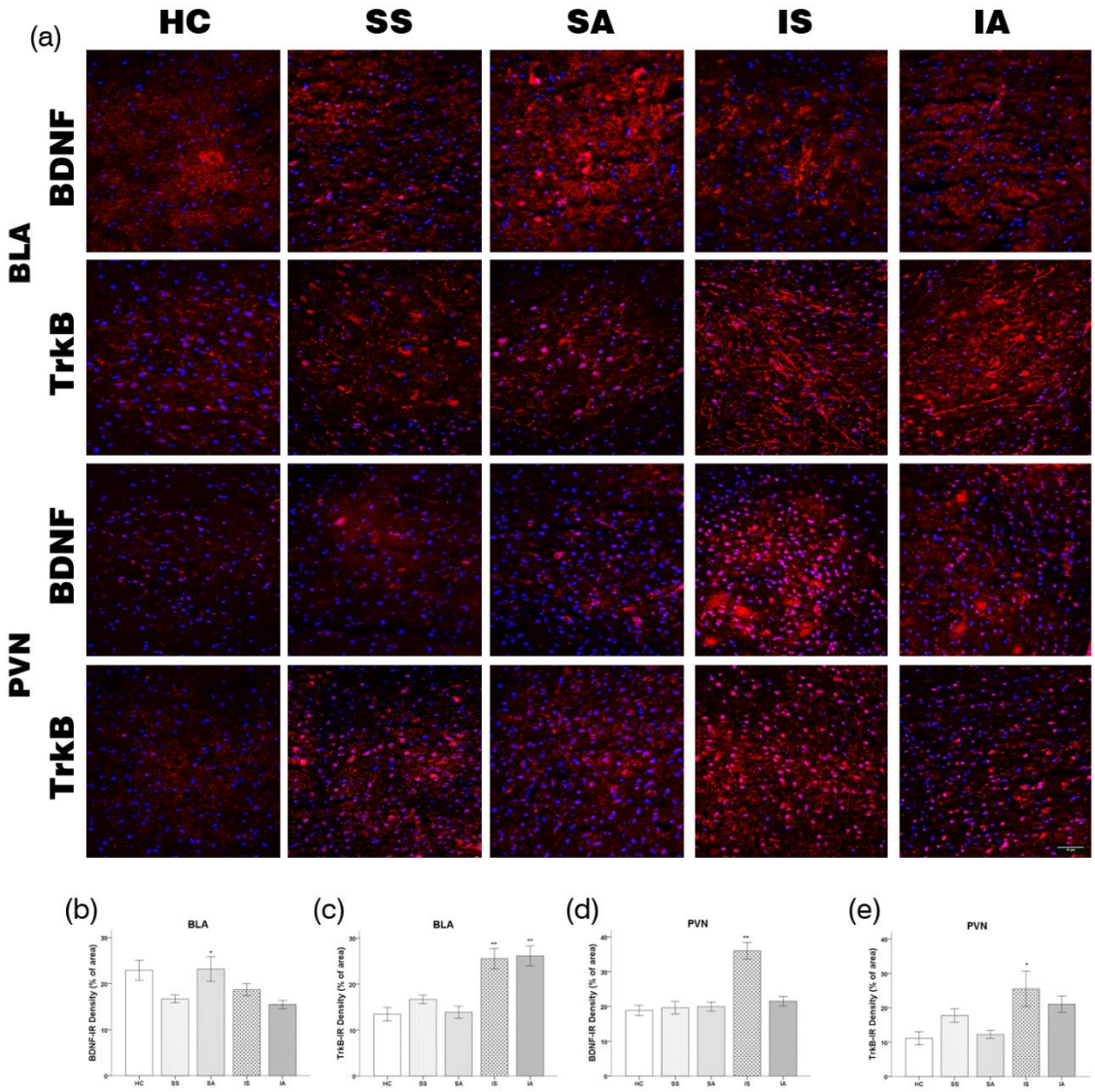

Fig. 11. Global ischemia significantly increased BDNF and TrkB-ir levels in the PVN, which were reduced by Antalarmin treatment. TrkB, but not BDNF, was elevated in BLA of ischemic groups. (a) Representative photomicrographs of BDNF (red), TrkB (red), and Hoescht (blue) immunopositive labeling within the BLA (top) and PVN (bottom) at 200X magnification. Histograms show the mean percent area optical densities for BDNF and TrkB in the BLA (b-c) and in the PVN (d-e), for each of the groups. Data are expressed as mean ± SEM. *$p < 0.05$, #$p < 0.05$, **$p < 0.01$.

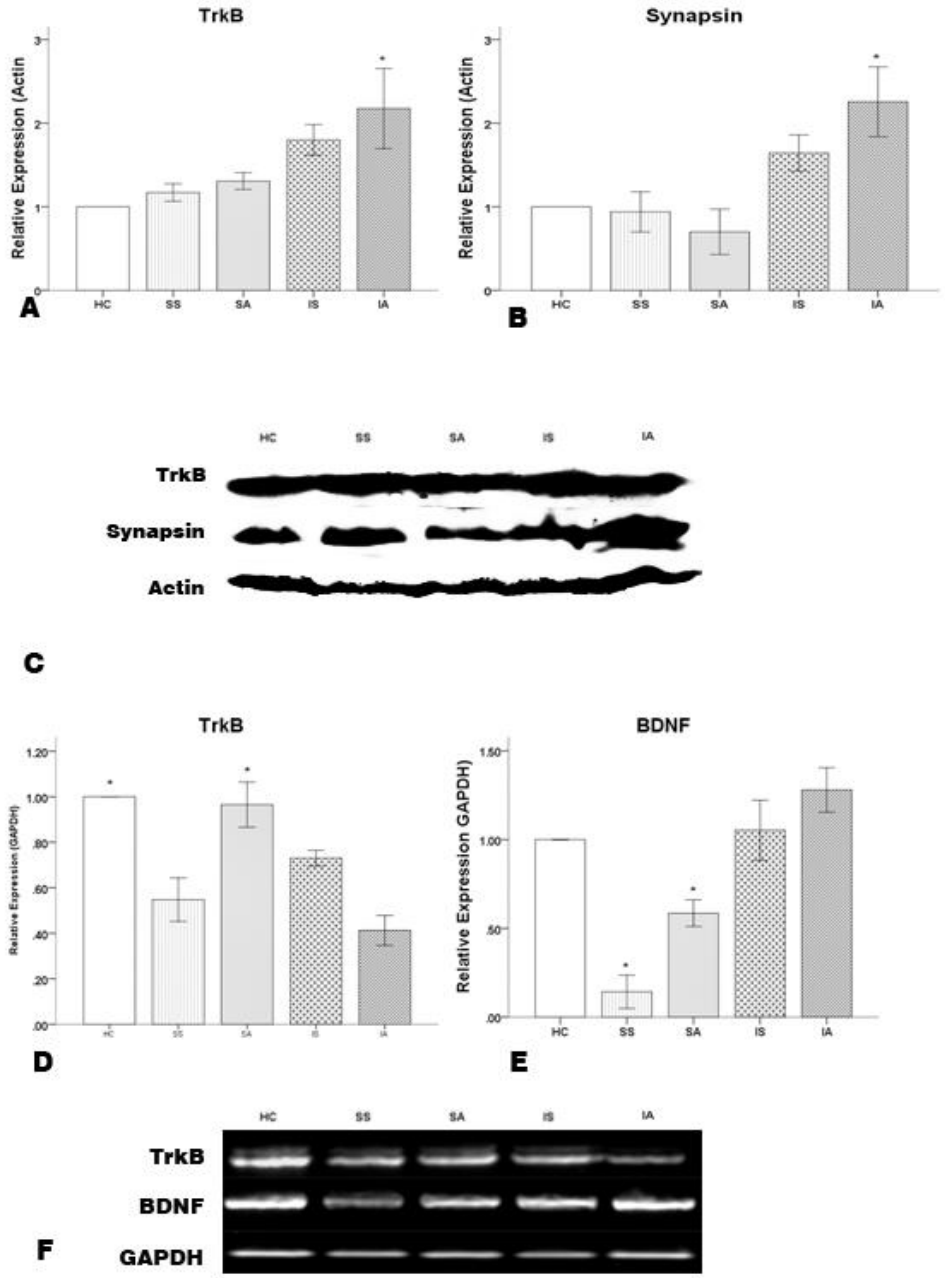

Fig. 12. Protein and mRNA expression in the amygdala 30 days after global cerebral ischemia. Average values for the optical density of immunoreactive bands, normalized relative to β-actin following Western blot for TrkB (A), Synapsin (B). Average values of the optical density of bands normalized relative to GAPDH following RT-PCR for BDNF (D) and TrkB (E). C & F shows representative protein and mRNA bands expressed as a ratio of β-actin or GAPDH, respectively, and normalized to the value of HC controls. Analyses revealed an increase in BDNF mRNA and reduction in TrkB mRNA in ischemic animals, as well as a significant increase in TrkB and synapsin protein levels in ischemic rats (n = 4-5 /group). Values represent means ± SEM, *$p < 0.05$.

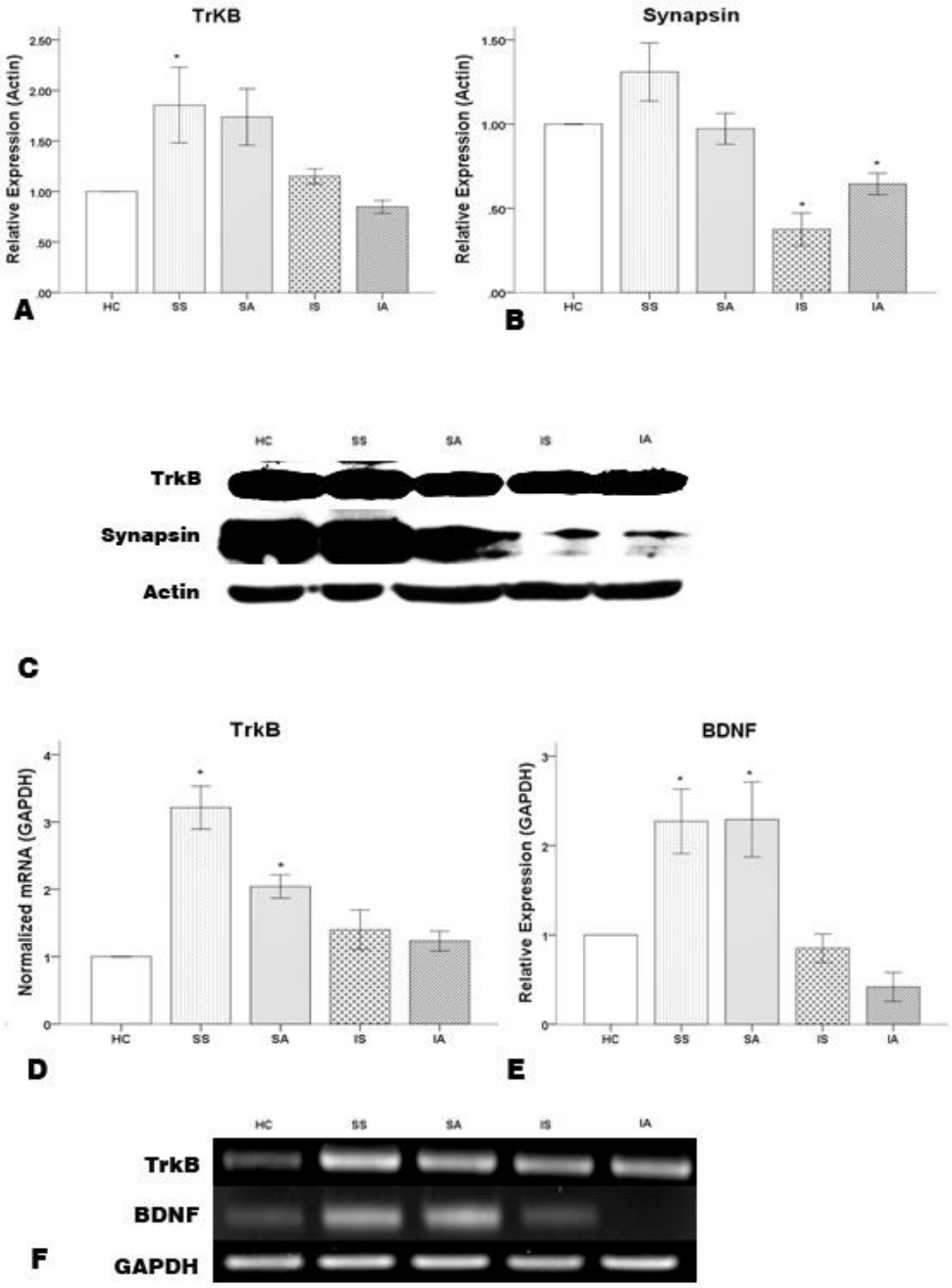

Fig. 13. Protein and mRNA expression in the dorsal hippocampus 30 days after global cerebral ischemia. Average values for the optical density of immunoreactive bands, normalized relative to β-actin following Western blot for TrkB (A), Synapsin (B). Average values of the optical density of bands normalized relative to GAPDH following RT-PCR for BDNF (D) and TrkB (E). C & F shows representative protein and mRNA bands expressed as a ratio of β-actin or GAPDH, respectively, and normalized to the value of HC controls. Analysis indicates reduced BDNF and TrkB mRNA expression in ischemic animals, and a significant reduction in TrkB and synapsin protein levels in ischemic rats (n = 4-5 /group). Values represent means ± SEM, *$p < 0.05$.

General Discussion

Cerebral ischemia initiates a complex sequence of events in the CNS which activates the HPA axis, ultimately culminating in brain damage (Radak et al., 2014). CRH and its related peptides and receptors have been identified over the last three decades and are fundamental molecular initiators of the stress response (Contoreggi, 2015). In response to stress, the brain shows changes in neuronal morphology and chemistry, which modify HPA axis function, and emotional and cognitive behavior (Leuner & Shors, 2013). Given the powerful influence of BDNF on neuronal plasticity, and its widespread distribution throughout the brain, it represents one of the prominent mechanism underlying neuronal and functional changes observed under normal and stressful conditions (Bennett & Lagopoulos, 2014). Recent reports show a relationship between BDNF and CRH in the context of stress, which may play a role in cardiovascular disease (Jung, Kim, Davis, Blair, & Cho, 2011). The focus of the present thesis is the CRHR1 signaling on CNS plasticity and pathology following global cerebral ischemia through a series of experiments addressing three main objectives. **The first thesis** objective assesses long-term changes in the expression of key regulators of the stress system in relation to spatial learning in the radial arm maze following global cerebral ischemia and the role of central CRHR1 blockade, using Antalarmin, on CORT secretion at multiple time intervals following global ischemia and after exposure to an acute stressor (Article 1). **The second objective** characterizes the impact of CRHR1 blockade on social and depressive-like behavior following global ischemia in relation to lasting effects of brain ischemia on expression of BDNF, TrkB and other synaptic plasticity markers in sub-regions of the mesocorticolimbic system using immunohistochemistry, Western blots and RT-PCR techniques (Article 2). **The third objective** aims to investigate possible effects of CRHR1 blockade against cognitive and anxiety-like

behavioral impairments, ischemic brain injury, and neuroinflammation, through changes in BDNF and TrkB expression in the hippocampus and amygdala using immunohistochemistry, Western blot, and RT-PCR techniques, 30 days post reperfusion (Article 3).

Cortisol concentrations are elevated in patients with Parkinson's disease, Alzheimer's disease, diabetes, major depression, and cerebral ischemia (Qiu et al., 2012; Tornhage et al., 2013; Zi & Shuai, 2013). In addition, for the majority of patients with epilepsy, stress exacerbates and/or triggers their seizures, with an elevated cortisol concentration further increased following seizures (van Campen et al., 2015), which can be reduced by Antalarmin treatment (Yuhas, Weizman, Chrousos, Ovadia, & Ashkenazi, 2004). Reducing CORT by adrenalectomy in a diabetic model attenuates learning deficits in the water maze and in a 14-unit T-maze (Stranahan, Arumugam, et al., 2008; Stranahan, Lee, et al., 2008). CRH, a peptide hormone, can influence the stress response, cardiovascular regulation, appetite control, immune function, glucose metabolism, visceral nociception and other physiological responses through its CRHR1 receptor (Bai, Shi, Zhang, Liu, & Yao, 2014; Su et al., 2015). It has been hypothesized that antagonism of CRHR1 may provide an effective pharmacological treatment for stress-related mental disorders (Valdez, 2006). It was thus suggested that modulation of CRHR1 signaling prior to ischemia may be an important factor in neuronal and behavioral function post ischemia. Therefore, CRH receptors were considered as a potential therapeutic target for post-ischemic emotional behavioral disorders. In the current thesis, the expectations were to observe that CRHR1 blockade a) reduces neuroendocrine reactivity in ischemic rats, b) inhibits neuronal injury and neuroinflammation, c) improves cognitive and emotional impairments following cerebral ischemia d) modulates key markers of neuroplasticity, namely BDNF and TrkB in stress-related regions of the mesocorticolimbic circuitry. Findings from the current thesis

demonstrate partial neuroprotection conferred by Antalarmin on ischemia-induced hippocampal damage, which was observed 30 days post ischemia. Possible mechanisms of action of Antalarmin could include downregulation of ischemia-induced CORT concentrations, measured at multiple long-term intervals post-ischemia and following acute stress exposure. Antalarmin also downregulated glial cell reactivity as well as expression of pro-inflammatory cytokine TNF-α in the CA1 and CA3 subfields of the hippocampus. Furthermore, the results of the present study highlight a possible role of Antalarmin in regulating BDNF activity within the most vulnerable region following brain ischemia by attenuating ischemia-induced alterations in neuroplasticity. Lastly, behavioral test results reveal a protective effect of Antalarmin on emotional and cognitive impairments in ischemic rats. Interestingly, ischemic animals showed heightened sociability, which was normalized by Antalarmin treatment, a behavioral trait that could be related to increased impulsive behavior post ischemia. This hypothesis remains to be tested. Together, these findings support a role for CRHR1 activation in the pathophysiology of global cerebral ischemia, and in the regulation of BDNF/TrkB signaling and socio-emotional cognitive impairments following cerebro-vascular accidents (See Table 1 in appendix B for a summary of the results of the present thesis).

1. CRH and Neuroprotection.

The current thesis demonstrates the therapeutic potential of Antalarmin on the injured brain at a delayed 30-day post ischemic interval, conferring ~20% greater neuronal survival than observed in vehicle-treated ischemic animals. This is consistent with neuroprotective effects of CRHR1 antagonists demonstrated at short intervals in various disease models, including epilepsy (O'Toole, Hooper, Wakefield, & Maguire, 2014), hypoglycemia-associated autonomic failure (Flanagan et al., 2003), irritable bowel syndrome (Fukudo, 2007), Alzheimer's (S. N. Campbell

et al., 2015; Carroll et al., 2011; Rissman et al., 2012), and stroke (Stevens et al., 2003). A dual role for CRH in the regulation of ischemic brain injury has been proposed, with the neuropeptide protecting brain tissue via CRHR2-mediated increased penumbral collateral blood flow, while neurotoxicity is reported via CRHR1 activation (De Michele et al., 2005).

Elevated CRH release acts as an excitatory neuromodulator, promoting over excitation of hippocampal neurons, excitotoxicity and delayed loss of CA1 pyramidal neurons (J. Wu, Ma, Ling, & Tang, 2012). Hippocampal cell death is observed following CRH-induced status epilepticus in infant rats, independently of glucocorticoids (Tallie Z. Baram & Hatalski, 1998; Brunson et al., 2001; Ribak & Baram, 1996). Systemic administration of CRHR1 antagonist reduces brain swelling and infarction after MCAO (Le Feuvre, Aisenthal, & Rothwell, 1991; Mackay et al., 2001), and reduced cerebral injury is reported in CRHR1 deficient mice (Stevens et al., 2003). In contrast, neuroprotective effects upon central administration of CRH antagonists have been reported following global and focal ischemia and NMDA-receptor-mediated excitotoxic damage (Lyons et al., 1991; Strijbos et al., 1994). The pro-apoptotic effect of CRH on PC12 cell involves activation of p38 MAPK (mitogen-activated protein kinase) and the Fas/Fas ligand system, which can be inhibited by application of CRHR1 antagonist, Antalarmin (Dermitzaki et al., 2002). In hippocampal slices of mice lacking CRHR1, synaptic potentiation is also attenuated in CA1 neurons due to a shift towards long-term depression (LTD), which is dependent on enhanced GABAergic activity (Schierloh, Deussing, Wurst, Zieglgansberger, & Rammes, 2007).

Furthermore, the CRH system plays a role in processes associated with blood vessel formation and cardiovascular function (Im, 2014). CRH regulates angiogenesis (vascular development) through CRHR2 signaling by increasing nitric oxide and vascular endothelial

growth factor (VEGF) levels (Bale et al., 2002; Bale, Giordano, & Vale, 2003). Inada and colleagues (2009) reported that CRHR1 blockade potentiate TNF-α-induced increased expression of vascular adhesion molecule-1 (VCAM-1) and E-selectin mRNA and protein levels in endothelial cells. CRH administered in the cerebral spinal fluid 20 minutes prior to cerebral ischemia, decreases permeability of the blood brain barrier (Hendryk, Jedrzejowska-Szypulka, Josko, Jarzab, & Dohler, 2002), by acting directly on brain microvessel endothelial cells through a cAMP pathway (Esposito, Basu, Letourneau, Jacobson, & Theoharides, 2003).

Proliferation of new cells has also been demonstrated in various stroke models (Lagace, 2012). Both global (J. Liu, Solway, Messing, & Sharp, 1998; Nakatomi et al., 2002) and focal ischemia (K. Jin, Minami, et al., 2001; Parent, Vexler, Gong, Derugin, & Ferriero, 2002; Zhu, Liu, Sun, & Lu, 2003) trigger compensatory neurogenesis involving migration of neuroblasts from the subgranular zone (SGZ) into the granule cell layer of the DG, to subsequently incorporate into the synaptic circuitry of the adult rodent brain. However, functions of these newborn cells remain to be established considering that the majority has been shown to eventually die. Nonetheless, within the first 2–4 weeks after focal ischemia, newly born, immature neurons have been reported to migrate in chains, with the assistance of reactive astrocytes and blood vessels, from the subventricular zone (SVZ) to incorporate in tissue adjacent to the degenerated striatum and form synapses (Thored et al., 2006; T. Yamashita et al., 2006; R. Zhang et al., 2004). Stroke also induces long-distance migration of newly born neurons from GFAP-expressing progenitor cells in the SVZ into a unique neurovascular niche in which enhanced angiogenesis promotes the survival and migration of new neurons in peri-infarct cortex (Ohab, Fleming, Blesch, & Carmichael, 2006). Activation of astrocytes represents both a protective response aimed at ameliorating and stopping the spread of damage from the primary

injury, as well as a pathological process that initiates secondary injury and profound disruptions to healthy function (Takano et al., 2014). In the current thesis, CRHR1 blockade significantly reduced GFAP expression in the injured CA1 and CA3 areas, which may suggest that Antalarmin conferred neuroprotection in part through downregulating reactive gliosis. This is consistent with studies indicating that exposure to acute or chronic stressors, likely potentiating CRH release, have effects to reduce neurogenesis in the DG at similar time intervals of the ischemic insult (S. Wang et al., 2012; S. H. Wang, Zhang, Guo, Teng, & Chen, 2008).

As alluded, CRH effects are strongly influenced by concentration. Thus, in contrast to deleterious effects, CRH administration also has the ability to inhibit glucocorticoid-mediated suppression of neuronal precursor cells proliferation and apoptosis via suppression of caspase-3 signaling pathways (Koutmani et al., 2013). Low CRH doses administered within 8 h of KCN-mediated ischemic insult significantly reduce cell death of cultured primary cortical neurons (Charron et al., 2009). In a similar manner, in vivo CRH administration within 8 h of a 10 min global cerebral ischemic episode significantly enhances CA1 neuronal survival and improves spatial memory in the radial maze, a phenomenon partly due to CRH activation of kappa- and delta-opioid receptors (Charron, Frechette, et al., 2008). Both delta opioid receptors and CRH are co-expressed in interneurons of the CA1, CA3, and dentate hilus of hippocampal sections, further supporting that CRH is anatomically positioned to act on opioid receptors (Williams & Milner, 2011). CRH-overexpression restricted to the CNS reduces seizure activity and excitotoxicity-induced neuronal cell death and inflammation in the CA1 and CA3 sub-regions of the hippocampus following an acute kainic acid injection in mice, through increased BDNF protein levels (Hanstein et al., 2008). Consistent with these observations, CRH has been shown to exert neuroprotective effects in vitro against various insults such as hypoxia (Fox, Anderson,

& Meyer, 1993), excitotoxicity (Elliott-Hunt et al., 2002), oxidative stress (Lezoualc'h, Engert, Berning, & Behl, 2000) and the toxic amyloid β peptide (Bayatti et al., 2003; Facci et al., 2003; Pedersen et al., 2001). CRH administration prevents neuronal apoptosis by suppressing procaspase-3 pathways and enhances neuronal survival via CRHR1 activation in cultured neurons exposed to DNA-damaging agent camptothecin (M. Radulovic, Hippel, & Spiess, 2003). In vitro, CRH and urocortin have also been shown to improve the viability of primary cortical neurons to glutamate exposure, an effect abolished by prior blockade of CRH receptors (Valadas et al., 2012). Similarly, CRH increases the survival of primary cerebellar granule neurons to apoptotic cell death induced through inhibition of cAMP-dependent PI-3-K secretion, a phenomenon blocked by pre-treatment with the selective CRHR1 antagonist, CP154,526 (Facci et al., 2003). Moreover, CRH activation of microglial cells induces the release of BDNF and nerve regeneration in spinal cord transection injury, through inhibition of oligodendrocyte glycoproteins, which are inhibitory molecules of axon regeneration (Yuan et al., 2010).

Considering these findings and contrasting dose-related effects of CRH, the partial neuroprotection conferred by CRHR1 blockade in the current study may be related to the normalization of the HPA axis as Antalarmin pre-treatment decreased CORT secretion post ischemia to comparable levels of sham groups. Similarly, Antalarmin treatment is sufficient to block the seizure-induced elevations in CORT levels and increase the latency to the first seizure and decrease the cumulative time exhibiting epileptiform activity (O'Toole et al., 2014). Acute brain injury strongly activates the stress response, boosting secretion of glucocorticoids in the bloodstream, which through central binding to GR and MR receptors in neurons, astrocytes, oligodendrocytes, and microglia play a determining role in neuronal survival (Sorrells, Caso, Munhoz, & Sapolsky, 2009). For example, MR and GR immunoreactivity is detected in

astrocytes and microglia shrouding the damaged CA1 region in the days after global cerebral ischemia (Hwang et al., 2006). Immobilization stress or glucocorticoid exposure decrease neurogenesis, but increases hippocampal oligodendrocytes formation as indicated by increased nestin-positive neuronal stem cells in the DG and upregulation of pro-oligodendrogenic genes, and increased nuclear localization of Olig1 protein, an effect that can be prevented by genetic blockade of GR expression (Chetty et al., 2014). Overexpression of glucocorticoids in myeloid and endothelial tissue aggravates cellular inflammation, worsening post ischemic cell death (Sorrells et al., 2013).

In the current thesis, Antalarmin attenuated post-ischemic IBA1, GFAP and TNFα immunoreactivity in the hippocampus. This is concordant with enhanced CRH expression under inflammatory conditions, which plays a direct immunomodulatory role as an endocrine or paracrine mediator in inflammatory sites (Karalis et al., 1991). CRH regulates the inflammatory response to CNS injury (Baigent, 2001; McCoy & Tansey, 2008; Stoll & Jander, 1999), closely linked to post stroke neurodegeneration (Stuller et al., 2012). Pro-inflammatory cytokines such as TNF-α and IL-1β can stimulate the release of CRH and then activate the HPA axis (Miller, Maletic, & Raison, 2009; Song, Zhang, & Manku, 2009). Increases in CRH mRNA in the PVN and CRH release into hypophyseal portal vessels have been observed after central or peripheral administration of IL-1 (Kakucska, Qi, Clark, & Lechan, 1993; R. Sapolsky, Rivier, Yamamoto, Plotsky, & Vale, 1987). Administration of the endotoxin lipopolysaccharide (LPS) reduces BDNF mRNA levels in the hippocampus (Haddad, Saade, & Safieh-Garabedian, 2002; Lapchak, Araujo, & Hefti, 1993) as well as induces depressive-like behavior (increased immobility in the FST, reduced sucrose preference, reduced exploration in the OFT) in females, associated with increased hypothalamic CRH, decreased BDNF, elevated COX-2 and accumulation of cytosolic

GR (Adzic et al., 2015). LPS administration also increases plasma ACTH and corticosterone levels, as well as CRH-labeled cFos neurons in the PVN (Juaneda et al., 1999; Juaneda et al., 2001; Lacroix & Rivest, 1996; Ribot et al., 2003; Rivest et al., 1995; Rivier, 2003; Rorato et al., 2009; Wei, Phillips, & Sternberg, 2002). Similarly, CRH co-labeled with cFos was higher in the PVN in parallel to high plasma ACTH and corticosterone during sepsis in the rat (Carlson, Chiu, Fiedler, & Hoffman, 2007). CRH mRNA is also elevated in the PVN and the CeA during colonic inflammation and 30 days following resolution of the inflammatory response (Greenwood-Van Meerveld, Johnson, Schulkin, & Myers, 2006) and CRH administration stimulates the release of pro-inflammatory cytokines and receptors (IL-6, IL-1, and IL-1R2) at the PVN, with substantial increases in IL-6 and COX-2 in the adrenal glands (Hueston & Deak, 2014; Leu & Singh, 1992). In a similar fashion, our experiments revealed elevated CRH, CRHR1 and GR immunoreactivity in the PVN 30 days post ischemia, while expression was decreased in the hippocampus, where cell death was observed. Our findings and that of others support bidirectional influence of the immune and stress systems, and involvement of both systems in affective disorder onset and progression (Bale, 2009), as well as a role regulating neuronal injury post stroke (Y. Chen et al., 2014).

Currently, hypotheses as to how Antalarmin provides neuroprotection include effects on oxidative stress through scavenging of reactive oxygen species (ROS) (Golab & Skwarlo-Sonta, 2007; Gougoura, Liakos, & Koukoulis, 2010) and decreasing inflammation (Webster et al., 2002; Wlk et al., 2002). ROS intracellular levels increase after incubating endothelial cells with CRH, and blocking CRHR1 with Antalarmin reverses this effect, whereas blocking CRHR2 with astressin-2β has no impact on ROS cell content (Gougoura et al., 2010). Genetic deletion of CRHR1 or Antalarmin administration promote intestinal inflammation, whereas deletion of

CRHR2 activity or astressin administration inhibit peripheral inflammatory response (Im et al., 2010). CRH through CRHR1 and UCN3 through CRHR2 increase mRNA and protein levels of vascular endothelial growth factor (VEGF)-A, boosting the inflammatory responses through angiogenesis in human colonic epithelial cells and primary mouse intestinal epithelial cells, through the cAMP/CREB pathway (Rhee et al., 2015).

In the current thesis (Articles 1 and 3), we report that CRH and BDNF are elevated in the PVN post ischemia, observations which are consistent with increased BDNF and CRH expression in PVN observed following mild (exposure to the Morris water Maze) and more severe stressors such as immobilization stress (Aguilar-Valles et al., 2005; Tapia-Arancibia, Rage, Givalois, & Arancibia, 2004). At the hippocampal CA1 layer, GR and CRHR1 were inhibited post ischemia, while BDNF remained elevated. This finding contrasts effects of stressors normally shown to reduce BDNF levels in the dissected hippocampus (Barbany & Persson, 1992; Schaaf, de Jong, de Kloet, & Vreugdenhil, 1998; M. A. Smith, Makino, Kvetnansky, et al., 1995; Ueyama et al., 1997b), although increased BDNF expression has been reported in the hippocampus and the cerebral cortex at short-term intervals following various brain insults, including kindling-induced seizures and cerebral ischemia (Katoh-Semba et al., 2001; Kokaia et al., 1998; Kokaia et al., 1994; Lindvall, Kokaia, Bengzon, Elmer, & Kokaia, 1994; Tsukahara, Iihara, Hashimoto, Nishijima, & Taniguchi, 1998). Our study is the first to explore longer term intervals post ischemia and our findings support sustained BDNF immunoreactivity 30 days post ischemia, despite extensive neuronal loss. Increased expression was attenuated by Antalarmin administration along with reduced cell damage. This is a finding worthy of note as BDNF injected directly in the brain has conferred neuroprotection in global and focal ischemia rat models (Beck et al., 1994; K. Yamashita, Wiessner, Lindholm, Thoenen,

& Hossmann, 1997). We have attempted co-labeling BDNF immunostaining with NeuN or GFAP to determine a possible source of BDNF expression in the CA1 considering neuronal attrition and the high expression of glial cells. However, findings were not conclusive as BDNF expression was minimally co-expressed with glial or neuronal markers. Microglial cells treated with CRH show increased BDNF levels in a dose-dependent manner and it is interesting to note that BDNF treatment inhibits oligodendrocyte glycoprotein levels, enabling a better axon outgrowth (Yuan et al., 2010). CRH levels increase in spinal tissue within 1–3 days after spinal cord transection injury and BDNF changes in accordance with CRH levels (Welling & Figueiredo, 2010). These findings strengthen the important role and interplay that BDNF and CRH play in the pathophysiological response to cerebral ischemia. They shed light on interrelated roles that these molecules play in regulating outgrowth of neuron axons and nerve regeneration. This knowledge could have fundamental importance in the development of clinical applications targeting neuronal recovery or protection.

2. CRHR1, Memory and Neuroplasticity

The current thesis also highlights a role for CRHR1 signaling as a contributor to cognitive deficits observed post global cerebral ischemia. Antalarmin-treated ischemic animals showed improved spatial learning and aversive learning in the Barnes Maze and Y Maze-passive avoidance task. Stress or emotional arousal are known to activate medial temporal lobe regions including the amygdala and hippocampus, which are modulated through inputs from several portions of the PFC (van Stegeren, 2009). Chronic stress leads to increases in glutamatergic signaling in the amygdala, resulting in enhanced BDNF expression and dendritic outgrowth, while in the hippocampus, a signaling mechanism downstream from the glutamate and upstream of BDNF results in decreased BDNF signaling (Boyle, 2013). In the current thesis (Article 3),

ischemic rats showed increased TrkB immunoreactivity in the BLA, elevated TrkB and synapsin protein levels in the amygdala, with increased BDNF mRNA levels. This increased expression of plasticity markers in the amygdala may underlie alteration in aversive learning in these animals (Chou, Huang, & Hsu, 2014). Yet, increased BDNF expression and hypertrophy of dendritic arborization at the BLA are associated with increased rather than reduced fear behavior following acute and chronic stress (Lakshminarasimhan & Chattarji, 2012). Nevertheless, damage or inactivation of the amygdala impairs fear learning (Rudy, Huff, & Matus-Amat, 2004; Schafe, Nader, Blair, & LeDoux, 2001), suggesting that reduced cells in the BLA may contribute to reduced passive avoidance in ischemic rats. In support with our results, impaired fear conditioning has been observed in gerbils and rats following global cerebral ischemia which has been associated to CA1 cell death and damage to the BLA (de la Tremblaye & Plamondon, 2011a; Henrich-Noack, Krautwald, Reymann, & Wetzel, 2011). Spatial memory impairments are commonly observed following forebrain ischemia, and associated with partial (Auer, Jensen, & Whishaw, 1989) and severe (Onodera, Araki, & Kogure, 1989) damage of hippocampal CA1 neurons, which likely affects negative feedback to the HPA axis system (Weidenfeld, Siegal, & Ovadia, 2013).

Multiple studies have correlated changes in neuronal damage to memory impairments post ischemia (Briones & Therrien, 2000; Gordan et al., 2012; Hodges et al., 1996; Jincai et al., 2014; Kiyota, Miyamoto, & Nagaoka, 1991; Meng, Wang, Liu, Gao, & Du, 2014; J. T. Neumann, Cohan, Dave, Wright, & Perez-Pinzon, 2013), but fewer have gone beyond neuronal damage to determine neurochemical mechanisms underlying ischemia-related impairments (Bowden, Woodbury, & Duncan, 2013). Among mechanisms proposed, post-ischemic memory function in the Morris water Maze has been shown to be improved by administration of

minocycline, a tetracycline derivative, which increases levels of CREB, pCREB, and BDNF in the hippocampus (Y. Zhao, Xiao, He, & Cai, 2015), which could be relevant for CRH action. In the current thesis, Antalarmin treatment attenuated ischemia-induced heightened BDNF immunoreactivity observed in the injured CA1 and CA3, but failed to affect decreased BDNF and TrkB protein and mRNA expression in the dissected dorsal portion of the hippocampus, indicating local action of CRHR1 blockade on ischemia-induced neuroplasticity in the hippocampus.

As briefly mentioned earlier and pertinent to CRH action on memory function, CRH is expressed in interneurons of the hippocampus, and released into the synaptic space to activate CRHR1 located on pyramidal cell-dendrites (Y. Chen, Hatalski, Brunson, & Baram, 2001), indicating that CRH is well positioned to influence changes in excitability of hippocampal neurons. CRH produces a long-lasting enhancement of synaptic efficacy in the rat hippocampus in vivo (H. L. Wang, Tsai, & Lee, 2000), and acts via CRHR1 to induce LTP or potentiate the magnitude of LTP (Blank et al., 2002; Schierloh et al., 2007). What seems crucial in determining its role are the bioavailable concentrations. Low-level activation of CRHR1 for short durations may enhance hippocampal function, whereas longer exposure to high levels of CRH may impair function (Adlard, Engesser-Cesar, & Cotman, 2011; Maras & Baram, 2012). Acute application of CORT and CRH augment cholinergic gamma oscillations in the CA3 region of ventral hippocampus slices via GR or CRHR1, respectively, which is important for formation of memory (Caliskan et al., 2015). CRH injection into the dorsal hippocampus of mice before training enhances context- and tone-dependent fear conditioning through CRHR1 (J. Radulovic, Ruhmann, Liepold, & Spiess, 1999b). When injected directly into the dentate gyrus, CRH improves the retention of an inhibitory avoidance task in rats (E. H. Lee et al., 1992; E. H. Lee et

al., 1993), while antisense oligodeoxynucleotides directed against CRH mRNA infused into the hippocampus impair passive avoidance retention (H. C. Wu, Chen, Lee, & Lee, 1997). CRH application facilitates (primes) LTP of population spikes in mouse hippocampal slices and acute stress (1 h of immobilization) enhances context-dependent fear conditioning which could be prevented by a non-selective CRH receptor antagonist (Blank et al., 2002). CRH receptor antagonist [9–41]-α-helical CRH administered into the BLA of male rats immediately after avoidance training produces a dose-dependent impairment of a 48-h retention performance (Roozendaal et al., 2002). Antalarmin (20 mg/kg·2 ml ip) impairs induction and expression of conditioned fear behavior (Deak et al., 1999). Using selective receptor antagonists, CRHR1 and CRHR2 show opposite effects, the former enhancing learning in a fear conditioning task, while stimulation of the latter impairs performance (J. Radulovic et al., 1999b). These studies highlight beneficial effect of CRH on memory consolidation at low doses.

Importantly, sustained delivery of CRH or of a CRHR1 agonist in hippocampal slices reduces the amplitude of hippocampal population spikes, preventing LTP onset (Rebaudo, Melani, Balestrino, & Izvarina, 2001). Moreover, excessive activation of CRHR1 negatively affects dendritic arborization, and is involved in CRH-induced neuron apoptosis, effects which are antagonized by prior CRHR1 blockade improving memory performance (Y. Chen et al., 2010; Ivy et al., 2010; Maras & Baram, 2012; Y. Zhang et al., 2012). CRH-overexpressing mice display reduced acoustic startle reactivity, impairments of prepulse inhibition, and deficits in spatial learning in the Morris water maze and in a forced alternation water T Maze task (Dirks et al., 2002; Heinrichs et al., 1996). These studies support the theory that the hypersensitivity of the HPA axis as evidenced by elevated CORT levels following acute restraint stress and high CRH

expression in the PVN may contribute to the impaired memory performance in ischemic rats of the current thesis, especially considering that CRHR1 reversed these memory deficits.

CRHR1 knockout studies further support a crucial role for this receptor in memory, with CRHR1 deletion leading to spatial learning deficits in a Y Maze test and anxiolytic behavior in the EPM and the black and white box tests (Contarino, Dellu, et al., 1999). The rs110402 and rs242924 polymorphisms in the CRHR1 gene are associated to decreased performance in working memory task for subjects with early-life stress exposure (Fuge et al., 2014), and have been identified as genetically driven subtypes of early working memory impairments in aged subjects due to alterations in hippocampal CRHR1 activation (Grimm et al., 2015). Central CRH administration impairs percentage correct responses in a two-choice spatial learning water maze discrimination task (Steckler & Holsboer, 2001), which can be reversed by CRHR1 antagonism (Zorrilla, Schulteis, et al., 2002). CRHR1 antagonist, SSR125543, normalizes corticosterone levels and attenuates cognitive deficits in the object recognition task, following an acute inescapable foot-shock stress (Philbert, Pichat, Palme, Belzung, & Griebel, 2012; Urani, Philbert, Cohen, & Griebel, 2011). Chronic administration of CP-154, 526 greatly attenuated defensive withdrawal behavior and decreased CRH mRNA in the PVN and made serum corticosterone concentration return to baseline level more quickly after airpuff startle (Arborelius et al., 2000). Chronic R121919 treatment is effective in attenuating CRH mRNA expression in the PVN following chronic social defeat stress in DBA/2 mice (Erhardt et al., 2009), and reverses reduced thermal nociception and hyperarousal in the acoustic startle response test following predator odor stress (Roltsch et al., 2014). Furthermore, repeated administration of Antalarmin ameliorates postnatal stress-induced dendritic outgrowth and spine formation abnormalities in CA3 pyramidal neurons and prevents reduced hippocampal expression of synapse-related proteins,

i.e., synaptophysin, PSD-95, nectin-1, and nectin-3, as well as attenuates postnatal stress-induced endocrine alterations (Liao et al., 2014). These studies further support of CRHR1 blockade in improving memory performance following global cerebral ischemia. In addition, our findings show reduced BDNF mRNA and protein levels in the mPFC of ischemic rats, which was attenuated by antalarmin treatment. This is interesting considering that CRH and CRHR1 are expressed in neurons of the mPFC (Kuhne et al., 2012), and CRHR1 blockade by systemic Antalarmin administration (20 μg/g of body weight) during early postnatal stress exposure ameliorates the development and plasticity of apical dendrites of layer V pyramidal neurons in the dorsal granular cingulate cortex and restores cognitive function in a temporal order object recognition test and in the Y Maze spontaneous alternation task (X. D. Yang et al., 2015). Therefore, the effects of Antalarmin in improving behavioral impairments of ischemic rats may be related to its role in the mPFC, especially considering that CRHR1 blockade partially reversed the ischemia-induced downregulation of BDNF and TrkB mRNA and protein expression in this region.

3. Heightened HPA Axis Activation and Memory Impairment

In the current thesis, Antalarmin reduced CORT concentrations and improved learning and memory performance in ischemic rats. It is possible that the blocking of the HPA axis by Antalarmin reduced cell death. This is plausible considering the association demonstrated between increased glucocorticoids and ischemia-induced CA1 injury (Antonawich, Miller, Rigsby, & Davis, 1999), consistent with dendritic atrophy and neuron loss induced by chronic corticosterone treatment (R. M. Sapolsky, Krey, & McEwen, 1985; Woolley, Gould, & Mcewen, 1990b). The hippocampus shows the highest density of corticosterone receptors in the brain (B. S. McEwen & Sapolsky, 1995), and excessive GR activation increases hippocampal neuronal

vulnerability to excitotoxicity, oxidative stress and ischemia (Goodman, Bruce, Cheng, & Mattson, 1996). In the presence of glucocorticoids, both in vitro and in vivo, hippocampal neurons become more vulnerable to ischemia insults (Morse & Davis, 1990; R. M. Sapolsky & Pulsinelli, 1985). Administration of the synthetic glucocorticoid dexamethasone worsens apoptosis and CA1 neuronal damage after ischemia (Charles et al., 2012), while adrenalectomy or infusion of the glucocorticoid synthesis inhibitor Metyrapone diminishes post ischemic hippocampal cell death (Morse & Davis, 1990; Smith-Swintosky et al., 1996).

Although these results support deleterious effects of glucocorticoids on post ischemic neurological outcomes and associated functional recovery (Caso et al., 2007; DeVries et al., 2001), timing is an important variable when examining the impact of HPA axis activation on sensitization of hippocampal cells to ischemic injury. On one hand, chronic stress treatment prior to stroke aggravates spatial memory impairments (Faraji, Ejaredar, Metz, & Sutherland, 2011; McDonald, Craig, & Hong, 2008). For example, rats exposed to seven days of variable stress prior to cerebral ischemia show increased corticosterone levels and increased hippocampal cell death associated with memory impairments in the Morris water Maze (McDonald et al., 2008). Repeated exposure to social stress or chronic treatment with exogenous corticosterone before induction of MCAO also leads to increased infarct size and cognitive impairments (Sugo et al., 2002). On the other hand, exposure to mild stressors or low corticosterone levels has been shown to reduce hippocampal infarct volume and improve recovery of spatial memory function following focal ischemia via corticosteroids anti-inflammatory effects (Faraji, Lehmann, Metz, & Sutherland, 2009). Of note, chronic post-treatment with dexamethasone reduces ischemic damage, while pre-treatment aggravates seizures and brain damage following ischemia (Koide, Wieloch, & Siesjo, 1986). Furthermore, glucocorticoid administration (40 mg/kg; subcutaneous

injection once per day for 3 days) at the termination of hypoxia-ischemia injury in rat pups promotes the development of dendrites and reduces cell death in the injured hippocampus although fails to alter spatial memory deficits (Y. D. Zhao et al., 2013). Corticosterone treatment over a 24 h period prior to hypoxia/ischemia similarly leads to reduced brain injury (Krugers, Knollema, Kemper, Ter Horst, & Korf, 1995). Even 21 days of chronic restraint-stress (5 h a day) prior to 5 min global cerebral ischemia increases corticosterone levels, and decreases cell death and microglia activation in the CA1 of the hippocampus through increased antioxidant levels (Yoo, Lee, Choi, et al., 2011; Yoo, Lee, Park, et al., 2011). Increased plasma concentrations of oxytocin and corticosterone through pre-ischemic forced swim exposure decreased neuronal injury (Moghimian, Faghihi, Karimian, & Imani, 2012). In contrast, acute stress exposure using the forced swim paradigm reduces doublecortin (DCX)-expressing cells and increases cell death in the retrosplenial cortex following 10 min BCCAO in rats (Kutsuna et al., 2013). In addition, left MCAO followed by chronic mild stress (18 days) increases cell death in the hippocampus by making the cells more vulnerable to apoptosis (S. Wang et al., 2012). These studies support time-dependent effects of glucocorticoids. At present, the number of studies reporting adverse effects of elevated post ischemic glucocorticoid levels outweighs reports supporting positive effects. However, these studies point to dysregulation of the HPA axis being a contributing factor to hippocampal injury and spatial memory impairment following cerebral ischemia.

4. Altered Mood and Emotional Responses in Animal Models of CVD

In Article 2, ischemic rats showed an increased immobility time in the FST, reduced sucrose preference, and reduced food intake and body weight gain. However, ischemic rats showed heightened sociability and preference for social novelty accompanied by reduced latency

to feed in the NSFT. In animal models, studies have reported increased immobility in the FST following forebrain ischemia, indicative of depressive-like behavior (Gaur & Kumar, 2010; Kato et al., 1997). Reduced preference for sucrose, body weight gain, and locomotor and exploratory activity are indicative of anhedonia, altered motivation, anxiety and/or increased stress in animal models of stroke (S. H. Wang et al., 2008; S. H. Wang et al., 2009), although mechanisms associated with these changes have only recently gained attention. Noteworthy, forebrain ischemic animals have shown reduced anxiety in the light/dark box test in spite of observed depressive-like behavior (B. Yan et al., 2007), which resembles results for article 2, where depressive-like behaviors were observed, despite reduced anxiety-like behavior in the three chamber social approach test and in the novelty suppressed feeding test. Conversely, mice subjected to focal ischemia via MCAO show despair in the FST and anhedonia in the SPT, but increased latency to feed in the novelty-suppressed feeding paradigm, suggesting heightened anxiety. Thus, global ischemia leads to a different emotional phenotype than rendered by focal ischemia.

These emotional impairments are reversed by antidepressant treatment with citalopram, and associated with reduced degeneration of midbrain TH-immunoreactive neurons (Kronenberg et al., 2012). Antidepressant administration similarly prevents depletion of norepinephrine and dopamine in the hippocampus and striatum and reduced immobility in the tail suspension and forced swim tests following forebrain ischemia (B. Yan et al., 2004). Reduced TH expression in the NAc and VTA has been reported 9 days following global ischemia (Dunbar et al., 2015). Interestingly, in Article 2, increased TH expression in the NAc and VTA was observed 30 days post ischemia, indicating possible time-related effects. In the context of animal models of stress-induced depressive-like behaviors, there is a potent activation of VTA dopamine neurons, which

stimulate dopaminergic transmission to its limbic targets including the NAc (Nestler & Carlezon, 2006). For example, high levels of immobility in the FST are associated with strong mesocortical dopamine activation in genetically susceptible individuals, and can be reduced and reversed, by bilateral mesocortical dopamine depletion (Scott, Cierpial, Kilts, & Weiss, 1996; Ventura, Cabib, & Puglisi-Allegra, 2002). Although the dorsolateral aspect of the striatum receives projections from the sensorimotor cortex, the ventromedial regions, including the NAc, receive afferents from the prefrontal cortex as well as limbic structures like the amygdala and hippocampus (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). The NAc thus exists at a "limbic-motor interface" and integrates memory with affectively valenced input from the amygdala and contextual information from the hippocampus (Mogenson, Jones, & Yim, 1980). It may then coordinate appropriate goal-directed behavioral responses by recruiting elements of the basal ganglia that are critical for motor output (Pennartz et al., 2009; Redgrave, Vautrelle, & Reynolds, 2011). DA transmission can increase in response to both rewarding and aversive stimuli (Brischoux, Chakraborty, Brierley, & Ungless, 2009; Carlezon & Thomas, 2009; Schultz, 2007). Notably, antibodies against BDNF or TrkB infused into the NAc attenuates dopamine release and behavioral abnormalities after methamphetamine administration underlining interactions of these signaling factors in regulating emotional outcome (Narita, Aoki, Takagi, Yajima, & Suzuki, 2003).

In article 2, we demonstrate increased BDNF mRNA and immunoreactivity in the NAc 30 days post ischemia, which Antalarmin successfully inhibited. Increased BDNF signaling in the ventral striatum and reduced BDNF expression in the hippocampus have been closely linked to depressive-like behaviors in humans and rodents exposed to chronic stress (Berton et al., 2006; Nestler & Carlezon, 2006). Emerging pieces of evidence suggest that alterations of BDNF

expression play an important role in depression (Dwivedi, 2009). The neurotrophic hypothesis of depression postulates that neurotrophin deficiency contributes to the pathology (atrophy) of the hippocampus and supplementing these deficits by antidepressant drugs reverses the symptoms of the disease (R. S. Duman, 2004, 2005; R. S. Duman & Monteggia, 2006). Postmortem studies in depressed patients show reduced expression of BDNF mRNA and protein levels in the hippocampus and PFC as well as in the serum and plasma (Dwivedi et al., 2003; Karege et al., 2002; Shimizu et al., 2003). In contrast, an increase in BDNF mRNA expression in the NAc (part of the ventral striatum) has been reported in depressed patients and in animal models of depression (Krishnan et al., 2007; Nestler, 2015). This pattern of BDNF expression in depressed patients resembles the site specific changes in BDNF expression observed in the current thesis, with ischemic animals showing reduced levels of BDNF protein and mRNA in the dorsal hippocampus and PFC, and an increase in the NAc, that could be attenuated by Antalarmin treatment. The action of Antalarmin treatment to site-specifically affect changes in brain plasticity post ischemia along with attenuation of delayed cognitive and emotional impairments may prove to be beneficial for post-stroke depressed patients.

This bi-directional CRH-BDNF interaction within discrete neuronal circuits and distinct changes associated with post ischemic recovery is intriguing and suggests that plasticity might be as important as neuronal protection. For instance, reduced BDNF expression in the hippocampus and elevated corticosterone levels have been associated with impaired functional recovery following cerebral ischemia (Ke et al., 2011), while centrally infused BDNF reduces ischemia-induced depressive-like behavior (M. K. Sun & Alkon, 2013). Consistently, reductions in hippocampal BDNF protein expression increases depressive-like behaviors 3 weeks following MCAO in both young and old rats, with increased brain edema, blood brain barrier disruption

and infarct volumes in the older rats (Boyko et al., 2013) . Interestingly, intravenous injection of neural progenitor cells restoring the BDNF-ERK-CREB signaling pathway in microsphere-embolized rats reduced depressive-like behaviors in the forced swim and sucrose preference tests without affecting neuronal damage (Moriyama, Takagi, & Tanonaka, 2011). In an animal model of Parkinson's disease, rats show increased immobility time in the FST indicating a depressive-like phenotype, and lower BDNF and TrkB mRNA levels in the CA1, CA3 and DG subfield of the hippocampus, and BLA, although no change was noted in the most affected dopamine regions including the caudate-putamen, substantia nigra, NAc (shell and core) and VTA (Berghauzen-Maciejewska et al., 2014; Berghauzen-Maciejewska et al., 2015; Kuter et al., 2011). In the current thesis, ischemic rats had reduced mRNA and protein levels of BDNF/TrkB among other plasticity markers in the hippocampus and the PFC, which corroborate decreased expression of these markers generally observed in ischemic models. Antalarmin treatment has minimal or no effect on these levels. However, CRHR1 blockade attenuated depressive-like behaviors together with region-specific changes in BDNF and TrkB immunoreactivity in the mesocorticolimbic circuitry, supporting a local action of CRHR1 activation on post ischemia regulation of these plasticity markers.

5. Stress, CRH System and Post Ischemic Depression

Stress aggravates depressive-like phenotype in ischemic animals. Findings from the current thesis provide the first demonstration of Antalarmin’s action to reduce ischemia-induced CORT secretion 30 days post ischemia as well as the impact of ischemia on various internal organs (thymus, seminal and adrenal glands). These findings further support a role for neuroendocrine dysregulation as a major contributor to emotional and cognitive impairments observed in survivors of cardiac arrest. Increasing evidence has indicated that the hyperactivity

of HPA axis is one of the main biochemical changes in major depression (S. J. Yang et al., 2014). Findings from animal models support that elevated CRH levels in extra hypothalamic regions contribute to dysregulation of glucocorticoid secretion, reduced motivation and elevated anxiety (Dore et al., 2013; S. K. Wood, McFadden, Grigoriadis, Bhatnagar, & Valentino, 2012). For instance, neonatal maternal separation leads to elevated CRH protein and mRNA levels in the PVN, CeA, BNST, and locus coeruleus in adulthood and increased GR at the hippocampus, while CRHR1 receptors are decreased in the CA1 and CeA, and CORT secretion and immobility in the FST increased (Diamantopoulou, Raftogianni, Stamatakis, Oitzl, & Stylianopoulou, 2013; Plotsky et al., 2005). Moreover, the therapeutic effects of six weeks of abscisic acid (ABA) (a derivative of carotenoids found in plants) administration in rats exposed to chronic unpredictable mild stress, as indicated by increased sucrose intake and swimming in the forced swim test, are characterized by lowered serum CORT concentrations, and reduced hypothalamic CRH and retinoic acid receptor alpha (RARα, member of the steroid/thyroid receptor family) mRNA expression (Qi et al., 2014). Notably, exposure to unpredictable mild stress reduces sucrose preference and increases immobility time in the forced swim test in focal ischemic rats, an effect that could be reversed by daily fluoxetine treatment (Y.-j. Guo, Zhang, Wang, Sui, & Sun, 2009; S. Wang et al., 2012). Reduced sucrose preference is associated with increased CRH expression in the hypothalamus in ischemic rats, which could be prevented by treatment with the selective serotonin reuptake inhibitor citalopram (S. S. Wang, Wang, Chen, Wu, & Xie, 2013). Increased levels of pro-inflammatory cytokines, such as TNF-α and IL-1β, have been found in both the periphery and brain of depressed patients (Maes et al., 2009). Olfactory bulbectomized rats exhibiting hyperactivity in open field, increased immobility time in TST and FST also show increased levels of serum corticosterone, CRH mRNA in the hypothalamus, and TNF-α and IL-

1β levels in hippocampus, and reduced GR and BDNF protein levels in the hippocampus (S. J. Yang et al., 2014). Herein, both stress and neuroinflammatory markers were increased in ischemic rats displaying depressive-like behaviors, suggesting that these systems are implicated in the pathophysiology of post stroke depression.

The role of CRH in mediating socially-induced stress responses following brain injury also appears an interesting avenue to investigate. Indeed, social isolation is a predictor of mortality within 1-year of acute myocardial infarction as strong as some of the classic physiological risk factors, including high blood cholesterol concentrations and hypertension (Mookadam & Arthur, 2004). Mice socially isolated 2 weeks prior to inducing an 8 min cardiac arrest show increased neuronal degeneration and microglial activation throughout the hippocampus, and increased mRNA expression of proinflammatory cytokine TNF-α and serum corticosterone concentrations 24 h after ischemia as compared to sham mice housed five per cage (Weil et al., 2008). The protective effect of social housing on infarct size, motor recovery, and neuroinflammation (reducing nuclear factor- kappa-B and IL-6 activation) is observed in focal ischemic male and female mice housed with an ovariectomized females (Craft et al., 2005; Karelina, Norman, Zhang, Morris, et al., 2009; Venna et al., 2012). Physical interaction for a minimum 12-day period prior to an ischemic event, in the absence of pre-ischemic or post-ischemic contact also promotes neuronal protection (Karelina, Norman, Zhang, & DeVries, 2009), an effect that can be mimicked by chronic central administration of the nonapeptide oxytocin, which is released during physical contact (Karelina et al., 2011). Noteworthy, central oxytocin administration inhibits stress-induced CRH mRNA expression via activation of GABAA receptors in the PVN (Bulbul et al., 2011). Post-stroke pair housing also increases BDNF levels and reduce immobility in the forced swim test (O'Keefe et al., 2014). Experiments

assessing the effects of focal ischemia, traumatic brain injury (TBI), and diffuse microembolic (ME) infarcts in rodents show impaired social interaction (Nemeth et al., 2012; D. K. Pandey et al., 2009; Verma et al., 2014). Post-stroke mice paired with either a sham or stroke partner show restored sucrose consumption and immobility in the tail suspension test. They also display increased sociability than isolated littermates that progressively become socially avoidant over 4 weeks post-stroke effects being observed from day 7. Reduced sociability thus appears as an additional effect of isolation and stroke shown in part mediated by neuronal inflammation and microglial activation (Verma et al., 2014). These findings support that ischemia-induced effects on stress responses and neuroinflammatory signaling contribute to emotional deficits and impaired social interaction with unfamiliar conspecifics.

These findings appears incongruent with socio-behavioral observations in the current thesis. Indeed, we observed enhanced social preference in a model of global cerebral ischemia, which could be reversed by Antalarmin treatment. In sham rats, CRHR1 blockade also had an impact, this time increasing social encounters. Previous studies have shown that Antalarmin treatment reduces the anxiogenic phenotype of isolated-reared rats, while also reversing the increased D2 receptor density in the CeA and Nac, without affecting BDNF and TrkB mRNA expression (Djouma, Card, Lodge, & Lawrence, 2006; Lodge & Lawrence, 2003). Noteworthy, chronic infusion of the CRHR1 antagonist Antalarmin has been shown to significantly attenuate cortisol secretion, arousal, vocalization and agitation following separation in long-term marmoset pairmates and increases sexual activity upon reunion (French et al., 2007). Considering that our ischemic rats' behavioral response is difficult to reconcile with these observations and that increased sociability does not either fit well with depressive phenotypes, we are contemplating that ischemic rats may be more impulsive in their actions and not gaging the risks in the same

manner. This could be partly mediated by impaired functioning of the PFC, which our findings tend to support. Studies using serial choice task, which are thought to more directly assess PFC function and impulsivity would provide important insights concerning this proposition.

6. CRHR1 and Depression

Clinical studies, supported by pharmacologic and genetic animal models, have demonstrated a prominent role of CRH in depressive and anxiety disorders (Groenink et al., 2003; Holsboer & Ising, 2008; Lloyd & Nemeroff, 2011), and a well acknowledged contribution in mediation of the valence, intensity, and learned flexibility of affective states (Heinrichs & Koob, 2004). Patients suffering from major (or melancholic) depression typically show hypercortisolism and a reduced HPA inhibitory feedback, although atypical depression is characterized by reduced HPA activity (Gold & Chrousos, 2002; Lamers et al., 2013). An atypical depression model through selective inbreeding of outbred CD-1 mice based on their anxiety-related behavior and passive/active coping style also showed that following an acute stressful stimulus, high anxiety responding mice have reduced corticosterone secretion and a blunted response in the Dex/CRH test, with higher expression of GR and decreased CRHR1 expression in the pituitary, compared to normal and low anxiety mice. These effects could be reversed by chronic CORT administration (Sotnikov et al., 2014). Notably, increased stimulation of the CRHR1 pathway characterizes posttraumatic stress disorder despite reduced plasma cortisol levels (Gold & Chrousos, 2002; Kasckow, Baker, & Geracioti, 2001; Kehne, 2007).

Nonetheless, CRH/dexamethasone studies suggest that hypersecretion of CRH is the driving force of HPA axis hyperactivity seen in depressed patients (Ising, Horstmann, et al., 2007; Keck & Holsboer, 2001). Specifically, upon CRH administration, plasma cortisol levels are increased in depressive patients, while ACTH is reduced (Gold et al., 1986). Patients

suffering from major depression also show elevated cerebrospinal fluid (CFS) CRH concentrations (Hartline, Owens, & Nemeroff, 1996; Heuser et al., 1998; Nemeroff et al., 1984) and increased numbers of both CRH-secreting neurons and CRH neurons that co-express arginine vasopressin (AVP) mRNA in the hypothalamus (Purba, Hoogendijk, Hofman, & Swaab, 1996; Raadsheer, Hoogendijk, Stam, Tilders, & Swaab, 1994). Other genes involved in the activation of CRH neuron such as CRHR1, estrogen receptor-alpha (AVPR1A), and MR are found to have high mRNA levels in the post-mortem hypothalamus of depressed patients (S. S. Wang et al., 2008). Elevated CRH-immunoreactivity is also observed in monoamine-containing neurons of the locus coeruleus, median and caudal dorsal raphe nucleus of depressed suicide patients (Austin, Janosky, & Murphy, 2003; Bissette, Klimek, Pan, Stockmeier, & Ordway, 2003; Merali et al., 2006). Reduced CRH receptor density in the frontal cortex is also reported in suicidal individuals (Nemeroff et al., 1988). CSF CRH levels are highest in depressed suicide victims, indicating that an increasing level of CRH hypersecretion may be associated with greater illness severity (Arborelius et al., 1999). Among other proposed mechanisms, CRH has been shown to colocalize with retinoic acid receptor alpha (RARα, member of the steroid/thyroid receptor family), and the density of CRH-RAR-α doubled-stained neurons as well as its ratio to the density of CRH neurons are increased in the PVN of patients with affective disorders (X. N. Chen et al., 2009). In the current thesis, ischemic animals showed elevated CRH expression in the PVN, increased TH expression in the locus coeruleus, and increased CORT response to stressful conditions, suggesting that the depressive-like behaviors in ischemic rats resemble the neuroendocrine changes observed in depressive patients.

Studies examining variations in single-nucleotide polymorphisms (SNP) of the CRHR1 gene in depressed patients have reinforced the link between CRHR1 and the pathology of

depression. Polymorphisms of CRHR1 gene have been associated with major depressive disorder in Han Chinese individuals in a recent study (Z. Liu et al., 2006), and with a stronger antidepressant response to treatment in major depressive Han Chinese patients (Z. Liu et al., 2007) and Mexican-American patients with high anxiety (Licinio et al., 2004). The rs242939 polymorphism of the CRHR1 gene is associated with early antidepressant effects and lower saliva cortisol levels in fluoxetine treated patients (Ventura-Junca et al., 2014). The CRHR1 rs110402 gene is associated with an increased risk to present a seasonal pattern and an early age of onset of the first depressive episode (Papiol et al., 2007). CRHR1SNP, rs4792887, has also been found in depressed men who react with high levels of HPA activity upon a low threshold of stress (Wasserman, Sokolowski, Rozanov, & Wasserman, 2008). Indeed, variants in the CRHR1 gene may be of importance in the prediction and treatment of depressed males at risk of suicidal behavior (Wasserman, Wasserman, Rozanov, & Sokolowski, 2009). Elevated levels of brooding (deep unhappy thoughts) are found in children of mothers with a history of major depression with variations in the CRHR1 TAT haplotype (Woody et al., 2015). Genetic variation in the CRH gene has also been linked to behavioral inhibition, a risk factor for panic and phobic anxiety disorders (Smoller et al., 2003; Smoller et al., 2005). Higher cortisol reactivity to a stressful task is observed in children with a haplotype of the CRHR1 gene, suggesting a role of CRH genes in normative HPA axis function and early neurodevelopment (Sheikh, Kryski, Smith, Hayden, & Singh, 2013). Variants of both CRHR1 and CRH-binding protein (CRHBP) have been shown to interact with early life stressors such as child abuse or maltreatment and predict the risk of depression and even suicidality later in life (Ben-Efraim, Wasserman, Wasserman, & Sokolowski, 2011; Bradley et al., 2008; Grabe et al., 2010; Heim et al., 2009; Roy, Hodgkinson, Deluca, Goldman, & Enoch, 2012; Tyrka et al., 2009). Genetic variation in CRHR1 gene is

related to the consolidation of memories of emotionally arousing experiences in adults and is thought to mediate the development of adult depression following childhood maltreatment (Polanczyk et al., 2009). Triple interactions between polymorphisms of genes for CRHR1 and the serotonin transporter (5-HTTLPR), with experience of child abuse, predict depressive symptoms in adulthood (Ressler et al., 2010). Polymorphisms in the CRHR1 alleles and 5-HTTLPR short alleles were associated with greater depressive reactivity to chronic stress for those also exposed to high levels of early-life adversities (Starr, Hammen, Conway, Raposa, & Brennan, 2014). BDNF Val66Met polymorphism has also been associated to higher ACTH and cortisol levels during the DEX/CRH test in depressed patients (Schule et al., 2006), and combined polymorphisms of the CRHR1 gene with variants of the BDNF gene (rs6265) increases the risk of recurrent major depression in a Chinese population (Xiao et al., 2011). Polymorphisms in 5-HTTLPR, BDNF, and CRHR1 were associated with depression and internalizing symptoms in children who were maltreated (Cicchetti & Rogosch, 2014). Variation in both GR and CRHR1 genes are associated psychosis measures but the CRHR1 gene contributed more to depression severity ratings (Schatzberg et al., 2014). Gene-gene interactions have also been found between arginine vasopressin (AVPR1b, rs28536160 and rs28373064) and CRHR1 (rs4076452 and rs110402) in patients with major depression (Szczepankiewicz et al., 2013). The CRHR1 SNP rs110402 found in depressed patients is associated with changes in blood oxygenation level-dependent signal in mood-related brain regions such as the subgenual cingulate during the viewing of negative versus neutral words (Hsu et al., 2012). In young rhesus monkeys, polymorphisms in the CRHR1 locus are significantly associated with an anxious temperament phenotype and individual variation in positron emission tomography metabolic activity of the anterior hippocampus and amygdala occurring during a human intruder challenge

(Rogers et al., 2013). Chronic stress-induced histone modifications at the promoter region of the CRHR1 gene in the hypothalamus is associated to a depressive phenotype in rodents, characterized by reduced locomotion and sucrose preference, with elevated CRHR1 mRNA and protein in the hypothalamus (Wan et al., 2014). Epigenetic modifications of the CRHR1 gene may therefore mediate permanent changes in brain function and contribute to the pathogenesis of psychiatric disorders (Hodes, 2013).

Similar to our acute CRHR1 blockade, administration of a CRHR1 antagonist, NBI30775, (Ivy et al., 2010) or a conditional forebrain CRHR1 deficiency occurring once the stressful episode has elapsed (X. D. Wang et al., 2012; X. D. Wang, G. Rammes, et al., 2011) prevents the emergence of anxiogenic effects and cognitive impairments induced by unstable maternal care. Treatment (7 days) with the CRHR1 antagonist, NBI30775, prevents the reduced social exploration and increased floating behaviors in the forced swim test, as well as the enhanced CRHR1 mRNA expression in the hippocampal CA1 and the CeA of the amygdala following one week of peripuberty stress (Veenit, Riccio, & Sandi, 2014). Administration of CRHR1 antagonists, SSR125543A (30 kg/kg) and Antalarmin (3, 10 and 30 mg/kg) prevents the occurrence of depressive-like behaviors in stressed mice (Griebel et al., 2002). Moreover, CRHR1 antagonist, SSR125543, administered for 14 days significantly increases swimming in the forced swim test in rats (Overstreet & Griebel, 2004). CRHR1 antagonist, CP-154, 526 (10-32 mg/kg), displays antidepressant-like effects in a learned helplessness animal model (Mansbach, Brooks, & Chen, 1997), and prevents anxiogenic-like activity in the EPM in the elevated zero-maze in rats (Lundkvist et al., 1996; Mallo et al., 2004). Acute administration of CP-154, 526 (Schulz et al., 1996) or Antalarmin (Deak et al., 1999) inhibits stress-induced rising of ACTH plasma and exhibits an anxiolytic effect in a fear-potentiated startle paradigm, and in

conditioned fear (Kikusui, Takeuchi, & Mori, 2000). Chronic CRHR1 blockade in adolescent rats exposed to inescapable electric foot shock similarly reverses anxiogenic responses in the open field and EPM as well as spatial memory impairment, and attenuated stress-induced elevations of CRHR1 expression in the hypothalamus, amygdala and the PFC (C. Li et al., 2015). These studies confirm the antidepressant and anxiolytic action of CRHR1 antagonist, which is consistent with Antalarmin's action in improving emotional impairments in ischemic rats.

In humans, the CRHR1 antagonist R121919 proved efficacious in reducing symptoms of depression and anxiety in a clinical study assessing 24 individuals with major depression (A. W. Zobel et al., 2000) as well as attenuating neuroendocrine responses to psychosocial stress without disrupting diurnal CRH evoked ACTH and CORT secretion (Ising, Zimmermann, et al., 2007). Rather, the treatment acted on key elements regulating the HPA axis including CRHR1, MR, GR protein and mRNA levels in the amygdala and hippocampus, among other brain structures implicated in emotional control and stress reactivity (Post et al., 2005). Of interest, Jutkiewicz et al. (2005) testing different CRHR1 antagonists revealed that LWH234 reduced immobility in the forced swim test, but without altering the swim-stress-induced ACTH response, while Antalarmin, CP154,526, and R121919 showed no antidepressant-like effects but significantly decreased swim-induced increases in ACTH. Similarly, a double-blind, placebo-controlled trial of a CRHR1 antagonist (CP-316,211) decreased urinary cortisol levels, but did not show an antidepressant action in depressed patients (Binneman et al., 2008). These findings may indicate that HPA axis hypersensitivity in ischemic rats contributes to the development of emotional phenotype, as Antalarmin was effective in normalising the HPA axis response to ischemia. Yet, the role of extra-hypothalamic sites for anti-depressive effects upon CRHR1 blockade may also be important, as Antalarmin did attenuated neuroplastic changes in the

mesocorticolimic circuitry. For example, the concurrent use of the non-peptide CRHR1 antagonists (CP154,526 or SSR125543) and arginine-vasopressin -V_{1B} - receptor antagonist (SSR149415) had no effect on anxiety, although combined submaximal dosages of the antagonists significantly reduced immobility in the forced swimming test, without ACTH suppression (Ramos, Homem, Suchecki, Tufik, & Troncone, 2014), suggesting a less important role of the HPA axis in mediating depressive-like behaviors. Taken together, CRHR1 blockade 30 min prior to global ischemia affected emotional behaviors in ischemic rats which could be related to altered neuroendocrine sensitivity and/or changes in brain regions regulating the emotional responses.

7. Hyperactivity following cerebral ischemia – Neuronal degeneration or hyperarousal?

In the current thesis, the EPM was performed prior to the open field test, favoring a measure of ischemia on anxiety-like behaviors in the EPM that was not influenced by prior exposure to the open field test. However, some behavioral neuroscientists recommend that animals should be placed in the OFT prior to the EPM to enhance exploration of rats from the enclosed arms to the open space (de Andrade et al., 2012; Teixeira et al., 2014). This may have contributed to ischemic animals showing heightened anxiety-like behavior in the EPM compared to sham groups in the current thesis, contrasting the anxiolytic effects observed in our animal ischemic cohorts in past studies. Interestingly, all surgical groups showed more anxiogenic behaviors than the HC group, and Antalarmin treatment reduced anxiety in both sham and ischemic animals. Global cerebral ischemia is accomplished via a two-day surgical procedure, which by itself is a potent stressful stimulus strongly activating the HPA axis in sham-operated and ischemic animals (Weidenfeld et al., 2011). Interestingly, despite anxious behavior in the EPM, we observed a trend for the ischemic animals to venture more in the anxiogenic centre

zone in the OFT and to show increased locomotion in the periphery compared to sham-vehicle treated animals. Antalarmin-treatment increased locomotion and exploration in the centre zone in both sham and ischemic groups, although home-cage animals ventured the most in the centre zone. In sum, there was a net effect of global ischemia on anxiety-like behavior and HPA axis function, which was attenuated by Antalarmin treatment.

Ischemia-induced hyperactivity has traditionally been related to CA1 neuronal injury following global ischemia (Andersen, Zimmer, & Sams-Dodd, 1997; Janac, Radenovic, Selakovic, & Prolic, 2006; Mileson & Schwartz, 1991), a proposition supported by observations that neuroprotective treatments reduce ischemia-induced locomotor hyperactivity (Ahn et al., 2009). Global ischemia has long-lasting effects on activity in the open field, which have been reported six months after cardiac arrest in male and female rats, although activity level receded by the 3rd test day (Kiryk et al., 2011). Hyperlocomotion has largely been interpreted as a transient response to neuronal degeneration, as elevated spontaneous locomotor activity is observed as of 24 h post ischemia and increases with occlusion time (J. C. Lee et al., 2013; D. K. Yu et al., 2012). Yet, there is evidence supporting that the stress system may be involved in the hyperactive phenotype following forebrain ischemia. Noteworthy, pre-exposure to the open field prior to global ischemia prevents increased locomotor activation in gerbils (D. Wang & Corbett, 1990). In addition, peak MR and GR protein expression coincides with days of peaked open field hyperactivity in neuroprotected ischemic animals (Yoo, Lee, Choi, et al., 2011). Of interest, CRHR1 blockade prevent ischemia-induced hyperactivity in the open-field without altering hippocampal injury (Plamondon & Khan, 2006). In this regard, ischemia-induced hyperactivity may reflect altered emotional reactivity to stressful environments following global cerebral ischemia. This is further supported by hyperactivity being prominent under bright (450 lux)

open-field illumination, while diminished when testing occurs under dim (40 lux) illumination, independently of hippocampal cell injury (M. Milot & Plamondon, 2008). In parallel, locomotor activity seems to be time-dependent as it is shown to increase after 1 and 5 days and decrease after 15 days, but re-appear after 30 days post reperfusion (Marc R. Milot & Plamondon, 2009). Similar discrepancies are also reported after cerebral hypoxic–ischemic (HI) injury, showing decreased (Charriaut-Marlangue et al., 2014; Gainotti, Azzoni, & Marra, 1999), increased (Delcour et al., 2012; Ming-Yan et al., 2012; Schlager et al., 2011; van der Kooij et al., 2010), or unaltered (Buwalda, Nyakas, Vosselman, & Luiten, 1995) open field locomotor activity. Interestingly, while 1 h restraint stress repeated for 7 days, last episode ending 24 h prior to focal ischemia, leads to reduced locomotion and increased CORT levels and infarct size, the opposite is found in 21 day-chronically stressed groups, which showed increased locomotion and plasma CORT levels but had reduced cell death (Madrigal et al., 2003).

Time-dependent variations in anxiety-like behavior have also been reported in the EPM. For example, anxiolytic behaviors in the EPM observed 7 days after 15 min 4VO, are replaced by anxiogenic responses 28 days after ischemia (de Oliveira et al., 2012). Early anxiogenic-like behavior within the initial 24 h reperfusion interval is replaced by anxiolytic EPM responses 5 days and a return to baseline levels 15 and 30 days following 10 min global ischemia (Marc R. Milot & Plamondon, 2009). Consistent with time-dependent effects, anxiogenic behavior in the EPM is apparent 72 h following 3 min cardiac arrest in mice (Menzebach et al., 2010). Rats subjected to 7 min cardiac arrest show increased anxiety in the EPM on days 2-5 post forebrain ischemia, which resumes to basal levels thereafter (Dhooper, Young, & Reid, 1997). At a longer 6-month intervals, 10 min cardiac arrest leads to increased time spent in the anxiogenic open arms (Kiryk et al., 2011). In contrast, male rats show reduced open arm time following two-

vessel occlusion (Kakehata et al., 2010). Bilateral common carotid arteries occlusion in mice also leads to decreased open-arm exploration (Nakashima, Ajiki, Nakashima, & Takahashi, 2003; Soares, Schiavon, Milani, & de Oliveira, 2013). Yet, several reports of global cerebral ischemia show increased time spent in the open arms of the EPM (Nelson, Lebessi, Sowinski, & Hodges, 1997; Plamondon & Khan, 2005; X. B. Yan, Wang, Hou, Ji, & Zhou, 2007), an effect associated with damage and/or altered function in the catecholamine system (Sanches et al., 2013). For example, reduced tyrosine hydroxylase-positive cells in the substantia nigra following mild and severe hypoxic/ischemic insults induced on postnatal day 7 have been associated with diminished anxiety on postnatal days 14, 21 and 28 (Hei et al., 2012; Ming-Yan et al., 2012). Together, these findings support the contribution of factors outside neuronal death in regulating behavioral responses following brain injury.

8. General Considerations

8.1 Age-dependent changes

Laboratory rats live about 2-3.5 years (average living expectancy is ~3 years), one human year almost equaling two rat weeks (13.8 rat days) if correlating their entire life span (Sengupta, 2013). In the current set of experiments, rats weighed between 250 and 320 g at time of surgery, indicating that rats used in this study were young adults. Currently, the majority of studies investigating stroke and cardiac arrest have utilized animals in their young adulthood (commonly aged 2–4 months), despite epidemiological studies reporting that the risk of cardiovascular disease increases as an individual ages (Cohan et al., 2015; J. I. Rojas, Zurru, Romano, Patrucco, & Cristiano, 2007; Rosamond, 2010). Consequently, these experiments may be less clinically relevant, impeding our ability to translate our results to the aged populations afflicted by brain ischemia. This is in part due to high mortality rate, even when using middle aged rats (10-12

months old), and increased difficulties in occluding the vertebral arteries as the animal ages and the skull becomes thicker leading to increased numbers of discarded animals due to unsuccessful vessel occlusion and incomplete ischemia. For these reasons very few studies have assessed stroke using aged animals although it represents a limitation that needs to be considered. Indeed, important cognitive functions and biological processes/parameters are affected by age. Even though the effects of aging on CRH regulation are far from clear, the majority of studies describe increased CRH production with advancing age (Aguilera, 2011; Swaab, Bao, & Lucassen, 2005). In contrast, BDNF plasma levels decrease in elderly humans, and BDNF expression is down-regulated in the hippocampus of aged rodents (Gibbons et al., 2014; Sallaberry et al., 2013; Tapia-Arancibia, Aliaga, Silhol, & Arancibia, 2008). Among healthy populations, elderly humans with prolonged cortisol elevations show reduced hippocampal volume and mild cognitive impairments (Knoops, Gerritsen, van der Graaf, Mali, & Geerlings, 2010; Lupien et al., 1998; Wolf, Convit, de Leon, Caraos, & Qadri, 2002). Following the combined DEX/CRH test, ACTH and cortisol values remain elevated in elderly compared to young adults, indicating altered HPA regulation in old age (Hatzinger, Brand, Herzig, & Holsboer-Trachsler, 2011). In both young and elderly subjects, the relationship between hippocampal volume and cortisol levels depends on the reactivity to stressful environments (Sindi et al., 2014), which is similar to the data found in the current thesis, whereby ischemic rats showed elevated CORT levels in response to the stressful conditions such as acute restraint stress and decapitation. Since many biochemical parameters change with aging (Anyanwu, 2007), a lessened ability of resistance to ischemic damage would be expected (Anuncibay-Soto et al., 2014). Cortical and striatal infarction volumes in the elderly rats (18 months) are substantially larger than in young animals (3 months) (Rosen, Dinapoli, Nagamine, & Crocco, 2005). Older rats not only suffer from larger

infarct volumes, but also have decreased functional recovery and larger blood–brain barrier disruption (DiNapoli, Huber, Houser, Li, & Rosen, 2008). However, there was no effect of rat age on the incidence of depressive-like behaviors post focal ischemia (Boyko et al., 2013), although depression is a prevalent psychiatric disorder in the elderly and is negatively correlated with serum BDNF levels (Hashizume et al., 2015). In light of the inherent effect of age on brain health, future investigations of the therapeutic interventions following brain ischemia in aging rodents could provide an interesting research venue.

8.2 Sex Differences

Women have a reduced incidence of stroke compared with men until well into old age, but poorer functional outcomes after stroke (Petrea et al., 2009; Rosamond, 2010). In combination with living longer, the postmenopausal phenomenon affecting hormonal secretion is part of the reasons for women being older at stroke onset and suffering more severe strokes (Haast, Gustafson, & Kiliaan, 2012). The phases of the estrus cycle affect stroke outcome (Carswell et al., 2000) as female sex hormones, especially estrogen, reduce the consequences of ischemia by multiple mechanisms and moderate inflammation (Cordeau, Lalancette-Hebert, Weng, & Kriz, 2008; Gibson, Gray, Murphy, & Bath, 2006; Murphy, McCullough, & Smith, 2004; Ritzel, Capozzi, & McCullough, 2013). Females have lower mortality rates following cardiac arrest when researchers controlled for differences in age, obesity and other variables (Greenberg et al., 2014). Depression is more common in women, but suicide more frequent in men (Endicott, 1998). Depressed suicide females have reduced BDNF protein in the PFC, while in males, a BDNF reduction has been observed in the hippocampus (Hayley et al., 2015). Women are twice as likely to suffer from mood and anxiety disorders as men (Bangasser & Valentino, 2012).

This pronounced sex difference has been proposed in part linked to heightened stress sensitivity in females (Parker & Brotchie, 2010). In both humans and rodents, females show a postpubertal increase in stress response magnitude and a more prolonged stress recovery time compared to males (Bale, 2009). Basal (i.e., unstressed) levels of corticosterone are elevated in female compared to male rats (Kitay, 1961; Weinstock, Razin, Schorer-Apelbaum, Men, & McCarty, 1998). Adult females have higher basal and stress-induced levels of ACTH and corticosterone than males (Handa, Burgess, Kerr, & O'Keefe, 1994), as well as increased CRH expression in the PVN, particularly when estrogen levels are elevated (Iwasaki-Sekino, Mano-Otagiri, Ohata, Yamauchi, & Shibasaki, 2009; Viau, Bingham, Davis, Lee, & Wong, 2005). Expression of immediate early gene cFos is more pronounced in females than males in CRH-positive PVN neurons following acute restraint stress (Babb, Masini, Day, & Campeau, 2013) and in the hippocampus after acute swim stress (Bohacek, Manuella, Roszkowski, & Mansuy, 2015). Yet, acute swim stress leads to increased intrinsic excitability and LTP in the DG of male compared to female rats (Zitman & Richter-Levin, 2013). During nicotine withdrawal, adult females display higher levels of anxiety-like behavior, plasma corticosterone, and CRH mRNA expression in the NAc relative to adult males, while during nicotine exposure, adult males exhibited higher levels of corticosterone and CRH mRNA in the amygdala relative to females (Gentile et al., 2011; Skwara, Karwoski, Czambel, Rubin, & Rhodes, 2012; Torres, Gentil, Natividad, Carcoba, & O'Dell, 2013).

There is an ongoing failure in research to include sex differences in study design and analysis because of concerns that hormonal cycles decrease the homogeneity of study populations (Holdcroft, 2007). Although, explicit comparison between the two sexes side by side within studies is ideal, it is appropriate to begin investigating effects of treatments using male

cohorts, and then investigation of such phenomenon using female subjects (Beery & Zucker, 2011). This would facilitate the discovery of sex differences and promote elaboration of mechanism of action of the sex-specific factors.

8.3 The use of behavioral tests "batteries" to study emotional/cognitive profiles in rodents

In the current thesis, a battery of behavioral tests was administered to experimental groups to measure different aspects of affective and cognitive domains. We took great care to place the order of tests so that animals were tested first in the least invasive test and subsequently in more invasive tests. For example, the NSFT was performed on day 27 post reperfusion to avoid the effects of food deprivation on stress levels (Gheibi, Saroukhani, & Azhdari-Zarmehri, 2013). Although, this is general practice (Venzala, Garcia-Garcia, Elizalde, & Tordera, 2013; Yousuf et al., 2015), it could be argued that testing history could influence the performance in the following tasks and that therefore treatment effects should be interpreted with caution. A study investigating order effects, revealed that some tasks are sensitive to test experience (Open Field, light-dark box, rotarod, hot plate), whereas other paradigms appeared to be insensitive (prepulse inhibition, acoustic startle habituation, conditioned fear, Morris water Maze)(McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001). Another study showing behavior in the FST and the OFT, but not in the zero maze, is sensitive to the order of the test in the test battery (Blokland et al., 2012). Yet, a study investigating the effects of inter-test interval on behavioral performance, showed there was no major difference in performance between mice of the standard test battery with 1 week inter-test intervals, and the rapid test battery with 1-2 day inter-test intervals (Paylor, Spencer, Yuva-Paylor, & Pieke-Dahl, 2006). Behavioral testing in itself is stressful (M. R. Milot & Plamondon, 2011a), and the administration of one test could influence the outcome of the next. For instance, in the current study, the FST (day 9) was

performed prior to the SPT (day 14), which may have impacted subsequent behavioral testing due to increased CORT levels following FST (Mishima et al., 2015). Although with several days elapsed between these tests, it should have been long enough for these effects to dissipate. Furthermore, handling is known to influence both the stress response and physiology of animals (Balcombe, Barnard, & Sandusky, 2004; Meijer, Sommer, Spruijt, van Zutphen, & Baumans, 2007). Repeated handling minimises aversion towards human contact and reduces anxiety-like behavior in the EPM (Gouveia & Hurst, 2013; Hurst & West, 2010). Handling stress increases cFos in both sexes, and blocks the effects of acute swim stress in males (Hanell & Marklund, 2014). The differential effect of stress on learning (harmful versus beneficial) depends on a number of variables, including: stimulus duration, intensity, number, the specific learning test (Shors & Servatius, 1997), sex (G. E. Wood & Shors, 1998), age (Adlard et al., 2011). In addition, both physical and cognitive stimulation have been shown to improve spatial memory performance in aged mice (Harburger, Nzerem, & Frick, 2007). Single housing is itself considered to be a form of social stress (Reinhardt, 2004), but was used considering the results of this study in the context of the existing literature (which used single-housing conditions), it is important to note this caveat. In general, it is important to have conditions that minimize stress in order to favor a reasonable degree of behavior, as manipulations tend to be anxiogenic (Overstreet, 2012). In the current thesis, biochemical and histological data from a group of naïve rats were compared to those from the control group, and although differences were observed on some occasions, they did not impede interpretation and clarity of the findings, suggesting that behavioral testing itself may have not altered the results in the biochemical analysis. In addition, the sham surgery in itself represents a stressful event and to make sure that absence or presence of statistically significant differences are not due to floor or ceiling effects in all the groups, the

HC control rats were included in statistical analysis, but was omitted from the Two-way analysis of variance to maintain a balanced general linear model of variance.

8.4 Challenges related to the western blotting procedure

In the current thesis, western blotting was performed to measure alterations in protein levels of TrkB, synapsin, PSD95 as well as β-actin, which was used as a control for normalization, in 7 different regions (dorsal & Ventral hippocampus, Amygdala, PFC, NAc, VTA and PVN), with 4-5 animals per group. Four regions as opposed to seven were reported in the current study to avoid lack of specificity of micro-punch dissections for the VTA and PVN. Film was used to expose the blots, which may have produced a poor dynamic range of quantitation, leading to reduced resolution and sensitivity of the bands. Future studies examining these protein levels may benefit from the next generation camera-based detection methods, improving both the sensitivity and linear dynamic range, permitting a much more accurate quantification of the relative density between samples (Taylor, Berkelman, Yadav, & Hammond, 2013). Optimization of protein loading in accordance with antibody concentration could also reduce the risk of high saturation or overexposure of the membrane, increasing sensitivity of the detection.

8.5 Impact on Mood Related Research

Post-stroke depression is an important psychological consequence of ischemic stroke, which affects around one third of stroke patients (Nabavi, Turner, Dean, Sureda, & Nabavi, 2014). Major depression is a complex disorder reflecting many subtypes and different aetiology. Much debate remains about the specific aetiology and pathophysiology of post-stroke depression. Post-stroke depression is likely determined by multiple factors possibly including size and location of the brain lesion, social handicap and the quality of family support but also prior history of depressive disorder (Provinciali et al., 2008). Despite some studies suggesting

that use of antidepressants among patients with a diagnosis of PSD is associated with improvement in depressive symptoms, little is known about antidepressant drugs that may induce a better response in such conditions (Y. Chen, Guo, Zhan, & Patel, 2006; M. L. Hackett, Anderson, House, & Xia, 2008; Starkstein, Mizrahi, & Power, 2008). The aetiology of PSD has been explained in two ways: (1) as a pathophysiological mechanism related to the brain injury (Bhogal, Teasell, Foley, & Speechley, 2004; Carson et al., 2000; Ku et al., 2013), particularly its site and location, and (2) as a psychological reaction to loss and social and psychological changes following stroke (N. Paul et al., 2013; Surtees et al., 2008). Others have emphasised that PSD is a multifactorial phenomenon (Whyte & Mulsant, 2002), being associated with post-stroke fatigue, sleep disturbance and bodily pain (Kouwenhoven, Gay, Bakken, & Lerdal, 2013).

The main theory underlying the pathogenesis of PSD is a series of abnormalities regarding monoamine neurotransmitters and endocrine and neurotropic factors following a stroke (Ji et al., 2014). For example, both depression and suicide are characterized by alterations in the stress response (Bao, Meynen, & Swaab, 2008; G. N. Pandey, 2013). Increased cortisol secretion and CRH expression in the hypothalamus as well as decreased levels of BDNF and TrkB have been frequently observed in relation to depression and suicide (Arborelius et al., 1999; Bao et al., 2008; Braquehais, Picouto, Casas, & Sher, 2012; Himmerich et al., 2006; Jokinen & Nordstrom, 2009; S. S. Wang et al., 2008; J. Zhao et al., 2015). Moreover, depressed patients show elevated pro-inflammatory levels of cytokines such as IL1β, IL6, and TNF-α (Connor & Leonard, 1998; Maes et al., 1999; Mikova, Yakimova, Bosmans, Kenis, & Maes, 2001; Pollak & Yirmiya, 2002; Sukoff Rizzo et al., 2012). Small hippocampal and amygdala volumes have been associated with major depression (Caetano et al., 2004; MacQueen & Frodl, 2011; Schuhmacher et al., 2012). Structural changes in the hippocampus (Adzic et al., 2015; S. Campbell & Macqueen, 2004), and

neuronal cell death and abnormal synaptic plasticity in the hippocampus (Oh, Park, & Kim, 2010) play a role in the pathophysiology of depression. Moreover, depressed patients in the remitted state are often impaired with respect to short- and long-term verbal memory, attention and cognitive speed as well as executive functioning in terms of set shifting and verbal fluency, which may be associated to elevated cortisol levels and decreased grey matter volume in the cingulate cortex (Behnken et al., 2013; Schlosser et al., 2013; Treadway et al., 2009). Depressive symptoms predict cognitive decline and dementia in the elderly (Verdelho et al., 2013).

The current thesis presents evidence regarding the underlying neurochemistry of post-stroke depression, commonalities between post-stroke depression and major depression disorder, in the hope to help develop treatment approaches. In the conducted studies, ischemic animals displayed hyperarousal of the stress system, depressive-like behaviors, memory impairments, increased impulsivity and sociability, which is unique to the global ischemic model. These results correspond to changes observed in animal models of depression which affects the mesocorticolimbic circuitry including the hippocampus, nucleus accumbens, amygdala and prefrontal cortex (Budni et al., 2013; Khemissi, Farooq, Le Guisquet, Sakly, & Belzung, 2014; Raone et al., 2007). Although genetics, biochemistry, and histology are very important tools for understanding underlying mechanisms of drug treatments, behavior represents the final output of the CNS and should be a foremost considered index in preclinical evaluations of successful treatment strategies (Hanell & Marklund, 2014). An individual's behavioral phenotype is influenced by a number of interacting factors such as neuroendocrine status, genetic variation and environmental effects among others (Sequeira-Cordero et al., 2014). Thus, behavioral research based on individual differences (i.e. classification of individuals according to systematic variations of specific behaviors) may prove to be a more informative approach to understand

mood disorders such as anxiety and depressive-like behaviors (Borta & Schwarting, 2005; Enriquez-Castillo et al., 2008; Gorisch & Schwarting, 2006; Herrero, Sandi, & Venero, 2006; Ho, Eichendorff, & Schwarting, 2002; Kazlauckas et al., 2005; Mallo et al., 2007; Naudon & Jay, 2005; Pawlak, Ho, & Schwarting, 2008; Sequeira-Cordero et al., 2014). Yet, the dysregulation of the stress response remains the most important etiological hypothesis of depression (Z. Liu et al., 2013; Pagliaccio et al., 2015).

9. Final Word

Stroke is a devastating condition afflicting mostly the elderly for which no effective medication exists to enhance neurorehabilitation. Great clinical benefits may accrue from targeting basic neurobiological mechanisms underlying CNS recovery, including the structural and functional alterations in the brain induced by dysregulation of the stress system, which was the focus of the current set of experiments. Our results confirm that global cerebral ischemia acts as a potent systemic stressor affecting body and brain responses. The internal stress response is an allosteric process, re-establishing homeostasis in response to a challenge (Bruce S. McEwen, 1998). The HPA axis is externally activated by stressful environments but also internally activated by the disruptions of homeostasis, triggered for instance by systemic stimuli or psychogenic stressors (Goldstein, 2010; Herman, Ostrander, Mueller, & Figueiredo, 2005; Pacak, 2000). The HPA axis has long been reported to participate in mediating behavioral responses to stressful events and cognitive performance in mammals (Cahill & McGaugh, 1996; Contarino, Heinrichs, et al., 1999). Stress modulates hippocampal-dependent learning and memory (Muravieva & Alberini, 2010; Vouimba et al., 2007). The relationship between stress intensity and memory function has been shown to follow an inverted U shaped curve response whereby memory performance is better in conditions of optimal stress or impaired under

conditions above or below optimal stress (Salehi et al., 2010). Despite the benefits of the stress response, stressors that are either prolonged or extreme, may result in abnormal changes in brain plasticity that, paradoxically, may impair the ability of the brain to appropriately regulate and respond to subsequent stressors (Radley & Morrison, 2005). While acute activation of brain arousal systems is essential to cope with stressors and enhances learning performance, prolonged activation of HPA axis has been hypothesized to underlie stress disorders and impair cognitive functions (B. S. McEwen & Sapolsky, 1995; Nemeroff, 1992). Dysregulation of the HPA axis and hippocampal and amygdalar circuitries are thought to regulate depression-like impairments observed in several neurological and affective disorders including schizophrenia, multiple sclerosis, Alzheimer's disease and epilepsy (A. Zobel et al., 2004). Our findings are supportive of studies suggesting that hippocampal neuronal loss and structural damage observed in depression may result from elevated levels of glucocorticoids (R. M. Sapolsky, 2000). For example, cognitive decline seen in both normal aging and Alzheimer's disease patients correlates with reduced hippocampal volume and deficits in memory tasks accompanied by prolonged cortisol elevations (Lupien et al., 1998; McAuley et al., 2009; Peavy et al., 2007). Similarly, in a study of 281 patients with ischemic stroke, functional outcome and mortality was predicted even one year after the ischemic episode by increased levels of cortisol corresponding to lesion size and neurological deficits, reflecting the severity of the stroke (Neidert et al., 2011). Recent experiments in our lab have demonstrated the beneficial effects of reducing corticosterone and norepinephrine levels, though pharmacological treatment, on memory performance of ischemic animals in spite of hippocampal degeneration at time of testing (M. R. Milot & Plamondon, 2011b), suggesting that changes in stress response and/or emotional reactivity play a significant role in ischemic impairments. In light of this, reducing stress in patients at risk for cardiovascular

disease may lead to significant improvements in functional recovery. There is thus a possibility that the CRH circuitry could play a role in ischemic behavioral impairments acting on mechanisms related to neuronal and/or glial functions but also likely on neuroplastic changes post-injury such as those related to BDNF signaling (see figure 1 in appendix B).

Appendix B

Study 1-Summary		
Behavior	Physiological Measures	Histological Measures
↓ Working memory (RAM)	↑ CORT up to 7 days post ischemia	↑ CRH, CRHR1, GR-ir in the PVN
	↑ CORT after acute restrain stress	↓ CRHR1 & GR -ir in the CA1
	27 days post ischemia	↓ CRH-ir in the CeA, but not significant in the CA1
		↑ TH-ir in the LC
		↓ Cell density in the CA1

Study 2-Summary		
Behavior	Physiological Measures	Histological, Protein, & mRNA Expression
↑ Sociability (SIT)	↓ Body Weight	↓ BDNF & TrkB-ir in the PFC
↑ Despair (FST)	↓ Food Intake	↑ BDNF & TH, but reduced TrkB-ir in the NAc
↑ Anhedonia (SPT)	↑ CORT	↓ BDNF & TrkB-ir in the VTA
↓ Anxiety (NSFT)	↑ Adrenal weight	↓ TrkB, Synapsin, PSD95 protein expression in the NAc
	↓ Thymus weight	↑ TrkB & BDNF mRNA expression in the NAc
	↓ Seminal vesicles weight	↓ TrkB, Synapsin, PSD95 protein expression in the PFC
		↓ TrkB & BDNF mRNA expression in the PFC

Study 3-Summary		
Behavior	Histology	Protein & mRNA Expression
↑ Anxiety (EPM)	↓ NeuN-ir in the CA1 & CA3	↓ TrkB & Synapsin protein expression in the hippocampus
↑ Exploration (OFT)	↓ Cell density in the CA1 & CA3	↓ TrkB & BDNF mRNA expression in the hippocampus
↓ Aversive learning (YM-PAT)	↑ GFAP, IBA1,TNF α- ir in the CA1 & CA3	↑ TrkB & Synapsin protein expression in the amygdala
↓ Spatial Memory (Barnes maze)	↑ BDNF, TrkB, pCREB-ir in the CA1 & CA3	↑ TrkB & BDNF mRNA expression in the amygdala
↑ CORT	↑ TrkB but not BDNF-ir in the BLA	
	↑ BDNF, TrkB-ir in the PVN	

Table 1. Summary of the results from the three studies in the current thesis (Articles 1, 2, & 3). CORT: corticosterone. ↓: decrease. ↑: increase.

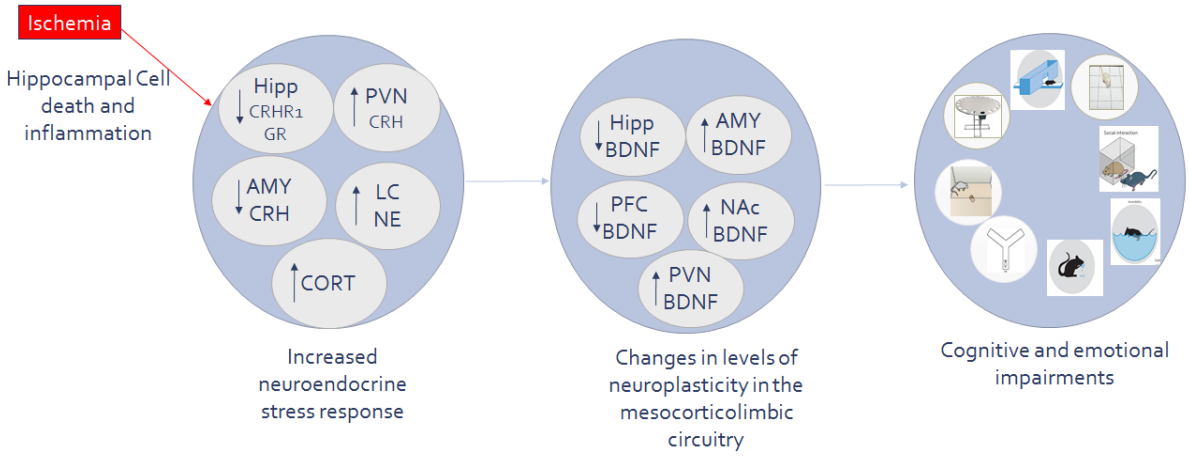

Figure 1. Simplified schematic diagram describing neuroendocrine and neuroplastic changes resulting in cognitive and emotional impairment post global cerebral ischemia. Forebrain ischemia triggers a physiological response involving autonomic and neuroendocrine stress system comprising the hypothalamus-pituitary-adrenal (HPA) axis. Neurons in the medial parvocellular region of the paraventricular nucleus of hypothalamus (PVN) release CRH, which triggers the subsequent secretion of ACTH from the pituitary gland, leading to the production of glucocorticoids (CORT) by the adrenal cortex. Norepinephrine is also increased in the locus coeruleus (LC). Normally, the hippocampus exerts an inhibitory influence over HPA function with its high density of MRs and GRs. However following ischemia, the CA1 and CA3 sub-regions of the hippocampus are injured resulting in increased HPA activity, as a consequence of impaired HPA axis negative feedback control. The corticomesolimbic circuitry is particularly sensitive to the deleterious effects of glucocorticoid excess, potentiating neuroplastic changes such as dendritic atrophy, reduced BDNF/TrkB expression in the hippocampus (Hipp) and the frontal cortex (PFC), while increased expression is observed in the amygdala (AMY) and the nucleus accumbens (NAc). These site-specific changes in neuroplasticity may contribute to depression, anxiety as well as decline in cognitive function post ischemia. In the current thesis, Antalarmin pre-treatment reduced HPA reactivity at short and long-term intervals, which reversed behavioral impairments by attenuating ischemia-induced alterations in corticomesolimbic BDNF/TrkB expression and neuronal injury.

References

Adamec, R. E., Blundell, J., & Burton, P. (2005). Neural circuit changes mediating lasting brain and behavioral response to predator stress. *Neurosci Biobehav Rev, 29*(8), 1225-1241. doi: S0149-7634(05)00101-6 [pii]10.1016/j.neubiorev.2005.05.007

Adamec, R. E., Blundell, J., & Collins, A. (2001). Neural plasticity and stress induced changes in defense in the rat. *Neurosci Biobehav Rev, 25*(7-8), 721-744. doi: S0149763401000537 [pii]

Adlard, P. A., Engesser-Cesar, C., & Cotman, C. W. (2011). Mild stress facilitates learning and exercise improves retention in aged mice. *Exp Gerontol, 46*(1), 53-59. doi: 10.1016/j.exger.2010.10.001

Adolphs, R. (2009). The social brain: neural basis of social knowledge. *Annu Rev Psychol, 60*, 693-716. doi: 10.1146/annurev.psych.60.110707.163514

Adzic, M., Djordjevic, J., Mitic, M., Brkic, Z., Lukic, I., & Radojcic, M. (2015). The contribution of hypothalamic neuroendocrine, neuroplastic and neuroinflammatory processes to lipopolysaccharide-induced depressive-like behaviour in female and male rats: Involvement of glucocorticoid receptor and C/EBP-beta. *Behav Brain Res, 291*, 130-139. doi: 10.1016/j.bbr.2015.05.029

Aguilar-Valles, A., Sanchez, E., de Gortari, P., Balderas, I., Ramirez-Amaya, V., Bermudez-Rattoni, F., & Joseph-Bravo, P. (2005). Analysis of the stress response in rats trained in the water-maze: differential expression of corticotropin-releasing hormone, CRH-R1, glucocorticoid receptors and brain-derived neurotrophic factor in limbic regions. *Neuroendocrinology, 82*(5-6), 306-319. doi: 10.1159/000093129

Aguilera, G. (2011). HPA axis responsiveness to stress: implications for healthy aging. *Exp Gerontol, 46*(2-3), 90-95. doi: 10.1016/j.exger.2010.08.023

Ahn, H. C., Yoo, K. Y., Hwang, I. K., Cho, J. H., Lee, C. H., Choi, J. H., . . . Won, M. H. (2009). Ischemia-related changes in naive and mutant forms of ubiquitin and neuroprotective effects of ubiquitin in the hippocampus following experimental transient ischemic damage. *Exp Neurol, 220*(1), 120-132. doi: 10.1016/j.expneurol.2009.07.031S0014-4886(09)00315-X [pii]

Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. *Physiol Rev, 89*(1), 121-145. doi: 10.1152/physrev.00017.2008

Alboni, S., Benatti, C., Capone, G., Corsini, D., Caggia, F., Tascedda, F., . . . Brunello, N. (2010). Time-dependent effects of escitalopram on brain derived neurotrophic factor (BDNF) and neuroplasticity related targets in the central nervous system of rats. *Eur J Pharmacol, 643*(2-3), 180-187. doi: 10.1016/j.ejphar.2010.06.028

Aldenhoff, J. B., Gruol, D. L., Rivier, J., Vale, W., & Siggins, G. R. (1983). Corticotropin releasing factor decreases postburst hyperpolarizations and excites hippocampal neurons. *Science, 221*(4613), 875-877.

Allan, S. M., & Rothwell, N. J. (2003). Inflammation in central nervous system injury. *Philos Trans R Soc Lond B Biol Sci, 358*(1438), 1669-1677. doi: 10.1098/rstb.2003.1358

Almeida, O. P., & Xiao, J. (2007). Mortality associated with incident mental health disorders after stroke. *Aust N Z J Psychiatry, 41*(3), 274-281. doi: 771210211 [pii] 10.1080/00048670601172772

Altar, C. A., Cai, N., Bliven, T., Juhasz, M., Conner, J. M., Acheson, A. L., . . . Wiegand, S. J. (1997). Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature, 389*(6653), 856-860. doi: 10.1038/39885

Altar, C. A., Siuciak, J. A., Wright, P., Ip, N. Y., Lindsay, R. M., & Wiegand, S. J. (1994). In situ hybridization of trkB and trkC receptor mRNA in rat forebrain and association with high-affinity binding of [125I]BDNF, [125I]NT-4/5 and [125I]NT-3. *European Journal of Neuroscience, 6*(9), 1389-1405.

Amantea, D., Nappi, G., Bernardi, G., Bagetta, G., & Corasaniti, M. T. (2009). Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. *FEBS J, 276*(1), 13-26. doi: 10.1111/j.1742-4658.2008.06766.x

Amat, J., Baratta, M. V., Paul, E., Bland, S. T., Watkins, L. R., & Maier, S. F. (2005). Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nat Neurosci, 8*(3), 365-371. doi: nn1399 [pii]
10.1038/nn1399

Anacker, C., Zunszain, P. A., Carvalho, L. A., & Pariante, C. M. (2011). The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology, 36*(3), 415-425. doi: 10.1016/j.psyneuen.2010.03.007

Anderova, M., Vorisek, I., Pivonkova, H., Benesova, J., Vargova, L., Cicanic, M., . . . Sykova, E. (2011). Cell death/proliferation and alterations in glial morphology contribute to changes in diffusivity in the rat hippocampus after hypoxia-ischemia. *J Cereb Blood Flow Metab, 31*(3), 894-907. doi: 10.1038/jcbfm.2010.168

Andersen, M. B., Zimmer, J., & Sams-Dodd, F. (1997). Postischemic hyperactivity in the Mongolian gerbil correlates with loss of hippocampal neurons. *Behav Neurosci, 111*(6), 1205-1216.

Andres, A. L., Regev, L., Phi, L., Seese, R. R., Chen, Y., Gall, C. M., & Baram, T. Z. (2013). NMDA receptor activation and calpain contribute to disruption of dendritic spines by the stress neuropeptide CRH. *J Neurosci, 33*(43), 16945-16960. doi: 10.1523/JNEUROSCI.1445-13.2013

Anne, M., Juha, K., Timo, M., Mikko, T., Olli, V., Kyosti, S., . . . Vilho, M. (2007). Neurohormonal activation in ischemic stroke: effects of acute phase disturbances on long-term mortality. *Curr Neurovasc Res, 4*(3), 170-175.

Annunziato, L., Boscia, F., & Pignataro, G. (2013). Ionic transporter activity in astrocytes, microglia, and oligodendrocytes during brain ischemia. *J Cereb Blood Flow Metab, 33*(7), 969-982. doi: 10.1038/jcbfm.2013.44

Antonawich, F. J., Miller, G., Rigsby, D. C., & Davis, J. N. (1999). Regulation of ischemic cell death by glucocorticoids and adrenocorticotropic hormone. *Neuroscience, 88*(1), 319-325.

Anuncibay-Soto, B., Perez-Rodriguez, D., Llorente, I. L., Regueiro-Purrinos, M., Gonzalo-Orden, J. M., & Fernandez-Lopez, A. (2014). Age-dependent modifications in vascular adhesion molecules and apoptosis after 48-h reperfusion in a rat global cerebral ischemia model. *Age (Dordr), 36*(5), 9703. doi: 10.1007/s11357-014-9703-7

Anyanwu, E. C. (2007). Neurochemical changes in the aging process: implications in medication in the elderly. *ScientificWorldJournal, 7*, 1603-1610. doi: 10.1100/tsw.2007.212

Arborelius, L., Owens, M. J., Plotsky, P. M., & Nemeroff, C. B. (1999). The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol, 160*(1), 1-12.

Arborelius, L., Skelton, K. H., Thrivikraman, K. V., Plotsky, P. M., Schulz, D. W., & Owens, M. J. (2000). Chronic administration of the selective corticotropin-releasing factor 1 receptor antagonist CP-154,526: behavioral, endocrine and neurochemical effects in the rat. *J Pharmacol Exp Ther, 294*(2), 588-597.

Arunrut, T., Alejandre, H., Chen, M., Cha, J., & Russo-Neustadt, A. (2009). Differential behavioral and neurochemical effects of exercise, reboxetine and citalopram with the forced swim test. *Life Sci, 84*(17-18), 584-589. doi: S0024-3205(09)00068-X [pii] 10.1016/j.lfs.2009.02.005

Auer, R. N., Jensen, M. L., & Whishaw, I. Q. (1989). Neurobehavioral deficit due to ischemic brain damage limited to half of the CA1 sector of the hippocampus. *J Neurosci, 9*(5), 1641-1647.

Austin, M. C., Janosky, J. E., & Murphy, H. A. (2003). Increased corticotropin-releasing hormone immunoreactivity in monoamine-containing pontine nuclei of depressed suicide men. *Mol Psychiatry, 8*(3), 324-332. doi: 10.1038/sj.mp.4001250 4001250 [pii]

Autry, A. E., & Monteggia, L. M. (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacol Rev, 64*(2), 238-258. doi: 10.1124/pr.111.005108

Avale, M. E., Chabout, J., Pons, S., Serreau, P., De Chaumont, F., Olivo-Marin, J. C., . . . Granon, S. (2011). Prefrontal nicotinic receptors control novel social interaction between mice. *Faseb Journal, 25*(7), 2145-2155. doi: Doi 10.1096/Fj.10-178558

Avignone, E., Frenguelli, B. G., & Irving, A. J. (2005). Differential responses to NMDA receptor activation in rat hippocampal interneurons and pyramidal cells may underlie enhanced pyramidal cell vulnerability. *European Journal of Neuroscience, 22*(12), 3077-3090. doi: 10.1111/j.1460-9568.2005.04497.x

Ayerbe, L., Ayis, S., Wolfe, C. D., & Rudd, A. G. (2013). Natural history, predictors and outcomes of depression after stroke: systematic review and meta-analysis. *Br J Psychiatry, 202*(1), 14-21. doi: 10.1192/bjp.bp.111.107664

Azogu, I., de la Tremblaye, P. B., Dunbar, M., Lebreton, M., LeMarec, N., & Plamondon, H. (2015). Acute sleep deprivation enhances avoidance learning and spatial memory and induces delayed alterations in neurochemical expression of GR, TH, DRD1, pCREB and Ki67 in rats. *Behavioural Brain Research, 279*(0), 177-190. doi: http://dx.doi.org/10.1016/j.bbr.2014.11.015

Babb, J. A., Masini, C. V., Day, H. E., & Campeau, S. (2013). Sex differences in activated corticotropin-releasing factor neurons within stress-related neurocircuitry and hypothalamic-pituitary-adrenocortical axis hormones following restraint in rats. *Neuroscience, 234*, 40-52. doi: 10.1016/j.neuroscience.2012.12.051

Badowska-Szalewska, E., Spodnik, E., Klejbor, I., & Morys, J. (2010). Effects of chronic forced swim stress on hippocampal brain-derived neutrophic factor (BDNF) and its receptor (TrkB) immunoreactive cells in juvenile and aged rats. *Acta Neurobiol Exp (Wars), 70*(4), 370-381.

Bah, T. M., Kaloustian, S., Rousseau, G., & Godbout, R. (2011). Pretreatment with pentoxifylline has antidepressant-like effects in a rat model of acute myocardial infarction. *Behavioural Pharmacology, 22*(8), 779-784. doi: Doi 10.1097/Fbp.0b013e32834d1385

Bai, Q., Shi, D., Zhang, Y., Liu, H., & Yao, X. (2014). Exploration of the antagonist CP-376395 escape pathway for the corticotropin-releasing factor receptor 1 by random acceleration

molecular dynamics simulations. *Mol Biosyst, 10*(7), 1958-1967. doi: 10.1039/c4mb00037d

Baigent, S. M. (2001). Peripheral corticotropin-releasing hormone and urocortin in the control of the immune response. *Peptides, 22*(5), 809-820.

Balcombe, J. P., Barnard, N. D., & Sandusky, C. (2004). Laboratory routines cause animal stress. *Contemp Top Lab Anim Sci, 43*(6), 42-51.

Bale, T. L. (2009). Neuroendocrine and immune influences on the CNS: it's a matter of sex. *Neuron, 64*(1), 13-16. doi: 10.1016/j.neuron.2009.09.036

Bale, T. L., Contarino, A., Smith, G. W., Chan, R., Gold, L. H., Sawchenko, P. E., . . . Lee, K. F. (2000). Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. *Nat Genet, 24*(4), 410-414. doi: 10.1038/74263

Bale, T. L., Giordano, F. J., Hickey, R. P., Huang, Y., Nath, A. K., Peterson, K. L., . . . Lee, K. F. (2002). Corticotropin-releasing factor receptor 2 is a tonic suppressor of vascularization. *Proc Natl Acad Sci U S A, 99*(11), 7734-7739. doi: DOI 10.1073/pnas.102187099

Bale, T. L., Giordano, F. J., & Vale, W. W. (2003). A new role for corticotropin-releasing factor receptor-2: suppression of vascularization. *Trends Cardiovasc Med, 13*(2), 68-71.

Bale, T. L., & Vale, W. W. (2003). Increased depression-like behaviors in corticotropin-releasing factor receptor-2-deficient mice: sexually dichotomous responses. *J Neurosci, 23*(12), 5295-5301. doi: 23/12/5295 [pii]

Banerjee, R., Hazra, S., Ghosh, A. K., & Mondal, A. C. (2014). Chronic administration of bacopa monniera increases BDNF protein and mRNA expressions: a study in chronic unpredictable stress induced animal model of depression. *Psychiatry Investig, 11*(3), 297-306. doi: 10.4306/pi.2014.11.3.297

Bangasser, D. A., & Valentino, R. J. (2012). Sex differences in molecular and cellular substrates of stress. *Cell Mol Neurobiol, 32*(5), 709-723. doi: 10.1007/s10571-012-9824-4

Bantsiele, G. B., Bentue-Ferrer, D., Amiot, N., Allain, H., Bourin, M., & Reymann, J. M. (2004). Does rat global transient cerebral ischemia serve as an appropriate model to study emotional disturbances? *Fundamental & Clinical Pharmacology, 18*(6), 685-692. doi: 10.1111/j.1472-8206.2004.00295.x

Bantsiele, G. B., Bentue-Ferrer, D., Saikali, S., Laviolle, B., Bourin, M., & Reymann, J. M. (2009a). Behavioral effects of four antidepressants on an ischemic rat model of emotional disturbances. *Behav Brain Res, 201*(2), 265-271. doi: S0166-4328(09)00121-1 [pii] 10.1016/j.bbr.2009.02.022

Bantsiele, G. B., Bentue-Ferrer, D., Saikali, S., Laviolle, B., Bourin, M., & Reymann, J. M. (2009b). Behavioral effects of four antidepressants on an ischemic rat model of emotional disturbances. *Behav Brain Res, 201*(2), 265-271. doi: 10.1016/j.bbr.2009.02.022

Bao, A. M., Meynen, G., & Swaab, D. F. (2008). The stress system in depression and neurodegeneration: focus on the human hypothalamus. *Brain Research Reviews, 57*(2), 531-553. doi: 10.1016/j.brainresrev.2007.04.005

Baram, T. Z., & Hatalski, C. G. (1998). Neuropeptide-mediated excitability: a key triggering mechanism for seizure generation in the developing brain. *Trends in Neurosciences, 21*(11), 471-476. doi: http://dx.doi.org/10.1016/S0166-2236(98)01275-2

Baram, T. Z., & Ribak, C. E. (1995). Peptide-induced infant status epilepticus causes neuronal death and synaptic reorganization. *Neuroreport, 6*(2), 277-280.

Barbany, G., & Persson, H. (1992). Regulation of Neurotrophin mRNA Expression in the Rat Brain by Glucocorticoids. *European Journal of Neuroscience, 4*(5), 396-403.

Barker-Collo, S. L. (2007). Depression and anxiety 3 months post stroke: prevalence and correlates. *Arch Clin Neuropsychol, 22*(4), 519-531. doi: S0887-6177(07)00080-7 [pii]10.1016/j.acn.2007.03.002

Barnett, H. J. (2010). Forty years of progress in stroke. *Stroke, 41*(6), 1068-1072. doi: 41/6/1068 [pii]10.1161/STROKEAHA.110.584680

Barrett, A. M. (2010). Rose-colored answers: neuropsychological deficits and patient-reported outcomes after stroke. *Behav Neurol, 22*(1-2), 17-23. doi: 10.3233/BEN-2009-0250 32U87V177X1P5113 [pii]

Bartsch, T., Dohring, J., Reuter, S., Finke, C., Rohr, A., Brauer, H., . . . Jansen, O. (2015). Selective neuronal vulnerability of human hippocampal CA1 neurons: lesion evolution, temporal course, and pattern of hippocampal damage in diffusion-weighted MR imaging. *J Cereb Blood Flow Metab, 35*(11), 1836-1845. doi: 10.1038/jcbfm.2015.137

Bartsch, T., Schonfeld, R., Muller, F. J., Alfke, K., Leplow, B., Aldenhoff, J., . . . Koch, J. M. (2010). Focal lesions of human hippocampal CA1 neurons in transient global amnesia impair place memory. *Science, 328*(5984), 1412-1415. doi: 10.1126/science.1188160

Bayatti, N., Hermann, H., Lutz, B., & Behl, C. (2005). Corticotropin-releasing hormone-mediated induction of intracellular signaling pathways and brain-derived neurotrophic factor expression is inhibited by the activation of the endocannabinoid system. *Endocrinology, 146*(3), 1205-1213. doi: 10.1210/en.2004-1154

Bayatti, N., Zschocke, J., & Behl, C. (2003). Brain region-specific neuroprotective action and signaling of corticotropin-releasing hormone in primary neurons. *Endocrinology, 144*(9), 4051-4060. doi: 10.1210/en.2003-0168

Beauchaine, T. P., Neuhaus, E., Zalewski, M., Crowell, S. E., & Potapova, N. (2011). The effects of allostatic load on neural systems subserving motivation, mood regulation, and social affiliation. *Dev Psychopathol, 23*(4), 975-999. doi: 10.1017/S0954579411000459 S0954579411000459 [pii]

Beck, T., Lindholm, D., Castren, E., & Wree, A. (1994). Brain-derived neurotrophic factor protects against ischemic cell damage in rat hippocampus. *J Cereb Blood Flow Metab, 14*(4), 689-692. doi: 10.1038/jcbfm.1994.86

Becker, K., Abraham, A., Kindler, J., Helmeke, C., & Braun, K. (2007). Exposure to neonatal separation stress alters exploratory behavior and corticotropin releasing factor expression in neurons in the amygdala and hippocampus. *Dev Neurobiol, 67*(5), 617-629. doi: 10.1002/dneu.20372

Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev, 35*(3), 565-572. doi: 10.1016/j.neubiorev.2010.07.002

Behl, C., & Clement, A. (2011). The Corticotropin-Releasing Hormone in Neuroprotection *Hormones in Neurodegeneration, Neuroprotection, and Neurogenesis* (pp. 237-249): Wiley-VCH Verlag GmbH & Co. KGaA.

Behnken, A., Bellingrath, S., Symanczik, J. P., Rieck, M. J., Zavorotnyy, M., Domschke, K., . . . Zwanzger, P. (2013). Associations between cognitive performance and cortisol reaction to the DEX/CRH test in patients recovered from depression. *Psychoneuroendocrinology, 38*(3), 447-454. doi: 10.1016/j.psyneuen.2012.07.005

Bejot, Y., Prigent-Tessier, A., Cachia, C., Giroud, M., Mossiat, C., Bertrand, N., . . . Marie, C. (2011). Time-dependent contribution of non neuronal cells to BDNF production after

ischemic stroke in rats. *Neurochem Int, 58*(1), 102-111. doi: 10.1016/j.neuint.2010.10.019

Bekinschtein, P., Cammarota, M., Igaz, L. M., Bevilaqua, L. R., Izquierdo, I., & Medina, J. H. (2007). Persistence of long-term memory storage requires a late protein synthesis- and BDNF- dependent phase in the hippocampus. *Neuron, 53*(2), 261-277. doi: S0896-6273(06)01026-9 [pii]10.1016/j.neuron.2006.11.025

Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, L., Rossato, J. I., Goldin, A., . . . Medina, J. H. (2008). BDNF is essential to promote persistence of long-term memory storage. *Proc Natl Acad Sci U S A, 105*(7), 2711-2716. doi: 10.1073/pnas.0711863105

Ben-Efraim, Y. J., Wasserman, D., Wasserman, J., & Sokolowski, M. (2011). Gene–environment interactions between CRHR1 variants and physical assault in suicide attempts. *Genes, Brain and Behavior, 10*(6), 663-672. doi: 10.1111/j.1601-183X.2011.00703.x

Bennett, M. R., & Lagopoulos, J. (2014). Stress and trauma: BDNF control of dendritic-spine formation and regression. *Prog Neurobiol, 112*, 80-99. doi: 10.1016/j.pneurobio.2013.10.005

Berendse, H. W., Galis-de Graaf, Y., & Groenewegen, H. J. (1992). Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *J Comp Neurol, 316*(3), 314-347. doi: 10.1002/cne.903160305

Berghauzen-Maciejewska, K., Kuter, K., Kolasiewicz, W., Glowacka, U., Dziubina, A., Ossowska, K., & Wardas, J. (2014). Pramipexole but not imipramine or fluoxetine reverses the "depressive-like" behaviour in a rat model of preclinical stages of Parkinson's disease. *Behav Brain Res, 271*, 343-353. doi: 10.1016/j.bbr.2014.06.029

Berghauzen-Maciejewska, K., Wardas, J., Kosmowska, B., Glowacka, U., Kuter, K., & Ossowska, K. (2015). Alterations of BDNF and trkB mRNA expression in the 6-hydroxydopamine-induced model of preclinical stages of Parkinson's disease: an influence of chronic pramipexole in rats. *PLoS One, 10*(3), e0117698. doi: 10.1371/journal.pone.0117698

Berton, O., McClung, C. A., Dileone, R. J., Krishnan, V., Renthal, W., Russo, S. J., . . . Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science, 311*(5762), 864-868. doi: 10.1126/science.1120972

Bessa, J. M., Morais, M., Marques, F., Pinto, L., Palha, J. A., Almeida, O. F., & Sousa, N. (2013). Stress-induced anhedonia is associated with hypertrophy of medium spiny neurons of the nucleus accumbens. *Transl Psychiatry, 3*, e266. doi: 10.1038/tp.2013.39

Bhogal, S. K., Teasell, R., Foley, N., & Speechley, M. (2004). Lesion location and poststroke depression - Systematic review of the methodological limitations in the literature. *Stroke, 35*(3), 794-802. doi: 10.1161/01.Str.0000117237.98749.26

Binneman, B., Feltner, D., Kolluri, S., Shi, Y., Qiu, R., & Stiger, T. (2008). A 6-week randomized, placebo-controlled trial of CP-316,311 (a selective CRH1 antagonist) in the treatment of major depression. *Am J Psychiatry, 165*(5), 617-620. doi: 10.1176/appi.ajp.2008.07071199

Bissette, G., Klimek, V., Pan, J., Stockmeier, C., & Ordway, G. (2003). Elevated concentrations of CRF in the locus coeruleus of depressed subjects. *Neuropsychopharmacology, 28*(7), 1328-1335. doi: 10.1038/sj.npp.13001911300191 [pii]

Bittencourt, J. C., & Sawchenko, P. E. (2000). Do centrally administered neuropeptides access cognate receptors?: an analysis in the central corticotropin-releasing factor system. *J Neurosci, 20*(3), 1142-1156.

Blanchard, D. C., Spencer, R. L., Weiss, S. M., Blanchard, R. J., McEwen, B., & Sakai, R. R. (1995). Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. *Psychoneuroendocrinology, 20*(2), 117-134. doi: 0306453094E0045B [pii]

Blank, T., Nijholt, I., Eckart, K., & Spiess, J. (2002). Priming of long-term potentiation in mouse hippocampus by corticotropin-releasing factor and acute stress: implications for hippocampus-dependent learning. *J Neurosci, 22*(9), 3788-3794. doi: 20026289

Bledsoe, A. C., Oliver, K. M., Scholl, J. L., & Forster, G. L. (2011). Anxiety states induced by post-weaning social isolation are mediated by CRF receptors in the dorsal raphe nucleus. *Brain Res Bull, 85*(3-4), 117-122. doi: S0361-9230(11)00080-3 [pii] 10.1016/j.brainresbull.2011.03.003

Blokland, A., ten Oever, S., van Gorp, D., van Draanen, M., Schmidt, T., Nguyen, E., . . . Klinkenberg, I. (2012). The use of a test battery assessing affective behavior in rats: Order effects. *Behav Brain Res, 228*(1), 16-21. doi: 10.1016/j.bbr.2011.11.042

Blum, S., Luchsinger, J. A., Manly, J. J., Schupf, N., Stern, Y., Brown, T. R., . . . Brickman, A. M. (2012). Memory after silent stroke Hippocampus and infarcts both matter. *Neurology, 78*(1), 38-46. doi: Doi 10.1212/Wnl.0b013e31823ed0cc

Bock, J., Gruss, M., Becker, S., & Braun, K. (2005). Experience-induced changes of dendritic spine densities in the prefrontal and sensory cortex: correlation with developmental time windows. *Cereb Cortex, 15*(6), 802-808. doi: 10.1093/cercor/bhh181bhh181 [pii]

Bohacek, J., Manuella, F., Roszkowski, M., & Mansuy, I. M. (2015). Hippocampal gene expression induced by cold swim stress depends on sex and handling. *Psychoneuroendocrinology, 52*, 1-12. doi: 10.1016/j.psyneuen.2014.10.026

Bonde, C., Noraberg, J., Noer, H., & Zimmer, J. (2005). Ionotropic glutamate receptors and glutamate transporters are involved in necrotic neuronal cell death induced by oxygen-glucose deprivation of hippocampal slice cultures. *Neuroscience, 136*(3), 779-794. doi: 10.1016/j.neuroscience.2005.07.020

Bonfiglio, J. J., Inda, C., Refojo, D., Holsboer, F., Arzt, E., & Silberstein, S. (2011). The corticotropin-releasing hormone network and the hypothalamic-pituitary-adrenal axis: molecular and cellular mechanisms involved. *Neuroendocrinology, 94*(1), 12-20. doi: 10.1159/000328226 000328226 [pii]

Bonfiglio, J. J., Inda, C., Senin, S., Maccarrone, G., Refojo, D., Giacomini, D., . . . Silberstein, S. (2013). B-Raf and CRHR1 internalization mediate biphasic ERK1/2 activation by CRH in hippocampal HT22 Cells. *Mol Endocrinol, 27*(3), 491-510. doi: 10.1210/me.2012-1359

Bornstein, S. R., Webster, E. L., Torpy, D. J., Richman, S. J., Mitsiades, N., Igel, M., . . . Chrousos, G. P. (1998). Chronic effects of a nonpeptide corticotropin-releasing hormone type I receptor antagonist on pituitary-adrenal function, body weight, and metabolic regulation. *Endocrinology, 139*(4), 1546-1555.

Borta, A., & Schwarting, R. K. (2005). Inhibitory avoidance, pain reactivity, and plus-maze behavior in Wistar rats with high versus low rearing activity. *Physiology & Behavior, 84*(3), 387-396. doi: 10.1016/j.physbeh.2005.01.009

Bourne, J. N., & Harris, K. M. (2008). Balancing structure and function at hippocampal dendritic spines. *Annu Rev Neurosci, 31*, 47-67. doi: 10.1146/annurev.neuro.31.060407.125646

Bowden, M. G., Woodbury, M. L., & Duncan, P. W. (2013). Promoting neuroplasticity and recovery after stroke: future directions for rehabilitation clinical trials. *Curr Opin Neurol, 26*(1), 37-42. doi: 10.1097/WCO.0b013e32835c5ba0

Boyko, M., Kutz, R., Gruenbaum, B. F., Cohen, H., Kozlovsky, N., Gruenbaum, S. E., . . . Zlotnik, A. (2013). The influence of aging on poststroke depression using a rat model via middle cerebral artery occlusion. *Cogn Affect Behav Neurosci, 13*(4), 847-859. doi: 10.3758/s13415-013-0177-3

Boyle, L. M. (2013). A neuroplasticity hypothesis of chronic stress in the basolateral amygdala. *Yale J Biol Med, 86*(2), 117-125.

Bozzini, S., Gambelli, P., Boiocchi, C., Schirinzi, S., Falcone, R., Buzzi, P., . . . Falcone, C. (2009). Coronary artery disease and depression: possible role of brain-derived neurotrophic factor and serotonin transporter gene polymorphisms. *Int J Mol Med, 24*(6), 813-818.

Bradley, R. G., Binder, E. B., Epstein, M. P., Tang, Y., Nair, H. P., Liu, W., . . . Ressler, K. J. (2008). Influence of child abuse on adult depression: moderation by the corticotropin-releasing hormone receptor gene. *Arch Gen Psychiatry, 65*(2), 190-200. doi: 10.1001/archgenpsychiatry.2007.26

Bramham, C. R., & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog Neurobiol, 76*(2), 99-125. doi: S0301-0082(05)00066-3 [pii] 10.1016/j.pneurobio.2005.06.003

Braquehais, M. D., Picouto, M. D., Casas, M., & Sher, L. (2012). Hypothalamic-pituitary-adrenal axis dysfunction as a neurobiological correlate of emotion dysregulation in adolescent suicide. *World J Pediatr, 8*(3), 197-206. doi: 10.1007/s12519-012-0358-0

Briones, T. L., & Therrien, B. (2000). Behavioral effects of transient cerebral ischemia. *Biol Res Nurs, 1*(4), 276-286.

Brischoux, F., Chakraborty, S., Brierley, D. I., & Ungless, M. A. (2009). Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. *Proc Natl Acad Sci U S A, 106*(12), 4894-4899. doi: 10.1073/pnas.0811507106

Briscoe, R. J., Cabrera, C. L., Baird, T. J., Rice, K. C., & Woods, J. H. (2000). Antalarmin blockade of corticotropin releasing hormone-induced hypertension in rats. *Brain Res, 881*(2), 204-207. doi: S0006-8993(00)02742-6 [pii]

Brisson, C. D., & Andrew, R. D. (2012). A neuronal population in hypothalamus that dramatically resists acute ischemic injury compared to neocortex. *J Neurophysiol, 108*(2), 419-430. doi: jn.00090.2012 [pii] 10.1152/jn.00090.2012

Brodaty, H., Withall, A., Altendorf, A., & Sachdev, P. S. (2007). Rates of depression at 3 and 15 months poststroke and their relationship with cognitive decline: the Sydney Stroke Study. *American Journal of Geriatric Psychiatry, 15*(6), 477-486. doi: Doi 10.1097/Jgp.0b013e3180590bca

Brown, C. E., & Murphy, T. H. (2008). Livin' on the edge: imaging dendritic spine turnover in the peri-infarct zone during ischemic stroke and recovery. *Neuroscientist, 14*(2), 139-146. doi: 1073858407309854 [pii] 10.1177/1073858407309854

Brown, C. E., Wong, C., & Murphy, T. H. (2008). Rapid morphologic plasticity of peri-infarct dendritic spines after focal ischemic stroke. *Stroke, 39*(4), 1286-1291. doi: STROKEAHA.107.498238 [pii]
10.1161/STROKEAHA.107.498238

Brown, E. S., Rush, A. J., & McEwen, B. S. (1999). Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. *Neuropsychopharmacology, 21*(4), 474-484. doi: 10.1016/S0893-133X(99)00054-8

Brown, S. M., Henning, S., & Wellman, C. L. (2005). Mild, short-term stress alters dendritic morphology in rat medial prefrontal cortex. *Cereb Cortex, 15*(11), 1714-1722. doi: bhi048 [pii]
10.1093/cercor/bhi048

Brunson, K. L., Eghbal-Ahmadi, M., Bender, R., Chen, Y., & Baram, T. Z. (2001). Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress. *Proc Natl Acad Sci U S A, 98*(15), 8856-8861. doi: 10.1073/pnas.151224898

Budni, J., Zomkowski, A. D., Engel, D., Santos, D. B., dos Santos, A. A., Moretti, M., . . . Rodrigues, A. L. (2013). Folic acid prevents depressive-like behavior and hippocampal antioxidant imbalance induced by restraint stress in mice. *Exp Neurol, 240*, 112-121. doi: 10.1016/j.expneurol.2012.10.024

Bulbul, M., Babygirija, R., Cerjak, D., Yoshimoto, S., Ludwig, K., & Takahashi, T. (2011). Hypothalamic oxytocin attenuates CRF expression via GABA(A) receptors in rats. *Brain Res, 1387*, 39-45. doi: 10.1016/j.brainres.2011.02.091

Buwalda, B., Nyakas, C., Vosselman, H. J., & Luiten, P. G. (1995). Effects of early postnatal anoxia on adult learning and emotion in rats. *Behav Brain Res, 67*(1), 85-90. doi: 016643289400108R [pii]

Caetano, S. C., Hatch, J. P., Brambilla, P., Sassi, R. B., Nicoletti, M., Mallinger, A. G., . . . Soares, J. C. (2004). Anatomical MRI study of hippocampus and amygdala in patients with current and remitted major depression. *Psychiatry Res, 132*(2), 141-147. doi: 10.1016/j.pscychresns.2004.08.002

Cahill, L., & McGaugh, J. L. (1996). Modulation of memory storage. *Curr Opin Neurobiol, 6*(2), 237-242. doi: S0959-4388(96)80078-X [pii]

Caliskan, G., Schulz, S. B., Gruber, D., Behr, J., Heinemann, U., & Gerevich, Z. (2015). Corticosterone and corticotropin-releasing factor acutely facilitate gamma oscillations in the hippocampus in vitro. *European Journal of Neuroscience, 41*(1), 31-44. doi: 10.1111/ejn.12750

Campbell, B. M., Morrison, J. L., Walker, E. L., & Merchant, K. M. (2004). Differential regulation of behavioral, genomic, and neuroendocrine responses by CRF infusions in rats. *Pharmacol Biochem Behav, 77*(3), 447-455. doi: 10.1016/j.pbb.2003.12.010 S0091305703003873 [pii]

Campbell, S., & Macqueen, G. (2004). The role of the hippocampus in the pathophysiology of major depression. *J Psychiatry Neurosci, 29*(6), 417-426.

Campbell, S. N., Zhang, C., Monte, L., Roe, A. D., Rice, K. C., Tache, Y., . . . Rissman, R. A. (2015). Increased tau phosphorylation and aggregation in the hippocampus of mice overexpressing corticotropin-releasing factor. *J Alzheimers Dis, 43*(3), 967-976. doi: 10.3233/JAD-141281

Carlezon, W. A., Jr., & Thomas, M. J. (2009). Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. *Neuropharmacology, 56 Suppl 1*, 122-132. doi: 10.1016/j.neuropharm.2008.06.075

Carlson, D. E., Chiu, W. C., Fiedler, S. M., & Hoffman, G. E. (2007). Central neural distribution of immunoreactive Fos and CRH in relation to plasma ACTH and corticosterone during sepsis in the rat. *Exp Neurol, 205*(2), 485-500. doi: 10.1016/j.expneurol.2007.03.015

Carroll, J. C., Iba, M., Bangasser, D. A., Valentino, R. J., James, M. J., Brunden, K. R., . . . Trojanowski, J. Q. (2011). Chronic Stress Exacerbates Tau Pathology, Neurodegeneration, and Cognitive Performance through a Corticotropin-Releasing Factor Receptor-Dependent Mechanism in a Transgenic Mouse Model of Tauopathy. *Journal of Neuroscience, 31*(40), 14436-14449. doi: 10.1523/Jneurosci.3836-11.2011

Carson, A. J., MacHale, S., Allen, K., Lawrie, S. M., Dennis, M., House, A., & Sharpe, M. (2000). Depression after stroke and lesion location: a systematic review. *Lancet, 356*(9224), 122-126. doi: 10.1016/S0140-6736(00)02448-X

Carswell, H. V., Anderson, N. H., Morton, J. J., McCulloch, J., Dominiczak, A. F., & Macrae, I. M. (2000). Investigation of estrogen status and increased stroke sensitivity on cerebral blood flow after a focal ischemic insult. *J Cereb Blood Flow Metab, 20*(6), 931-936. doi: 10.1097/00004647-200006000-00005

Carty, M. L., Wixey, J. A., Kesby, J., Reinebrant, H. E., Colditz, P. B., Gobe, G., & Buller, K. M. (2010). Long-term losses of amygdala corticotropin-releasing factor neurons are associated with behavioural outcomes following neonatal hypoxia-ischemia. *Behav Brain Res, 208*(2), 609-618. doi: S0166-4328(10)00024-0 [pii] 10.1016/j.bbr.2010.01.007

Casals, J. B., Pieri, N. C. G., Feitosa, M. L. T., Ercolin, A. C. M., Roballo, K. C. S., Barreto, R. S. N., . . . Ambrósio, C. E. (2011). The Use of Animal Models for Stroke Research: A Review. *Comparative Medicine, 61*(4), 305-313.

Caso, J. R., Moro, M. A., Lorenzo, P., Lizasoain, I., & Leza, J. C. (2007). Involvement of IL-1beta in acute stress-induced worsening of cerebral ischaemia in rats. *Eur Neuropsychopharmacol, 17*(9), 600-607. doi: S0924-977X(07)00026-0 [pii] 10.1016/j.euroneuro.2007.02.009

Castren, E. (2005). Is mood chemistry? *Nat Rev Neurosci, 6*(3), 241-246. doi: nrn1629 [pii] 10.1038/nrn1629

Castren, E., Voikar, V., & Rantamaki, T. (2007). Role of neurotrophic factors in depression. *Curr Opin Pharmacol, 7*(1), 18-21. doi: S1471-4892(06)00171-8 [pii] 10.1016/j.coph.2006.08.009

Chalmers, D. T., Lovenberg, T. W., & De Souza, E. B. (1995). Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. *J Neurosci, 15*(10), 6340-6350.

Chang, D. J., Lee, N., Choi, C., Jeon, I., Oh, S. H., Shin, D. A., . . . Song, J. (2012). Therapeutic Effect of BDNF-overexpressing Human Neural Stem Cells (HB1.F3.BDNF) in a Rodent Model of Middle Cerebral Artery Occlusion. *Cell Transplant*. doi: ct0835chang [pii]10.3727/096368912X657323

Charles, M. S., Ostrowski, R. P., Manaenko, A., Duris, K., Zhang, J. H., & Tang, J. (2012). Role of the pituitary-adrenal axis in granulocyte-colony stimulating factor-induced neuroprotection against hypoxia-ischemia in neonatal rats. *Neurobiol Dis, 47*(1), 29-37.

Charriaut-Marlangue, C., Nguyen, T., Bonnin, P., Duy, A. P., Leger, P. L., Csaba, Z., . . . Baud, O. (2014). Sildenafil mediates blood-flow redistribution and neuroprotection after neonatal hypoxia-ischemia. *Stroke, 45*(3), 850-856. doi: 10.1161/STROKEAHA.113.003606 STROKEAHA.113.003606 [pii]

Charron, C., Frechette, S., Proulx, G., & Plamondon, H. (2008). In vivo administration of corticotropin-releasing hormone at remote intervals following ischemia enhances CA1 neuronal survival and recovery of spatial memory impairments: a role for opioid receptors. *Behav Brain Res, 188*(1), 125-135. doi: S0166-4328(07)00571-2 [pii] 10.1016/j.bbr.2007.10.027

Charron, C., Fréchette, S., Proulx, G., & Plamondon, H. (2008). In vivo administration of corticotropin-releasing hormone at remote intervals following ischemia enhances CA1 neuronal survival and recovery of spatial memory impairments: A role for opioid receptors. *Behav Brain Res, 188*(1), 125-135. doi: http://dx.doi.org/10.1016/j.bbr.2007.10.027

Charron, C., Schock, S. C., Proulx, G., Thompson, C. S., Hakim, A. M., & Plamondon, H. (2009). Protection conferred by Corticotropin-releasing hormone in rat primary cortical neurons against chemical ischemia involves opioid receptor activation. *Brain Res, 1257*, 117-127. doi: 10.1016/j.brainres.2008.12.053

Chatterjee, K., Fall, S., & Barer, D. (2010). Mood after stroke: a case control study of biochemical, neuro-imaging and socio-economic risk factors for major depression in stroke survivors. *BMC Neurol, 10*, 125. doi: 10.1186/1471-2377-10-125

Chen, A., Xiong, L. J., Tong, Y., & Mao, M. (2013). The neuroprotective roles of BDNF in hypoxic ischemic brain injury. *Biomed Rep, 1*(2), 167-176. doi: 10.3892/br.2012.48

Chen, B., Dowlatshahi, D., MacQueen, G. M., Wang, J. F., & Young, L. T. (2001). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry, 50*(4), 260-265.

Chen, D. Y., Bambah-Mukku, D., Pollonini, G., & Alberini, C. M. (2012). Glucocorticoid receptors recruit the CaMKIIalpha-BDNF-CREB pathways to mediate memory consolidation. *Nat Neurosci, 15*(12), 1707-1714. doi: 10.1038/nn.3266

Chen, J., Qin, J., Su, Q., Liu, Z., & Yang, J. (2012). Treadmill rehabilitation treatment enhanced BDNF-TrkB but not NGF-TrkA signaling in a mouse intracerebral hemorrhage model. *Neurosci Lett, 529*(1), 28-32. doi: 10.1016/j.neulet.2012.09.021 S0304-3940(12)01239-6 [pii]

Chen, J. H., Kuo, H. C., Lee, K. F., & Tsai, T. H. (2014). Magnolol protects neurons against ischemia injury via the downregulation of p38/MAPK, CHOP and nitrotyrosine. *Toxicol Appl Pharmacol, 279*(3), 294-302. doi: 10.1016/j.taap.2014.07.005

Chen, J. H., Kuo, H. C., Lee, K. F., & Tsai, T. H. (2015). Global proteomic analysis of brain tissues in transient ischemia brain damage in rats. *Int J Mol Sci, 16*(6), 11873-11891. doi: 10.3390/ijms160611873

Chen, L. Y., Rex, C. S., Casale, M. S., Gall, C. M., & Lynch, G. (2007). Changes in synaptic morphology accompany actin signaling during LTP. *J Neurosci, 27*(20), 5363-5372. doi: 10.1523/JNEUROSCI.0164-07.2007

Chen, X. N., Meng, Q. Y., Bao, A. M., Swaab, D. F., Wang, G. H., & Zhou, J. N. (2009). The involvement of retinoic acid receptor-alpha in corticotropin-releasing hormone gene

expression and affective disorders. *Biol Psychiatry, 66*(9), 832-839. doi: 10.1016/j.biopsych.2009.05.031

Chen, X. Q., Du, J. Z., & Wang, Y. S. (2004). Regulation of hypoxia-induced release of corticotropin-releasing factor in the rat hypothalamus by norepinephrine. *Regul Pept, 119*(3), 221-228. doi: 10.1016/j.regpep.2004.02.005 S0167011504000424 [pii]

Chen, X. Q., Xu, N. Y., Du, J. Z., Wang, Y., & Duan, C. (2005). Corticotropin-releasing factor receptor subtype 1 and somatostatin modulating hypoxia-caused downregulated mRNA of pituitary growth hormone and upregulated mRNA of hepatic insulin-like growth factor-I of rats. *Mol Cell Endocrinol, 242*(1-2), 50-58. doi: S0303-7207(05)00253-4 [pii] 10.1016/j.mce.2005.07.005

Chen, Y., Andres, A. L., Frotscher, M., & Baram, T. Z. (2012). Tuning synaptic transmission in the hippocampus by stress: the CRH system. *Front Cell Neurosci, 6*, 13. doi: 10.3389/fncel.2012.00013

Chen, Y., Bender, R. A., Frotscher, M., & Baram, T. Z. (2001). Novel and transient populations of corticotropin-releasing hormone-expressing neurons in developing hippocampus suggest unique functional roles: a quantitative spatiotemporal analysis. *J Neurosci, 21*(18), 7171-7181.

Chen, Y., Brunson, K. L., Adelmann, G., Bender, R. A., Frotscher, M., & Baram, T. Z. (2004). Hippocampal corticotropin releasing hormone: pre- and postsynaptic location and release by stress. *Neuroscience, 126*(3), 533-540. doi: 10.1016/j.neuroscience.2004.03.036 S0306452204002088 [pii]

Chen, Y., Brunson, K. L., Muller, M. B., Cariaga, W., & Baram, T. Z. (2000). Immunocytochemical distribution of corticotropin-releasing hormone receptor type-1 (CRF(1))-like immunoreactivity in the mouse brain: light microscopy analysis using an antibody directed against the C-terminus. *J Comp Neurol, 420*(3), 305-323. doi: 10.1002/(SICI)1096-9861(20000508)420:3<305::AID-CNE3>3.0.CO;2-8 [pii]

Chen, Y., Dube, C. M., Rice, C. J., & Baram, T. Z. (2008). Rapid loss of dendritic spines after stress involves derangement of spine dynamics by corticotropin-releasing hormone. *J Neurosci, 28*(11), 2903-2911. doi: 10.1523/JNEUROSCI.0225-08.2008

Chen, Y., Fenoglio, K. A., Dube, C. M., Grigoriadis, D. E., & Baram, T. Z. (2006). Cellular and molecular mechanisms of hippocampal activation by acute stress are age-dependent. *Mol Psychiatry, 11*(11), 992-1002. doi: 4001863 [pii] 10.1038/sj.mp.4001863

Chen, Y., Guo, J. J., Zhan, S., & Patel, N. C. (2006). Treatment effects of antidepressants in patients with post-stroke depression: a meta-analysis. *Ann Pharmacother, 40*(12), 2115-2122. doi: 10.1345/aph.1H389

Chen, Y., Hatalski, C. G., Brunson, K. L., & Baram, T. Z. (2001). Rapid phosphorylation of the CRE binding protein precedes stress-induced activation of the corticotropin releasing hormone gene in medial parvocellular hypothalamic neurons of the immature rat. *Brain Res Mol Brain Res, 96*(1-2), 39-49.

Chen, Y., Kramar, E. A., Chen, L. Y., Babayan, A. H., Andres, A. L., Gall, C. M., . . . Baram, T. Z. (2012). Impairment of synaptic plasticity by the stress mediator CRH involves selective destruction of thin dendritic spines via RhoA signaling. *Mol Psychiatry*. doi: 10.1038/mp.2012.17mp201217 [pii]

Chen, Y., Kramar, E. A., Chen, L. Y., Babayan, A. H., Andres, A. L., Gall, C. M., . . . Baram, T. Z. (2013). Impairment of synaptic plasticity by the stress mediator CRH involves selective destruction of thin dendritic spines via RhoA signaling. *Mol Psychiatry, 18*(4), 485-496. doi: 10.1038/mp.2012.17

Chen, Y., Liu, J., Wu, X., & Nice, E. C. (2013). Xiangshao Granule Exerts Antidepressive Effects in a Depression Mouse Model by Ameliorating Deficits in Hippocampal BDNF and TrkB. *Evid Based Complement Alternat Med, 2013*, 309262. doi: 10.1155/2013/309262

Chen, Y., Rex, C. S., Rice, C. J., Dube, C. M., Gall, C. M., Lynch, G., & Baram, T. Z. (2010). Correlated memory defects and hippocampal dendritic spine loss after acute stress involve corticotropin-releasing hormone signaling. *Proc Natl Acad Sci U S A, 107*(29), 13123-13128. doi: 10.1073/pnas.1003825107

Chen, Y., Won, S. J., Xu, Y., & Swanson, R. A. (2014). Targeting microglial activation in stroke therapy: pharmacological tools and gender effects. *Curr Med Chem, 21*(19), 2146-2155.

Cheng, Y. D., Al-Khoury, L., & Zivin, J. A. (2004). Neuroprotection for ischemic stroke: two decades of success and failure. *NeuroRx, 1*(1), 36-45. doi: 10.1602/neurorx.1.1.36

Chetty, S., Friedman, A. R., Taravosh-Lahn, K., Kirby, E. D., Mirescu, C., Guo, F., . . . Kaufer, D. (2014). Stress and glucocorticoids promote oligodendrogenesis in the adult hippocampus. *Mol Psychiatry, 19*(12), 1275-1283. doi: Doi 10.1038/Mp.2013.190

Chi, P., Greengard, P., & Ryan, T. A. (2001). Synapsin dispersion and reclustering during synaptic activity. *Nat Neurosci, 4*(12), 1187-1193. doi: 10.1038/nn756

Choi, D. W. (1996). Ischemia-induced neuronal apoptosis. *Curr Opin Neurobiol, 6*(5), 667-672. doi: S0959-4388(96)80101-2 [pii]

Chollet, F. (2013). Pharmacologic approaches to cerebral aging and neuroplasticity: insights from the stroke model. *Dialogues Clin Neurosci, 15*(1), 67-76.

Chou, D., Huang, C. C., & Hsu, K. S. (2014). Brain-derived neurotrophic factor in the amygdala mediates susceptibility to fear conditioning. *Exp Neurol, 255*, 19-29. doi: 10.1016/j.expneurol.2014.02.016

Christensen, H., Boysen, G., & Johannesen, H. H. (2004). Serum-cortisol reflects severity and mortality in acute stroke. *J Neurol Sci, 217*(2), 175-180.

Cibelli, G., Corsi, P., Diana, G., Vitiello, F., & Thiel, G. (2001). Corticotropin-releasing factor triggers neurite outgrowth of a catecholaminergic immortalized neuron via cAMP and MAP kinase signalling pathways. *Eur J Neurosci, 13*(7), 1339-1348. doi: ejn1510 [pii]

Cicchetti, D., & Rogosch, F. A. (2014). Genetic moderation of child maltreatment effects on depression and internalizing symptoms by serotonin transporter linked polymorphic region (5-HTTLPR), brain-derived neurotrophic factor (BDNF), norepinephrine transporter (NET), and corticotropin releasing hormone receptor 1 (CRHR1) genes in African American children. *Dev Psychopathol, 26*(4 Pt 2), 1219-1239. doi: 10.1017/S0954579414000984

Cintra, A., Fuxe, K., Harfstrand, A., Agnati, L. F., Wikstrom, A. C., Okret, S., . . . Gustafsson, J. A. (1987). Presence of glucocorticoid receptor immunoreactivity in corticotrophin releasing factor and in growth hormone releasing factor immunoreactive neurons of the rat di- and telencephalon. *Neurosci Lett, 77*(1), 25-30. doi: 0304-3940(87)90601-X [pii]

Clarke, R. M., Lyons, A., O'Connell, F., Deighan, B. F., Barry, C. E., Anyakoha, N. G., . . . Lynch, M. A. (2008). A pivotal role for interleukin-4 in atorvastatin-associated neuroprotection in rat brain. *J Biol Chem, 283*(4), 1808-1817. doi: M707442200 [pii]

10.1074/jbc.M707442200
Cleck, J. N., Ecke, L. E., & Blendy, J. A. (2008). Endocrine and gene expression changes following forced swim stress exposure during cocaine abstinence in mice. *Psychopharmacology (Berl), 201*(1), 15-28. doi: 10.1007/s00213-008-1243-3
Cohan, C. H., Neumann, J. T., Dave, K. R., Alekseyenko, A., Binkert, M., Stransky, K., . . . Perez-Pinzon, M. A. (2015). Effect of cardiac arrest on cognitive impairment and hippocampal plasticity in middle-aged rats. *PLoS One, 10*(5), e0124918. doi: 10.1371/journal.pone.0124918
Colbourne, F., Sutherland, G. R., & Auer, R. N. (1999). Electron microscopic evidence against apoptosis as the mechanism of neuronal death in global ischemia. *Journal of Neuroscience, 19*(11), 4200-4210.
Cole, J., Costafreda, S. G., McGuffin, P., & Fu, C. H. (2011). Hippocampal atrophy in first episode depression: a meta-analysis of magnetic resonance imaging studies. *J Affect Disord, 134*(1-3), 483-487. doi: S0165-0327(11)00319-3 [pii] 10.1016/j.jad.2011.05.057
Colla, M., Kronenberg, G., Deuschle, M., Meichel, K., Hagen, T., Bohrer, M., & Heuser, I. (2007). Hippocampal volume reduction and HPA-system activity in major depression. *J Psychiatr Res, 41*(7), 553-560. doi: S0022-3956(06)00121-X [pii] 10.1016/j.jpsychires.2006.06.011
Collingridge, G. L., Peineau, S., Howland, J. G., & Wang, Y. T. (2010). Long-term depression in the CNS. *Nat Rev Neurosci, 11*(7), 459-473. doi: 10.1038/nrn2867
Comelli, M. C., Seren, M. S., Guidolin, D., Manev, R. M., Favaron, M., Rimland, J. M., . . . Manev, H. (1992). Photochemical stroke and brain-derived neurotrophic factor (BDNF) mRNA expression. *Neuroreport, 3*(6), 473-476.
Conner, J. M., Lauterborn, J. C., Yan, Q., Gall, C. M., & Varon, S. (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J Neurosci, 17*(7), 2295-2313.
Connor, T. J., & Leonard, B. E. (1998). Depression, stress and immunological activation: the role of cytokines in depressive disorders. *Life Sci, 62*(7), 583-606.
Conrad, C. D., Galea, L. A., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine pretreatment. *Behav Neurosci, 110*(6), 1321-1334.
Contarino, A., Dellu, F., Koob, G. F., Smith, G. W., Lee, K. F., Vale, W., & Gold, L. H. (1999). Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. *Brain Res, 835*(1), 1-9.
Contarino, A., Heinrichs, S. C., & Gold, L. H. (1999). Understanding corticotropin releasing factor neurobiology: contributions from mutant mice. *Neuropeptides, 33*(1), 1-12. doi: 10.1054/npep.1999.0001
Contoreggi, C. (2015). Corticotropin releasing hormone and imaging, rethinking the stress axis. *Nucl Med Biol, 42*(4), 323-339. doi: 10.1016/j.nucmedbio.2014.11.008
Cook, S. C., & Wellman, C. L. (2004). Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol, 60*(2), 236-248. doi: 10.1002/neu.20025
Cordeau, P., Jr., Lalancette-Hebert, M., Weng, Y. C., & Kriz, J. (2008). Live imaging of neuroinflammation reveals sex and estrogen effects on astrocyte response to ischemic injury. *Stroke, 39*(3), 935-942. doi: 10.1161/STROKEAHA.107.501460

Correia Bacarin, C., Mori, M. A., Dias Fiuza Ferreira, E., Valerio Romanini, C., Weffort de Oliveira, R. M., & Milani, H. (2013). Fish oil provides robust and sustained memory recovery after cerebral ischemia: Influence of treatment regimen. *Physiol Behav, 119*, 61-71. doi: 10.1016/j.physbeh.2013.06.001 S0031-9384(13)00187-X [pii]

Cortes-Mendoza, J., Diaz de Leon-Guerrero, S., Pedraza-Alva, G., & Perez-Martinez, L. (2013). Shaping synaptic plasticity: the role of activity-mediated epigenetic regulation on gene transcription. *Int J Dev Neurosci, 31*(6), 359-369. doi: 10.1016/j.ijdevneu.2013.04.003

Coull, J. A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., . . . De Koninck, Y. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature, 438*(7070), 1017-1021. doi: 10.1038/nature04223

Craft, T. K., & Devries, A. C. (2009). Vulnerability to stroke: implications of perinatal programming of the hypothalamic-pituitary-adrenal axis. *Front Behav Neurosci, 3*, 54. doi: 10.3389/neuro.08.054.2009

Craft, T. K., Glasper, E. R., McCullough, L., Zhang, N., Sugo, N., Otsuka, T., . . . DeVries, A. C. (2005). Social interaction improves experimental stroke outcome. *Stroke, 36*(9), 2006-2011. doi: 01.STR.0000177538.17687.54 [pii] 10.1161/01.STR.0000177538.17687.54

Cregg, J. M., DePaul, M. A., Filous, A. R., Lang, B. T., Tran, A., & Silver, J. (2014). Functional regeneration beyond the glial scar. *Exp Neurol, 253*, 197-207. doi: 10.1016/j.expneurol.2013.12.024

Cummings, S., Elde, R., Ells, J., & Lindall, A. (1983). Corticotropin-releasing factor immunoreactivity is widely distributed within the central nervous system of the rat: an immunohistochemical study. *J Neurosci, 3*(7), 1355-1368.

Cunha, C., Brambilla, R., & Thomas, K. L. (2010). A simple role for BDNF in learning and memory? *Front Mol Neurosci, 3*, 1. doi: 10.3389/neuro.02.001.2010

Dahlstrom, A., & Fuxe, K. (1964). Localization of monoamines in the lower brain stem. *Experientia, 20*(7), 398-399.

Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2011). Impulsivity, compulsivity, and top-down cognitive control. *Neuron, 69*(4), 680-694. doi: 10.1016/j.neuron.2011.01.020

Dalley, J. W., Mar, A. C., Economidou, D., & Robbins, T. W. (2008). Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. *Pharmacol Biochem Behav, 90*(2), 250-260. doi: 10.1016/j.pbb.2007.12.021

Dalley, J. W., & Roiser, J. P. (2012). Dopamine, serotonin and impulsivity. *Neuroscience, 215*, 42-58. doi: 10.1016/j.neuroscience.2012.03.065

Dancause, N., & Nudo, R. J. (2011). Shaping plasticity to enhance recovery after injury. *Prog Brain Res, 192*, 273-295. doi: B978-0-444-53355-5.00015-4 [pii] 10.1016/B978-0-444-53355-5.00015-4

Dautzenberg, F. M., & Hauger, R. L. (2002). The CRF peptide family and their receptors: yet more partners discovered. *Trends in Pharmacological Sciences, 23*(2), 71-77. doi: 10.1016/s0165-6147(02)01946-6

Davis, H. P., Baranowski, J. R., Pulsinelli, W. A., & Volpe, B. T. (1987). Retention of reference memory following ischemic hippocampal damage. *Physiol Behav, 39*(6), 783-786.

Davis, J. N., & Antonawich, F. J. (1997). Role of apoptotic proteins in ischemic hippocampal damage. *Ann N Y Acad Sci, 835*, 309-320.

de Andrade, T. M., de Melo, A. S., Dias, R. G., Varela, E. L., de Oliveira, F. R., Vieira, J. L., . . . Maia Cdo, S. (2012). Potential behavioral and pro-oxidant effects of Petiveria alliacea L. extract in adult rats. *J Ethnopharmacol, 143*(2), 604-610. doi: 10.1016/j.jep.2012.07.020

de Jong, M. F. C., Beishuizen, A., de Jong, M. J., Girbes, A. R. J., & Groeneveld, A. B. J. (2008). The pituitary-adrenal axis is activated more in non-survivors than in survivors of cardiac arrest, irrespective of therapeutic hypothermia. *Resuscitation, 78*(3), 281-288. doi: 10.1016/j.resuscitation.2008.03.227

de la Tremblaye, P. B., & Plamondon, H. (2011a). Impaired conditioned emotional response and object recognition are concomitant to neuronal damage in the amygdala and perirhinal cortex in middle-aged ischemic rats. *Behav Brain Res, 219*(2), 227-233. doi: 10.1016/j.bbr.2011.01.009

de la Tremblaye, P. B., & Plamondon, H. (2011b). Impaired conditioned emotional response and object recognition are concomitant to neuronal damage in the amygdala and perirhinal cortex in middle-aged ischemic rats. *Behav Brain Res*. doi: S0166-4328(11)00034-9 [pii] 10.1016/j.bbr.2011.01.009

de la Tremblaye, P. B., Raymond, J., Milot, M. R., Merali, Z., & Plamondon, H. (2014). Evidence of lasting dysregulation of neuroendocrine and HPA axis function following global cerebral ischemia in male rats and the effect of Antalarmin on plasma corticosterone level. *Horm Behav, 65*(3), 273-284. doi: 10.1016/j.yhbeh.2014.01.003

De Michele, M., Touzani, O., Foster, A. C., Fieschi, C., Sette, G., & McCulloch, J. (2005). Corticotropin-releasing factor: effect on cerebral blood flow in physiologic and ischaemic conditions. *Exp Brain Res, 165*(3), 375-382. doi: 10.1007/s00221-005-2303-0

de Oliveira Coelho, C. A., Ferreira, T. L., Soares, J. C., & Oliveira, M. G. (2013). Hippocampal NMDA receptor blockade impairs CREB phosphorylation in amygdala after contextual fear conditioning. *Hippocampus, 23*(7), 545-551. doi: 10.1002/hipo.22118

de Oliveira, R. M., Martin, S., de Oliveira, C. L., Milani, H., Schiavon, A. P., Joca, S., . . . Del Bel, E. A. (2012). Eag1, Eag2, and SK3 potassium channel expression in the rat hippocampus after global transient brain ischemia. *J Neurosci Res, 90*(3), 632-640. doi: 10.1002/jnr.22772

De Souza, E. B., & Van Loon, G. R. (1982). Stress-induced inhibition of the plasma corticosterone response to a subsequent stress in rats: a nonadrenocorticotropin-mediated mechanism. *Endocrinology, 110*(1), 23-33.

Deak, T., Nguyen, K. T., Ehrlich, A. L., Watkins, L. R., Spencer, R. L., Maier, S. F., . . . Gold, P. W. (1999). The impact of the nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. *Endocrinology, 140*(1), 79-86. doi: 10.1210/endo.140.1.6415

Debure, L. I., Moyse, E., Fevre-Montange, M., Hardin, H., Belin, M. F., Rousset, C., . . . Weissmann, D. (1992). Somatotopic organization of tyrosine hydroxylase expression in the rat locus coeruleus: long term effect of RU24722. *Brain Res, 581*(1), 19-32. doi: 0006-8993(92)90339-B [pii]

Delcour, M., Russier, M., Amin, M., Baud, O., Paban, V., Barbe, M. F., & Coq, J. O. (2012). Impact of prenatal ischemia on behavior, cognitive abilities and neuroanatomy in adult rats with white matter damage. *Behav Brain Res, 232*(1), 233-244. doi: 10.1016/j.bbr.2012.03.029 S0166-4328(12)00224-0 [pii]

Denes, A., Vidyasagar, R., Feng, J., Narvainen, J., McColl, B. W., Kauppinen, R. A., & Allan, S. M. (2007). Proliferating resident microglia after focal cerebral ischaemia in mice. *J Cereb Blood Flow Metab, 27*(12), 1941-1953. doi: 10.1038/sj.jcbfm.9600495

Deplanque, D., Venna, V. R., & Bordet, R. (2011). Brain ischemia changes the long term response to antidepressant drugs in mice. *Behavioural Brain Research*. doi: 10.1016/j.bbr.2011.01.003

Dermitzaki, E., Tsatsanis, C., Gravanis, A., & Margioris, A. N. (2002). Corticotropin-releasing hormone induces Fas ligand production and apoptosis in PC12 cells via activation of p38 mitogen-activated protein kinase. *J Biol Chem, 277*(14), 12280-12287. doi: 10.1074/jbc.M111236200

DeVries, A. C., Joh, H. D., Bernard, O., Hattori, K., Hurn, P. D., Traystman, R. J., & Alkayed, N. J. (2001). Social stress exacerbates stroke outcome by suppressing Bcl-2 expression. *Proc Natl Acad Sci U S A, 98*(20), 11824-11828. doi: 10.1073/pnas.201215298 201215298 [pii]

Dhooper, A., Young, C., & Reid, K. H. (1997). Ischemia-induced anxiety following cardiac arrest in the rat. *Behav Brain Res, 84*(1-2), 57-62.

Diamantopoulou, A., Raftogianni, A., Stamatakis, A., Oitzl, M. S., & Stylianopoulou, F. (2013). Effects of denial of reward through maternal contact in the neonatal period on adult hypothalamic-pituitary-adrenal axis function in the rat. *Psychoneuroendocrinology, 38*(6), 830-841. doi: DOI 10.1016/j.psyneuen.2012.09.007

Dias, B. G., Banerjee, S. B., Duman, R. S., & Vaidya, V. A. (2003). Differential regulation of brain derived neurotrophic factor transcripts by antidepressant treatments in the adult rat brain. *Neuropharmacology, 45*(4), 553-563.

DiNapoli, V. A., Huber, J. D., Houser, K., Li, X. L., & Rosen, C. L. (2008). Early disruptions of the blood-brain barrier may contribute to exacerbated neuronal damage and prolonged functional recovery following stroke in aged rats. *Neurobiol Aging, 29*(5), 753-764. doi: 10.1016/j.neurobiolaging.2006.12.007

Diorio, D., Viau, V., & Meaney, M. J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci, 13*(9), 3839-3847.

Dirks, A., Groenink, L., Schipholt, M. I., van der Gugten, J., Hijzen, T. H., Geyer, M. A., & Olivier, B. (2002). Reduced startle reactivity and plasticity in transgenic mice overexpressing corticotropin-releasing hormone. *Biol Psychiatry, 51*(7), 583-590.

Dirnagl, U., Iadecola, C., & Moskowitz, M. A. (1999). Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci, 22*(9), 391-397.

Djouma, E., Card, K., Lodge, D. J., & Lawrence, A. J. (2006). The CRF1 receptor antagonist, antalarmin, reverses isolation-induced up-regulation of dopamine D2 receptors in the amygdala and nucleus accumbens of fawn-hooded rats. *Eur J Neurosci, 23*(12), 3319-3327. doi: 10.1111/j.1460-9568.2006.04864.x

Dluzniewska, J., Beresewicz, M., Wojewodzka, U., Gajkowska, B., & Zablocka, B. (2005). Transient cerebral ischemia induces delayed proapoptotic Bad translocation to mitochondria in CA1 sector of hippocampus. *Brain Res Mol Brain Res, 133*(2), 274-280. doi: S0169-328X(04)00503-0 [pii]10.1016/j.molbrainres.2004.10.013

Dong, J. Y., Zhang, Y. H., Tong, J., & Qin, L. Q. (2012). Depression and risk of stroke: a meta-analysis of prospective studies. *Stroke, 43*(1), 32-37. doi: 10.1161/STROKEAHA.111.630871

Dore, R., Iemolo, A., Smith, K. L., Wang, X., Cottone, P., & Sabino, V. (2013). CRF mediates the anxiogenic and anti-rewarding, but not the anorectic effects of PACAP. *Neuropsychopharmacology, 38*(11), 2160-2169. doi: 10.1038/npp.2013.113 npp2013113 [pii]

Drevets, W. C., Price, J. L., & Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct, 213*(1-2), 93-118. doi: 10.1007/s00429-008-0189-x

Duman, C. H., & Duman, R. S. (2015). Spine synapse remodeling in the pathophysiology and treatment of depression. *Neurosci Lett, 601*, 20-29. doi: 10.1016/j.neulet.2015.01.022

Duman, R. S. (2004). Role of neurotrophic factors in the etiology and treatment of mood disorders. *Neuromolecular Med, 5*(1), 11-25. doi: 10.1385/NMM:5:1:011

Duman, R. S. (2005). Neurotrophic factors and regulation of mood: role of exercise, diet and metabolism. *Neurobiol Aging, 26 Suppl 1*, 88-93. doi: 10.1016/j.neurobiolaging.2005.08.018

Duman, R. S., & Monteggia, L. M. (2006). A neurotrophic model for stress-related mood disorders. *Biol Psychiatry, 59*(12), 1116-1127. doi: 10.1016/j.biopsych.2006.02.013

Dunbar, M., de la Tremblaye, P. B., Azogu, I., & Plamondon, H. (2015). Endocannabinoid CB1 receptor activation upon global ischemia adversely impact recovery of reward and stress signaling molecules, neuronal survival and behavioral impulsivity. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. doi: http://dx.doi.org/10.1016/j.pnpbp.2015.10.010

Dunn, A. J., & File, S. E. (1987). Corticotropin-Releasing Factor Has an Anxiogenic Action in the Social-Interaction Test. *Hormones and Behavior, 21*(2), 193-202. doi: Doi 10.1016/0018-506x(87)90044-4

Dwivedi, Y. (2009). Brain-derived neurotrophic factor: role in depression and suicide. *Neuropsychiatr Dis Treat, 5*, 433-449.

Dwivedi, Y., Rizavi, H. S., Conley, R. R., Roberts, R. C., Tamminga, C. A., & Pandey, G. N. (2003). Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch Gen Psychiatry, 60*(8), 804-815. doi: 10.1001/archpsyc.60.8.804

Eisch, A. J., Bolanos, C. A., de Wit, J., Simonak, R. D., Pudiak, C. M., Barrot, M., . . . Nestler, E. J. (2003). Brain-derived neurotrophic factor in the ventral midbrain-nucleus accumbens pathway: a role in depression. *Biol Psychiatry, 54*(10), 994-1005.

Ekdahl, C. T., Kokaia, Z., & Lindvall, O. (2009). Brain inflammation and adult neurogenesis: the dual role of microglia. *Neuroscience, 158*(3), 1021-1029. doi: 10.1016/j.neuroscience.2008.06.052

Elkabes, S., DiCicco-Bloom, E. M., & Black, I. B. (1996). Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *J Neurosci, 16*(8), 2508-2521.

Elkabes, S., DiCiccoBloom, E. M., & Black, I. B. (1996). Brain microglia macrophages express neurotrophins that selectively regulate microglial proliferation and function. *Journal of Neuroscience, 16*(8), 2508-2521.

Elliott-Hunt, C. R., Kazlauskaite, J., Wilde, G. J., Grammatopoulos, D. K., & Hillhouse, E. W. (2002). Potential signalling pathways underlying corticotrophin-releasing hormone-mediated neuroprotection from excitotoxicity in rat hippocampus. *J Neurochem, 80*(3), 416-425.

Empana, J. P., Sykes, D. H., Luc, G., Juhan-Vague, I., Arveiler, D., Ferrieres, J., . . . Ducimetiere, P. (2005). Contributions of depressive mood and circulating inflammatory markers to coronary heart disease in healthy European men: the Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Circulation, 111*(18), 2299-2305. doi: 01.CIR.0000164203.54111.AE [pii] 10.1161/01.CIR.0000164203.54111.AE

Endicott, J. (1998). Gender similarities and differences in the course of depression. *J Gend Specif Med, 1*(3), 40-43.

Endres, M., Fan, G., Hirt, L., Fujii, M., Matsushita, K., Liu, X., . . . Moskowitz, M. A. (2000). Ischemic brain damage in mice after selectively modifying BDNF or NT4 gene expression. *J Cereb Blood Flow Metab, 20*(1), 139-144. doi: 10.1097/00004647-200001000-00018

Enriquez-Castillo, A., Alamilla, J., Barral, J., Gourbiere, S., Flores-Serrano, A. G., Gongora-Alfaro, J. L., & Pineda, J. C. (2008). Differential effects of caffeine on the antidepressant-like effect of amitriptyline in female rat subpopulations with low and high immobility in the forced swimming test. *Physiology & Behavior, 94*(3), 501-509. doi: 10.1016/j.physbeh.2008.03.004

Erhardt, A., Muller, M. B., Rodel, A., Welt, T., Ohl, F., Holsboer, F., & Keck, M. E. (2009). Consequences of chronic social stress on behaviour and vasopressin gene expression in the PVN of DBA/2OlaHsd mice--influence of treatment with the CRHR1-antagonist R121919/NBI 30775. *J Psychopharmacol, 23*(1), 31-39. doi: 10.1177/0269881108089813

Erkut, Z. A., Pool, C., & Swaab, D. F. (1998). Glucocorticoids suppress corticotropin-releasing hormone and vasopressin expression in human hypothalamic neurons. *J Clin Endocrinol Metab, 83*(6), 2066-2073.

Escartin, C., & Bonvento, G. (2008). Targeted activation of astrocytes: a potential neuroprotective strategy. *Mol Neurobiol, 38*(3), 231-241. doi: 10.1007/s12035-008-8043-y

Esposito, P., Basu, S., Letourneau, R., Jacobson, S., & Theoharides, T. C. (2003). Corticotropin-releasing factor (CRF) can directly affect brain microvessel endothelial cells. *Brain Research, 968*(2), 192-198. doi: Doi 10.1016/S0006-8993(03)02237-6

Ewa, B. S., Beata, L., Ilona, K., Dariusz, S., & Janusz, M. (2012). Brain derived neurotrophic factor (BDNF) containing neurons in the hypothalamic paraventricular and supraoptic nuclei of juvenile and middle-aged rats after chronic stress. *Int J Dev Neurosci, 30*(2), 139-146. doi: 10.1016/j.ijdevneu.2011.12.001

Facci, L., Stevens, D. A., Pangallo, M., Franceschini, D., Skaper, S. D., & Strijbos, P. J. (2003). Corticotropin-releasing factor (CRF) and related peptides confer neuroprotection via type 1 CRF receptors. *Neuropharmacology, 45*(5), 623-636.

Fanous, S., Hammer, R. P., Jr., & Nikulina, E. M. (2010). Short- and long-term effects of intermittent social defeat stress on brain-derived neurotrophic factor expression in mesocorticolimbic brain regions. *Neuroscience, 167*(3), 598-607. doi: 10.1016/j.neuroscience.2010.02.064

Fanous, S., Terwilliger, E. F., Hammer, R. P., Jr., & Nikulina, E. M. (2011). Viral depletion of VTA BDNF in rats modulates social behavior, consequences of intermittent social defeat stress, and long-term weight regulation. *Neurosci Lett, 502*(3), 192-196. doi: 10.1016/j.neulet.2011.07.043

Faraji, J., Ejaredar, M., Metz, G. A., & Sutherland, R. J. (2011). Chronic stress prior to hippocampal stroke enhances post-stroke spatial deficits in the ziggurat task. *Neurobiol Learn Mem, 95*(3), 335-345. doi: 10.1016/j.nlm.2011.01.006

Faraji, J., Lehmann, H., Metz, G. A., & Sutherland, R. J. (2009). Stress and corticosterone enhance cognitive recovery from hippocampal stroke in rats. *Neurosci Lett, 462*(3), 248-252. doi: S0304-3940(09)00883-0 [pii] 10.1016/j.neulet.2009.06.096

Fassbender, K., Schmidt, R., Mossner, R., Daffertshofer, M., & Hennerici, M. (1994). Pattern of activation of the hypothalamic-pituitary-adrenal axis in acute stroke. Relation to acute confusional state, extent of brain damage, and clinical outcome. *Stroke, 25*(6), 1105-1108.

Faustino, J. V., Wang, X., Johnson, C. E., Klibanov, A., Derugin, N., Wendland, M. F., & Vexler, Z. S. (2011). Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. *J Neurosci, 31*(36), 12992-13001. doi: 10.1523/JNEUROSCI.2102-11.2011

Feibel, J. H., Hardy, P. M., Campbell, R. G., Goldstein, M. N., & Joynt, R. J. (1977). Prognostic value of the stress response following stroke. *JAMA, 238*(13), 1374-1376.

Feldman, S., & Conforti, N. (1985). Modifications of adrenocortical responses following frontal cortex simulation in rats with hypothalamic deafferentations and medial forebrain bundle lesions. *Neuroscience, 15*(4), 1045-1047. doi: 0306-4522(85)90253-2 [pii]

Feldman, S., Conforti, N., & Weidenfeld, J. (1995). Limbic pathways and hypothalamic neurotransmitters mediating adrenocortical responses to neural stimuli. *Neurosci Biobehav Rev, 19*(2), 235-240. doi: 0149763494000626 [pii]

Feldman, S., & Weidenfeld, J. (1999). Glucocorticoid receptor antagonists in the hippocampus modify the negative feedback following neural stimuli. *Brain Res, 821*(1), 33-37. doi: S0006-8993(99)01054-9 [pii]

Fenoglio, K. A., Brunson, K. L., & Baram, T. Z. (2006). Hippocampal neuroplasticity induced by early-life stress: functional and molecular aspects. *Front Neuroendocrinol, 27*(2), 180-192. doi: 10.1016/j.yfrne.2006.02.001

Ferdman, N., Murmu, R. P., Bock, J., Braun, K., & Leshem, M. (2007). Weaning age, social isolation, and gender, interact to determine adult explorative and social behavior, and dendritic and spine morphology in prefrontal cortex of rats. *Behav Brain Res, 180*(2), 174-182. doi: S0166-4328(07)00154-4 [pii] 10.1016/j.bbr.2007.03.011

Ferland, J. M. N., Zeeb, F. D., Yu, K., Kaur, S., Taves, M. D., & Winstanley, C. A. (2014). Greater sensitivity to novelty in rats is associated with increased motor impulsivity following repeated exposure to a stimulating environment: implications for the etiology of impulse control deficits. *European Journal of Neuroscience, 40*(12), 3746-3756. doi: 10.1111/ejn.12748

Ferrini, F., & De Koninck, Y. (2013). Microglia control neuronal network excitability via BDNF signalling. *Neural Plast, 2013*, 429815. doi: 10.1155/2013/429815

Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T., & Lu, B. (1996). Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature, 381*(6584), 706-709. doi: 10.1038/381706a0

File, S. E. (1980). The Use of Social-Interaction as a Method for Detecting Anxiolytic Activity of Chlordiazepoxide-Like Drugs. *Journal of Neuroscience Methods, 2*(3), 219-238. doi: Doi 10.1016/0165-0270(80)90012-6

File, S. E., & Hyde, J. R. G. (1978). Can Social-Interaction Be Used to Measure Anxiety. *British Journal of Pharmacology, 62*(1), 19-24.

Filho, C. B., Jesse, C. R., Donato, F., Giacomeli, R., Del Fabbro, L., da Silva Antunes, M., . . . Souza, L. C. (2015). Chronic unpredictable mild stress decreases BDNF and NGF levels and Na(+),K(+)-ATPase activity in the hippocampus and prefrontal cortex of mice: antidepressant effect of chrysin. *Neuroscience, 289*, 367-380. doi: 10.1016/j.neuroscience.2014.12.048

Fix, A. S., Horn, J. W., Wightman, K. A., Johnson, C. A., Long, G. G., Storts, R. W., . . . Olney, J. W. (1993). Neuronal vacuolization and necrosis induced by the noncompetitive N-methyl-D-aspartate (NMDA) antagonist MK(+)801 (dizocilpine maleate): a light and electron microscopic evaluation of the rat retrosplenial cortex. *Exp Neurol, 123*(2), 204-215. doi: 10.1006/exnr.1993.1153

Flanagan, D. E., Keshavarz, T., Evans, M. L., Flanagan, S., Fan, X., Jacob, R. J., & Sherwin, R. S. (2003). Role of corticotrophin-releasing hormone in the impairment of counterregulatory responses to hypoglycemia. *Diabetes, 52*(3), 605-613.

Flandreau, E. I., Ressler, K. J., Owens, M. J., & Nemeroff, C. B. (2012). Chronic overexpression of corticotropin-releasing factor from the central amygdala produces HPA axis hyperactivity and behavioral anxiety associated with gene-expression changes in the hippocampus and paraventricular nucleus of the hypothalamus. *Psychoneuroendocrinology, 37*(1), 27-38. doi: 10.1016/j.psyneuen.2011.04.014

Fox, M. W., Anderson, R. E., & Meyer, F. B. (1993). Neuroprotection by corticotropin releasing factor during hypoxia in rat brain. *Stroke, 24*(7), 1072-1075; discussion 1075-1076.

French, J. A., Fite, J. E., Jensen, H., Oparowski, K., Rukstalis, M. R., Fix, H., . . . Schulkin, J. (2007). Treatment with CRH-1 antagonist antalarmin reduces behavioral and endocrine responses to social stressors in marmosets (Callithrix kuhlii). *Am J Primatol, 69*(8), 877-889. doi: 10.1002/ajp.20385

Frenkel, D., Huang, Z., Maron, R., Koldzic, D. N., Hancock, W. W., Moskowitz, M. A., & Weiner, H. L. (2003). Nasal vaccination with myelin oligodendrocyte glycoprotein reduces stroke size by inducing IL-10-producing CD4+ T cells. *J Immunol, 171*(12), 6549-6555.

Freund, N., MacGillivilray, H. T., Thompson, B. S., Lukkes, J. L., Stanis, J. J., Brenhouse, H. C., & Andersen, S. L. (2014). Sex-dependent changes in ADHD-like behaviors in juvenile rats following cortical dopamine depletion. *Behav Brain Res, 270*, 357-363. doi: 10.1016/j.bbr.2014.05.024

Fuchikami, M., Morinobu, S., Kurata, A., Yamamoto, S., & Yamawaki, S. (2009). Single immobilization stress differentially alters the expression profile of transcripts of the brain-derived neurotrophic factor (BDNF) gene and histone acetylation at its promoters in the rat hippocampus. *Int J Neuropsychopharmacol, 12*(1), 73-82. doi: S1461145708008997 [pii]10.1017/S1461145708008997

Fuge, P., Aust, S., Fan, Y., Weigand, A., Gartner, M., Feeser, M., . . . Grimm, S. (2014). Interaction of early life stress and corticotropin-releasing hormone receptor gene: effects on working memory. *Biol Psychiatry, 76*(11), 888-894. doi: 10.1016/j.biopsych.2014.04.016

Fukudo, S. (2007). Role of corticotropin-releasing hormone in irritable bowel syndrome and intestinal inflammation. *J Gastroenterol, 42 Suppl 17*, 48-51. doi: 10.1007/s00535-006-1942-7

Fultz, N. H., Ofstedal, M. B., Herzog, A. R., & Wallace, R. B. (2003). Additive and interactive effects of comorbid physical and mental conditions on functional health. *J Aging Health, 15*(3), 465-481.

Fure, B., Wyller, T. B., Engedal, K., & Thommessen, B. (2006). Emotional symptoms in acute ischemic stroke. *International Journal of Geriatric Psychiatry, 21*(4), 382-387. doi: 10.1002/gps.1482

Gadek-Michalska, A., Tadeusz, J., Rachwalska, P., & Bugajski, J. (2013). Cytokines, prostaglandins and nitric oxide in the regulation of stress-response systems. *Pharmacol Rep, 65*(6), 1655-1662.

Gainotti, G., Acciarri, A., Bizzarro, A., Marra, C., Masullo, C., Misciagna, S., . . . Colosimo, C. (2004). The role of brain infarcts and hippocampal atrophy in subcortical ischaemic vascular dementia. *Neurol Sci, 25*(4), 192-197. doi: 10.1007/s10072-004-0321-5

Gainotti, G., Azzoni, A., & Marra, C. (1999). Frequency, phenomenology and anatomical-clinical correlates of major post-stroke depression. *Br J Psychiatry, 175*, 163-167.

Gallagher, J. P., Orozco-Cabal, L. F., Liu, J., & Shinnick-Gallagher, P. (2008). Synaptic physiology of central CRH system. *Eur J Pharmacol, 583*(2-3), 215-225. doi: 10.1016/j.ejphar.2007.11.075

Gaur, V., & Kumar, A. (2010). Behavioral, biochemical and cellular correlates in the protective effect of sertraline against transient global ischemia induced behavioral despair: possible involvement of nitric oxide-cyclic guanosine monophosphate study pathway. *Brain Res Bull, 82*(1-2), 57-64. doi: 10.1016/j.brainresbull.2010.01.010 S0361-9230(10)00029-8 [pii]

Gehlert, D. R., Shekhar, A., Morin, S. M., Hipskind, P. A., Zink, C., Gackenheimer, S. L., . . . Sajdyk, T. J. (2005). Stress and central Urocortin increase anxiety-like behavior in the social interaction test via the CRF1 receptor. *Eur J Pharmacol, 509*(2-3), 145-153. doi: 10.1016/j.ejphar.2004.12.030

Gehrmann, J., Banati, R. B., Wiessner, C., Hossmann, K. A., & Kreutzberg, G. W. (1995). Reactive microglia in cerebral ischaemia: an early mediator of tissue damage? *Neuropathol Appl Neurobiol, 21*(4), 277-289.

Gentile, N. E., Andrekanic, J. D., Karwoski, T. E., Czambel, R. K., Rubin, R. T., & Rhodes, M. E. (2011). Sexually diergic hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine infusion, and nicotine withdrawal by mecamylamine in rats. *Brain Res Bull, 85*(3-4), 145-152. doi: 10.1016/j.brainresbull.2011.03.001

Gheibi, N., Saroukhani, M., & Azhdari-Zarmehri, H. (2013). The effect of food deprivation on nociception in formalin test and plasma levels of noradrenaline and corticosterone in rats. *Basic Clin Neurosci, 4*(4), 341-347.

Ghitza, U. E., Zhai, H., Wu, P., Airavaara, M., Shaham, Y., & Lu, L. (2010). Role of BDNF and GDNF in drug reward and relapse: a review. *Neurosci Biobehav Rev, 35*(2), 157-171. doi: 10.1016/j.neubiorev.2009.11.009

Gibbons, T. E., Pence, B. D., Petr, G., Ossyra, J. M., Mach, H. C., Bhattacharya, T. K., . . . Woods, J. A. (2014). Voluntary wheel running, but not a diet containing (-)-epigallocatechin-3-gallate and beta-alanine, improves learning, memory and hippocampal

neurogenesis in aged mice. *Behav Brain Res, 272*, 131-140. doi: 10.1016/j.bbr.2014.05.049

Gibson, C. L., Gray, L. J., Murphy, S. P., & Bath, P. M. (2006). Estrogens and experimental ischemic stroke: a systematic review. *J Cereb Blood Flow Metab, 26*(9), 1103-1113. doi: 10.1038/sj.jcbfm.9600270

Gillardon, F., Kiprianova, I., Sandkuhler, J., Hossmann, K. A., & Spranger, M. (1999). Inhibition of caspases prevents cell death of hippocampal CA1 neurons, but not impairment of hippocampal long-term potentiation following global ischemia. *Neuroscience, 93*(4), 1219-1222.

Ginsberg, M. D. (2008). Neuroprotection for ischemic stroke: past, present and future. *Neuropharmacology, 55*(3), 363-389. doi: 10.1016/j.neuropharm.2007.12.007

Gionet, T. X., Thomas, J. D., Warner, D. S., Goodlett, C. R., Wasserman, E. A., & West, J. R. (1991). Forebrain ischemia induces selective behavioral impairments associated with hippocampal injury in rats. *Stroke, 22*(8), 1040-1047.

Girbovan, C., Morin, L., & Plamondon, H. (2011). Repeated resveratrol administration confers lasting protection against neuronal damage but induces dose-related alterations of behavioral impairments after global ischemia. *Behav Pharmacol*. doi: 10.1097/FBP.0b013e32834eafa3

Girbovan, C., & Plamondon, H. (2015). Resveratrol downregulates type-1 glutamate transporter expression and microglia activation in the hippocampus following cerebral ischemia reperfusion in rats. *Brain Res, 1608*, 203-214. doi: 10.1016/j.brainres.2015.02.038

Givalois, L., Naert, G., Rage, F., Ixart, G., Arancibia, S., & Tapia-Arancibia, L. (2004). A single brain-derived neurotrophic factor injection modifies hypothalamo-pituitary-adrenocortical axis activity in adult male rats. *Mol Cell Neurosci, 27*(3), 280-295. doi: 10.1016/j.mcn.2004.07.002

Globus, M. Y., Busto, R., Dietrich, W. D., Martinez, E., Valdes, I., & Ginsberg, M. D. (1989). Direct evidence for acute and massive norepinephrine release in the hippocampus during transient ischemia. *J Cereb Blood Flow Metab, 9*(6), 892-896.

Gobbo, O. L., & O'Mara, S. M. (2004). Impact of enriched-environment housing on brain-derived neurotrophic factor and on cognitive performance after a transient global ischemia. *Behav Brain Res, 152*(2), 231-241. doi: 10.1016/j.bbr.2003.10.017

Goerdt, S., Politz, O., Schledzewski, K., Birk, R., Gratchev, A., Guillot, P., . . . Orfanos, C. E. (1999). Alternative versus classical activation of macrophages. *Pathobiology, 67*(5-6), 222-226. doi: 28096 [pii]
28096

Golab, M., & Skwarlo-Sonta, K. (2007). Mechanisms involved in the anti-inflammatory action of inhaled tea tree oil in mice. *Exp Biol Med (Maywood), 232*(3), 420-426.

Gold, P. W., & Chrousos, G. P. (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol Psychiatry, 7*(3), 254-275. doi: 10.1038/sj.mp.4001032

Gold, P. W., Loriaux, D. L., Roy, A., Kling, M. A., Calabrese, J. R., Kellner, C. H., . . . et al. (1986). Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. Pathophysiologic and diagnostic implications. *N Engl J Med, 314*(21), 1329-1335. doi: 10.1056/NEJM198605223142101

Goldstein, D. S. (2010). Adrenal responses to stress. *Cell Mol Neurobiol, 30*(8), 1433-1440. doi: 10.1007/s10571-010-9606-9

Goldwater, D. S., Pavlides, C., Hunter, R. G., Bloss, E. B., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2009). Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience, 164*(2), 798-808. doi: 10.1016/j.neuroscience.2009.08.053 S0306-4522(09)01404-3 [pii]

Goodman, Y., Bruce, A. J., Cheng, B., & Mattson, M. P. (1996). Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. *J Neurochem, 66*(5), 1836-1844.

Gordan, M. L., Jungwirth, B., Ohl, F., Kellermann, K., Kochs, E. F., & Blobner, M. (2012). Evaluation of neurobehavioral deficits following different severities of cerebral ischemia in rats: A comparison between the modified hole board test and the Morris water maze test. *Behav Brain Res, 235*(1), 7-20. doi: 10.1016/j.bbr.2012.07.027

Gorisch, J., & Schwarting, R. K. (2006). Wistar rats with high versus low rearing activity differ in radial maze performance. *Neurobiol Learn Mem, 86*(2), 175-187. doi: 10.1016/j.nlm.2006.02.004

Gothe, F., Enache, D., Wahlund, L. O., Winblad, B., Crisby, M., Lokk, J., & Aarsland, D. (2012). Cerebrovascular diseases and depression: epidemiology, mechanisms and treatment. *Panminerva Med, 54*(3), 161-170. doi: R41122731 [pii]

Gougoura, S., Liakos, P., & Koukoulis, G. N. (2010). Effect of CRH on NO bioavailability, ROS production and antioxidant defense systems in endothelial EAhy926 cells. *Free Radic Res, 44*(7), 803-812. doi: 10.3109/10715762.2010.485988

Gounko, N. V., Swinny, J. D., Kalicharan, D., Jafari, S., Corteen, N., Seifi, M., . . . van der Want, J. J. (2012). Corticotropin-releasing factor and urocortin regulate spine and synapse formation: structural basis for stress-induced neuronal remodeling and pathology. *Mol Psychiatry, 18*(1), 86-92. doi: 10.1038/mp.2012.43 mp201243 [pii]

Gouveia, K., & Hurst, J. L. (2013). Reducing mouse anxiety during handling: effect of experience with handling tunnels. *PLoS One, 8*(6), e66401. doi: 10.1371/journal.pone.0066401

Govindarajan, A., Rao, B. S., Nair, D., Trinh, M., Mawjee, N., Tonegawa, S., & Chattarji, S. (2006). Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. *Proc Natl Acad Sci U S A, 103*(35), 13208-13213. doi: 10.1073/pnas.0605180103

Grabe, H. J., Schwahn, C., Appel, K., Mahler, J., Schulz, A., Spitzer, C., . . . Volzke, H. (2010). Childhood maltreatment, the corticotropin-releasing hormone receptor gene and adult depression in the general population. *Am J Med Genet B Neuropsychiatr Genet, 153B*(8), 1483-1493. doi: 10.1002/ajmg.b.31131

Graham, D. L., Krishnan, V., Larson, E. B., Graham, A., Edwards, S., Bachtell, R. K., . . . Self, D. W. (2009). Tropomyosin-related kinase B in the mesolimbic dopamine system: region-specific effects on cocaine reward. *Biol Psychiatry, 65*(8), 696-701. doi: 10.1016/j.biopsych.2008.09.032

Graves, A. R., Moore, S. J., Bloss, E. B., Mensh, B. D., Kath, W. L., & Spruston, N. (2012). Hippocampal pyramidal neurons comprise two distinct cell types that are countermodulated by metabotropic receptors. *Neuron, 76*(4), 776-789. doi: 10.1016/j.neuron.2012.09.036

Greenberg, M. R., Ahnert, A. M., Patel, N. C., Bennett, C. E., Elliott, N., Lundquist, M., . . . Burmeister, D. B. (2014). Sex differences in cardiac arrest survivors who receive therapeutic hypothermia. *Am J Emerg Med, 32*(6), 545-548. doi: 10.1016/j.ajem.2014.02.004

Greenwood-Van Meerveld, B., Johnson, A. C., Schulkin, J., & Myers, D. A. (2006). Long-term expression of corticotropin-releasing factor (CRF) in the paraventricular nucleus of the hypothalamus in response to an acute colonic inflammation. *Brain Res, 1071*(1), 91-96. doi: 10.1016/j.brainres.2005.11.071

Greetfeld, M., Schmidt, M. V., Ganea, K., Sterlemann, V., Liebl, C., & Muller, M. B. (2009). A single episode of restraint stress regulates central corticotrophin- releasing hormone receptor expression and binding in specific areas of the mouse brain. *J Neuroendocrinol, 21*(5), 473-480. doi: JNE1865 [pii] 10.1111/j.1365-2826.2009.01865.x

Gregus, A., Wintink, A. J., Davis, A. C., & Kalynchuk, L. E. (2005). Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behav Brain Res, 156*(1), 105-114. doi: 10.1016/j.bbr.2004.05.013

Griebel, G., Simiand, J., Steinberg, R., Jung, M., Gully, D., Roger, P., . . . Soubrie, P. (2002). 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]5-methyl-N-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization in rodent models of stress-related disorders. *Journal of Pharmacology and Experimental Therapeutics, 301*(1), 333-345. doi: Unsp 4642/974299 Doi 10.1124/Jpet.301.1.333

Grimm, S., Gartner, M., Fuge, P., Fan, Y., Weigand, A., Feeser, M., . . . Bajbouj, M. (2015). Variation in the corticotropin-releasing hormone receptor 1 (CRHR1) gene modulates age effects on working memory. *J Psychiatr Res, 61*, 57-63. doi: 10.1016/j.jpsychires.2014.12.001

Groenink, L., Pattij, T., De Jongh, R., Van der Gugten, J., Oosting, R. S., Dirks, A., & Olivier, B. (2003). 5-HT1A receptor knockout mice and mice overexpressing corticotropin-releasing hormone in models of anxiety. *Eur J Pharmacol, 463*(1-3), 185-197.

Guan, J., Tong, W., Ding, W., Du, S., Xiao, Z., Han, Q., . . . Wang, R. (2012). Neuronal regeneration and protection by collagen-binding BDNF in the rat middle cerebral artery occlusion model. *Biomaterials, 33*(5), 1386-1395. doi: 10.1016/j.biomaterials.2011.10.073

Guiraud, V., Amor, M. B., Mas, J. L., & Touze, E. (2010). Triggers of ischemic stroke: a systematic review. *Stroke, 41*(11), 2669-2677. doi: 10.1161/STROKEAHA.110.597443

Guo, S., Som, A. T., Waeber, C., & Lo, E. H. (2012). Vascular neuroprotection via TrkB- and Akt-dependent cell survival signaling. *J Neurochem, 123 Suppl 2*, 58-64. doi: 10.1111/j.1471-4159.2012.07944.x

Guo, Y.-j., Zhang, Z.-j., Wang, S.-h., Sui, Y.-x., & Sun, Y. (2009). Notch1 signaling, hippocampal neurogenesis and behavioral responses to chronic unpredicted mild stress in adult ischemic rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 33*(4), 688-694. doi: 10.1016/j.pnpbp.2009.03.022

Gustafson, I., Westerberg, E. J., & Wieloch, T. (1991). Extracellular brain cortical levels of noradrenaline in ischemia: effects of desipramine and postischemic administration of idazoxan. *Exp Brain Res, 86*(3), 555-561.

Guzowski, J. F., & McGaugh, J. L. (1997). Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. *Proc Natl Acad Sci U S A, 94*(6), 2693-2698.

Haast, R. A. M., Gustafson, D. R., & Kiliaan, A. J. (2012). Sex differences in stroke. *Journal of Cerebral Blood Flow and Metabolism, 32*(12), 2100-2107. doi: 10.1038/jcbfm.2012.141

Hackett, M. L., Anderson, C. S., House, A., & Xia, J. (2008). Interventions for treating depression after stroke. *Cochrane Database Syst Rev*(4), CD003437. doi: 10.1002/14651858.CD003437.pub3

Hackett, M. L., Yapa, C., Parag, V., & Anderson, C. S. (2005). Frequency of Depression After Stroke. *Stroke, 36*(6), 1330-1340. doi: 10.1161/01.str.0000165928.19135.35

Haddad, J. J., Saade, N. E., & Safieh-Garabedian, B. (2002). Cytokines and neuro-immune-endocrine interactions: a role for the hypothalamic-pituitary-adrenal revolving axis. *J Neuroimmunol, 133*(1-2), 1-19.

Hadidi, N., Treat-Jacobson, D. J., & Lindquist, R. (2009). Poststroke depression and functional outcome: a critical review of literature. *Heart Lung, 38*(2), 151-162. doi: S0147-9563(08)00097-6 [pii]
10.1016/j.hrtlng.2008.05.002

Hadley, G., Papadakis, M., & Buchan, A. M. (2014). A method of inducing global cerebral ischemia. *Methods Mol Biol, 1135*, 111-120. doi: 10.1007/978-1-4939-0320-7_10

Hagan, J. J., & Beaughard, M. (1990). The effects of forebrain ischaemia on spatial learning. *Behav Brain Res, 41*(2), 151-160. doi: 0166-4328(90)90150-D [pii]

Hall, J., Thomas, K. L., & Everitt, B. J. (2000). Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nat Neurosci, 3*(6), 533-535. doi: 10.1038/75698

Hama, S., Yamashita, H., Shigenobu, M., Watanabe, A., Kurisu, K., Yamawaki, S., & Kitaoka, T. (2007). Post-stroke affective or apathetic depression and lesion location: left frontal lobe and bilateral basal ganglia. *Eur Arch Psychiatry Clin Neurosci, 257*(3), 149-152. doi: 10.1007/s00406-006-0698-7

Han, F., Ozawa, H., Matsuda, K. I., Lu, H., De Kloet, E. R., & Kawata, M. (2007). Changes in the expression of corticotrophin-releasing hormone, mineralocorticoid receptor and glucocorticoid receptor mRNAs in the hypothalamic paraventricular nucleus induced by fornix transection and adrenalectomy. *J Neuroendocrinol, 19*(4), 229-238. doi: JNE1519 [pii]
10.1111/j.1365-2826.2006.01519.x

Handa, R. J., Burgess, L. H., Kerr, J. E., & O'Keefe, J. A. (1994). Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Horm Behav, 28*(4), 464-476. doi: 10.1006/hbeh.1994.1044

Hanell, A., & Marklund, N. (2014). Structured evaluation of rodent behavioral tests used in drug discovery research. *Frontiers in Behavioral Neuroscience, 8*. doi: Artn 252
10.3389/Fnbeh.2014.00252

Hanstein, R., Lu, A., Wurst, W., Holsboer, F., Deussing, J. M., Clement, A. B., & Behl, C. (2008). Transgenic overexpression of corticotropin releasing hormone provides partial protection against neurodegeneration in an in vivo model of acute excitotoxic stress. *Neuroscience, 156*(3), 712-721. doi: 10.1016/j.neuroscience.2008.07.034

Harburger, L. L., Nzerem, C. K., & Frick, K. M. (2007). Single enrichment variables differentially reduce age-related memory decline in female mice. *Behav Neurosci, 121*(4), 679-688. doi: 10.1037/0735-7044.121.4.679

Harrison, F. E., Reiserer, R. S., Tomarken, A. J., & McDonald, M. P. (2006). Spatial and nonspatial escape strategies in the Barnes maze. *Learn Mem, 13*(6), 809-819. doi: 10.1101/lm.334306

Hartley, C. A., & Phelps, E. A. (2010). Changing fear: the neurocircuitry of emotion regulation. *Neuropsychopharmacology, 35*(1), 136-146. doi: 10.1038/npp.2009.121

Hartline, K. M., Owens, M. J., & Nemeroff, C. B. (1996). Postmortem and cerebrospinal fluid studies of corticotropin-releasing factor in humans. *Ann N Y Acad Sci, 780*, 96-105.

Hartman, R., Lee, J., Zipfel, G., & Wozniak, D. (2005). Characterizing learning deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. *Brain Research, 1043*(1-2), 48-56. doi: 10.1016/j.brainres.2005.02.030

Hartman, R. E., Lee, J. M., Zipfel, G. J., & Wozniak, D. F. (2005). Characterizing learning deficits and hippocampal neuron loss following transient global cerebral ischemia in rats. *Brain Res, 1043*(1-2), 48-56. doi: S0006-8993(05)00298-2 [pii] 10.1016/j.brainres.2005.02.030

Harukuni, I., & Bhardwaj, A. (2006). Mechanisms of brain injury after global cerebral ischemia. *Neurol Clin, 24*(1), 1-21. doi: 10.1016/j.ncl.2005.10.004

Hashizume, M., Hachisu, M., Yoshida, H., Kim, M., Kim, H. K., Amano, Y., . . . Ihara, K. (2015). Serum brain-derived neurotrophic factor level in elderly women depression: a community-based study. *Prog Neuropsychopharmacol Biol Psychiatry, 56*, 109-116. doi: 10.1016/j.pnpbp.2014.08.012

Hatzinger, M., Brand, S., Herzig, N., & Holsboer-Trachsler, E. (2011). In healthy young and elderly adults, hypothalamic-pituitary-adrenocortical axis reactivity (HPA AR) varies with increasing pharmacological challenge and with age, but not with gender. *J Psychiatr Res, 45*(10), 1373-1380. doi: 10.1016/j.jpsychires.2011.05.006

Hauger, R. L., Risbrough, V., Oakley, R. H., Olivares-Reyes, J. A., & Dautzenberg, F. M. (2009). Role of CRF receptor signaling in stress vulnerability, anxiety, and depression. *Ann N Y Acad Sci, 1179*, 120-143. doi: 10.1111/j.1749-6632.2009.05011.x

Hawley, D. F., Morch, K., Christie, B. R., & Leasure, J. L. (2012). Differential response of hippocampal subregions to stress and learning. *PLoS One, 7*(12), e53126. doi: 10.1371/journal.pone.0053126

Hayes, D. M., Knapp, D. J., Breese, G. R., & Thiele, T. E. (2005). Comparison of basal neuropeptide Y and corticotropin releasing factor levels between the high ethanol drinking C57BL/6J and low ethanol drinking DBA/2J inbred mouse strains. *Alcohol Clin Exp Res, 29*(5), 721-729. doi: 00000374-200505000-00004 [pii]

Hayley, S., Du, L., Litteljohn, D., Palkovits, M., Faludi, G., Merali, Z., . . . Anisman, H. (2015). Gender and brain regions specific differences in brain derived neurotrophic factor protein levels of depressed individuals who died through suicide. *Neurosci Lett, 600*, 12-16. doi: 10.1016/j.neulet.2015.05.052

Hehar, H., Yeates, K., Kolb, B., Esser, M. J., & Mychasiuk, R. (2015). Impulsivity and Concussion in Juvenile Rats: Examining Molecular and Structural Aspects of the Frontostriatal Pathway. *PLoS One, 10*(10), e0139842. doi: 10.1371/journal.pone.0139842

Hei, M. Y., Luo, Y. L., Zhang, X. C., Liu, H., Gao, R., & Wu, J. J. (2012). Hypoxic-ischemic injury decreases anxiety-like behavior in rats when associated with loss of tyrosine-

hydroxylase immunoreactive neurons of the substantia nigra. *Brazilian Journal of Medical and Biological Research, 45*(1), 13-19. doi: Doi 10.1590/S0100-879x2011007500161

Heim, C., Bradley, B., Mletzko, T. C., Deveau, T. C., Musselman, D. L., Nemeroff, C. B., . . . Binder, E. B. (2009). Effect of Childhood Trauma on Adult Depression and Neuroendocrine Function: Sex-Specific Moderation by CRH Receptor 1 Gene. *Front Behav Neurosci, 3*, 41. doi: 10.3389/neuro.08.041.2009

Heinrichs, S. C. (2003). Modulation of social learning in rats by brain corticotropin-releasing factor. *Brain Res, 994*(1), 107-114. doi: S0006899303037442 [pii]

Heinrichs, S. C., & Koob, G. F. (2004). Corticotropin-releasing factor in brain: a role in activation, arousal, and affect regulation. *J Pharmacol Exp Ther, 311*(2), 427-440. doi: 10.1124/jpet.103.052092
jpet.103.052092 [pii]

Heinrichs, S. C., Lapsansky, J., Lovenberg, T. W., De Souza, E. B., & Chalmers, D. T. (1997). Corticotropin-releasing factor CRF1, but not CRF2, receptors mediate anxiogenic-like behavior. *Regul Pept, 71*(1), 15-21. doi: S0167-0115(97)01005-7 [pii]

Heinrichs, S. C., Stenzel-Poore, M. P., Gold, L. H., Battenberg, E., Bloom, F. E., Koob, G. F., . . . Pich, E. M. (1996). Learning impairment in transgenic mice with central overexpression of corticotropin-releasing factor. *Neuroscience, 74*(2), 303-311.

Heiss, W. D. (2012). The ischemic penumbra: how does tissue injury evolve? *Ann N Y Acad Sci, 1268*, 26-34. doi: 10.1111/j.1749-6632.2012.06668.x

Helgager, J., Liu, G., & McNamara, J. O. (2013). The cellular and synaptic location of activated TrkB in mouse hippocampus during limbic epileptogenesis. *J Comp Neurol, 521*(3), 499-521, Spc491. doi: 10.1002/cne.23225

Helmeke, C., Ovtscharoff, W., Jr., Poeggel, G., & Braun, K. (2001). Juvenile emotional experience alters synaptic inputs on pyramidal neurons in the anterior cingulate cortex. *Cereb Cortex, 11*(8), 717-727.

Helmeke, C., Poeggel, G., & Braun, K. (2001). Differential emotional experience induces elevated spine densities on basal dendrites of pyramidal neurons in the anterior cingulate cortex of Octodon degus. *Neuroscience, 104*(4), 927-931. doi: S0306452201002019 [pii]

Hendryk, S., Jedrzejowska-Szypulka, H., Josko, J., Jarzab, B., & Dohler, K. D. (2002). Influence of the corticotropin releasing hormone (CRH) on the brain-blood barrier permeability in cerebral ischemia in rats. *J Physiol Pharmacol, 53*(1), 85-94.

Hennigan, A., O'Callaghan, R. M., & Kelly, A. M. (2007). Neurotrophins and their receptors: roles in plasticity, neurodegeneration and neuroprotection. *Biochemical Society Transactions, 35*, 424-427.

Henrich-Noack, P., Krautwald, K., Reymann, K. G., & Wetzel, W. (2011). Effects of transient global ischaemia on freezing behaviour and activity in a context-dependent fear conditioning task - Implications for memory investigations. *Brain Res Bull, 85*(6), 346-353. doi: 10.1016/j.brainresbull.2011.04.001

Hering, H., & Sheng, M. (2001). Dendritic spines: structure, dynamics and regulation. *Nat Rev Neurosci, 2*(12), 880-888. doi: 10.1038/35104061

Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry, 29*(8), 1201-1213. doi: S0278-5846(05)00269-1 [pii]

10.1016/j.pnpbp.2005.08.006
Hermes, G., Li, N., Duman, C., & Duman, R. (2011). Post-weaning chronic social isolation produces profound behavioral dysregulation with decreases in prefrontal cortex synaptic-associated protein expression in female rats. *Physiol Behav, 104*(2), 354-359. doi: 10.1016/j.physbeh.2010.12.019
S0031-9384(10)00466-X [pii]
Herrero, A. I., Sandi, C., & Venero, C. (2006). Individual differences in anxiety trait are related to spatial learning abilities and hippocampal expression of mineralocorticoid receptors. *Neurobiol Learn Mem, 86*(2), 150-159. doi: 10.1016/j.nlm.2006.02.001
Heuser, I., Bissette, G., Dettling, M., Schweiger, U., Gotthardt, U., Schmider, J., . . . Holsboer, F. (1998). Cerebrospinal fluid concentrations of corticotropin-releasing hormone, vasopressin, and somatostatin in depressed patients and healthy controls: response to amitriptyline treatment. *Depress Anxiety, 8*(2), 71-79.
Himmerich, H., Binder, E. B., Kunzel, H. E., Schuld, A., Lucae, S., Uhr, M., . . . Ising, M. (2006). Successful antidepressant therapy restores the disturbed interplay between TNF-alpha system and HPA axis. *Biol Psychiatry, 60*(8), 882-888. doi: 10.1016/j.biopsych.2006.03.075
Ho, Y. J., Eichendorff, J., & Schwarting, R. K. (2002). Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behav Brain Res, 136*(1), 1-12.
Hock, C., Heese, K., Hulette, C., Rosenberg, C., & Otten, U. (2000). Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch Neurol, 57*(6), 846-851. doi: noc90083 [pii]
Hodes, G. E. (2013). Sex, stress, and epigenetics: regulation of behavior in animal models of mood disorders. *Biology of Sex Differences, 4*. doi: Artn 1
Doi 10.1186/2042-6410-4-1
Hodges, H., Sowinski, P., Fleming, P., Kershaw, T. R., Sinden, J. D., Meldrum, B. S., & Gray, J. A. (1996). Contrasting effects of fetal CA1 and CA3 hippocampal grafts on deficits in spatial learning and working memory induced by global cerebral ischaemia in rats. *Neuroscience, 72*(4), 959-988.
Hofer, M., Pagliusi, S. R., Hohn, A., Leibrock, J., & Barde, Y. A. (1990). Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO J, 9*(8), 2459-2464.
Holdcroft, A. (2007). Gender bias in research: how does it affect evidence based medicine? *Journal of the Royal Society of Medicine, 100*(1), 2-3. doi: Doi 10.1258/Jrsm.100.1.2
Hollrigel, G. S., Chen, K., Baram, T. Z., & Soltesz, I. (1998). The pro-convulsant actions of corticotropin-releasing hormone in the hippocampus of infant rats. *Neuroscience, 84*(1), 71-79.
Holsboer, F., & Barden, N. (1996). Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr Rev, 17*(2), 187-205.
Holsboer, F., & Ising, M. (2008). Central CRH system in depression and anxiety--evidence from clinical studies with CRH1 receptor antagonists. *Eur J Pharmacol, 583*(2-3), 350-357. doi: 10.1016/j.ejphar.2007.12.032
Holtmaat, A., & Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat Rev Neurosci, 10*(9), 647-658. doi: 10.1038/nrn2699

Horch, H. W., Kruttgen, A., Portbury, S. D., & Katz, L. C. (1999). Destabilization of cortical dendrites and spines by BDNF. *Neuron, 23*(2), 353-364.

Horger, B. A., Iyasere, C. A., Berhow, M. T., Messer, C. J., Nestler, E. J., & Taylor, J. R. (1999). Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J Neurosci, 19*(10), 4110-4122.

Hostetler, C. M., & Ryabinin, A. E. (2013). The CRF system and social behavior: a review. *Front Neurosci, 7*, 92. doi: 10.3389/fnins.2013.00092

Howells, D. W., Porritt, M. J., Wong, J. Y., Batchelor, P. E., Kalnins, R., Hughes, A. J., & Donnan, G. A. (2000). Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Exp Neurol, 166*(1), 127-135. doi: 10.1006/exnr.2000.7483

Hsu, D. T., Chen, F. L., Takahashi, L. K., & Kalin, N. H. (1998). Rapid stress-induced elevations in corticotropin-releasing hormone mRNA in rat central amygdala nucleus and hypothalamic paraventricular nucleus: an in situ hybridization analysis. *Brain Res, 788*(1-2), 305-310. doi: S0006-8993(98)00032-8 [pii]

Hsu, D. T., Mickey, B. J., Langenecker, S. A., Heitzeg, M. M., Love, T. M., Wang, H., . . . Zubieta, J. K. (2012). Variation in the corticotropin-releasing hormone receptor 1 (CRHR1) gene influences fMRI signal responses during emotional stimulus processing. *J Neurosci, 32*(9), 3253-3260. doi: 10.1523/JNEUROSCI.5533-11.2012

Huang, E. J., & Reichardt, L. F. (2001). Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci, 24*, 677-736. doi: 10.1146/annurev.neuro.24.1.677 24/1/677 [pii]

Hueston, C. M., & Deak, T. (2014). The inflamed axis: The interaction between stress, hormones, and the expression of inflammatory-related genes within key structures comprising the hypothalamic-pituitary-adrenal axis. *Physiology & Behavior, 124*, 77-91. doi: 10.1016/j.physbeh.2013.10.035

Hurst, J. L., & West, R. S. (2010). Taming anxiety in laboratory mice. *Nat Methods, 7*(10), 825-826. doi: 10.1038/nmeth.1500

Hwang, I. K., Yoo, K. Y., Nam, Y. S., Choi, J. H., Lee, I. S., Kwon, Y. G., . . . Won, M. H. (2006). Mineralocorticoid and glucocorticoid receptor expressions in astrocytes and microglia in the gerbil hippocampal CA1 region after ischemic insult. *Neurosci Res, 54*(4), 319-327. doi: 10.1016/j.neures.2005.12.012

Im, E. (2014). Corticotropin-releasing Hormone and Its Biological Diversity toward Angiogenesis. *Intest Res, 12*(2), 96-102. doi: 10.5217/ir.2014.12.2.96

Im, E., Rhee, S. H., Park, Y. S., Fiocchi, C., Tache, Y., & Pothoulakis, C. (2010). Corticotropin-releasing hormone family of peptides regulates intestinal angiogenesis. *Gastroenterology, 138*(7), 2457-2467, 2467 e2451-2455. doi: 10.1053/j.gastro.2010.02.055

Imaki, T., Katsumata, H., Miyata, M., Naruse, M., Imaki, J., & Minami, S. (2001). Expression of corticotropin-releasing hormone type 1 receptor in paraventricular nucleus after acute stress. *Neuroendocrinology, 73*(5), 293-301. doi: 54646 [pii] 54646

Inada, Y., Ikeda, K., Tojo, K., Sakamoto, M., Takada, Y., & Tajima, N. (2009). Possible involvement of corticotropin-releasing factor receptor signaling on vascular inflammation. *Peptides, 30*(2), 365-372. doi: 10.1016/j.peptides.2008.10.015

Ising, M., Horstmann, S., Kloiber, S., Lucae, S., Binder, E. B., Kern, N., . . . Holsboer, F. (2007). Combined dexamethasone/corticotropin releasing hormone test predicts treatment

response in major depression - a potential biomarker? *Biol Psychiatry, 62*(1), 47-54. doi: S0006-3223(06)01002-X [pii]
10.1016/j.biopsych.2006.07.039

Ising, M., Zimmermann, U. S., Kunzel, H. E., Uhr, M., Foster, A. C., Learned-Coughlin, S. M., . . . Grigoriadis, D. E. (2007). High-affinity CRF1 receptor antagonist NBI-34041: preclinical and clinical data suggest safety and efficacy in attenuating elevated stress response. *Neuropsychopharmacology, 32*(9), 1941-1949. doi: 1301328 [pii]
10.1038/sj.npp.1301328

Itoi, K., Mouri, T., Takahashi, K., Murakami, O., Imai, Y., Sasaki, S., . . . Sasano, N. (1987). Suppression by glucocorticoid of the immunoreactivity of corticotropin-releasing factor and vasopressin in the paraventricular nucleus of rat hypothalamus. *Neurosci Lett, 73*(3), 231-236.

Ivy, A. S., Rex, C. S., Chen, Y., Dube, C., Maras, P. M., Grigoriadis, D. E., . . . Baram, T. Z. (2010). Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. *J Neurosci, 30*(39), 13005-13015. doi: 10.1523/JNEUROSCI.1784-10.2010

Iwasaki-Sekino, A., Mano-Otagiri, A., Ohata, H., Yamauchi, N., & Shibasaki, T. (2009). Gender differences in corticotropin and corticosterone secretion and corticotropin-releasing factor mRNA expression in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala in response to footshock stress or psychological stress in rats. *Psychoneuroendocrinology, 34*(2), 226-237. doi: 10.1016/j.psyneuen.2008.09.003

Janac, B., Radenovic, L., Selakovic, V., & Prolic, Z. (2006). Time course of motor behavior changes in Mongolian gerbils submitted to different durations of cerebral ischemia. *Behav Brain Res, 175*(2), 362-373. doi: S0166-4328(06)00515-8 [pii]
10.1016/j.bbr.2006.09.008

Jang, S. H. (2013). Motor function-related maladaptive plasticity in stroke: A review. *NeuroRehabilitation, 32*(2), 311-316. doi: 10.3233/NRE-130849
V286855411163562 [pii]

Janssen, H., Bernhardt, J., Collier, J. M., Sena, E. S., McElduff, P., Attia, J., . . . Spratt, N. J. (2010). An enriched environment improves sensorimotor function post-ischemic stroke. *Neurorehabil Neural Repair, 24*(9), 802-813. doi: 10.1177/1545968310372092
1545968310372092 [pii]

Jaszberenyi, M., Bagosi, Z., Thurzo, B., Foldesi, I., Szabo, G., & Telegdy, G. (2009). Endocrine, behavioral and autonomic effects of neuropeptide AF. *Horm Behav, 56*(1), 24-34. doi: S0018-506X(09)00046-4 [pii]
10.1016/j.yhbeh.2009.02.006

Jeanneteau, F. D., Lambert, W. M., Ismaili, N., Bath, K. G., Lee, F. S., Garabedian, M. J., & Chao, M. V. (2012). BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. *Proc Natl Acad Sci U S A, 109*(4), 1305-1310. doi: 10.1073/pnas.1114122109

Ji, X. W., Wu, C. L., Wang, X. C., Liu, J., Bi, J. Z., & Wang, D. Y. (2014). Monoamine neurotransmitters and fibroblast growth factor-2 in the brains of rats with post-stroke depression. *Exp Ther Med, 8*(1), 159-164. doi: 10.3892/etm.2014.1674

Jia, H., Zhang, X. M., Zhang, B. A., Liu, Y., & Li, J. M. (2012). Dendritic morphology of neurons in medial prefrontal cortex and hippocampus in 2VO rats. *Neurol Sci, 33*(5), 1063-1070. doi: 10.1007/s10072-011-0898-4

Jiang, Y., Wei, N., Lu, T., Zhu, J., Xu, G., & Liu, X. (2011). Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats. *Neuroscience, 172*, 398-405. doi: 10.1016/j.neuroscience.2010.10.054

Jiang, Y., Wei, N., Zhu, J., Lu, T., Chen, Z., Xu, G., & Liu, X. (2010). Effects of brain-derived neurotrophic factor on local inflammation in experimental stroke of rat. *Mediators Inflamm, 2010*, 372423. doi: 10.1155/2010/372423

Jin, K., Mao, X. O., Simon, R. P., & Greenberg, D. A. (2001). Cyclic AMP response element binding protein (CREB) and CREB binding protein (CBP) in global cerebral ischemia. *J Mol Neurosci, 16*(1), 49-56. doi: 10.1385/JMN:16:1:49

Jin, K., Minami, M., Lan, J. Q., Mao, X. O., Batteur, S., Simon, R. P., & Greenberg, D. A. (2001). Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. *Proc Natl Acad Sci U S A, 98*(8), 4710-4715. doi: 10.1073/pnas.081011098
98/8/4710 [pii]

Jin, R., Yang, G., & Li, G. (2010). Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. *J Leukoc Biol, 87*(5), 779-789. doi: 10.1189/jlb.1109766

Jincai, W., Tingfang, D., Yongheng, Z., Zhongmin, L., Kaihua, Z., & Xiaohong, L. (2014). Effects of vinpocetine and ozagrel on behavioral recovery of rats after global brain ischemia. *J Clin Neurosci, 21*(4), 661-663. doi: 10.1016/j.jocn.2013.07.039

Joels, M., & Baram, T. Z. (2009). The neuro-symphony of stress. *Nat Rev Neurosci, 10*(6), 459-466. doi: 10.1038/nrn2632

Joels, M., Karst, H., DeRijk, R., & de Kloet, E. R. (2008). The coming out of the brain mineralocorticoid receptor. *Trends Neurosci, 31*(1), 1-7. doi: S0166-2236(07)00296-2 [pii]
10.1016/j.tins.2007.10.005

Johansen, F. F. (1993). Interneurons in rat hippocampus after cerebral ischemia. Morphometric, functional, and therapeutic investigations. *Acta Neurol Scand Suppl, 150*, 1-32.

Johansson, A., Olsson, T., Carlberg, B., Karlsson, K., & Fagerlund, M. (1997). Hypercortisolism after stroke - Partly cytokine-mediated? *Journal of the Neurological Sciences, 147*(1), 43-47.

Johansson, B. B., & Ohlsson, A. L. (1996). Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. *Experimental Neurology, 139*(2), 322-327. doi: DOI 10.1006/exnr.1996.0106

Johnson, J. D., O'Connor, K. A., Deak, T., Stark, M., Watkins, L. R., & Maier, S. F. (2002). Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain Behav Immun, 16*(4), 461-476. doi: S0889159101906385 [pii]

Johnson, S. A., Fournier, N. M., & Kalynchuk, L. E. (2006). Effect of different doses of corticosterone on depression-like behavior and HPA axis responses to a novel stressor. *Behav Brain Res, 168*(2), 280-288. doi: 10.1016/j.bbr.2005.11.019

Johnston, C. E., Herschel, D. J., Lasek, A. W., Hammer, R. P., Jr., & Nikulina, E. M. (2015). Knockdown of ventral tegmental area mu-opioid receptors in rats prevents effects of social defeat stress: implications for amphetamine cross-sensitization, social avoidance, weight regulation and expression of brain-derived neurotrophic factor. *Neuropharmacology, 89*, 325-334. doi: 10.1016/j.neuropharm.2014.10.010

Jokinen, J., & Nordstrom, P. (2009). HPA axis hyperactivity and attempted suicide in young adult mood disorder inpatients. *J Affect Disord, 116*(1-2), 117-120. doi: 10.1016/j.jad.2008.10.015

Joynt, K. E., Whellan, D. J., & O'Connor, C. M. (2003). Depression and cardiovascular disease: mechanisms of interaction. *Biol Psychiatry, 54*(3), 248-261.

Juaneda, C., Lafon-Dubourg, P., Ciofi, P., Sarrieau, A., Corio, M., & Tramu, G. (1999). Immune challenge-stimulated hypophysiotropic corticotropin-releasing hormone messenger RNA expression is associated with an induction of neurotensin messenger RNAs without alteration of vasopressin messenger RNAs. *Neuroscience, 93*(1), 393-400. doi: Doi 10.1016/S0306-4522(99)00133-5

Juaneda, C., Lafon-Dubourg, P., Ciofi, P., Sarrieau, A., Wenger, T., Tramu, G., & Corio, M. (2001). CCK mRNA expression in neuroendocrine CRH neurons is increased in rats subjected to an immune challenge. *Brain Res, 901*(1-2), 277-280. doi: Doi 10.1016/S0006-8993(01)02365-4

Jung, S. H., Kim, J., Davis, J. M., Blair, S. N., & Cho, H. C. (2011). Association among basal serum BDNF, cardiorespiratory fitness and cardiovascular disease risk factors in untrained healthy Korean men. *European Journal of Applied Physiology, 111*(2), 303-311. doi: 10.1007/s00421-010-1658-5

Jutkiewicz, E. M., Wood, S. K., Houshyar, H., Hsin, L. W., Rice, K. C., & Woods, J. H. (2005). The effects of CRF antagonists, antalarmin, CP154,526, LWH234, and R121919, in the forced swim test and on swim-induced increases in adrenocorticotropin in rats. *Psychopharmacology (Berl), 180*(2), 215-223. doi: 10.1007/s00213-005-2164-z

Kadar, T., Dachir, S., Shukitt-Hale, B., & Levy, A. (1998). Sub-regional hippocampal vulnerability in various animal models leading to cognitive dysfunction. *J Neural Transm (Vienna), 105*(8-9), 987-1004.

Kakehata, J., Yamaguchi, T., Togashi, H., Sakuma, I., Otani, H., Morimoto, Y., & Yoshioka, M. (2010). Therapeutic potentials of an artificial oxygen-carrier, liposome-encapsulated hemoglobin, for ischemia/reperfusion-induced cerebral dysfunction in rats. *J Pharmacol Sci, 114*(2), 189-197. doi: JST.JSTAGE/jphs/10115FP [pii]

Kakucska, I., Qi, Y. P., Clark, B. D., & Lechan, R. M. (1993). Endotoxin-Induced Corticotropin-Releasing Hormone Gene-Expression in the Hypothalamic Paraventricular Nucleus Is Mediated Centrally by Interleukin-1. *Endocrinology, 133*(2), 815-821. doi: Doi 10.1210/En.133.2.815

Kalliokoski, O., Abelson, K. S., Koch, J., Boschian, A., Thormose, S. F., Fauerby, N., . . . Hau, J. (2010). The effect of voluntarily ingested buprenorphine on rats subjected to surgically induced global cerebral ischaemia. *In Vivo, 24*(5), 641-646.

Kang, H., Welcher, A. A., Shelton, D., & Schuman, E. M. (1997). Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. *Neuron, 19*(3), 653-664. doi: S0896-6273(00)80378-5 [pii]

Karalis, K., Sano, H., Redwine, J., Listwak, S., Wilder, R. L., & Chrousos, G. P. (1991). Autocrine or paracrine inflammatory actions of corticotropin-releasing hormone in vivo. *Science, 254*(5030), 421-423.

Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J. M., & Bertschy, G. (2005). Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biol Psychiatry, 57*(9), 1068-1072. doi: S0006-3223(05)00051-X [pii]

10.1016/j.biopsych.2005.01.008
Karege, F., Perret, G., Bondolfi, G., Schwald, M., Bertschy, G., & Aubry, J. M. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res, 109*(2), 143-148.
Karege, F., Vaudan, G., Schwald, M., Perroud, N., & La Harpe, R. (2005). Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. *Brain Res Mol Brain Res, 136*(1-2), 29-37. doi: S0169-328X(04)00634-5 [pii]
10.1016/j.molbrainres.2004.12.020
Karelina, K., Norman, G. J., Zhang, N., & DeVries, A. C. (2009). Social contact influences histological and behavioral outcomes following cerebral ischemia. *Exp Neurol, 220*(2), 276-282. doi: 10.1016/j.expneurol.2009.08.022
S0014-4886(09)00361-6 [pii]
Karelina, K., Norman, G. J., Zhang, N., Morris, J. S., Peng, H., & DeVries, A. C. (2009). Social isolation alters neuroinflammatory response to stroke. *Proc Natl Acad Sci U S A, 106*(14), 5895-5900. doi: 10.1073/pnas.0810737106
0810737106 [pii]
Karelina, K., Stuller, K. A., Jarrett, B., Zhang, N., Wells, J., Norman, G. J., & DeVries, A. C. (2011). Oxytocin mediates social neuroprotection after cerebral ischemia. *Stroke, 42*(12), 3606-3611. doi: 10.1161/STROKEAHA.111.628008
STROKEAHA.111.628008 [pii]
Kasahara, M., Groenink, L., Kas, M. J., Bijlsma, E. Y., Olivier, B., & Sarnyai, Z. (2011). Influence of transgenic corticotropin-releasing factor (CRF) over-expression on social recognition memory in mice. *Behav Brain Res, 218*(2), 357-362. doi: 10.1016/j.bbr.2010.12.029
Kasahara, M., Groenink, L., Olivier, B., & Sarnyai, Z. (2011). Corticotropin-releasing factor (CRF) over-expression down-regulates hippocampal dopamine receptor protein expression and CREB activation in mice. *Neuro Endocrinol Lett, 32*(2), 193-198.
Kasai, H., Fukuda, M., Watanabe, S., Hayashi-Takagi, A., & Noguchi, J. (2010). Structural dynamics of dendritic spines in memory and cognition. *Trends Neurosci, 33*(3), 121-129. doi: 10.1016/j.tins.2010.01.001
Kasckow, J. W., Baker, D., & Geracioti, T. D., Jr. (2001). Corticotropin-releasing hormone in depression and post-traumatic stress disorder. *Peptides, 22*(5), 845-851. doi: S0196-9781(01)00399-0 [pii]
Kato, M., Iwata, H., Katayama, T., Asai, H., Narita, H., & Endo, T. (1997). Possible therapeutic effect of T-794, a novel reversible inhibitor of monoamine oxidase-A, on post-stroke emotional disturbances, assessed in animal models of depression. *Biol Pharm Bull, 20*(4), 349-353.
Katoh-Semba, R., Takeuchi, I. K., Inaguma, Y., Ichisaka, S., Hata, Y., Tsumoto, T., . . . Kato, K. (2001). Induction of brain-derived neurotrophic factor by convulsant drugs in the rat brain: involvement of region-specific voltage-dependent calcium channels. *J Neurochem, 77*(1), 71-83.
Kazlauckas, V., Schuh, J., Dall'Igna, O. P., Pereira, G. S., Bonan, C. D., & Lara, D. R. (2005). Behavioral and cognitive profile of mice with high and low exploratory phenotypes. *Behav Brain Res, 162*(2), 272-278. doi: 10.1016/j.bbr.2005.03.021

Ke, Z., Yip, S. P., Li, L., Zheng, X. X., & Tong, K. Y. (2011). The effects of voluntary, involuntary, and forced exercises on brain-derived neurotrophic factor and motor function recovery: a rat brain ischemia model. *PLoS One, 6*(2), e16643. doi: 10.1371/journal.pone.0016643

Keck, M. E., & Holsboer, F. (2001). Hyperactivity of CRH neuronal circuits as a target for therapeutic interventions in affective disorders. *Peptides, 22*(5), 835-844. doi: S0196-9781(01)00398-9 [pii]

Kehne, J. H. (2007). The CRF1 receptor, a novel target for the treatment of depression, anxiety, and stress-related disorders. *CNS Neurol Disord Drug Targets, 6*(3), 163-182.

Kellendonk, C., Gass, P., Kretz, O., Schutz, G., & Tronche, F. (2002). Corticosteroid receptors in the brain: gene targeting studies. *Brain Res Bull, 57*(1), 73-83. doi: S0361923001006384 [pii]

Kesslak, J. P., So, V., Choi, J., Cotman, C. W., & Gomez-Pinilla, F. (1998). Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? *Behav Neurosci, 112*(4), 1012-1019.

Kettenmann, H., Hanisch, U. K., Noda, M., & Verkhratsky, A. (2011). Physiology of microglia. *Physiol Rev, 91*(2), 461-553. doi: 10.1152/physrev.00011.2010

Khan, S., Milot, M., Lecompte-Collin, J., & Plamondon, H. (2004). Time-dependent changes in CRH concentrations and release in discrete brain regions following global ischemia: effects of MK-801 pretreatment. *Brain Res, 1016*(1), 48-57. doi: 10.1016/j.brainres.2004.04.062

Khemissi, W., Farooq, R. K., Le Guisquet, A. M., Sakly, M., & Belzung, C. (2014). Dysregulation of the hypothalamus-pituitary-adrenal axis predicts some aspects of the behavioral response to chronic fluoxetine: association with hippocampal cell proliferation. *Frontiers in Behavioral Neuroscience, 8*, 340. doi: 10.3389/fnbeh.2014.00340

Kielian, T., & Esen, N. (2004). Effects of neuroinflammation on glia-glia gap junctional intercellular communication: a perspective. *Neurochem Int, 45*(2-3), 429-436. doi: 10.1016/j.neuint.2003.09.010
S019701860300305X [pii]

Kikusui, T., Takeuchi, Y., & Mori, Y. (2000). Involvement of corticotropin-releasing factor in the retrieval process of fear-conditioned ultrasonic vocalization in rats. *Physiology & Behavior, 71*(3-4), 323-328.

Kim, G., & Kim, E. (2013). The Effects of Antecedent Exercise on Motor Function Recovery and Brain-derived Neurotrophic Factor Expression after Focal Cerebral Ischemia in Rats. *J Phys Ther Sci, 25*(5), 553-556. doi: 10.1589/jpts.25.553

Kim, J. J., & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nat Rev Neurosci, 3*(6), 453-462. doi: 10.1038/nrn849

Kim, J. M., Stewart, R., Bae, K. Y., Kim, S. W., Kang, H. J., Shin, I. S., . . . Yoon, J. S. (2012). Serotonergic and BDNF genes and risk of depression after stroke. *J Affect Disord, 136*(3), 833-840. doi: 10.1016/j.jad.2011.09.029
S0165-0327(11)00555-6 [pii]

Kim, J. M., Stewart, R., Kang, H. J., Kim, S. Y., Kim, S. W., Shin, I. S., . . . Yoon, J. S. (2013). A longitudinal study of BDNF promoter methylation and genotype with poststroke depression. *J Affect Disord, 149*(1-3), 93-99. doi: 10.1016/j.jad.2013.01.008
S0165-0327(13)00060-8 [pii]

Kim, J. M., Stewart, R., Kim, S. W., Yang, S. J., Shina, I. S., Kim, Y. H., & Yoon, J. S. (2008). BDNF genotype potentially modifying the association between incident stroke and depression. *Neurobiology of Aging, 29*(5), 789-792. doi: DOI 10.1016/j.neurobiolaging.2006.11.021

Kim, M. W., Bang, M. S., Han, T. R., Ko, Y. J., Yoon, B. W., Kim, J. H., . . . Kim, M. H. (2005). Exercise increased BDNF and trkB in the contralateral hemisphere of the ischemic rat brain. *Brain Res, 1052*(1), 16-21. doi: 10.1016/j.brainres.2005.05.070

Kim, S. Y., & Jones, T. A. (2010). Lesion size-dependent synaptic and astrocytic responses in cortex contralateral to infarcts in middle-aged rats. *Synapse, 64*(9), 659-671. doi: 10.1002/syn.20777

Kiprianova, I., Freiman, T. M., Desiderato, S., Schwab, S., Galmbacher, R., Gillardon, F., & Spranger, M. (1999). Brain-derived neurotrophic factor prevents neuronal death and glial activation after global ischemia in the rat. *Journal of Neuroscience Research, 56*(1), 21-27.

Kirino, T. (2000). Delayed neuronal death. *Neuropathology, 20 Suppl*, S95-97.

Kiryk, A., Pluta, R., Figiel, I., Mikosz, M., Ulamek, M., Niewiadomska, G., . . . Kaczmarek, L. (2011). Transient brain ischemia due to cardiac arrest causes irreversible long-lasting cognitive injury. *Behavioural Brain Research, 219*(1), 1-7. doi: 10.1016/j.bbr.2010.12.004

Kishi, Y., Kosier, J. T., & Robinson, R. G. (1996). Suicidal plans in patients with acute stroke. *J Nerv Ment Dis, 184*(5), 274-280.

Kishi, Y., Robinson, R. G., & Kosier, J. T. (1996). Suicidal plans in patients with stroke: comparison between acute-onset and delayed-onset suicidal plans. *Int Psychogeriatr, 8*(4), 623-634.

Kishimoto, T., Radulovic, J., Radulovic, M., Lin, C. R., Schrick, C., Hooshmand, F., . . . Spiess, J. (2000). Deletion of crhr2 reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. *Nat Genet, 24*(4), 415-419. doi: 10.1038/74271

Kitay, J. I. (1961). Sex differences in adrenal cortical secretion in the rat. *Endocrinology, 68*, 818-824. doi: 10.1210/endo-68-5-818

Kiyota, Y., Miyamoto, M., & Nagaoka, A. (1991). Relationship between Brain-Damage and Memory Impairment in Rats Exposed to Transient Forebrain Ischemia. *Brain Res, 538*(2), 295-302. doi: Doi 10.1016/0006-8993(91)90443-Y

Knoops, A. J., Gerritsen, L., van der Graaf, Y., Mali, W. P., & Geerlings, M. I. (2010). Basal hypothalamic pituitary adrenal axis activity and hippocampal volumes: the SMART-Medea study. *Biol Psychiatry, 67*(12), 1191-1198. doi: 10.1016/j.biopsych.2010.01.025

Koide, T., Wieloch, T. W., & Siesjo, B. K. (1986). Chronic dexamethasone pretreatment aggravates ischemic neuronal necrosis. *J Cereb Blood Flow Metab, 6*(4), 395-404. doi: 10.1038/jcbfm.1986.72

Kokaia, Z., Andsberg, G., Yan, Q., & Lindvall, O. (1998). Rapid alterations of BDNF protein levels in the rat brain after focal ischemia: evidence for increased synthesis and anterograde axonal transport. *Exp Neurol, 154*(2), 289-301. doi: 10.1006/exnr.1998.6888

Kokaia, Z., Metsis, M., Kokaia, M., Bengzon, J., Elmer, E., Smith, M. L., . . . Lindvall, O. (1994). Brain insults in rats induce increased expression of the BDNF gene through differential use of multiple promoters. *European Journal of Neuroscience, 6*(4), 587-596.

Kokaia, Z., Nawa, H., Uchino, H., Elmer, E., Kokaia, M., Carnahan, J., . . . Lindvall, O. (1996). Regional brain-derived neurotrophic factor mRNA and protein levels following transient forebrain ischemia in the rat. *Brain Res Mol Brain Res, 38*(1), 139-144.

Kolber, B. J., Roberts, M. S., Howell, M. P., Wozniak, D. F., Sands, M. S., & Muglia, L. J. (2008). Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning. *Proc Natl Acad Sci U S A, 105*(33), 12004-12009. doi: 0803216105 [pii]
10.1073/pnas.0803216105

Konishi, S., Kasagi, Y., Katsumata, H., Minami, S., & Imaki, T. (2003). Regulation of corticotropin-releasing factor (CRF) type-1 receptor gene expression by CRF in the hypothalamus. *Endocr J, 50*(1), 21-36.

Koob, G. F. (1999). Corticotropin-releasing factor, norepinephrine, and stress. *Biol Psychiatry, 46*(9), 1167-1180. doi: S0006-3223(99)00164-X [pii]

Koob, G. F., Cole, B. J., Swerdlow, N. R., Le Moal, M., & Britton, K. T. (1990). Stress, performance, and arousal: focus on CRF. *NIDA Res Monogr, 97*, 163-176.

Koponen, E., Rantamaki, T., Voikar, V., Saarelainen, T., MacDonald, E., & Castren, E. (2005). Enhanced BDNF signaling is associated with an antidepressant-like behavioral response and changes in brain monoamines. *Cellular and Molecular Neurobiology, 25*(6), 973-980. doi: DOI 10.1007/s10571-005-8468-z

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., & Bonhoeffer, T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A, 92*(19), 8856-8860.

Korte, M., Staiger, V., Griesbeck, O., Thoenen, H., & Bonhoeffer, T. (1996). The involvement of brain-derived neurotrophic factor in hippocampal long-term potentiation revealed by gene targeting experiments. *J Physiol Paris, 90*(3-4), 157-164. doi: S0928-4257(97)81415-5 [pii]

Koutmani, Y., Politis, P. K., Elkouris, M., Agrogiannis, G., Kemerli, M., Patsouris, E., . . . Karalis, K. P. (2013). Corticotropin-releasing hormone exerts direct effects on neuronal progenitor cells: implications for neuroprotection. *Mol Psychiatry, 18*(3), 300-307. doi: 10.1038/mp.2012.198

Kouwenhoven, S. E., Gay, C. L., Bakken, L. N., & Lerdal, A. (2013). Depressive symptoms in acute stroke: A cross-sectional study of their association with sociodemographics and clinical factors. *Neuropsychological Rehabilitation, 23*(5), 658-677. doi: 10.1080/09602011.2013.801778

Kouwenhoven, S. E., Kirkevold, M., Engedal, K., & Kim, H. S. (2011). Depression in acute stroke: prevalence, dominant symptoms and associated factors. A systematic literature review. *Disabil Rehabil, 33*(7), 539-556. doi: 10.3109/09638288.2010.505997

Kovacs, K. J., & Makara, G. B. (1988). Corticosterone and dexamethasone act at different brain sites to inhibit adrenalectomy-induced adrenocorticotropin hypersecretion. *Brain Res, 474*(2), 205-210. doi: 0006-8993(88)90435-0 [pii]

Kovalenko, T., Osadchenko, I., Nikonenko, A., Lushnikova, I., Voronin, K., Nikonenko, I., . . . Skibo, G. (2006). Ischemia-induced modifications in hippocampal CA1 stratum radiatum excitatory synapses. *Hippocampus, 16*(10), 814-825. doi: 10.1002/hipo.20211

Kozlovsky, N., Matar, M. A., Kaplan, Z., Kotler, M., Zohar, J., & Cohen, H. (2007). Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with

PTSD-like behavioural stress response. *Int J Neuropsychopharmacol, 10*(6), 741-758. doi: S1461145707007560 [pii]
10.1017/S1461145707007560
Kozlovsky, N., Matar, M. A., Kaplan, Z., Kotler, M., Zohar, J., & Cohen, H. (2008). The immediate early gene Arc is associated with behavioral resilience to stress exposure in an animal model of posttraumatic stress disorder. *Eur Neuropsychopharmacol, 18*(2), 107-116. doi: S0924-977X(07)00098-3 [pii]
10.1016/j.euroneuro.2007.04.009
Kozlovsky, N., Zohar, J., Kaplan, Z., & Cohen, H. (2012). Microinfusion of a corticotrophin-releasing hormone receptor 1 antisense oligodeoxynucleotide into the dorsal hippocampus attenuates stress responses at specific times after stress exposure. *J Neuroendocrinol, 24*(3), 489-503. doi: 10.1111/j.1365-2826.2011.02267.x
Krafft, P. R., Bailey, E. L., Lekic, T., Rolland, W. B., Altay, O., Tang, J., . . . Sudlow, C. L. (2012). Etiology of stroke and choice of models. *Int J Stroke, 7*(5), 398-406. doi: 10.1111/j.1747-4949.2012.00838.x
Kratzer, S., Mattusch, C., Metzger, M. W., Dedic, N., Noll-Hussong, M., Kafitz, K. W., . . . Rammes, G. (2013). Activation of CRH receptor type 1 expressed on glutamatergic neurons increases excitability of CA1 pyramidal neurons by the modulation of voltage-gated ion channels. *Front Cell Neurosci, 7*, 91. doi: 10.3389/fncel.2013.00091
Kreutzberg, G. W. (1996). Microglia: a sensor for pathological events in the CNS. *Trends Neurosci, 19*(8), 312-318.
Krishnan, V., Han, M. H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., . . . Nestler, E. J. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell, 131*(2), 391-404. doi: 10.1016/j.cell.2007.09.018
Kriz, J. (2006). Inflammation in ischemic brain injury: timing is important. *Crit Rev Neurobiol, 18*(1-2), 145-157.
Kronenberg, G., Balkaya, M., Prinz, V., Gertz, K., Ji, S., Kirste, I., . . . Endres, M. (2012). Exofocal Dopaminergic Degeneration as Antidepressant Target in Mouse Model of Poststroke Depression. *Biol Psychiatry, 72*(4), 273-281. doi: 10.1016/j.biopsych.2012.02.026
Krugers, H. J., Knollema, S., Kemper, R. H., Ter Horst, G. J., & Korf, J. (1995). Down-regulation of the hypothalamo-pituitary-adrenal axis reduces brain damage and number of seizures following hypoxia/ischaemia in rats. *Brain Res, 690*(1), 41-47.
Ku, H. L., Chen, C. H., Yang, Y. T., Hu, C. J., Wu, D., Chen, C. C., . . . Chi, N. F. (2013). Association Between Cerebral Lesions and Emotional Changes in Acute Ischemic Stroke Patients. *Journal of Nervous and Mental Disease, 201*(5), 400-406. doi: 10.1097/NMD.0b013e31828e0fe9
Kuhne, C., Puk, O., Graw, J., de Angelis, M. H., Schutz, G., Wurst, W., & Deussing, J. M. (2012). Visualizing Corticotropin-Releasing Hormone Receptor Type 1 Expression and Neuronal Connectivities in the Mouse Using a Novel Multifunctional Allele. *Journal of Comparative Neurology, 520*(14), 3150-3180. doi: 10.1002/cne.23082
Kuramoto, N., Azuma, K., Inoue, K., Ogita, K., Mitani, A., Zhang, L., . . . Yoneda, Y. (1998). Correlation between potentiation of AP1 DNA binding and expression of c-Fos in association with phosphorylation of CREB at serine133 in thalamus of gerbils with ischemia. *Brain Research, 806*(2), 152-164. doi: Doi 10.1016/S0006-8993(98)00693-3

Kuter, K., Kolasiewicz, W., Golembiowska, K., Dziubina, A., Schulze, G., Berghauzen, K., . . . Ossowska, K. (2011). Partial lesion of the dopaminergic innervation of the ventral striatum induces "depressive-like" behavior of rats. *Pharmacological Reports, 63*(6), 1383-1392.

Kutsuna, N., Eriguchi, T., Oshima, H., Suma, T., Sakatani, K., Yoshino, A., & Katayama, Y. (2013). Acute stress exposure preceding global brain ischemia accelerates decreased doublecortin expression in the rat retrosplenial cortex. *Adv Exp Med Biol, 789*, 65-71. doi: 10.1007/978-1-4614-7411-1_10

Lacroix, S., & Rivest, S. (1996). Role of cyclo-oxygenase pathways in the stimulatory influence of immune challenge on the transcription of a specific CRF receptor subtype in the rat brain. *J Chem Neuroanat, 10*(1), 53-71.

Ladd, C. O., Huot, R. L., Thrivikraman, K. V., Nemeroff, C. B., & Plotsky, P. M. (2004). Long-term adaptations in glucocorticoid receptor and mineralocorticoid receptor mRNA and negative feedback on the hypothalamo-pituitary-adrenal axis following neonatal maternal separation. *Biol Psychiatry, 55*(4), 367-375. doi: 10.1016/j.biopsych.2003.10.007
S0006322303010953 [pii]

Lagace, D. C. (2012). Does the endogenous neurogenic response alter behavioral recovery following stroke? *Behav Brain Res, 227*(2), 426-432. doi: http://dx.doi.org/10.1016/j.bbr.2011.08.045

Lai, A. Y., & Todd, K. G. (2006). Microglia in cerebral ischemia: molecular actions and interactions. *Can J Physiol Pharmacol, 84*(1), 49-59. doi: 10.1139/Y05-143

Lai, A. Y., & Todd, K. G. (2008). Differential regulation of trophic and proinflammatory microglial effectors is dependent on severity of neuronal injury. *Glia, 56*(3), 259-270. doi: 10.1002/glia.20610

Laird, M. D., Vender, J. R., & Dhandapani, K. M. (2008). Opposing roles for reactive astrocytes following traumatic brain injury. *Neurosignals, 16*(2-3), 154-164. doi: 10.1159/000111560

Lakshminarasimhan, H., & Chattarji, S. (2012). Stress leads to contrasting effects on the levels of brain derived neurotrophic factor in the hippocampus and amygdala. *PLoS One, 7*(1), e30481. doi: 10.1371/journal.pone.0030481

Lalonde, C. C., & Mielke, J. G. (2014). Selective vulnerability of hippocampal sub-fields to oxygen-glucose deprivation is a function of animal age. *Brain Res, 1543*, 271-279. doi: 10.1016/j.brainres.2013.10.056

Lambert, K. G., Buckelew, S. K., Staffiso-Sandoz, G., Gaffga, S., Carpenter, W., Fisher, J., & Kinsley, C. H. (1998). Activity-stress induces atrophy of apical dendrites of hippocampal pyramidal neurons in male rats. *Physiol Behav, 65*(1), 43-49. doi: S0031-9384(98)00114-0 [pii]

Lamers, F., Vogelzangs, N., Merikangas, K. R., de Jonge, P., Beekman, A. T., & Penninx, B. W. (2013). Evidence for a differential role of HPA-axis function, inflammation and metabolic syndrome in melancholic versus atypical depression. *Mol Psychiatry, 18*(6), 692-699. doi: 10.1038/mp.2012.144

Lapchak, P. A., Araujo, D. M., & Hefti, F. (1993). Systemic interleukin-1 beta decreases brain-derived neurotrophic factor messenger RNA expression in the rat hippocampal formation. *Neuroscience, 53*(2), 297-301.

Larsson, I. M., Wallin, E., Rubertsson, S., & Kristofferzon, M. L. (2014). Health-related quality of life improves during the first six months after cardiac arrest and hypothermia treatment. *Resuscitation, 85*(2), 215-220. doi: 10.1016/j.resuscitation.2013.09.017

Laryea, G., Arnett, M. G., & Muglia, L. J. (2012). Behavioral Studies and Genetic Alterations in Corticotropin-Releasing Hormone (CRH) Neurocircuitry: Insights into Human Psychiatric Disorders. *Behav Sci (Basel), 2*(2), 135-171. doi: 10.3390/bs2020135

Le Feuvre, R. A., Aisenthal, L., & Rothwell, N. J. (1991). Involvement of corticotrophin releasing factor (CRF) in the thermogenic and anorexic actions of serotonin (5-HT) and related compounds. *Brain Res, 555*(2), 245-250. doi: 0006-8993(91)90348-Y [pii]

Le Mevel, J. C., Abitbol, S., Beraud, G., & Maniey, J. (1979). Temporal changes in plasma adrenocorticotropin concentration after repeated neurotropic stress in male and female rats. *Endocrinology, 105*(3), 812-817.

Lee, B., Shim, I., Lee, H., & Hahm, D. H. (2013). Fucoidan prevents depression-like behavior in rats exposed to repeated restraint stress. *J Nat Med, 67*(3), 534-544. doi: 10.1007/s11418-012-0712-5

Lee, B., Sur, B., Yeom, M., Shim, I., Lee, H., & Hahm, D. H. (2014). Alpha-Asarone, a Major Component of Acorus gramineus, Attenuates Corticosterone-Induced Anxiety-Like Behaviours via Modulating TrkB Signaling Process. *Korean J Physiol Pharmacol, 18*(3), 191-200. doi: 10.4196/kjpp.2014.18.3.191

Lee, E. H., Hung, H. C., Lu, K. T., Chen, W. H., & Chen, H. Y. (1992). Protein synthesis in the hippocampus associated with memory facilitation by corticotropin-releasing factor in rats. *Peptides, 13*(5), 927-937.

Lee, E. H., Lee, C. P., Wang, H. I., & Lin, W. R. (1993). Hippocampal CRF, NE, and NMDA system interactions in memory processing in the rat. *Synapse, 14*(2), 144-153. doi: 10.1002/syn.890140207

Lee, J. C., Ahn, J. H., Lee, D. H., Yan, B. C., Park, J. H., Kim, I. H., . . . Won, M. H. (2013). Neuronal damage and gliosis in the somatosensory cortex induced by various durations of transient cerebral ischemia in gerbils. *Brain Research, 1510*, 78-88. doi: DOI 10.1016/j.brainres.2013.03.008

Lee, J. H., Shin, H. K., Park, S. Y., Kim, C. D., Lee, W. S., & Hong, K. W. (2009). Cilostazol preserves CA1 hippocampus and enhances generation of immature neuroblasts in dentate gyrus after transient forebrain ischemia in rats. *Exp Neurol, 215*(1), 87-94. doi: 10.1016/j.expneurol.2008.09.013

Lee, T., Saruta, J., Sasaguri, K., Sato, S., & Tsukinoki, K. (2008). Allowing animals to bite reverses the effects of immobilization stress on hippocampal neurotrophin expression. *Brain Res, 1195*, 43-49. doi: S0006-8993(07)02920-4 [pii] 10.1016/j.brainres.2007.12.013

Lee, T. H., Kato, H., Chen, S. T., Kogure, K., & Itoyama, Y. (2002). Expression disparity of brain-derived neurotrophic factor immunoreactivity and mRNA in ischemic hippocampal neurons. *Neuroreport, 13*(17), 2271-2275. doi: 10.1097/01.wnr.0000043410.7262

Lehotsky, J., Burda, J., Danielisova, V., Gottlieb, M., Kaplan, P., & Saniova, B. (2009). Ischemic tolerance: the mechanisms of neuroprotective strategy. *Anat Rec (Hoboken), 292*(12), 2002-2012. doi: 10.1002/ar.20970

Lemos, J. C., Wanat, M. J., Smith, J. S., Reyes, B. A., Hollon, N. G., Van Bockstaele, E. J., . . . Phillips, P. E. (2012). Severe stress switches CRF action in the nucleus accumbens from appetitive to aversive. *Nature, 490*(7420), 402-406. doi: 10.1038/nature11436

Leu, S. J., & Singh, V. K. (1992). Stimulation of interleukin-6 production by corticotropin-releasing factor. *Cell Immunol, 143*(1), 220-227.

Leuner, B., & Shors, T. J. (2013). Stress, anxiety, and dendritic spines: what are the connections? *Neuroscience, 251*, 108-119. doi: 10.1016/j.neuroscience.2012.04.021

Lezoualc'h, F., Engert, S., Berning, B., & Behl, C. (2000). Corticotropin-releasing hormone-mediated neuroprotection against oxidative stress is associated with the increased release of non-amyloidogenic amyloid beta precursor protein and with the suppression of nuclear factor-kappaB. *Mol Endocrinol, 14*(1), 147-159. doi: 10.1210/mend.14.1.0403

Li, B., Piao, C. S., Liu, X. Y., Guo, W. P., Xue, Y. Q., Duan, W. M., . . . Zhao, L. R. (2010). Brain self-protection: the role of endogenous neural progenitor cells in adult brain after cerebral cortical ischemia. *Brain Res, 1327*, 91-102. doi: 10.1016/j.brainres.2010.02.030

Li, C., Liu, Y., Yin, S., Lu, C., Liu, D., Jiang, H., & Pan, F. (2015). Long-term effects of early adolescent stress: dysregulation of hypothalamic-pituitary-adrenal axis and central corticotropin releasing factor receptor 1 expression in adult male rats. *Behav Brain Res, 288*, 39-49. doi: 10.1016/j.bbr.2015.04.007

Li, S., Wang, C., Wang, W., Dong, H., Hou, P., & Tang, Y. (2008). Chronic mild stress impairs cognition in mice: from brain homeostasis to behavior. *Life Sci, 82*(17-18), 934-942. doi: 10.1016/j.lfs.2008.02.010

Li, S. J., Liu, W., Wang, J. L., Zhang, Y., Zhao, D. J., Wang, T. J., & Li, Y. Y. (2014). The role of TNF-alpha, IL-6, IL-10, and GDNF in neuronal apoptosis in neonatal rat with hypoxic-ischemic encephalopathy. *Eur Rev Med Pharmacol Sci, 18*(6), 905-909. doi: 7155 [pii]

Li, Y. C., Wang, L. L., Pei, Y. Y., Shen, J. D., Li, H. B., Wang, B. Y., & Bai, M. (2015). Baicalin decreases SGK1 expression in the hippocampus and reverses depressive-like behaviors induced by corticosterone. *Neuroscience*. doi: 10.1016/j.neuroscience.2015.10.023

Liao, X. M., Yang, X. D., Jia, J., Li, J. T., Xie, X. M., Su, Y. A., . . . Wang, X. D. (2014). Blockade of corticotropin-releasing hormone receptor 1 attenuates early-life stress-induced synaptic abnormalities in the neonatal hippocampus. *Hippocampus, 24*(5), 528-540. doi: 10.1002/hipo.22254

Licinio, J., O'Kirwan, F., Irizarry, K., Merriman, B., Thakur, S., Jepson, R., . . . Wong, M. L. (2004). Association of a corticotropin-releasing hormone receptor 1 haplotype and antidepressant treatment response in Mexican-Americans. *Mol Psychiatry, 9*(12), 1075-1082. doi: 10.1038/sj.mp.4001587

Liebetrau, M., Steen, B., & Skoog, I. (2008). Depression as a risk factor for the incidence of first-ever stroke in 85-year-olds. *Stroke, 39*(7), 1960-1965. doi: Doi 10.1161/Strokeaha.107.490797

Liebsch, G., Landgraf, R., Engelmann, M., Lorscher, P., & Holsboer, F. (1999). Differential behavioural effects of chronic infusion of CRH 1 and CRH 2 receptor antisense oligonucleotides into the rat brain. *J Psychiatr Res, 33*(2), 153-163. doi: S0022-3956(98)80047-2 [pii]

Liguz-Lecznar, M., & Kossut, M. (2013). Influence of inflammation on poststroke plasticity. *Neural Plast, 2013*, 258582. doi: 10.1155/2013/258582

Lim, C., Alexander, M. P., LaFleche, G., Schnyer, D. M., & Verfaellie, M. (2004). The neurological and cognitive sequelae of cardiac arrest. *Neurology, 63*(10), 1774-1778. doi: 63/10/1774 [pii]

Lim, H., Jang, S., Lee, Y., Moon, S., Kim, J., & Oh, S. (2012). Enhancement of Anxiety and Modulation of TH and pERK Expressions in Amygdala by Repeated Injections of Corticosterone. *Biomol Ther (Seoul), 20*(4), 418-424. doi: 10.4062/biomolther.2012.20.4.418

Lindvall, O., Ernfors, P., Bengzon, J., Kokaia, Z., Smith, M. L., Siesjo, B. K., & Persson, H. (1992). Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the adult rat brain following cerebral ischemia and hypoglycemic coma. *Proc Natl Acad Sci U S A, 89*(2), 648-652.

Lindvall, O., Kokaia, Z., Bengzon, J., Elmer, E., & Kokaia, M. (1994). Neurotrophins and brain insults. *Trends Neurosci, 17*(11), 490-496.

Linnarsson, S., Bjorklund, A., & Ernfors, P. (1997). Learning deficit in BDNF mutant mice. *European Journal of Neuroscience, 9*(12), 2581-2587.

Lipsky, R. H., & Marini, A. M. (2007). Brain-derived neurotrophic factor in neuronal survival and behavior-related plasticity. *Ann N Y Acad Sci, 1122*, 130-143. doi: 10.1196/annals.1403.009

Lipton, P. (1999). Ischemic cell death in brain neurons. *Physiol Rev, 79*(4), 1431-1568.

Liu, G., Gu, B., He, X. P., Joshi, R. B., Wackerle, H. D., Rodriguiz, R. M., . . . McNamara, J. O. (2013). Transient inhibition of TrkB kinase after status epilepticus prevents development of temporal lobe epilepsy. *Neuron, 79*(1), 31-38. doi: 10.1016/j.neuron.2013.04.027

Liu, I. Y., Lyons, W. E., Mamounas, L. A., & Thompson, R. F. (2004). Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning. *J Neurosci, 24*(36), 7958-7963. doi: 10.1523/JNEUROSCI.1948-04.2004

Liu, J., Solway, K., Messing, R. O., & Sharp, F. R. (1998). Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. *J Neurosci, 18*(19), 7768-7778.

Liu, W., & Zhou, C. (2012). Corticosterone reduces brain mitochondrial function and expression of mitofusin, BDNF in depression-like rodents regardless of exercise preconditioning. *Psychoneuroendocrinology, 37*(7), 1057-1070. doi: S0306-4530(11)00355-6 [pii] 10.1016/j.psyneuen.2011.12.003

Liu, X., Wellman, L. L., Yang, L., Ambrozewicz, M. A., Tang, X., & Sanford, L. D. (2011). Antagonizing corticotropin-releasing factor in the central nucleus of the amygdala attenuates fear-induced reductions in sleep but not freezing. *Sleep, 34*(11), 1539-1549. doi: 10.5665/sleep.1394

Liu, Z., Liu, W., Yao, L., Yang, C., Xiao, L., Wan, Q., . . . Xiao, Z. (2013). Negative life events and corticotropin-releasing-hormone receptor1 gene in recurrent major depressive disorder. *Sci Rep, 3*, 1548. doi: 10.1038/srep01548

Liu, Z., Zhu, F., Wang, G., Xiao, Z., Tang, J., Liu, W., . . . Li, W. (2007). Association study of corticotropin-releasing hormone receptor1 gene polymorphisms and antidepressant response in major depressive disorders. *Neurosci Lett, 414*(2), 155-158. doi: 10.1016/j.neulet.2006.12.013

Liu, Z., Zhu, F., Wang, G., Xiao, Z., Wang, H., Tang, J., . . . Li, W. (2006). Association of corticotropin-releasing hormone receptor1 gene SNP and haplotype with major depression. *Neurosci Lett, 404*(3), 358-362. doi: 10.1016/j.neulet.2006.06.016

Livingston-Thomas, J. M., McGuire, E. P., Doucette, T. A., & Tasker, R. A. (2014). Voluntary forced use of the impaired limb following stroke facilitates functional recovery in the rat. *Behav Brain Res, 261*, 210-219. doi: 10.1016/j.bbr.2013.12.032

Lloyd, R. B., & Nemeroff, C. B. (2011). The role of corticotropin-releasing hormone in the pathophysiology of depression: therapeutic implications. *Curr Top Med Chem, 11*(6), 609-617.

Lodge, D. J., & Lawrence, A. J. (2003). The CRF1 receptor antagonist antalarmin reduces volitional ethanol consumption in isolation-reared fawn-hooded rats. *Neuroscience, 117*(2), 243-247.

Loiseau, F., & Milian, M. J. (2009). Blockade of dopamine D-3 receptors in frontal cortex, but not in sub-cortical structures, enhances social recognition in rats: Similar actions of D-1 receptor agonists, but not of D-2 antagonists. *European Neuropsychopharmacology, 19*(1), 23-33. doi: 10.1016/j.euroneuro.2008.07.012

Lorentz, C. U., Parrish, D. C., Alston, E. N., Pellegrino, M. J., Woodward, W. R., Hempstead, B. L., & Habecker, B. A. (2013). Sympathetic denervation of peri-infarct myocardium requires the p75 neurotrophin receptor. *Exp Neurol, 249*, 111-119. doi: 10.1016/j.expneurol.2013.08.015

Love, S. (2003). Apoptosis and brain ischaemia. *Prog Neuropsychopharmacol Biol Psychiatry, 27*(2), 267-282. doi: 10.1016/S0278-5846(03)00022-8

Lovenberg, T. W., Liaw, C. W., Grigoriadis, D. E., Clevenger, W., Chalmers, D. T., De Souza, E. B., & Oltersdorf, T. (1995). Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci U S A, 92*(3), 836-840.

Lu, B., & Chow, A. (1999). Neurotrophins and hippocampal synaptic transmission and plasticity. *J Neurosci Res, 58*(1), 76-87. doi: 10.1002/(SICI)1097-4547(19991001)58:1<76::AID-JNR8>3.0.CO;2-0 [pii]

Lu, B., & Gottschalk, W. (2000). Modulation of hippocampal synaptic transmission and plasticity by neurotrophins. *Prog Brain Res, 128*, 231-241. doi: S0079-6123(00)28020-5 [pii]

10.1016/S0079-6123(00)28020-5

Lu, F., Nakamura, T., Toyoshima, T., Liu, Y., Shinomiya, A., Hirooka, K., . . . Itano, T. (2014). Neuroprotection of granulocyte colony-stimulating factor during the acute phase of transient forebrain ischemia in gerbils. *Brain Res, 1548*, 49-55. doi: 10.1016/j.brainres.2013.12.010

Luine, V., Villegas, M., Martinez, C., & McEwen, B. S. (1994). Repeated stress causes reversible impairments of spatial memory performance. *Brain Res, 639*(1), 167-170. doi: 0006-8993(94)91778-7 [pii]

Lundgren-Nilsson, A., Rosen, H., Hofgren, C., & Sunnerhagen, K. S. (2005). The first year after successful cardiac resuscitation: function, activity, participation and quality of life. *Resuscitation, 66*(3), 285-289. doi: 10.1016/j.resuscitation.2005.04.001

Lundkvist, J., Chai, Z., Teheranian, R., Hasanvan, H., Bartfai, T., Jenck, F., . . . Moreau, J. L. (1996). A non peptidic corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. *Eur J Pharmacol, 309*(2), 195-200.

Luo, L., Liu, X. L., Mu, R. H., Wu, Y. J., Liu, B. B., Geng, D., . . . Yi, L. T. (2015). Hippocampal BDNF signaling restored with chronic asiaticoside treatment in depression-like mice. *Brain Res Bull, 114*, 62-69. doi: 10.1016/j.brainresbull.2015.03.006

Lupien, S. J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N. P., . . . Meaney, M. J. (1998). Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci, 1*(1), 69-73. doi: 10.1038/271

Lynch, G., Rex, C. S., Chen, L. Y., & Gall, C. M. (2008). The substrates of memory: defects, treatments, and enhancement. *Eur J Pharmacol, 585*(1), 2-13. doi: 10.1016/j.ejphar.2007.11.082

Lyons, M. K., Anderson, R. E., & Meyer, F. B. (1991). Corticotropin releasing factor antagonist reduces ischemic hippocampal neuronal injury. *Brain Res, 545*(1-2), 339-342.

Ma, Y. L., Chen, K. Y., Wei, C. L., & Lee, E. H. Y. (1999). Corticotropin-releasing factor enhances brain-derived neurotrophic factor gene expression to facilitate memory retention in rats. *Chinese Journal of Physiology, 42*(2), 73-81.

Ma, Y. L., Wang, H. L., Wu, H. C., Wei, C. L., & Lee, E. H. (1998). Brain-derived neurotrophic factor antisense oligonucleotide impairs memory retention and inhibits long-term potentiation in rats. *Neuroscience, 82*(4), 957-967. doi: S0306452297003254 [pii]

Mackay, K. B., Bozigian, H., Grigoriadis, D. E., Loddick, S. A., Verge, G., & Foster, A. C. (2001). Neuroprotective effects of the CRF1 antagonist R121920 after permanent focal ischemia in the rat. *J Cereb Blood Flow Metab, 21*(10), 1208-1214. doi: 10.1097/00004647-200110000-00009

Maclellan, C. L., Keough, M. B., Granter-Button, S., Chernenko, G. A., Butt, S., & Corbett, D. (2011). A Critical Threshold of Rehabilitation Involving Brain-Derived Neurotrophic Factor Is Required for Poststroke Recovery. *Neurorehabil Neural Repair*. doi: 1545968311407517 [pii] 10.1177/1545968311407517

MacQueen, G., & Frodl, T. (2011). The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? *Mol Psychiatry, 16*(3), 252-264. doi: 10.1038/mp.2010.80

Madinier, A., Bertrand, N., Mossiat, C., Prigent-Tessier, A., Beley, A., Marie, C., & Garnier, P. (2009). Microglial involvement in neuroplastic changes following focal brain ischemia in rats. *PLoS One, 4*(12), e8101. doi: 10.1371/journal.pone.0008101

Madinier, A., Bertrand, N., Rodier, M., Quirie, A., Mossiat, C., Prigent-Tessier, A., . . . Garnier, P. (2013). Ipsilateral versus contralateral spontaneous post-stroke neuroplastic changes: involvement of BDNF? *Neuroscience, 231*, 169-181. doi: 10.1016/j.neuroscience.2012.11.054

Madrigal, J. L., Caso, J. R., de Cristobal, J., Cardenas, A., Leza, J. C., Lizasoain, I., . . . Moro, M. A. (2003). Effect of subacute and chronic immobilisation stress on the outcome of permanent focal cerebral ischaemia in rats. *Brain Res, 979*(1-2), 137-145. doi: S0006899303028920 [pii]

Maes, M., Lin, A. H., Delmeire, L., Van Gastel, A., Kenis, G., De Jongh, R., & Bosmans, E. (1999). Elevated serum interleukin-6 (IL-6) and IL-6 receptor concentrations in posttraumatic stress disorder following accidental man-made traumatic events. *Biol Psychiatry, 45*(7), 833-839.

Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., . . . Maj, M. (2009). The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis, 24*(1), 27-53. doi: 10.1007/s11011-008-9118-1

Magarinos, A. M., Orchinik, M., & McEwen, B. S. (1998). Morphological changes in the hippocampal CA3 region induced by non-invasive glucocorticoid administration: a paradox. *Brain Res, 809*(2), 314-318. doi: S0006-8993(98)00882-8 [pii]

Magarinos, A. M., Somoza, G., & De Nicola, A. F. (1987). Glucocorticoid negative feedback and glucocorticoid receptors after hippocampectomy in rats. *Horm Metab Res, 19*(3), 105-109.

Makino, S., Schulkin, J., Smith, M. A., Pacak, K., Palkovits, M., & Gold, P. W. (1995). Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. *Endocrinology, 136*(10), 4517-4525.

Malek, M., Duszczyk, M., Zyszkowski, M., Ziembowicz, A., & Salinska, E. (2013). Hyperbaric oxygen and hyperbaric air treatment result in comparable neuronal death reduction and improved behavioral outcome after transient forebrain ischemia in the gerbil. *Exp Brain Res, 224*(1), 1-14. doi: 10.1007/s00221-012-3283-5

Maletic, V., Robinson, M., Oakes, T., Iyengar, S., Ball, S. G., & Russell, J. (2007). Neurobiology of depression: an integrated view of key findings. *Int J Clin Pract, 61*(12), 2030-2040. doi: IJCP1602 [pii] 10.1111/j.1742-1241.2007.01602.x

Mallo, T., Alttoa, A., Koiv, K., Tonissaar, M., Eller, M., & Harro, J. (2007). Rats with persistently low or high exploratory activity: behaviour in tests of anxiety and depression, and extracellular levels of dopamine. *Behav Brain Res, 177*(2), 269-281. doi: 10.1016/j.bbr.2006.11.022

Mallo, T., Berggard, C., Eller, M., Damberg, M., Oreland, L., & Harro, J. (2004). Effect of long-term blockade of CRF(1) receptors on exploratory behaviour, monoamines and transcription factor AP-2. *Pharmacol Biochem Behav, 77*(4), 855-865. doi: 10.1016/j.pbb.2004.02.016

Mangus, D. B., Huang, L., Applegate, P. M., Gatling, J. W., Zhang, J., & Applegate, R. L., 2nd. (2014). A systematic review of neuroprotective strategies after cardiac arrest: from bench to bedside (Part I - Protection via specific pathways). *Med Gas Res, 4*, 9. doi: 10.1186/2045-9912-4-9

Maniam, J., & Morris, M. J. (2012). The link between stress and feeding behaviour. *Neuropharmacology, 63*(1), 97-110. doi: 10.1016/j.neuropharm.2012.04.017 S0028-3908(12)00153-0 [pii]

Mansbach, R. S., Brooks, E. N., & Chen, Y. L. (1997). Antidepressant-like effects of CP-154,526, a selective CRF(1) receptor antagonist. *Eur J Pharmacol, 323*(1), 21-26. doi: Doi 10.1016/S0014-2999(97)00025-3

Maras, P. M., & Baram, T. Z. (2012). Sculpting the hippocampus from within: stress, spines, and CRH. *Trends Neurosci, 35*(5), 315-324. doi: 10.1016/j.tins.2012.01.005

Marin, M. F., Hupbach, A., Maheu, F. S., Nader, K., & Lupien, S. J. (2011). Metyrapone administration reduces the strength of an emotional memory trace in a long-lasting manner. *J Clin Endocrinol Metab, 96*(8), E1221-1227. doi: 10.1210/jc.2011-0226 jc.2011-0226 [pii]

Marmigere, F., Givalois, L., Rage, F., Arancibia, S., & Tapia-Arancibia, L. (2003). Rapid induction of BDNF expression in the hippocampus during immobilization stress challenge in adult rats. *Hippocampus, 13*(5), 646-655. doi: 10.1002/hipo.10109

Marta-Moreno, J., Mostacero, E., Lopez del Val, J., & Morales-Asin, F. (1997). [Hormonal response to stress after cerebrovascular accident: relation to type, size and site of the lesion]. *Rev Neurol, 25*(140), 535-540.

Marti, O., Marti, J., & Armario, A. (1994). Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. *Physiol Behav, 55*(4), 747-753. doi: 0031-9384(94)90055-8 [pii]

Martin, S., Henley, J. M., Holman, D., Zhou, M., Wiegert, O., van Spronsen, M., . . . Krugers, H. J. (2009). Corticosterone alters AMPAR mobility and facilitates bidirectional synaptic plasticity. *PLoS One, 4*(3), e4714. doi: 10.1371/journal.pone.0004714

Martinez-Tellez, R. I., Hernandez-Torres, E., Gamboa, C., & Flores, G. (2009). Prenatal stress alters spine density and dendritic length of nucleus accumbens and hippocampus neurons in rat offspring. *Synapse, 63*(9), 794-804. doi: 10.1002/syn.20664

Martone, M. E., Hu, B. R., & Ellisman, M. H. (2000). Alterations of hippocampal postsynaptic densities following transient ischemia. *Hippocampus, 10*(5), 610-616. doi: 10.1002/1098-1063(2000)10:5<610::AID-HIPO12>3.0.CO;2-E

Masana, M., Castane, A., Santana, N., Bortolozzi, A., & Artigas, F. (2012). Noradrenergic antidepressants increase cortical dopamine: Potential use in augmentation strategies. *Neuropharmacology, 63*(4), 675-684. doi: S0028-3908(12)00218-3 [pii] 10.1016/j.neuropharm.2012.05.020

McAllister, A. K., Katz, L. C., & Lo, D. C. (1997). Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. *Neuron, 18*(5), 767-778.

McAllister, A. K., Lo, D. C., & Katz, L. C. (1995). Neurotrophins regulate dendritic growth in developing visual cortex. *Neuron, 15*(4), 791-803.

McAuley, M. T., Kenny, R. A., Kirkwood, T. B., Wilkinson, D. J., Jones, J. J., & Miller, V. M. (2009). A mathematical model of aging-related and cortisol induced hippocampal dysfunction. *BMC Neurosci, 10*, 26. doi: 1471-2202-10-26 [pii] 10.1186/1471-2202-10-26

McCoy, M. K., & Tansey, M. G. (2008). TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. *J Neuroinflammation, 5*, 45. doi: 10.1186/1742-2094-5-45 1742-2094-5-45 [pii]

McDevitt, R. A., Szot, P., Baratta, M. V., Bland, S. T., White, S. S., Maier, S. F., & Neumaier, J. F. (2009). Stress-induced activity in the locus coeruleus is not sensitive to stressor controllability. *Brain Res, 1285*, 109-118. doi: S0006-8993(09)01168-8 [pii] 10.1016/j.brainres.2009.06.017

McDonald, R. J., Craig, L. A., & Hong, N. S. (2008). Enhanced cell death in hippocampus and emergence of cognitive impairments following a localized mini-stroke in hippocampus if preceded by a previous episode of acute stress. *European Journal of Neuroscience, 27*(8), 2197-2209. doi: DOI 10.1111/j.1460-9568.2008.06151.x

McEwen, B. S. (1998). Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Annals of the New York Academy of Sciences, 840*(1), 33-44. doi: 10.1111/j.1749-6632.1998.tb09546.x

McEwen, B. S. (2010). Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. *Ann N Y Acad Sci, 1204 Suppl*, E38-59. doi: 10.1111/j.1749-6632.2010.05568.x

McEwen, B. S., & Sapolsky, R. M. (1995). Stress and cognitive function. *Curr Opin Neurobiol, 5*(2), 205-216. doi: 0959-4388(95)80028-X [pii]

McGaugh, J. L. (2004). The Amygdala Modulates the Consolidation of Memories of Emotionally Arousing Experiences. *Annual Review of Neuroscience, 27*(1), 1-28. doi: 10.1146/annurev.neuro.27.070203.144157

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). The use of behavioral test batteries: Effects of training history. *Physiology & Behavior, 73*(5), 705-717. doi: Doi 10.1016/S0031-9384(01)00528-5

Meijer, M. K., Sommer, R., Spruijt, B. M., van Zutphen, L. F., & Baumans, V. (2007). Influence of environmental enrichment and handling on the acute stress response in individually housed mice. *Lab Anim, 41*(2), 161-173. doi: 10.1258/002367707780378168

Melkas, S., Vataja, R., Oksala, N. K., Jokinen, H., Pohjasvaara, T., Oksala, A., . . . Erkinjuntti, T. (2010). Depression-executive dysfunction syndrome relates to poor poststroke survival. *Am J Geriatr Psychiatry, 18*(11), 1007-1016. doi: 10.1097/JGP.0b013e3181d695d7

Meng, F., Wang, Y., Liu, R., Gao, M., & Du, G. (2014). Pinocembrin alleviates memory impairment in transient global cerebral ischemic rats. *Exp Ther Med, 8*(4), 1285-1290. doi: 10.3892/etm.2014.1923

Menzebach, A., Bergt, S., von Waldthausen, P., Dinu, C., Noldge-Schomburg, G., & Vollmar, B. (2010). A comprehensive study of survival, tissue damage, and neurological dysfunction in a murine model of cardiopulmonary resuscitation after potassium-induced cardiac arrest. *Shock, 33*(2), 189-196. doi: 10.1097/SHK.0b013e3181ad59a3

Merali, Z., Kent, P., Du, L., Hrdina, P., Palkovits, M., Faludi, G., . . . Anisman, H. (2006). Corticotropin-releasing hormone, arginine vasopressin, gastrin-releasing peptide, and neuromedin B alterations in stress-relevant brain regions of suicides and control subjects. *Biol Psychiatry, 59*(7), 594-602. doi: S0006-3223(05)00993-5 [pii] 10.1016/j.biopsych.2005.08.008

Merali, Z., McIntosh, J., Kent, P., Michaud, D., & Anisman, H. (1998). Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. *J Neurosci, 18*(12), 4758-4766.

Merlo Pich, E., Lorang, M., Yeganeh, M., Rodriguez de Fonseca, F., Raber, J., Koob, G. F., & Weiss, F. (1995). Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci, 15*(8), 5439-5447.

Meyer-Lindenberg, A., & Tost, H. (2012). Neural mechanisms of social risk for psychiatric disorders. *Nat Neurosci, 15*(5), 663-668. doi: 10.1038/nn.3083

Michelsen, K. A., van den Hove, D. L., Schmitz, C., Segers, O., Prickaerts, J., & Steinbusch, H. W. (2007). Prenatal stress and subsequent exposure to chronic mild stress influence dendritic spine density and morphology in the rat medial prefrontal cortex. *BMC Neurosci, 8*, 107. doi: 1471-2202-8-107 [pii] 10.1186/1471-2202-8-107

Mikova, O., Yakimova, R., Bosmans, E., Kenis, G., & Maes, M. (2001). Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis. *Eur Neuropsychopharmacol, 11*(3), 203-208.

Mileson, B. E., & Schwartz, R. D. (1991). The Use of Locomotor-Activity as a Behavioral Screen for Neuronal Damage Following Transient Forebrain Ischemia in Gerbils. *Neuroscience Letters, 128*(1), 71-76. doi: Doi 10.1016/0304-3940(91)90762-I

Miller, A. H., Maletic, V., & Raison, C. L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry, 65*(9), 732-741. doi: 10.1016/j.biopsych.2008.11.029

Milot, M., & Plamondon, H. (2008). Ischemia-induced hyperactivity: effects of dim versus bright illumination on open-field exploration and habituation following global ischemia in rats. *Behav Brain Res, 192*(2), 166-172. doi: S0166-4328(08)00185-X [pii] 10.1016/j.bbr.2008.03.044

Milot, M. R., James, J. S., Merali, Z., & Plamondon, H. (2012). A refined blood collection method for quantifying corticosterone. *Lab Anim (NY), 41*(3), 77-83. doi: 10.1038/laban0312-77

Milot, M. R., & Plamondon, H. (2009). Time-dependent effects of global cerebral ischemia on anxiety, locomotion, and habituation in rats. *Behavioural Brain Research, 200*(1), 173-180. doi: 10.1016/j.bbr.2009.01.009

Milot, M. R., & Plamondon, H. (2011a). Changes in HPA reactivity and noradrenergic functions regulate spatial memory impairments at delayed time intervals following cerebral ischemia. *Horm Behav, 59*(4), 594-604. doi: 10.1016/j.yhbeh.2011.02.017

Milot, M. R., & Plamondon, H. (2011b). Changes in HPA reactivity and noradrenergic functions regulate spatial memory impairments at delayed time intervals following cerebral ischemia. *Horm Behav*. doi: S0018-506X(11)00058-4 [pii] 10.1016/j.yhbeh.2011.02.017

Ming-Yan, H., Luo, Y. L., Zhang, X. C., Liu, H., Gao, R., & Wu, J. J. (2012). Hypoxic-ischemic injury decreases anxiety-like behavior in rats when associated with loss of tyrosine-hydroxylase immunoreactive neurons of the substantia nigra. *Braz J Med Biol Res, 45*(1), 13-19. doi: S0100-879X2011007500161 [pii]

Minichiello, L., Calella, A. M., Medina, D. L., Bonhoeffer, T., Klein, R., & Korte, M. (2002). Mechanism of TrkB-mediated hippocampal long-term potentiation. *Neuron, 36*(1), 121-137.

Miranda, D. R. (1994). Quality of life after cardiopulmonary resuscitation. *Chest, 106*(2), 524-530.

Mishima, Y., Shinoda, Y., Sadakata, T., Kojima, M., Wakana, S., & Furuichi, T. (2015). Lack of stress responses to long-term effects of corticosterone in Caps2 knockout mice. *Sci Rep, 5*, 8932. doi: 10.1038/srep08932

Mitra, R., Adamec, R., & Sapolsky, R. (2009). Resilience against predator stress and dendritic morphology of amygdala neurons. *Behav Brain Res, 205*(2), 535-543. doi: S0166-4328(09)00483-5 [pii] 10.1016/j.bbr.2009.08.014

Mitra, R., Jadhav, S., McEwen, B. S., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proc Natl Acad Sci U S A, 102*(26), 9371-9376. doi: 0504011102 [pii] 10.1073/pnas.0504011102

Mizoguchi, K., Ikeda, R., Shoji, H., Tanaka, Y., Maruyama, W., & Tabira, T. (2009). Aging attenuates glucocorticoid negative feedback in rat brain. *Neuroscience, 159*(1), 259-270. doi: 10.1016/j.neuroscience.2008.12.020

S0306-4522(08)01797-1 [pii]

Mizoguchi, K., Ishige, A., Aburada, M., & Tabira, T. (2003). Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. *Neuroscience, 119*(3), 887-897. doi: S0306452203001052 [pii]

Mizumatsu, S., Monje, M. L., Morhardt, D. R., Rola, R., Palmer, T. D., & Fike, J. R. (2003). Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. *Cancer Res, 63*(14), 4021-4027.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., & Nabeshima, T. (2000). Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *J Neurosci, 20*(18), 7116-7121. doi: 20/18/7116 [pii]

Mogenson, G. J., Jones, D. L., & Yim, C. Y. (1980). From motivation to action: functional interface between the limbic system and the motor system. *Prog Neurobiol, 14*(2-3), 69-97.

Moghimian, M., Faghihi, M., Karimian, S. M., & Imani, A. (2012). The effect of acute stress exposure on ischemia and reperfusion injury in rat heart: role of oxytocin. *Stress, 15*(4), 385-392. doi: 10.3109/10253890.2011.630436

Monteggia, L. M., Barrot, M., Powell, C. M., Berton, O., Galanis, V., Gemelli, T., . . . Nestler, E. J. (2004). Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc Natl Acad Sci U S A, 101*(29), 10827-10832. doi: 10.1073/pnas.0402141101
0402141101 [pii]

Mookadam, F., & Arthur, H. M. (2004). Social support and its relationship to morbidity and mortality after acute myocardial infarction: systematic overview. *Arch Intern Med, 164*(14), 1514-1518. doi: 10.1001/archinte.164.14.1514
164/14/1514 [pii]

Moran, G. M., Fletcher, B., Feltham, M. G., Calvert, M., Sackley, C., & Marshall, T. (2014). Fatigue, psychological and cognitive impairment following transient ischaemic attack and minor stroke: a systematic review. *Eur J Neurol, 21*(10), 1258-1267. doi: 10.1111/ene.12469

Morioka, T., Kalehua, A. N., & Streit, W. J. (1991). The microglial reaction in the rat dorsal hippocampus following transient forebrain ischemia. *J Cereb Blood Flow Metab, 11*(6), 966-973. doi: 10.1038/jcbfm.1991.162

Moriyama, Y., Takagi, N., & Tanonaka, K. (2011). Intravenous injection of neural progenitor cells improved depression-like behavior after cerebral ischemia. *Transl Psychiatry, 1*, e29. doi: 10.1038/tp.2011.32
tp201132 [pii]

Morris, R. G., Moser, E. I., Riedel, G., Martin, S. J., Sandin, J., Day, M., & O'Carroll, C. (2003). Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. *Philos Trans R Soc Lond B Biol Sci, 358*(1432), 773-786. doi: 10.1098/rstb.2002.1264

Morse, J. K., & Davis, J. N. (1990). Regulation of ischemic hippocampal damage in the gerbil: adrenalectomy alters the rate of CA1 cell disappearance. *Exp Neurol, 110*(1), 86-92.

Moulaert, V. R., Verbunt, J. A., van Heugten, C. M., & Wade, D. T. (2009). Cognitive impairments in survivors of out-of-hospital cardiac arrest: a systematic review. *Resuscitation, 80*(3), 297-305. doi: 10.1016/j.resuscitation.2008.10.034

Moulaert, V. R., Wachelder, E. M., Verbunt, J. A., Wade, D. T., & van Heugten, C. M. (2010). Determinants of quality of life in survivors of cardiac arrest. *J Rehabil Med, 42*(6), 553-558. doi: 10.2340/16501977-0547

Mu, J. S., Li, W. P., Yao, Z. B., & Zhou, X. F. (1999). Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Res, 835*(2), 259-265. doi: S0006-8993(99)01592-9 [pii]

Müller, M. B., & Wurst, W. (2004). Getting closer to affective disorders: the role of CRH receptor systems. *Trends in Molecular Medicine, 10*(8), 409-415. doi: 10.1016/j.molmed.2004.06.007

Muller, M. B., Zimmermann, S., Sillaber, I., Hagemeyer, T. P., Deussing, J. M., Timpl, P., . . . Wurst, W. (2003). Limbic corticotropin-releasing hormone receptor 1 mediates anxiety-related behavior and hormonal adaptation to stress. *Nat Neurosci, 6*(10), 1100-1107. doi: 10.1038/nn1123

Murakami, S., Imbe, H., Morikawa, Y., Kubo, C., & Senba, E. (2005). Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci Res, 53*(2), 129-139. doi: 10.1016/j.neures.2005.06.008

Muravieva, E. V., & Alberini, C. M. (2010). Limited efficacy of propranolol on the reconsolidation of fear memories. *Learn Mem, 17*(6), 306-313. doi: 17/6/306 [pii] 10.1101/lm.1794710

Murmu, M. S., Salomon, S., Biala, Y., Weinstock, M., Braun, K., & Bock, J. (2006). Changes of spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy. *Eur J Neurosci, 24*(5), 1477-1487. doi: EJN5024 [pii]
10.1111/j.1460-9568.2006.05024.x

Murphy, S. J., McCullough, L. D., & Smith, J. M. (2004). Stroke in the female: Role of biological sex and estrogen. *Ilar Journal, 45*(2), 147-159.

Murros, K., Fogelholm, R., Kettunen, S., & Vuorela, A. L. (1993). Serum cortisol and outcome of ischemic brain infarction. *J Neurol Sci, 116*(1), 12-17.

Myers, B., & Greenwood-Van Meerveld, B. (2010). Elevated corticosterone in the amygdala leads to persistent increases in anxiety-like behavior and pain sensitivity. *Behav Brain Res, 214*(2), 465-469. doi: 10.1016/j.bbr.2010.05.049
S0166-4328(10)00423-7 [pii]

Naarding, P., de Koning, I., van Kooten, F., Janzing, J. G. E., Beekman, A. T. F., & Koudstaal, P. J. (2007). Post-stroke dementia and depression: frontosubcortical dysfunction as missing link? *International Journal of Geriatric Psychiatry, 22*(1), 1-8. doi: Doi 10.1002/Gps.1599

Nabavi, S. F., Turner, A., Dean, O., Sureda, A., & Nabavi, S. M. (2014). Post-stroke Depression Therapy: Where are we now? *Curr Neurovasc Res, 11*(3), 279-289.

Naert, G., Ixart, G., Maurice, T., Tapia-Arancibia, L., & Givalois, L. (2011). Brain-derived neurotrophic factor and hypothalamic-pituitary-adrenal axis adaptation processes in a depressive-like state induced by chronic restraint stress. *Mol Cell Neurosci, 46*(1), 55-66. doi: 10.1016/j.mcn.2010.08.006

Naert, G., Ixart, G., Tapia-Arancibia, L., & Givalois, L. (2006). Continuous i.c.v. infusion of brain-derived neurotrophic factor modifies hypothalamic-pituitary-adrenal axis activity, locomotor activity and body temperature rhythms in adult male rats. *Neuroscience, 139*(2), 779-789. doi: 10.1016/j.neuroscience.2005.12.028

Nakajima, K., Tohyama, Y., Kohsaka, S., & Kurihara, T. (2002). Ceramide activates microglia to enhance the production/secretion of brain-derived neurotrophic factor (BDNF) without induction of deleterious factors in vitro. *J Neurochem, 80*(4), 697-705.

Nakashima, M. N., Ajiki, K., Nakashima, K., & Takahashi, M. (2003). Possible role of nitric oxide in anxiety following transient cerebral ischemia in mice. *J Pharmacol Sci, 91*(1), 47-52.

Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., . . . Nakafuku, M. (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell, 110*(4), 429-441. doi: S0092867402008620 [pii]

Narantuya, D., Nagai, A., Sheikh, A. M., Masuda, J., Kobayashi, S., Yamaguchi, S., & Kim, S. U. (2010). Human microglia transplanted in rat focal ischemia brain induce neuroprotection and behavioral improvement. *PLoS One, 5*(7), e11746. doi: 10.1371/journal.pone.0011746

Narita, M., Aoki, K., Takagi, M., Yajima, Y., & Suzuki, T. (2003). Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine. *Neuroscience, 119*(3), 767-775.

Naudon, L., & Jay, T. M. (2005). Opposite behaviours in the forced swimming test are linked to differences in spatial working memory performances in the rat. *Neuroscience, 130*(2), 285-293. doi: 10.1016/j.neuroscience.2004.09.009

Neidert, S., Katan, M., Schuetz, P., Fluri, F., Ernst, A., Bingisser, R., . . . Christ-Crain, M. (2011). Anterior pituitary axis hormones and outcome in acute ischaemic stroke. *J Intern Med, 269*(4), 420-432. doi: 10.1111/j.1365-2796.2010.02327.x

Neigh, G. N., Glasper, E. R., Kofler, J., Traystman, R. J., Mervis, R. F., Bachstetter, A., & DeVries, A. C. (2004). Cardiac arrest with cardiopulmonary resuscitation reduces dendritic spine density in CA1 pyramidal cells and selectively alters acquisition of spatial memory. *Eur J Neurosci, 20*(7), 1865-1872. doi: 10.1111/j.1460-9568.2004.03649.x

Neigh, G. N., Karelina, K., Glasper, E. R., Bowers, S. L., Zhang, N., Popovich, P. G., & DeVries, A. C. (2009). Anxiety after cardiac arrest/cardiopulmonary resuscitation: exacerbated by stress and prevented by minocycline. *Stroke, 40*(11), 3601-3607. doi: 10.1161/STROKEAHA.109.564146

Neigh, G. N., Karelina, K., Zhang, N., Glasper, E. R., Owens, M. J., Plotsky, P. M., . . . Devries, A. C. (2009). Cardiac arrest and cardiopulmonary resuscitation dysregulates the hypothalamic-pituitary-adrenal axis. *J Cereb Blood Flow Metab, 29*(10), 1673-1682. doi: 10.1038/jcbfm.2009.85

Nelson, A., Lebessi, A., Sowinski, P., & Hodges, H. (1997). Comparison of effects of global cerebral ischaemia on spatial learning in the standard and radial water maze: relationship of hippocampal damage to performance. *Behav Brain Res, 85*(1), 93-115. doi: S0166432896001672 [pii]

Nemeroff, C. B. (1992). New vistas in neuropeptide research in neuropsychiatry: focus on corticotropin-releasing factor. *Neuropsychopharmacology, 6*(2), 69-75.

Nemeroff, C. B., Krishnan, K. R., Reed, D., Leder, R., Beam, C., & Dunnick, N. R. (1992). Adrenal gland enlargement in major depression. A computed tomographic study. *Arch Gen Psychiatry, 49*(5), 384-387.

Nemeroff, C. B., Owens, M. J., Bissette, G., Andorn, A. C., & Stanley, M. (1988). Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry, 45*(6), 577-579.

Nemeroff, C. B., Widerlov, E., Bissette, G., Walleus, H., Karlsson, I., Eklund, K., . . . Vale, W. (1984). Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science, 226*(4680), 1342-1344.

Nemeth, C. L., Shurte, M. S., McTigue, D. M., Nemeroff, C. B., & Neigh, G. N. (2012). Microembolism infarcts lead to delayed changes in affective-like behaviors followed by spatial memory impairment. *Behav Brain Res, 234*(2), 259-266. doi: 10.1016/j.bbr.2012.06.014
S0166-4328(12)00427-5 [pii]

Nestler, E. J. (2015). Role of the Brain's Reward Circuitry in Depression: Transcriptional Mechanisms. *Int Rev Neurobiol, 124*, 151-170. doi: 10.1016/bs.irn.2015.07.003

Nestler, E. J., & Carlezon, W. A., Jr. (2006). The mesolimbic dopamine reward circuit in depression. *Biol Psychiatry, 59*(12), 1151-1159. doi: 10.1016/j.biopsych.2005.09.018

Neumann, J., Gunzer, M., Gutzeit, H. O., Ullrich, O., Reymann, K. G., & Dinkel, K. (2006). Microglia provide neuroprotection after ischemia. *FASEB J, 20*(6), 714-716. doi: 05-4882fje [pii]10.1096/fj.05-4882fje

Neumann, J. T., Cohan, C. H., Dave, K. R., Wright, C. B., & Perez-Pinzon, M. A. (2013). Global Cerebral Ischemia: Synaptic and Cognitive Dysfunction. *Curr Drug Targets, 14*(1), 20-35.

Nguyen, K. T., Deak, T., Owens, S. M., Kohno, T., Fleshner, M., Watkins, L. R., & Maier, S. F. (1998). Exposure to acute stress induces brain interleukin-1beta protein in the rat. *J Neurosci, 18*(6), 2239-2246.

Nibuya, M., Morinobu, S., & Duman, R. S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci, 15*(11), 7539-7547.

Nibuya, M., Takahashi, M., Russell, D. S., & Duman, R. S. (1999). Repeated stress increases catalytic TrkB mRNA in rat hippocampus. *Neurosci Lett, 267*(2), 81-84. doi: S0304-3940(99)00335-3 [pii]

Nikulina, E. M., Lacagnina, M. J., Fanous, S., Wang, J., & Hammer, R. P., Jr. (2012). Intermittent social defeat stress enhances mesocorticolimbic DeltaFosB/BDNF co-expression and persistently activates corticotegmental neurons: implication for vulnerability to psychostimulants. *Neuroscience, 212*, 38-48. doi: 10.1016/j.neuroscience.2012.04.012

Nishiyama, Y., Komaba, Y., Ueda, M., Nagayama, H., Amemiya, S., & Katayama, Y. (2010). Early Depressive Symptoms after Ischemic Stroke Are Associated with a Left Lenticulocapsular Area Lesion. *Journal of Stroke and Cerebrovascular Diseases, 19*(3), 184-189. doi: 10.1016/j.jstrokecerebrovasdis.2009.04.002

Nitatori, T., Sato, N., Waguri, S., Karasawa, Y., Araki, H., Shibanai, K., . . . Uchiyama, Y. (1995). Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. *J Neurosci, 15*(2), 1001-1011.

Nudo, R. J. (2011). Neural bases of recovery after brain injury. *J Commun Disord*. doi: S0021-9924(11)00026-8 [pii]
10.1016/j.jcomdis.2011.04.004

O'Connor, K. A., Ginsberg, A. B., Maksimova, E., Wieseler Frank, J. L., Johnson, J. D., Spencer, R. L., . . . Maier, S. F. (2004). Stress-induced sensitization of the hypothalamic-pituitary adrenal axis is associated with alterations of hypothalamic and pituitary gene expression. *Neuroendocrinology, 80*(4), 252-263. doi: 82876 [pii] 10.1159/000082876

O'Connor, K. A., Johnson, J. D., Hammack, S. E., Brooks, L. M., Spencer, R. L., Watkins, L. R., & Maier, S. F. (2003). Inescapable shock induces resistance to the effects of dexamethasone. *Psychoneuroendocrinology, 28*(4), 481-500. doi: S0306453002000355 [pii]

O'Keefe, L. M., Doran, S. J., Mwilambwe-Tshilobo, L., Conti, L. H., Venna, V. R., & McCullough, L. D. (2014). Social isolation after stroke leads to depressive-like behavior and decreased BDNF levels in mice. *Behav Brain Res, 260*, 162-170. doi: 10.1016/j.bbr.2013.10.047 S0166-4328(13)00672-4 [pii]

O'Neill, P. A., Davies, I., Fullerton, K. J., & Bennett, D. (1991). Stress hormone and blood glucose response following acute stroke in the elderly. *Stroke, 22*(7), 842-847.

O'Toole, K. K., Hooper, A., Wakefield, S., & Maguire, J. (2014). Seizure-induced disinhibition of the HPA axis increases seizure susceptibility. *Epilepsy Res, 108*(1), 29-43. doi: 10.1016/j.eplepsyres.2013.10.013

Oh, D. H., Park, Y. C., & Kim, S. H. (2010). Increased glycogen synthase kinase-3beta mRNA level in the hippocampus of patients with major depression: a study using the stanley neuropathology consortium integrative database. *Psychiatry Investig, 7*(3), 202-207. doi: 10.4306/pi.2010.7.3.202

Ohab, J. J., Fleming, S., Blesch, A., & Carmichael, S. T. (2006). A Neurovascular Niche for Neurogenesis after Stroke. *Journal of Neuroscience, 26*(50), 13007-13016. doi: 10.1523/jneurosci.4323-06.2006

Okazawa, H., Murata, M., Watanabe, M., Kamei, M., & Kanazawa, I. (1992). Dopaminergic stimulation up-regulates the in vivo expression of brain-derived neurotrophic factor (BDNF) in the striatum. *FEBS Lett, 313*(2), 138-142. doi: 0014-5793(92)81430-T [pii]

Olsson, T. (1990). Urinary free cortisol excretion shortly after ischaemic stroke. *J Intern Med, 228*(2), 177-181.

Olsson, T., Astrom, M., Eriksson, S., & Forssell, A. (1989). Hypercortisolism revealed by the dexamethasone suppression test in patients [corrected] with acute ischemic stroke. *Stroke, 20*(12), 1685-1690.

Onodera, H., Araki, T., & Kogure, K. (1989). Protein kinase C activity in the rat hippocampus after forebrain ischemia: autoradiographic analysis by [3H]phorbol 12,13-dibutyrate. *Brain Res, 481*(1), 1-7. doi: 0006-8993(89)90478-2 [pii]

Oto, J., Suzue, A., Inui, D., Fukuta, Y., Hosotsubo, K., Torii, M., . . . Nishimura, M. (2008). Plasma proinflammatory and anti-inflammatory cytokine and catecholamine concentrations as predictors of neurological outcome in acute stroke patients. *J Anesth, 22*(3), 207-212. doi: 10.1007/s00540-008-0639-x

Ou, L. C., & Gean, P. W. (2006). Regulation of amygdala-dependent learning by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol-3-kinase. *Neuropsychopharmacology, 31*(2), 287-296. doi: 1300830 [pii]10.1038/sj.npp.1300830

Ou, L. C., Yeh, S. H., & Gean, P. W. (2010). Late expression of brain-derived neurotrophic factor in the amygdala is required for persistence of fear memory. *Neurobiol Learn Mem, 93*(3), 372-382. doi: 10.1016/j.nlm.2009.12.003

Overstreet, D. H. (2012). Modeling depression in animal models. *Methods Mol Biol, 829*, 125-144. doi: 10.1007/978-1-61779-458-2_7

Overstreet, D. H., & Griebel, G. (2004). Antidepressant-like effects of CRF1 receptor antagonist SSR125543 in an animal model of depression. *Eur J Pharmacol, 497*(1), 49-53. doi: 10.1016/j.ejphar.2004.06.035

Ovtscharoff, W., Jr., & Braun, K. (2001). Maternal separation and social isolation modulate the postnatal development of synaptic composition in the infralimbic cortex of Octodon degus. *Neuroscience, 104*(1), 33-40. doi: S0306-4522(01)00059-8 [pii]

Pacak, K. (2000). Stressor-specific activation of the hypothalamic-pituitary-adrenocortical axis. *Physiol Res, 49 Suppl 1*, S11-17.

Pagliaccio, D., Luby, J. L., Bogdan, R., Agrawal, A., Gaffrey, M. S., Belden, A. C., . . . Barch, D. M. (2015). HPA axis genetic variation, pubertal status, and sex interact to predict amygdala and hippocampus responses to negative emotional faces in school-age children. *Neuroimage, 109*, 1-11. doi: 10.1016/j.neuroimage.2015.01.017

Pandey, D. K., Yadav, S. K., Mahesh, R., & Rajkumar, R. (2009). Depression-like and anxiety-like behavioural aftermaths of impact accelerated traumatic brain injury in rats: a model of comorbid depression and anxiety? *Behav Brain Res, 205*(2), 436-442. doi: 10.1016/j.bbr.2009.07.027
S0166-4328(09)00447-1 [pii]

Pandey, G. N. (2013). Biological basis of suicide and suicidal behavior. *Bipolar Disord, 15*(5), 524-541. doi: 10.1111/bdi.12089

Papiol, S., Arias, B., Gasto, C., Gutierrez, B., Catalan, R., & Fananas, L. (2007). Genetic variability at HPA axis in major depression and clinical response to antidepressant treatment. *Journal of Affective Disorders, 104*(1-3), 83-90. doi: DOI 10.1016/j.jad.2007.02.017

Parent, J. M., Vexler, Z. S., Gong, C., Derugin, N., & Ferriero, D. M. (2002). Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Ann Neurol, 52*(6), 802-813. doi: 10.1002/ana.10393

Pariante, C. M., & Lightman, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends Neurosci, 31*(9), 464-468. doi: S0166-2236(08)00164-1 [pii]
10.1016/j.tins.2008.06.006

Parker, G., & Brotchie, H. (2010). Gender differences in depression. *Int Rev Psychiatry, 22*(5), 429-436. doi: 10.3109/09540261.2010.492391

Paul, C., Stratil, C., Hofmann, F., & Kleppisch, T. (2010). cGMP-dependent protein kinase type I promotes CREB/CRE-mediated gene expression in neurons of the lateral amygdala. *Neurosci Lett, 473*(2), 82-86. doi: 10.1016/j.neulet.2010.02.020

Paul, N., Das, S., Hazra, A., Ghosal, M. K., Ray, B. K., Banerjee, T. K., . . . Das, S. K. (2013). Depression Among Stroke Survivors: A Community-based, Prospective Study from Kolkata, India. *American Journal of Geriatric Psychiatry, 21*(9), 821-831. doi: 10.1016/j.jagp.2013.03.013

Paul, S. L., Srikanth, V. K., & Thrift, A. G. (2007). The large and growing burden of stroke. *Curr Drug Targets, 8*(7), 786-793.

Pawlak, C. R., Ho, Y. J., & Schwarting, R. K. (2008). Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. *Neurosci Biobehav Rev, 32*(8), 1544-1568. doi: 10.1016/j.neubiorev.2008.06.007

Paxinos, G., & Watson, C. (1997). The Rat Brainin Stereotaxic Coordinates. *Academic Press, 3rd Edition*(San Diego).

Paylor, R., Spencer, C. M., Yuva-Paylor, L. A., & Pieke-Dahl, S. (2006). The use of behavioral test batteries, II: Effect of test interval. *Physiology & Behavior, 87*(1), 95-102. doi: 10.1016/j.physbeh.2005.09.002

Pearn, J. (2000). Successful cardiopulmonary resuscitation outcome reviews. *Resuscitation, 47*(3), 311-316.

Peavy, G. M., Lange, K. L., Salmon, D. P., Patterson, T. L., Goldman, S., Gamst, A. C., . . . Galasko, D. (2007). The effects of prolonged stress and APOE genotype on memory and cortisol in older adults. *Biol Psychiatry, 62*(5), 472-478. doi: S0006-3223(07)00256-9 [pii]
10.1016/j.biopsych.2007.03.013

Pedersen, W. A., McCullers, D., Culmsee, C., Haughey, N. J., Herman, J. P., & Mattson, M. P. (2001). Corticotropin-releasing hormone protects neurons against insults relevant to the pathogenesis of Alzheimer's disease. *Neurobiol Dis, 8*(3), 492-503. doi: 10.1006/nbdi.2001.0395

Pekny, M., & Nilsson, M. (2005). Astrocyte activation and reactive gliosis. *Glia, 50*(4), 427-434. doi: 10.1002/glia.20207

Peng, S., Wuu, J., Mufson, E. J., & Fahnestock, M. (2005). Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *J Neurochem, 93*(6), 1412-1421. doi: 10.1111/j.1471-4159.2005.03135.x

Pennartz, C. M., Berke, J. D., Graybiel, A. M., Ito, R., Lansink, C. S., van der Meer, M., . . . Voorn, P. (2009). Corticostriatal Interactions during Learning, Memory Processing, and Decision Making. *J Neurosci, 29*(41), 12831-12838. doi: 10.1523/JNEUROSCI.3177-09.2009

Petit-Demouliere, B., Chenu, F., & Bourin, M. (2005). Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology (Berl), 177*(3), 245-255. doi: 10.1007/s00213-004-2048-7

Petito, C. K., Feldmann, E., Pulsinelli, W. A., & Plum, F. (1987). Delayed hippocampal damage in humans following cardiorespiratory arrest. *Neurology, 37*(8), 1281-1286.

Petito, C. K., Olarte, J. P., Roberts, B., Nowak, T. S., Jr., & Pulsinelli, W. A. (1998). Selective glial vulnerability following transient global ischemia in rat brain. *J Neuropathol Exp Neurol, 57*(3), 231-238.

Petrea, R. E., Beiser, A. S., Seshadri, S., Kelly-Hayes, M., Kase, C. S., & Wolf, P. A. (2009). Gender differences in stroke incidence and poststroke disability in the Framingham heart study. *Stroke, 40*(4), 1032-1037. doi: 10.1161/STROKEAHA.108.542894

Philbert, J., Belzung, C., & Griebel, G. (2013). The CRF1 receptor antagonist SSR125543 prevents stress-induced cognitive deficit associated with hippocampal dysfunction: Comparison with paroxetine and D-cycloserine. *Psychopharmacology (Berl), 228*(1), 97-107. doi: DOI 10.1007/s00213-013-3020-1

Philbert, J., Pichat, P., Palme, R., Belzung, C., & Griebel, G. (2012). The CRF(1) receptor antagonist SSR125543 attenuates long-term cognitive deficit induced by acute

inescapable stress in mice, independently from the hypothalamic pituitary adrenal axis. *Pharmacol Biochem Behav, 102*(3), 415-422. doi: 10.1016/j.pbb.2012.05.013

Pich, E. M., Grimaldi, R., Zini, I., Frasoldati, A., Marrama, P., & Agnati, L. F. (1993). Involvement of alpha 2-receptors in the analgesia induced by transient forebrain ischemia in rats. *Pharmacol Biochem Behav, 45*(3), 607-614.

Pierce, R. C., & Bari, A. A. (2001). The role of neurotrophic factors in psychostimulant-induced behavioral and neuronal plasticity. *Rev Neurosci, 12*(2), 95-110.

Pitts, M. W., & Takahashi, L. K. (2011). The central amygdala nucleus via corticotropin-releasing factor is necessary for time-limited consolidation processing but not storage of contextual fear memory. *Neurobiol Learn Mem, 95*(1), 86-91. doi: S1074-7427(10)00192-9 [pii]
10.1016/j.nlm.2010.11.006

Pitts, M. W., Todorovic, C., Blank, T., & Takahashi, L. K. (2009). The central nucleus of the amygdala and corticotropin-releasing factor: insights into contextual fear memory. *J Neurosci, 29*(22), 7379-7388. doi: 29/22/7379 [pii]
10.1523/JNEUROSCI.0740-09.2009

Pizarro, J. M., Lumley, L. A., Medina, W., Robison, C. L., Chang, W. E., Alagappan, A., . . . Meyerhoff, J. L. (2004). Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. *Brain Res, 1025*(1-2), 10-20. doi: 10.1016/j.brainres.2004.06.085

Plamondon, H., & Khan, S. (2005). Characterization of anxiety and habituation profile following global ischemia in rats. *Physiol Behav, 84*(4), 543-552. doi: S0031-9384(05)00040-5 [pii]
10.1016/j.physbeh.2005.02.001

Plamondon, H., & Khan, S. (2006). The CRH1 antagonist CP154,526 failed to alter ischemia-induced neurodegeneration and spatial memory deficits in rats but inhibited behavioral activity in the novel open field. *Behav Brain Res, 166*(1), 85-92. doi: 10.1016/j.bbr.2005.07.031

Platenik, J., Fisar, Z., Buchal, R., Jirak, R., Kitzlerova, E., Zverova, M., & Raboch, J. (2013). GSK3beta, CREB, and BDNF in peripheral blood of patients with Alzheimer's disease and depression. *Prog Neuropsychopharmacol Biol Psychiatry, 50C*, 83-93. doi: S0278-5846(13)00270-4 [pii]
10.1016/j.pnpbp.2013.12.001

Plotsky, P. M., Thrivikraman, K. V., Nemeroff, C. B., Caldji, C., Sharma, S., & Meaney, M. J. (2005). Long-term consequences of neonatal rearing on central corticotropin-releasing factor systems in adult male rat offspring. *Neuropsychopharmacology, 30*(12), 2192-2204. doi: 10.1038/sj.npp.1300769

Ploughman, M., Attwood, Z., White, N., Dore, J. J., & Corbett, D. (2007). Endurance exercise facilitates relearning of forelimb motor skill after focal ischemia. *Eur J Neurosci, 25*(11), 3453-3460. doi: EJN5591 [pii]
10.1111/j.1460-9568.2007.05591.x

Ploughman, M., Windle, V., MacLellan, C. L., White, N., Dore, J. J., & Corbett, D. (2009). Brain-derived neurotrophic factor contributes to recovery of skilled reaching after focal ischemia in rats. *Stroke, 40*(4), 1490-1495. doi: STROKEAHA.108.531806 [pii]
10.1161/STROKEAHA.108.531806

Poeggel, G., Helmeke, C., Abraham, A., Schwabe, T., Friedrich, P., & Braun, K. (2003). Juvenile emotional experience alters synaptic composition in the rodent cortex,

hippocampus, and lateral amygdala. *Proc Natl Acad Sci U S A, 100*(26), 16137-16142. doi: 10.1073/pnas.2434663100
2434663100 [pii]

Polanczyk, G., Caspi, A., Williams, B., Price, T. S., Danese, A., Sugden, K., . . . Moffitt, T. E. (2009). Protective effect of CRHR1 gene variants on the development of adult depression following childhood maltreatment: replication and extension. *Arch Gen Psychiatry, 66*(9), 978-985. doi: 10.1001/archgenpsychiatry.2009.114

Pollak, Y., & Yirmiya, R. (2002). Cytokine-induced changes in mood and behaviour: implications for "depression due to a general medical condition", immunotherapy and antidepressive treatment. *International Journal of Neuropsychopharmacology, 5*(4), 389-399. doi: 10.1017/S1461145702003152

Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol, 47*(4), 379-391.

Porte, Y., Trifilieff, P., Wolff, M., Micheau, J., Buhot, M. C., & Mons, N. (2011). Extinction of spatial memory alters CREB phosphorylation in hippocampal CA1. *Hippocampus, 21*(11), 1169-1179. doi: 10.1002/hipo.20844

Post, A., Ohl, F., Almeida, O. F., Binder, E. B., Rucker, M., Welt, S., . . . Sillaber, I. (2005). Identification of molecules potentially involved in mediating the in vivo actions of the corticotropin-releasing hormone receptor 1 antagonist, NBI30775 (R121919). *Psychopharmacology (Berl), 180*(1), 150-158. doi: 10.1007/s00213-004-2134-x

Poulos, A. M., Li, V., Sterlace, S. S., Tokushige, F., Ponnusamy, R., & Fanselow, M. S. (2009). Persistence of fear memory across time requires the basolateral amygdala complex. *Proceedings of the National Academy of Sciences, 106*(28), 11737-11741. doi: 10.1073/pnas.0905257106

Preil, J., Muller, M. B., Gesing, A., Reul, J. M., Sillaber, I., van Gaalen, M. M., . . . Wurst, W. (2001). Regulation of the hypothalamic-pituitary-adrenocortical system in mice deficient for CRH receptors 1 and 2. *Endocrinology, 142*(11), 4946-4955. doi: 10.1210/endo.142.11.8507

Prewitt, C. M., & Herman, J. P. (1997). Hypothalamo-Pituitary-Adrenocortical Regulation Following Lesions of the Central Nucleus of the Amygdala. *Stress, 1*(4), 263-280. doi: I405N970032 [pii]

Provinciali, L., Paolucci, S., Torta, R., Toso, V., Gobbi, B., & Gandolfo, C. (2008). Depression after First-Ever Ischemic Stroke: The Prognostic Role of Neuroanatomic Subtypes in Clinical Practice. *Cerebrovascular Diseases, 26*(6), 592-599. doi: 10.1159/000165112

Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol, 463*(1-3), 3-33. doi: S001429990301272X [pii]

Pu, L., Liu, Q. S., & Poo, M. M. (2006). BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. *Nat Neurosci, 9*(5), 605-607. doi: 10.1038/nn1687

Pu, S., Yamada, T., Yokoyama, K., Matsumura, H., Mitani, H., Adachi, A., . . . Nakagome, K. (2012). Reduced prefrontal cortex activation during the working memory task associated with poor social functioning in late-onset depression: multi-channel near-infrared spectroscopy study. *Psychiatry Res, 203*(2-3), 222-228. doi: 10.1016/j.pscychresns.2012.01.007
S0925-4927(12)00027-3 [pii]

Pulsinelli, W. A., & Brierley, J. B. (1979). A new model of bilateral hemispheric ischemia in the unanesthetized rat. *Stroke, 10*(3), 267-272.

Pulsinelli, W. A., Brierley, J. B., & Plum, F. (1982). Temporal profile of neuronal damage in a model of transient forebrain ischemia. *Ann Neurol, 11*(5), 491-498. doi: 10.1002/ana.410110509

Purba, J. S., Hoogendijk, W. J., Hofman, M. A., & Swaab, D. F. (1996). Increased number of vasopressin- and oxytocin-expressing neurons in the paraventricular nucleus of the hypothalamus in depression. *Arch Gen Psychiatry, 53*(2), 137-143.

Qi, C. C., Zhang, Z., Fang, H., Liu, J., Zhou, N., Ge, J. F., . . . Zhou, J. N. (2014). Antidepressant effects of abscisic Acid mediated by the downregulation of corticotrophin-releasing hormone gene expression in rats. *Int J Neuropsychopharmacol, 18*(4). doi: 10.1093/ijnp/pyu006

Qiao, H., An, S. C., Ren, W., & Ma, X. M. (2014). Progressive alterations of hippocampal CA3-CA1 synapses in an animal model of depression. *Behav Brain Res, 275*, 191-200. doi: 10.1016/j.bbr.2014.08.040

Qiu, G., Spangler, E. L., Wan, R. Q., Miller, M., Mattson, M. P., So, K. F., . . . Ingram, D. K. (2012). Neuroprotection provided by dietary restriction in rats is further enhanced by reducing glucocortocoids. *Neurobiol Aging, 33*(10), 2398-2410. doi: 10.1016/j.neurobiolaging.2011.11.025

Raadsheer, F. C., Hoogendijk, W. J., Stam, F. C., Tilders, F. J., & Swaab, D. F. (1994). Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology, 60*(4), 436-444.

Radak, D., Resanovic, I., & Isenovic, E. R. (2014). Changes in hypothalamus-pituitary-adrenal axis following transient ischemic attack. *Angiology, 65*(8), 723-732. doi: 10.1177/0003319713503487

Radecki, D. T., Brown, L. M., Martinez, J., & Teyler, T. J. (2005). BDNF protects against stress-induced impairments in spatial learning and memory and LTP. *Hippocampus, 15*(2), 246-253. doi: 10.1002/hipo.20048

Radley, J. J., & Morrison, J. H. (2005). Repeated stress and structural plasticity in the brain. *Ageing Res Rev, 4*(2), 271-287. doi: S1568-1637(05)00009-7 [pii] 10.1016/j.arr.2005.03.004

Radley, J. J., Rocher, A. B., Miller, M., Janssen, W. G., Liston, C., Hof, P. R., . . . Morrison, J. H. (2006). Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cereb Cortex, 16*(3), 313-320. doi: bhi104 [pii]
10.1093/cercor/bhi104

Radley, J. J., Sisti, H. M., Hao, J., Rocher, A. B., McCall, T., Hof, P. R., . . . Morrison, J. H. (2004). Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience, 125*(1), 1-6. doi: 10.1016/j.neuroscience.2004.01.006 S0306452204000284 [pii]

Radulovic, J., Ruhmann, A., Liepold, T., & Spiess, J. (1999a). Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. *J Neurosci, 19*(12), 5016-5025.

Radulovic, J., Ruhmann, A., Liepold, T., & Spiess, J. (1999b). Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: Differential roles of CRF receptors 1 and 2. *Journal of Neuroscience, 19*(12), 5016-5025.

Radulovic, M., Hippel, C., & Spiess, J. (2003). Corticotropin-releasing factor (CRF) rapidly suppresses apoptosis by acting upstream of the activation of caspases. *J Neurochem, 84*(5), 1074-1085.

Raff, H., Jacobson, L., & Cullinan, W. E. (2007). Augmented hypothalamic corticotrophin-releasing hormone mRNA and corticosterone responses to stress in adult rats exposed to perinatal hypoxia. *J Neuroendocrinol, 19*(11), 907-912. doi: JNE1595 [pii] 10.1111/j.1365-2826.2007.01595.x

Ramos, A. d. T., Homem, K. S. d. C., Suchecki, D., Tufik, S., & Troncone, L. R. P. (2014). Drug-induced suppression of ACTH secretion does not promote anti-depressive or anxiolytic effects. *Behavioural Brain Research, 265*(0), 69-75. doi: http://dx.doi.org/10.1016/j.bbr.2014.02.024

Rao, R. V., & Bredesen, D. E. (2004). Misfolded proteins, endoplasmic reticulum stress and neurodegeneration. *Curr Opin Cell Biol, 16*(6), 653-662. doi: 10.1016/j.ceb.2004.09.012

Raone, A., Cassanelli, A., Scheggi, S., Rauggi, R., Danielli, B., & De Montis, M. G. (2007). Hypothalamus-pituitary-adrenal modifications consequent to chronic stress exposure in an experimental model of depression in rats. *Neuroscience, 146*(4), 1734-1742. doi: 10.1016/j.neuroscience.2007.03.027

Rattiner, L. M., Davis, M., French, C. T., & Ressler, K. J. (2004). Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *J Neurosci, 24*(20), 4796-4806. doi: 10.1523/JNEUROSCI.5654-03.2004

Rattiner, L. M., Davis, M., & Ressler, K. J. (2004). Differential regulation of brain-derived neurotrophic factor transcripts during the consolidation of fear learning. *Learn Mem, 11*(6), 727-731. doi: 10.1101/lm.83304

Rebaudo, R., Melani, R., Balestrino, M., & Izvarina, N. (2001). Electrophysiological effects of sustained delivery of CRF and its receptor agonists in hippocampal slices. *Brain Res, 922*(1), 112-117.

Redgrave, P., Vautrelle, N., & Reynolds, J. N. (2011). Functional properties of the basal ganglia's re-entrant loop architecture: selection and reinforcement. *Neuroscience, 198*, 138-151. doi: 10.1016/j.neuroscience.2011.07.060

Refojo, D., Schweizer, M., Kuehne, C., Ehrenberg, S., Thoeringer, C., Vogl, A. M., . . . Deussing, J. M. (2011). Glutamatergic and dopaminergic neurons mediate anxiogenic and anxiolytic effects of CRHR1. *Science, 333*(6051), 1903-1907. doi: 10.1126/science.1202107

Reich, P., Regestein, Q. R., Murawski, B. J., DeSilva, R. A., & Lown, B. (1983). Unrecognized organic mental disorders in survivors of cardiac arrest. *Am J Psychiatry, 140*(9), 1194-1197.

Reinhardt, V. (2004). Common husbandry-related variables in biomedical research with animals. *Lab Anim, 38*(3), 213-235. doi: 10.3201/eid0905.020537

Ressler, K. J., Bradley, B., Mercer, K. B., Deveau, T. C., Smith, A. K., Gillespie, C. F., . . . Binder, E. B. (2010). Polymorphisms in CRHR1 and the serotonin transporter loci: gene x gene x environment interactions on depressive symptoms. *Am J Med Genet B Neuropsychiatr Genet, 153B*(3), 812-824. doi: 10.1002/ajmg.b.31052

Reul, J. M., & de Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology, 117*(6), 2505-2511. doi: 10.1210/endo-117-6-2505

Reul, J. M. H. M., & Holsboer, F. (2002). Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. *Current Opinion in Pharmacology, 2*(1), 23-33. doi: 10.1016/s1471-4892(01)00117-5

Reyes, B. A., Valentino, R. J., & Van Bockstaele, E. J. (2008). Stress-induced intracellular trafficking of corticotropin-releasing factor receptors in rat locus coeruleus neurons. *Endocrinology, 149*(1), 122-130. doi: en.2007-0705 [pii] 10.1210/en.2007-0705

Rhee, S. H., Ma, E. L., Lee, Y., Tache, Y., Pothoulakis, C., & Im, E. (2015). Corticotropin Releasing Hormone and Urocortin 3 Stimulate Vascular Endothelial Growth Factor Expression through the cAMP/CREB Pathway. *J Biol Chem, 290*(43), 26194-26203. doi: 10.1074/jbc.M115.678979

Ribak, C. E., & Baram, T. Z. (1996). Selective death of hippocampal CA3 pyramidal cells with mossy fiber afferents after CRH-induced status epilepticus in infant rats. *Brain Res Dev Brain Res, 91*(2), 245-251.

Ribot, E., Lafon, P., Ciofi, P., Sarrieau, A., Tramu, G., & Corio, M. (2003). Prostaglandins mediate lipopolysaccharide effects upon cholecystokinin and neurotensin phenotypes in neuroendocrine corticotropin-releasing hormone neurons: In situ hybridization evidence in the rat. *Neuroscience, 120*(3), 643-648. doi: 10.1016/S0306-4522(03)00357-9

Rickhag, M., Teilum, M., & Wieloch, T. (2007). Rapid and long-term induction of effector immediate early genes (BDNF, Neuritin and Arc) in peri-infarct cortex and dentate gyrus after ischemic injury in rat brain. *Brain Res, 1151*, 203-210. doi: 10.1016/j.brainres.2007.03.005

Ridder, S., Chourbaji, S., Hellweg, R., Urani, A., Zacher, C., Schmid, W., . . . Gass, P. (2005). Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. *J Neurosci, 25*(26), 6243-6250. doi: 25/26/6243 [pii] 10.1523/JNEUROSCI.0736-05.2005

Rissman, R. A., Staup, M. A., Lee, A. R., Justice, N. J., Rice, K. C., Vale, W., & Sawchenko, P. E. (2012). Corticotropin-releasing factor receptor-dependent effects of repeated stress on tau phosphorylation, solubility, and aggregation. *Proc Natl Acad Sci U S A, 109*(16), 6277-6282. doi: 10.1073/pnas.1203140109

Ritzel, R. M., Capozzi, L. A., & McCullough, L. D. (2013). Sex, stroke, and inflammation: the potential for estrogen-mediated immunoprotection in stroke. *Horm Behav, 63*(2), 238-253. doi: 10.1016/j.yhbeh.2012.04.007

Rivest, S., Laflamme, N., & Nappi, R. E. (1995). Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *J Neurosci, 15*(4), 2680-2695.

Rivier, C. (2003). Role of nitric oxide in regulating the rat hypothalamic-pituitary-adrenal axis response to endotoxemia. *Ann N Y Acad Sci, 992*, 72-85.

Roberge, M. C., Hotte-Bernard, J., Messier, C., & Plamondon, H. (2008). Food restriction attenuates ischemia-induced spatial learning and memory deficits despite extensive CA1 ischemic injury. *Behav Brain Res, 187*(1), 123-132. doi: S0166-4328(07)00464-0 [pii] 10.1016/j.bbr.2007.09.002

Roberge, M. C., Messier, C., Staines, W. A., & Plamondon, H. (2008). Food restriction induces long-lasting recovery of spatial memory deficits following global ischemia in delayed

matching and non-matching-to-sample radial arm maze tasks. *Neuroscience, 156*(1), 11-29. doi: 10.1016/j.neuroscience.2008.05.062
S0306-4522(08)01082-8 [pii]

Robinson, R. G., & Bloom, F. E. (1977). Pharmacological treatment following experimental cerebral infarction: implications for understanding psychological symptoms of human stroke. *Biol Psychiatry, 12*(5), 669-680.

Robinson, R. G., Kubos, K. L., Starr, L. B., Rao, K., & Price, T. R. (1984). Mood disorders in stroke patients. Importance of location of lesion. *Brain, 107 (Pt 1)*, 81-93.

Robinson, R. G., & Spalletta, G. (2010). Poststroke depression: a review. *Can J Psychiatry, 55*(6), 341-349.

Rodriguez-Tebar, A., Dechant, G., & Barde, Y. A. (1990). Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron, 4*(4), 487-492.

Roe, S. Y., McGowan, E. M., & Rothwell, N. J. (1998). Evidence for the involvement of corticotrophin-releasing hormone in the pathogenesis of traumatic brain injury. *European Journal of Neuroscience, 10*(2), 553-559.

Rogers, J., Raveendran, M., Fawcett, G. L., Fox, A. S., Shelton, S. E., Oler, J. A., . . . Kalin, N. H. (2013). CRHR1 genotypes, neural circuits and the diathesis for anxiety and depression. *Mol Psychiatry, 18*(6), 700-707. doi: 10.1038/mp.2012.152

Rojas, J. I., Zurru, M. C., Romano, M., Patrucco, L., & Cristiano, E. (2007). Acute ischemic stroke and transient ischemic attack in the very old - risk factor profile and stroke subtype between patients older than 80 years and patients aged less than 80 years. *Eur J Neurol, 14*(8), 895-899. doi: 10.1111/j.1468-1331.2007.01841.x

Rojas, J. J., Deniz, B. F., Miguel, P. M., Diaz, R., Hermel Edo, E., Achaval, M., . . . Pereira, L. O. (2013). Effects of daily environmental enrichment on behavior and dendritic spine density in hippocampus following neonatal hypoxia-ischemia in the rat. *Exp Neurol, 241*, 25-33. doi: 10.1016/j.expneurol.2012.11.026

Roltsch, E. A., Baynes, B. B., Mayeux, J. P., Whitaker, A. M., Baiamonte, B. A., & Gilpin, N. W. (2014). Predator odor stress alters corticotropin-releasing factor-1 receptor (CRF1R)-dependent behaviors in rats. *Neuropharmacology, 79*, 83-89. doi: 10.1016/j.neuropharm.2013.11.005

Roozendaal, B., Brunson, K. L., Holloway, B. L., McGaugh, J. L., & Baram, T. Z. (2002). Involvement of stress-released corticotropin-releasing hormone in the basolateral amygdala in regulating memory consolidation. *Proc Natl Acad Sci U S A, 99*(21), 13908-13913. doi: 10.1073/pnas.212504599

Roozendaal, B., Schelling, G., & McGaugh, J. L. (2008). Corticotropin-releasing factor in the basolateral amygdala enhances memory consolidation via an interaction with the beta-adrenoceptor-cAMP pathway: dependence on glucocorticoid receptor activation. *J Neurosci, 28*(26), 6642-6651. doi: 28/26/6642 [pii]
10.1523/JNEUROSCI.1336-08.2008

Rorato, R., Menezes, A. M., Giusti-Paiva, A., de Castro, M., Antunes-Rodrigues, J., & Elias, L. L. (2009). Prostaglandin mediates endotoxaemia-induced hypophagia by activation of pro-opiomelanocortin and corticotrophin-releasing factor neurons in rats. *Exp Physiol, 94*(3), 371-379. doi: 10.1113/expphysiol.2008.045435

Rosamond. (2010). Heart Disease and Stroke Statistics-2008 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee

(vol 117, pg e25, 2008). *Circulation, 122*(1), E10-E10. doi: 10.1161/CIR.0b013e3181e65b27

Rosen, C. L., Dinapoli, V. A., Nagamine, T., & Crocco, T. (2005). Influence of age on stroke outcome following transient focal ischemia. *Journal of Neurosurgery, 103*(4), 687-694. doi: 10.3171/jns.2005.103.4.0687

Row, B. W., & Dohanich, G. P. (2008). Post-training administration of corticotropin-releasing hormone (CRH) enhances retention of a spatial memory through a noradrenergic mechanism in male rats. *Neurobiol Learn Mem, 89*(4), 370-378. doi: S1074-7427(07)00171-2 [pii] 10.1016/j.nlm.2007.10.008

Roy, A., Hodgkinson, C. A., Deluca, V., Goldman, D., & Enoch, M. A. (2012). Two HPA axis genes, CRHBP and FKBP5, interact with childhood trauma to increase the risk for suicidal behavior. *J Psychiatr Res, 46*(1), 72-79. doi: 10.1016/j.jpsychires.2011.09.009

Ruan, Y. W., Han, X. J., Shi, Z. S., Lei, Z. G., & Xu, Z. C. (2012). Remodeling of synapses in the CA1 area of the hippocampus after transient global ischemia. *Neuroscience, 218*, 268-277. doi: S0306-4522(12)00468-X [pii] 10.1016/j.neuroscience.2012.05.035

Rubin, R. T., Phillips, J. J., McCracken, J. T., & Sadow, T. F. (1996). Adrenal gland volume in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. *Biol Psychiatry, 40*(2), 89-97. doi: 0006-3223(95)00358-4 [pii]
10.1016/0006-3223(95)00358-4

Rudy, J. W., Huff, N. C., & Matus-Amat, P. (2004). Understanding contextual fear conditioning: insights from a two-process model. *Neurosci Biobehav Rev, 28*(7), 675-685. doi: 10.1016/j.neubiorev.2004.09.004

Ryan, B. K., Vollmayr, B., Klyubin, I., Gass, P., & Rowan, M. J. (2010). Persistent inhibition of hippocampal long-term potentiation in vivo by learned helplessness stress. *Hippocampus, 20*(6), 758-767. doi: 10.1002/hipo.20677

Salehi, B., Cordero, M. I., & Sandi, C. (2010). Learning under stress: the inverted-U-shape function revisited. *Learn Mem, 17*(10), 522-530. doi: 17/10/522 [pii] 10.1101/lm.1914110

Sallaberry, C., Nunes, F., Costa, M. S., Fioreze, G. T., Ardais, A. P., Botton, P. H., . . . Porciuncula, L. O. (2013). Chronic caffeine prevents changes in inhibitory avoidance memory and hippocampal BDNF immunocontent in middle-aged rats. *Neuropharmacology, 64*, 153-159. doi: 10.1016/j.neuropharm.2012.07.010

Sanches, E. F., Arteni, N. S., Nicola, F., Boisserand, L., Willborn, S., & Netto, C. A. (2013). Early hypoxia-ischemia causes hemisphere and sex-dependent cognitive impairment and histological damage. *Neuroscience, 237*, 208-215. doi: 10.1016/j.neuroscience.2013.01.066
S0306-4522(13)00110-3 [pii]

Santos, C. O., Caeiro, L., Ferro, J. M., & Figueira, M. L. (2012). A study of suicidal thoughts in acute stroke patients. *J Stroke Cerebrovasc Dis, 21*(8), 749-754. doi: 10.1016/j.jstrokecerebrovasdis.2011.04.001

Santos, M., Gold, G., Kovari, E., Herrmann, F. R., Bozikas, V. P., Bouras, C., & Giannakopoulos, P. (2009). Differential impact of lacunes and microvascular lesions on poststroke depression. *Stroke, 40*(11), 3557-3562. doi: 10.1161/STROKEAHA.109.548545

Sapolsky, R., Rivier, C., Yamamoto, G., Plotsky, P., & Vale, W. (1987). Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science, 238*(4826), 522-524.

Sapolsky, R. M. (2000). Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry, 57*(10), 925-935. doi: ynv20127 [pii]

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1985). Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J Neurosci, 5*(5), 1222-1227.

Sapolsky, R. M., & Pulsinelli, W. A. (1985). Glucocorticoids potentiate ischemic injury to neurons: therapeutic implications. *Science, 229*(4720), 1397-1400.

Sarabdjitsingh, R. A., Meijer, O. C., Schaaf, M. J., & de Kloet, E. R. (2009). Subregion-specific differences in translocation patterns of mineralocorticoid and glucocorticoid receptors in rat hippocampus. *Brain Res, 1249*, 43-53. doi: 10.1016/j.brainres.2008.10.048

Sato, Y., Chin, Y., Kato, T., Tanaka, Y., Tozuka, Y., Mase, M., . . . Hisatsune, T. (2009). White matter activated glial cells produce BDNF in a stroke model of monkeys. *Neurosci Res, 65*(1), 71-78. doi: 10.1016/j.neures.2009.05.010

Schaaf, M. J., de Jong, J., de Kloet, E. R., & Vreugdenhil, E. (1998). Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. *Brain Res, 813*(1), 112-120.

Schaaf, M. J., De Kloet, E. R., & Vreugdenhil, E. (2000). Corticosterone effects on BDNF expression in the hippocampus. Implications for memory formation. *Stress, 3*(3), 201-208.

Schabitz, W. R., Berger, C., Kollmar, R., Seitz, M., Tanay, E., Kiessling, M., . . . Sommer, C. (2004). Effect of brain-derived neurotrophic factor treatment and forced arm use on functional motor recovery after small cortical ischemia. *Stroke, 35*(4), 992-997. doi: 10.1161/01.STR.0000119754.85848.0D

01.STR.0000119754.85848.0D [pii]

Schabitz, W. R., Steigleder, T., Cooper-Kuhn, C. M., Schwab, S., Sommer, C., Schneider, A., & Kuhn, H. G. (2007). Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. *Stroke, 38*(7), 2165-2172. doi: 10.1161/STROKEAHA.106.477331

Schachtrup, C., Ryu, J. K., Helmrick, M. J., Vagena, E., Galanakis, D. K., Degen, J. L., . . . Akassoglou, K. (2010). Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGF-beta after vascular damage. *J Neurosci, 30*(17), 5843-5854. doi: 10.1523/JNEUROSCI.0137-10.2010
30/17/5843 [pii]

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of Pavlovian fear conditioning: a cellular and molecular perspective. *Trends Neurosci, 24*(9), 540-546.

Schatzberg, A. F., Keller, J., Tennakoon, L., Lembke, A., Williams, G., Kraemer, F. B., . . . Murphy, G. M. (2014). HPA axis genetic variation, cortisol and psychosis in major depression. *Mol Psychiatry, 19*(2), 220-227. doi: 10.1038/mp.2013.129

Schierloh, A., Deussing, J., Wurst, W., Zieglgansberger, W., & Rammes, G. (2007). Corticotropin-releasing factor (CRF) receptor type 1-dependent modulation of synaptic plasticity. *Neurosci Lett, 416*(1), 82-86. doi: 10.1016/j.neulet.2007.01.047

Schinder, A. F., & Poo, M. (2000). The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci, 23*(12), 639-645. doi: S0166223600016726 [pii]

Schlager, G. W., Griesmaier, E., Wegleiter, K., Neubauer, V., Urbanek, M., Kiechl-Kohlendorfer, U., . . . Keller, M. (2011). Systemic G-CSF treatment does not improve long-term outcomes after neonatal hypoxic-ischaemic brain injury. *Exp Neurol, 230*(1), 67-74. doi: 10.1016/j.expneurol.2010.11.021 S0014-4886(10)00422-X [pii]

Schlosser, N., Wolf, O. T., Fernando, S. C., Terfehr, K., Otte, C., Spitzer, C., . . . Wingenfeld, K. (2013). Effects of acute cortisol administration on response inhibition in patients with major depression and healthy controls. *Psychiatry Res, 209*(3), 439-446. doi: 10.1016/j.psychres.2012.12.019

Schmidt-Kastner, R., & Freund, T. F. (1991). Selective vulnerability of the hippocampus in brain ischemia. *Neuroscience, 40*(3), 599-636.

Schmidt, H. D., & Duman, R. S. (2010). Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. *Neuropsychopharmacology, 35*(12), 2378-2391. doi: npp2010114 [pii] 10.1038/npp.2010.114

Schuhmacher, A., Mossner, R., Jessen, F., Scheef, L., Block, W., Belloche, A. C., . . . Zobel, A. (2012). Association of amygdala volumes with cortisol secretion in unipolar depressed patients. *Psychiatry Res, 202*(2), 96-103. doi: 10.1016/j.pscychresns.2011.09.007

Schule, C., Zill, P., Baghai, T. C., Eser, D., Zwanzger, P., Wenig, N., . . . Bondy, B. (2006). Brain-derived neurotrophic factor Val66Met polymorphism and dexamethasone/CRH test results in depressed patients. *Psychoneuroendocrinology, 31*(8), 1019-1025. doi: 10.1016/j.psyneuen.2006.06.002

Schultz, W. (2007). Multiple dopamine functions at different time courses. *Annu Rev Neurosci, 30*, 259-288. doi: 10.1146/annurev.neuro.28.061604.135722

Schulz, D. W., Mansbach, R. S., Sprouse, J., Braselton, J. P., Collins, J., Corman, M., . . . Heym, J. (1996). CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc Natl Acad Sci U S A, 93*(19), 10477-10482.

Scott, P. A., Cierpial, M. A., Kilts, C. D., & Weiss, J. M. (1996). Susceptibility and resistance of rats to stress-induced decreases in swim-test activity: a selective breeding study. *Brain Res, 725*(2), 217-230.

Sen, S., Duman, R., & Sanacora, G. (2008). Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry, 64*(6), 527-532. doi: 10.1016/j.biopsych.2008.05.005

Sengupta, P. (2013). The Laboratory Rat: Relating Its Age With Human's. *Int J Prev Med, 4*(6), 624-630.

Sequeira-Cordero, A., Mora-Gallegos, A., Cuenca-Berger, P., & Fornaguera-Trias, J. (2014). Individual differences in the forced swimming test and neurochemical kinetics in the rat brain. *Physiology & Behavior, 128*, 60-69. doi: 10.1016/j.physbeh.2014.01.037

Serafini, G. (2012). Neuroplasticity and major depression, the role of modern antidepressant drugs. *World J Psychiatry, 2*(3), 49-57. doi: 10.5498/wjp.v2.i3.49

Seroogy, K. B., Lundgren, K. H., Tran, T. M., Guthrie, K. M., Isackson, P. J., & Gall, C. M. (1994). Dopaminergic neurons in rat ventral midbrain express brain-derived neurotrophic factor and neurotrophin-3 mRNAs. *J Comp Neurol, 342*(3), 321-334. doi: 10.1002/cne.903420302

Sesack, S. R., Deutch, A. Y., Roth, R. H., & Bunney, B. S. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-

tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol, 290*(2), 213-242. doi: 10.1002/cne.902900205

Shao, S. H., Shi, S. S., Li, Z. L., Zhao, M. S., Xie, S. Y., & Pan, F. (2010). Aging effects on the BDNF mRNA and TrkB mRNA expression of the hippocampus in different durations of stress. *Chin J Physiol, 53*(5), 285-293.

Sharma, B. K., & Kumar, K. (1998). Role of proinflammatory cytokines in cerebral ischemia: a review. *Metab Brain Dis, 13*(1), 1-8.

Sheikh, H. I., Kryski, K. R., Smith, H. J., Hayden, E. P., & Singh, S. M. (2013). Corticotropin-releasing hormone system polymorphisms are associated with children's cortisol reactivity. *Neuroscience, 229*, 1-11. doi: 10.1016/j.neuroscience.2012.10.056

Sheng, H., Xu, Y., Chen, Y., Zhang, Y., Xu, X., He, C., & Ni, X. (2012). CRH-R1 and CRH-R2 differentially modulate dendritic outgrowth of hippocampal neurons. *Endocrine, 41*(3), 458-464. doi: 10.1007/s12020-012-9603-5

Shepard, J. D., Barron, K. W., & Myers, D. A. (2000). Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. *Brain Res, 861*(2), 288-295. doi: S0006-8993(00)02019-9 [pii]

Sher, L., Oquendo, M. A., Burke, A. K., Cooper, T. B., & Mann, J. J. (2013). Combined dexamethasone suppression-corticotrophin-releasing hormone stimulation test in medication-free major depression and healthy volunteers. *J Affect Disord, 151*(3), 1108-1112. doi: 10.1016/j.jad.2013.06.049

S0165-0327(13)00529-6 [pii]

Shi, S. S., Shao, S. H., Yuan, B. P., Pan, F., & Li, Z. L. (2010). Acute stress and chronic stress change brain-derived neurotrophic factor (BDNF) and tyrosine kinase-coupled receptor (TrkB) expression in both young and aged rat hippocampus. *Yonsei Med J, 51*(5), 661-671. doi: 201009661 [pii]
10.3349/ymj.2010.51.5.661

Shimizu, E., Hashimoto, K., Okamura, N., Koike, K., Komatsu, N., Kumakiri, C., . . . Iyo, M. (2003). Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry, 54*(1), 70-75.

Shirayama, Y., Yang, C., Zhang, J. C., Ren, Q., Yao, W., & Hashimoto, K. (2015). Alterations in brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in the brain regions of a learned helplessness rat model and the antidepressant effects of a TrkB agonist and antagonist. *Eur Neuropsychopharmacol*. doi: 10.1016/j.euroneuro.2015.09.002

Shors, T. J., & Servatius, R. J. (1997). The contribution of stressor intensity, duration, and context to the stress-induced facilitation of associative learning. *Neurobiol Learn Mem, 68*(1), 92-96. doi: 10.1006/nlme.1997.3763

Silberman, Y., & Winder, D. G. (2013). Corticotropin releasing factor and catecholamines enhance glutamatergic neurotransmission in the lateral subdivision of the central amygdala. *Neuropharmacology, 70*, 316-323. doi: 10.1016/j.neuropharm.2013.02.014

Silva-Gomez, A. B., Rojas, D., Juarez, I., & Flores, G. (2003). Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Res, 983*(1-2), 128-136. doi: S0006899303030427 [pii]

Sindi, S., Fiocco, A. J., Juster, R. P., Lord, C., Pruessner, J., & Lupien, S. J. (2014). Now you see it, now you don't: Testing environments modulate the association between hippocampal

volume and cortisol levels in young and older adults. *Hippocampus, 24*(12), 1623-1632. doi: 10.1002/hipo.22341

Singh, A., Black, S. E., Herrmann, N., Leibovitch, F. S., Ebert, P. L., Lawrence, J., & Szalai, J. P. (2000). Functional and neuroanatomic correlations in poststroke depression: the Sunnybrook Stroke Study. *Stroke, 31*(3), 637-644.

Singh, K. K., Park, K. J., Hong, E. J., Kramer, B. M., Greenberg, M. E., Kaplan, D. R., & Miller, F. D. (2008). Developmental axon pruning mediated by BDNF-p75NTR-dependent axon degeneration. *Nat Neurosci, 11*(6), 649-658. doi: 10.1038/nn.2114

Siuciak, J. A., Lewis, D. R., Wiegand, S. J., & Lindsay, R. M. (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol Biochem Behav, 56*(1), 131-137. doi: 10.1016/S0091-3057(96)00169-4

Skelton, K. H., Nemeroff, C. B., Knight, D. L., & Owens, M. J. (2000). Chronic administration of the triazolobenzodiazepine alprazolam produces opposite effects on corticotropin-releasing factor and urocortin neuronal systems. *J Neurosci, 20*(3), 1240-1248.

Skwara, A. J., Karwoski, T. E., Czambel, R. K., Rubin, R. T., & Rhodes, M. E. (2012). Influence of environmental enrichment on hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine by osmotic mini-pumps, and nicotine withdrawal by mecamylamine in male and female rats. *Behav Brain Res, 234*(1), 1-10. doi: 10.1016/j.bbr.2012.06.003

Slattery, D. A., & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols, 7*(6), 1009-1014. doi: 10.1038/nprot.2012.044

Slowik, A., Turaj, W., Pankiewicz, J., Dziedzic, T., Szermer, P., & Szczudlik, A. (2002). Hypercortisolemia in acute stroke is related to the inflammatory response. *Journal of the Neurological Sciences, 196*(1-2), 27-32. doi: Pii S0022-510x(02)00018-7 Doi 10.1016/S0022-510x(02)00018-7

Smith-Swintosky, V. L., Pettigrew, L. C., Sapolsky, R. M., Phares, C., Craddock, S. D., Brooke, S. M., & Mattson, M. P. (1996). Metyrapone, an inhibitor of glucocorticoid production, reduces brain injury induced by focal and global ischemia and seizures. *J Cereb Blood Flow Metab, 16*(4), 585-598. doi: 10.1097/00004647-199607000-00008

Smith, G. W., Aubry, J. M., Dellu, F., Contarino, A., Bilezikjian, L. M., Gold, L. H., . . . Lee, K. F. (1998). Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron, 20*(6), 1093-1102. doi: S0896-6273(00)80491-2 [pii]

Smith, M. A., & Cizza, G. (1996). Stress-induced changes in brain-derived neurotrophic factor expression are attenuated in aged Fischer 344/N rats. *Neurobiol Aging, 17*(6), 859-864. doi: S0197458096000668 [pii]

Smith, M. A., Makino, S., Kim, S. Y., & Kvetnansky, R. (1995). Stress increases brain-derived neurotropic factor messenger ribonucleic acid in the hypothalamus and pituitary. *Endocrinology, 136*(9), 3743-3750. doi: 10.1210/endo.136.9.7649080

Smith, M. A., Makino, S., Kvetnansky, R., & Post, R. M. (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci, 15*(3 Pt 1), 1768-1777.

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues Clin Neurosci, 8*(4), 383-395.

Smoller, J. W., Rosenbaum, J. F., Biederman, J., Kennedy, J., Dai, D., Racette, S. R., . . . Slaugenhaupt, S. A. (2003). Association of a genetic marker at the corticotropin-releasing

hormone locus with behavioral inhibition. *Biol Psychiatry, 54*(12), 1376-1381. doi: Doi 10.1016/S0006-3223(03)00598-5

Smoller, J. W., Yamaki, L. H., Fagerness, J. A., Biederman, J., Racette, S., Laird, N. M., . . . Sklar, P. B. (2005). The corticotropin-releasing hormone gene and behavioral inhibition in children at risk for panic disorder. *Biol Psychiatry, 57*(12), 1485-1492. doi: DOI 10.1016/j.biopsych.2005.02.018

Soares, L. M., Schiavon, A. P., Milani, H., & de Oliveira, R. M. (2013). Cognitive impairment and persistent anxiety-related responses following bilateral common carotid artery occlusion in mice. *Behav Brain Res, 249*, 28-37. doi: 10.1016/j.bbr.2013.04.010 S0166-4328(13)00210-6 [pii]

Sofroniew, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci, 32*(12), 638-647. doi: 10.1016/j.tins.2009.08.002

Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathol, 119*(1), 7-35. doi: 10.1007/s00401-009-0619-8

Song, C., Zhang, X. Y., & Manku, M. (2009). Increased phospholipase A2 activity and inflammatory response but decreased nerve growth factor expression in the olfactory bulbectomized rat model of depression: effects of chronic ethyl-eicosapentaenoate treatment. *J Neurosci, 29*(1), 14-22. doi: 10.1523/JNEUROSCI.3569-08.2009

Sorrells, S. F., Caso, J. R., Munhoz, C. D., Hu, C. K., Tran, K. V., Miguel, Z. D., . . . Sapolsky, R. M. (2013). Glucocorticoid signaling in myeloid cells worsens acute CNS injury and inflammation. *J Neurosci, 33*(18), 7877-7889. doi: 10.1523/JNEUROSCI.4705-12.2013

Sorrells, S. F., Caso, J. R., Munhoz, C. D., & Sapolsky, R. M. (2009). The Stressed CNS: When Glucocorticoids Aggravate Inflammation. *Neuron, 64*(1), 33-39. doi: DOI 10.1016/j.neuron.2009.09.032

Sotnikov, S., Wittmann, A., Bunck, M., Bauer, S., Deussing, J., Schmidt, M., . . . Czibere, L. (2014). Blunted HPA axis reactivity reveals glucocorticoid system dysbalance in a mouse model of high anxiety-related behavior. *Psychoneuroendocrinology, 48*(0), 41-51. doi: http://dx.doi.org/10.1016/j.psyneuen.2014.06.006

Spalletta, G., Guida, G., De Angelis, D., & Caltagirone, C. (2002). Predictors of cognitive level and depression severity are different in patients with left and right hemispheric stroke within the first year of illness. *Journal of Neurology, 249*(11), 1541-1551. doi: DOI 10.1007/s00415-002-0885-z

Spijker, A. T., & van Rossum, E. F. (2012). Glucocorticoid sensitivity in mood disorders. *Neuroendocrinology, 95*(3), 179-186. doi: 10.1159/000329846 000329846 [pii]

Spikman, J. M., Timmerman, M. E., Milders, M. V., Veenstra, W. S., & van der Naalt, J. (2012). Social Cognition Impairments in Relation to General Cognitive Deficits, Injury Severity, and Prefrontal Lesions in Traumatic Brain Injury Patients. *Atlanta (GA): Centers for Disease Control and Prevention, National Center for Injury Prevention and Control*.

Stanciu, M., Radulovic, J., & Spiess, J. (2001). Phosphorylated cAMP response element binding protein in the mouse brain after fear conditioning: relationship to Fos production. *Brain Res Mol Brain Res, 94*(1-2), 15-24.

Starkstein, S. E., Mizrahi, R., & Power, B. D. (2008). Antidepressant therapy in post-stroke depression. *Expert Opin Pharmacother, 9*(8), 1291-1298. doi: 10.1517/14656566.9.8.1291

Starr, L. R., Hammen, C., Conway, C. C., Raposa, E., & Brennan, P. A. (2014). Sensitizing effect of early adversity on depressive reactions to later proximal stress: Moderation by polymorphisms in serotonin transporter and corticotropin releasing hormone receptor genes in a 20-year longitudinal study. *Dev Psychopathol, 26*(4 Pt 2), 1241-1254. doi: 10.1017/S0954579414000996

Steckler, T., & Holsboer, F. (2001). Interaction between the cholinergic system and CRH in the modulation of spatial discrimination learning in mice. *Brain Res, 906*(1-2), 46-59. doi: Doi 10.1016/S0006-8993(01)02555-0

Stenzel-Poore, M. P., Duncan, J. E., Rittenberg, M. B., Bakke, A. C., & Heinrichs, S. C. (1996). CRH overproduction in transgenic mice: behavioral and immune system modulation. *Ann N Y Acad Sci, 780*, 36-48.

Stern, C. M., Meitzen, J., & Mermelstein, P. G. (2011). Corticotropin-releasing factor and urocortin I activate CREB through functionally selective Gbetagamma signaling in hippocampal pyramidal neurons. *Eur J Neurosci, 34*(5), 671-681. doi: 10.1111/j.1460-9568.2011.07812.x

Stevens, S. L., Shaw, T. E., Dykhuizen, E., Lessov, N. S., Hill, J. K., Wurst, W., & Stenzel-Poore, M. P. (2003). Reduced cerebral injury in CRH-R1 deficient mice after focal ischemia: a potential link to microglia and atrocytes that express CRH-R1. *J Cereb Blood Flow Metab, 23*(10), 1151-1159. doi: 10.1097/01.WCB.0000086957.72078.D4

Stoll, G., & Jander, S. (1999). The role of microglia and macrophages in the pathophysiology of the CNS. *Prog Neurobiol, 58*(3), 233-247. doi: S0301008298000835 [pii]

Stranahan, A. M., Arumugam, T. V., Cutler, R. G., Lee, K., Egan, J. M., & Mattson, M. P. (2008). Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nat Neurosci, 11*(3), 309-317. doi: 10.1038/nn2055

Stranahan, A. M., Lee, K., Pistell, P. J., Nelson, C. M., Readal, N., Miller, M. G., . . . Mattson, M. P. (2008). Accelerated cognitive aging in diabetic rats is prevented by lowering corticosterone levels. *Neurobiol Learn Mem, 90*(2), 479-483. doi: 10.1016/j.nlm.2008.05.005

Strijbos, P. J., Relton, J. K., & Rothwell, N. J. (1994). Corticotrophin-releasing factor antagonist inhibits neuronal damage induced by focal cerebral ischaemia or activation of NMDA receptors in the rat brain. *Brain Res, 656*(2), 405-408.

Stuller, K. A., Jarrett, B., & DeVries, A. C. (2012). Stress and social isolation increase vulnerability to stroke. *Exp Neurol, 233*(1), 33-39. doi: 10.1016/j.expneurol.2011.01.016 S0014-4886(11)00030-6 [pii]

Su, J., Tanaka, Y., Muratsubaki, T., Kano, M., Kanazawa, M., & Fukudo, S. (2015). Injection of corticotropin-releasing hormone into the amygdala aggravates visceral nociception and induces noradrenaline release in rats. *Neurogastroenterology and Motility, 27*(1), 30-39. doi: 10.1111/nmo.12462

Sugo, N., Hurn, P. D., Morahan, M. B., Hattori, K., Traystman, R. J., & DeVries, A. C. (2002). Social stress exacerbates focal cerebral ischemia in mice. *Stroke, 33*(6), 1660-1664.

Sukoff Rizzo, S. J., Neal, S. J., Hughes, Z. A., Beyna, M., Rosenzweig-Lipson, S., Moss, S. J., & Brandon, N. J. (2012). Evidence for sustained elevation of IL-6 in the CNS as a key contributor of depressive-like phenotypes. *Transl Psychiatry, 2*, e199. doi: 10.1038/tp.2012.120

Sulejczak, D., Ziemlinska, E., Czarkowska-Bauch, J., Nosecka, E., Strzalkowski, R., & Skup, M. (2007a). Focal photothrombotic lesion of the rat motor cortex increases BDNF levels in

motor-sensory cortical areas not accompanied by recovery of forelimb motor skills. *Journal of Neurotrauma, 24*(8), 1362-1377. doi: DOI 10.1089/neu.2006.0261

Sulejczak, D., Ziemlinska, E., Czarkowska-Bauch, J., Nosecka, E., Strzalkowski, R., & Skup, M. (2007b). Focal photothrombotic lesion of the rat motor cortex increases BDNF levels in motor-sensory cortical areas not accompanied by recovery of forelimb motor skills. *J Neurotrauma, 24*(8), 1362-1377. doi: 10.1089/neu.2006.0261

Sullivan, R. M. (2004). Hemispheric asymmetry in stress processing in rat prefrontal cortex and the role of mesocortical dopamine. *Stress, 7*(2), 131-143. doi: 4BQ6AUCHQLGVY6NR [pii]
10.1080/10253890410001679310

Sun, B. Z., Chen, L., Wu, Q., Wang, H. L., Wei, X. B., Xiang, Y. X., & Zhang, X. M. (2014). Suppression of inflammatory response by flurbiprofen following focal cerebral ischemia involves the NF-kappa B signaling pathway. *International Journal of Clinical and Experimental Medicine, 7*(9), 3087-3095.

Sun, M. K., & Alkon, D. L. (2013). Cerebral ischemia-induced difference in sensitivity to depression and potential therapeutics in rats. *Behav Pharmacol, 24*(3), 222-228. doi: 10.1097/FBP.0b013e3283618afe

Surtees, P. G., Wainwright, N. W., Luben, R. N., Wareham, N. J., Bingham, S. A., & Khaw, K. T. (2008). Depression and ischemic heart disease mortality: evidence from the EPIC-Norfolk United Kingdom prospective cohort study. *Am J Psychiatry, 165*(4), 515-523. doi: 10.1176/appi.ajp.2007.07061018

Sutherland, R., Gibb, R., & Kolb, B. (2010). The hippocampus makes a significant contribution to experience-dependent neocortical plasticity. *Behav Brain Res, 214*(1), 121-124. doi: S0166-4328(10)00425-0 [pii]
10.1016/j.bbr.2010.05.051

Swaab, D. F., Bao, A. M., & Lucassen, P. J. (2005). The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev, 4*(2), 141-194. doi: 10.1016/j.arr.2005.03.003

Swanson, L. W., Sawchenko, P. E., Rivier, J., & Vale, W. W. (1983). Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology, 36*(3), 165-186.

Swindle, M. M., Makin, A., Herron, A. J., Clubb, F. J., Jr., & Frazier, K. S. (2012). Swine as models in biomedical research and toxicology testing. *Vet Pathol, 49*(2), 344-356. doi: 10.1177/0300985811402846

Szczepankiewicz, A., Leszczynska-Rodziewicz, A., Pawlak, J., Rajewska-Rager, A., Wilkosc, M., Zaremba, D., . . . Hauser, J. (2013). Epistatic interaction between CRHR1 and AVPR1b variants as a predictor of major depressive disorder. *Psychiatric Genetics, 23*(6), 239-246. doi: Doi 10.1097/Ypg.0000000000000007

Takano, T., He, W., Han, X. N., Wang, F. S., Xu, Q. W., Wang, X. H., . . . Nedergaard, M. (2014). Rapid Manifestation of Reactive Astrogliosis in Acute Hippocampal Brain Slices. *Glia, 62*(1), 78-95. doi: 10.1002/glia.22588

Takeda, A., & Tamano, H. (2012). Proposed glucocorticoid-mediated zinc signaling in the hippocampus. *Metallomics, 4*(7), 614-618. doi: 10.1039/c2mt20018j

Takeshima, Y., Nakamura, M., Miyake, H., Tamaki, R., Inui, T., Horiuchi, K., . . . Nakase, H. (2011). Neuroprotection with intraventricular brain-derived neurotrophic factor in rat

venous occlusion model. *Neurosurgery, 68*(5), 1334-1341. doi: 10.1227/NEU.0b013e31820c048e

Tanaka, K. (2001). Alteration of second messengers during acute cerebral ischemia - adenylate cyclase, cyclic AMP-dependent protein kinase, and cyclic AMP response element binding protein. *Prog Neurobiol, 65*(2), 173-207.

Tapia-Arancibia, L., Aliaga, E., Silhol, M., & Arancibia, S. (2008). New insights into brain BDNF function in normal aging and Alzheimer disease. *Brain Research Reviews, 59*(1), 201-220. doi: 10.1016/j.brainresrev.2008.07.007

Tapia-Arancibia, L., Rage, F., Givalois, L., & Arancibia, S. (2004). Physiology of BDNF: focus on hypothalamic function. *Front Neuroendocrinol, 25*(2), 77-107. doi: 10.1016/j.yfrne.2004.04.001

Taraszewska, A., Zelman, I. B., Ogonowska, W., & Chrzanowska, H. (2002). The pattern of irreversible brain changes after cardiac arrest in humans. *Folia Neuropathol, 40*(3), 133-141.

Tarcic, N., Ovadia, H., Weiss, D. W., & Weidenfeld, J. (1998). Restraint stress-induced thymic involution and cell apoptosis are dependent on endogenous glucocorticoids. *J Neuroimmunol, 82*(1), 40-46. doi: S0165-5728(97)00186-0 [pii]

Tardito, D., Perez, J., Tiraboschi, E., Musazzi, L., Racagni, G., & Popoli, M. (2006). Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. *Pharmacol Rev, 58*(1), 115-134. doi: 10.1124/pr.58.1.7

Tateno, A., Kimura, M., & Robinson, R. G. (2002). Phenomenological characteristics of poststroke depression: early- versus late-onset. *Am J Geriatr Psychiatry, 10*(5), 575-582.

Taylor, S. C., Berkelman, T., Yadav, G., & Hammond, M. (2013). A defined methodology for reliable quantification of Western blot data. *Mol Biotechnol, 55*(3), 217-226. doi: 10.1007/s12033-013-9672-6

Teixeira, F. B., Fernandes, R. M., Farias-Junior, P. M., Costa, N. M., Fernandes, L. M., Santana, L. N., . . . Lima, R. R. (2014). Evaluation of the effects of chronic intoxication with inorganic mercury on memory and motor control in rats. *Int J Environ Res Public Health, 11*(9), 9171-9185. doi: 10.3390/ijerph110909171

Telegdy, G., & Adamik, A. (2008). Involvement of CRH receptors in urocortin-induced hyperthermia. *Peptides, 29*(11), 1937-1942. doi: S0196-9781(08)00318-5 [pii] 10.1016/j.peptides.2008.07.028

Texel, S. J., Zhang, J., Camandola, S., Unger, E. L., Taub, D. D., Koehler, R. C., . . . Mattson, M. P. (2011). Ceruloplasmin deficiency reduces levels of iron and BDNF in the cortex and striatum of young mice and increases their vulnerability to stroke. *PLoS One, 6*(9), e25077. doi: 10.1371/journal.pone.0025077 PONE-D-11-08824 [pii]

Thoeringer, C. K., Henes, K., Eder, M., Dahlhoff, M., Wurst, W., Holsboer, F., . . . Wotjak, C. T. (2012). Consolidation of remote fear memories involves Corticotropin-Releasing Hormone (CRH) receptor type 1-mediated enhancement of AMPA receptor GluR1 signaling in the dentate gyrus. *Neuropsychopharmacology, 37*(3), 787-796. doi: 10.1038/npp.2011.256 npp2011256 [pii]

Thored, P., Arvidsson, A., Cacci, E., Ahlenius, H., Kallur, T., Darsalia, V., . . . Lindvall, O. (2006). Persistent production of neurons from adult brain stem cells during recovery after stroke. *Stem Cells, 24*(3), 739-747. doi: 2005-0281 [pii] 10.1634/stemcells.2005-0281

Tian, X., Guo, J., Zhu, M., Li, M., Wu, G., & Xia, Y. (2013). delta-Opioid receptor activation rescues the functional TrkB receptor and protects the brain from ischemia-reperfusion injury in the rat. *Plos One, 8*(7), e69252. doi: 10.1371/journal.pone.0069252

Tidey, J. W., & Miczek, K. A. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. *Brain Res, 721*(1-2), 140-149. doi: 0006-8993(96)00159-X [pii]

Timpl, P., Spanagel, R., Sillaber, I., Kresse, A., Reul, J. M., Stalla, G. K., . . . Wurst, W. (1998). Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nat Genet, 19*(2), 162-166. doi: 10.1038/520

Titus, A. D. J., Rao, B. S. S., Harsha, H. N., Ramkumar, K., Srikumar, B. N., Singh, S. B., . . . Raju, T. R. (2007). Hypobaric hypoxia-induced dendritic atrophy of hippocampal neurons is associated with cognitive impairment in adult rats. *Neuroscience, 145*(1), 265-278. doi: DOI 10.1016/j.neuroscience.2006.11.037

Tombaugh, G. C., Yang, S. H., Swanson, R. A., & Sapolsky, R. M. (1992). Glucocorticoids exacerbate hypoxic and hypoglycemic hippocampal injury in vitro: biochemical correlates and a role for astrocytes. *J Neurochem, 59*(1), 137-146.

Tonissaar, M., Mallo, T., Eller, M., Haidkind, R., Koiv, K., & Harro, J. (2008). Rat behavior after chronic variable stress and partial lesioning of 5-HT-ergic neurotransmission: effects of citalopram. *Prog Neuropsychopharmacol Biol Psychiatry, 32*(1), 164-177. doi: S0278-5846(07)00271-0 [pii] 10.1016/j.pnpbp.2007.08.001

Toriya, M., Maekawa, F., Maejima, Y., Onaka, T., Fujiwara, K., Nakagawa, T., . . . Yada, T. (2010). Long-term infusion of brain-derived neurotrophic factor reduces food intake and body weight via a corticotrophin-releasing hormone pathway in the paraventricular nucleus of the hypothalamus. *Journal of Neuroendocrinology, 22*(9), 987-995. doi: 10.1111/j.1365-2826.2010.02039.x

Tornhage, C. J., Skogar, O., Borg, A., Larsson, B., Robertsson, L., Andersson, L., . . . Lokk, J. (2013). Short- and long-term effects of tactile massage on salivary cortisol concentrations in Parkinson's disease: a randomised controlled pilot study. *BMC Complement Altern Med, 13*, 357. doi: 10.1186/1472-6882-13-357

Torres, O. V., Gentil, L. G., Natividad, L. A., Carcoba, L. M., & O'Dell, L. E. (2013). Behavioral, Biochemical, and Molecular Indices of Stress are Enhanced in Female Versus Male Rats Experiencing Nicotine Withdrawal. *Front Psychiatry, 4*, 38. doi: 10.3389/fpsyt.2013.00038

Tortora, G. J., & Amitrano, R. J. (2013). Anatomy and physiology laboratory manual. *Belmont, CA : Brooks/Cole, Sengage Learning*(Seventh edition).

Toth, I., & Neumann, I. D. (2013). Animal models of social avoidance and social fear. *Cell Tissue Res, 354*(1), 107-118. doi: 10.1007/s00441-013-1636-4

Towfighi, A., & Saver, J. L. (2011). Stroke declines from third to fourth leading cause of death in the United States: historical perspective and challenges ahead. *Stroke, 42*(8), 2351-2355. doi: 10.1161/STROKEAHA.111.621904

Traver, S., Marien, M., Martin, E., Hirsch, E. C., & Michel, P. P. (2006). The phenotypic differentiation of locus ceruleus noradrenergic neurons mediated by brain-derived neurotrophic factor is enhanced by corticotropin releasing factor through the activation of a cAMP-dependent signaling pathway. *Molecular Pharmacology, 70*(1), 30-40. doi: DOI 10.1124/mol.106.022715

Treadway, M. T., Grant, M. M., Ding, Z. H., Hollon, S. D., Gore, J. C., & Shelton, R. C. (2009). Early Adverse Events, HPA Activity and Rostral Anterior Cingulate Volume in MDD. *PLoS One, 4*(3). doi: ARTN e4887 10.1371/journal.pone.0004887

Trifilieff, P., Herry, C., Vanhoutte, P., Caboche, J., Desmedt, A., Riedel, G., . . . Micheau, J. (2006). Foreground contextual fear memory consolidation requires two independent phases of hippocampal ERK/CREB activation. *Learn Mem, 13*(3), 349-358. doi: 10.1101/lm.80206

Tsukahara, T., Iihara, K., Hashimoto, N., Nishijima, T., & Taniguchi, T. (1998). Increases in levels of brain-derived neurotrophic factor mRNA and its promoters after transient forebrain ischemia in the rat brain. *Neurochem Int, 33*(2), 201-207.

Tsukahara, T., Yonekawa, Y., Tanaka, K., Ohara, O., Wantanabe, S., Kimura, T., . . . Taniguchi, T. (1994). The role of brain-derived neurotrophic factor in transient forebrain ischemia in the rat brain. *Neurosurgery, 34*(2), 323-331; discussion 331.

Tsutsui-Kimura, I., Yoshida, T., Ohmura, Y., Izumi, T., & Yoshioka, M. (2015). Milnacipran remediates impulsive deficits in rats with lesions of the ventromedial prefrontal cortex. *Int J Neuropsychopharmacol, 18*(5). doi: 10.1093/ijnp/pyu083

Tu, W. J., Dong, X., Zhao, S. J., Yang, D. G., & Chen, H. (2013). Prognostic value of plasma neuroendocrine biomarkers in patients with acute ischaemic stroke. *J Neuroendocrinol, 25*(9), 771-778. doi: 10.1111/jne.12052

Tyler, W. J., & Pozzo-Miller, L. (2003). Miniature synaptic transmission and BDNF modulate dendritic spine growth and form in rat CA1 neurones. *J Physiol, 553*(Pt 2), 497-509. doi: 10.1113/jphysiol.2003.052639

Tyrka, A. R., Price, L. H., Gelernter, J., Schepker, C., Anderson, G. M., & Carpenter, L. L. (2009). Interaction of Childhood Maltreatment with the Corticotropin-Releasing Hormone Receptor Gene: Effects on Hypothalamic-Pituitary-Adrenal Axis Reactivity. *Biol Psychiatry, 66*(7), 681-685. doi: DOI 10.1016/j.biopsych.2009.05.012

Uchino, H., Lindvall, O., Siesjo, B. K., & Kokaia, Z. (1997). Hyperglycemia and hypercapnia suppress BDNF gene expression in vulnerable regions after transient forebrain ischemia in the rat. *J Cereb Blood Flow Metab, 17*(12), 1303-1308. doi: 10.1097/00004647-199712000-00005

Ueyama, T., Kawai, Y., Nemoto, K., Sekimoto, M., Tone, S., & Senba, E. (1997a). Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. *Neurosci Res, 28*(2), 103-110. doi: S0168-0102(97)00030-8 [pii]

Ueyama, T., Kawai, Y., Nemoto, K., Sekimoto, M., Tone, S., & Senba, E. (1997b). Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. *Neurosci Res, 28*(2), 103-110. doi: Doi 10.1016/S0168-0102(97)00030-8

Uht, R. M., McKelvy, J. F., Harrison, R. W., & Bohn, M. C. (1988). Demonstration of glucocorticoid receptor-like immunoreactivity in glucocorticoid-sensitive vasopressin and corticotropin-releasing factor neurons in the hypothalamic paraventricular nucleus. *J Neurosci Res, 19*(4), 405-411, 468-409.

Urani, A., Philbert, J., Cohen, C., & Griebel, G. (2011). The corticotropin-releasing factor 1 receptor antagonist, SSR125543, and the vasopressin 1b receptor antagonist, SSR149415, prevent stress-induced cognitive impairment in mice. *Pharmacol Biochem Behav, 98*(3), 425-431. doi: 10.1016/j.pbb.2011.02.019

Uys, J. D., Muller, C. J., Marais, L., Harvey, B. H., Stein, D. J., & Daniels, W. M. (2006). Early life trauma decreases glucocorticoid receptors in rat dentate gyrus upon adult re-stress: reversal by escitalopram. *Neuroscience, 137*(2), 619-625. doi: S0306-4522(05)00933-4 [pii]
10.1016/j.neuroscience.2005.08.089

Vaccarino, V., Johnson, B. D., Sheps, D. S., Reis, S. E., Kelsey, S. F., Bittner, V., . . . Bairey Merz, C. N. (2007). Depression, inflammation, and incident cardiovascular disease in women with suspected coronary ischemia: the National Heart, Lung, and Blood Institute-sponsored WISE study. *J Am Coll Cardiol, 50*(21), 2044-2050. doi: S0735-1097(07)02714-3 [pii]
10.1016/j.jacc.2007.07.069

Valadas, J. S., Batalha, V. L., Ferreira, D. G., Gomes, R., Coelho, J. E., Sebastiao, A. M., . . . Lopes, L. V. (2012). Neuroprotection afforded by adenosine A2A receptor blockade is modulated by corticotrophin-releasing factor (CRF) in glutamate injured cortical neurons. *Journal of Neurochemistry, 123*(6), 1030-1040. doi: Doi 10.1111/Jnc.12050

Valdez, G. R. (2006). Development of CRF1 receptor antagonists as antidepressants and anxiolytics - Progress to date. *CNS Drugs, 20*(11), 887-896. doi: Doi 10.2165/00023210-200620110-00002

van Campen, J. S., Jansen, F. E., Pet, M. A., Otte, W. M., Hillegers, M. H. J., Joels, M., & Braun, K. P. J. (2015). Relation between stress-precipitated seizures and the stress response in childhood epilepsy. *Brain, 138*, 2234-2248. doi: 10.1093/brain/awv157

Van de Kar, L. D., & Blair, M. L. (1999). Forebrain pathways mediating stress-induced hormone secretion. *Front Neuroendocrinol, 20*(1), 1-48. doi: 10.1006/frne.1998.0172

van der Kooij, M. A., Ohl, F., Arndt, S. S., Kavelaars, A., van Bel, F., & Heijnen, C. J. (2010). Mild neonatal hypoxia-ischemia induces long-term motor- and cognitive impairments in mice. *Brain Behav Immun, 24*(5), 850-856. doi: 10.1016/j.bbi.2009.09.003 S0889-1591(09)00425-5 [pii]

van Kerkhof, L. W. M., Damsteegt, R., Trezza, V., Voorn, P., & Vanderschuren, L. J. M. J. (2013). Social Play Behavior in Adolescent Rats is Mediated by Functional Activity in Medial Prefrontal Cortex and Striatum. *Neuropsychopharmacology, 38*(10), 1899-1909. doi: 10.1038/npp.2013.83

Van Pett, K., Viau, V., Bittencourt, J. C., Chan, R. K., Li, H. Y., Arias, C., . . . Sawchenko, P. E. (2000). Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *J Comp Neurol, 428*(2), 191-212.

Van Pett, K., Viau, V., Bittencourt, J. C., Chan, R. K. W., Li, H.-Y., Arias, C., . . . Sawchenko, P. E. (2000). Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *The Journal of Comparative Neurology, 428*(2), 191-212. doi: 10.1002/1096-9861(20001211)428:2<191::aid-cne1>3.0.co;2-u

van Praag, H., Christie, B. R., Sejnowski, T. J., & Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci U S A, 96*(23), 13427-13431.

van Stegeren, A. H. (2009). Imaging stress effects on memory: a review of neuroimaging studies. *Can J Psychiatry, 54*(1), 16-27.

Varin, A., & Gordon, S. (2009). Alternative activation of macrophages: immune function and cellular biology. *Immunobiology, 214*(7), 630-641. doi: 10.1016/j.imbio.2008.11.009 S0171-2985(08)00148-4 [pii]

Vataja, R., Leppavuori, A., Pohjasvaara, T., Mantyla, R., Aronen, H. J., Salonen, O., . . . Erkinjuntti, T. (2004). Poststroke depression and lesion location revisited. *J Neuropsychiatry Clin Neurosci, 16*(2), 156-162. doi: 10.1176/appi.neuropsych.16.2.156 16/2/156 [pii]

Vataja, R., Pohjasvaara, T., Leppavuori, A., Mantyla, R., Aronen, H. J., Salonen, O., . . . Erkinjuntti, T. (2001). Magnetic resonance imaging correlates of depression after ischemic stroke. *Arch Gen Psychiatry, 58*(10), 925-931.

Veenit, V., Riccio, O., & Sandi, C. (2014). CRHR1 links peripuberty stress with deficits in social and stress-coping behaviors. *J Psychiatr Res, 53*, 1-7. doi: 10.1016/j.jpsychires.2014.02.015

Venna, V. R., Weston, G., Benashski, S. E., Tarabishy, S., Liu, F., Li, J., . . . McCullough, L. D. (2012). NF-kappaB contributes to the detrimental effects of social isolation after experimental stroke. *Acta Neuropathol, 124*(3), 425-438. doi: 10.1007/s00401-012-0990-8

Venna, V. R., Xu, Y., Doran, S. J., Patrizz, A., & McCullough, L. D. (2014). Social interaction plays a critical role in neurogenesis and recovery after stroke. *Transl Psychiatry, 4*, e351. doi: 10.1038/tp.2013.128 tp2013128 [pii]

Ventura-Junca, R., Symon, A., Lopez, P., Fiedler, J. L., Rojas, G., Heskia, C., . . . Herrera, L. (2014). Relationship of cortisol levels and genetic polymorphisms to antidepressant response to placebo and fluoxetine in patients with major depressive disorder: a prospective study. *BMC Psychiatry, 14*, 220. doi: 10.1186/s12888-014-0220-0

Ventura, R., Cabib, S., & Puglisi-Allegra, S. (2002). Genetic susceptibility of mesocortical dopamine to stress determines liability to inhibition of mesoaccumbens dopamine and to behavioral 'despair' in a mouse model of depression. *Neuroscience, 115*(4), 999-1007.

Venzala, E., Garcia-Garcia, A. L., Elizalde, N., & Tordera, R. M. (2013). Social vs. environmental stress models of depression from a behavioural and neurochemical approach. *Eur Neuropsychopharmacol, 23*(7), 697-708. doi: 10.1016/j.euroneuro.2012.05.010

Verdelho, A., Madureira, S., Moleiro, C., Ferro, J. M., O'Brien, J. T., Poggesi, A., . . . Study, L. (2013). Depressive symptoms predict cognitive decline and dementia in older people independently of cerebral white matter changes: the LADIS study. *J Neurol Neurosurg Psychiatry, 84*(11), 1250-1254. doi: 10.1136/jnnp-2012-304191

Verma, R., Friedler, B. D., Harris, N. M., & McCullough, L. D. (2014). Pair housing reverses post-stroke depressive behavior in mice. *Behav Brain Res, 269C*, 155-163. doi: S0166-4328(14)00273-3 [pii] 10.1016/j.bbr.2014.04.044

Viau, V., Bingham, B., Davis, J., Lee, P., & Wong, M. (2005). Gender and puberty interact on the stress-induced activation of parvocellular neurosecretory neurons and corticotropin-releasing hormone messenger ribonucleic acid expression in the rat. *Endocrinology, 146*(1), 137-146. doi: 10.1210/en.2004-0846

Vila, N., Castillo, J., Davalos, A., & Chamorro, A. (2000). Proinflammatory cytokines and early neurological worsening in ischemic stroke. *Stroke, 31*(10), 2325-2329.

Vitale, J. E. (2011). Emotion facilitation and passive avoidance learning in psychopathic female offenders. *Crim Justice Behav, 38*(7), 641-658. doi: 10.1177/0093854811403590

von Euler, M., Bendel, O., Bueters, T., Sandin, J., & von Euler, G. (2006). Profound but transient deficits in learning and memory after global ischemia using a novel water maze test. *Behav Brain Res, 166*(2), 204-210. doi: S0166-4328(05)00335-9 [pii] 10.1016/j.bbr.2005.07.016

Voorn, P., Vanderschuren, L. J., Groenewegen, H. J., Robbins, T. W., & Pennartz, C. M. (2004). Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci, 27*(8), 468-474. doi: 10.1016/j.tins.2004.06.006

Vouimba, R. M., Yaniv, D., & Richter-Levin, G. (2007). Glucocorticoid receptors and beta-adrenoceptors in basolateral amygdala modulate synaptic plasticity in hippocampal dentate gyrus, but not in area CA1. *Neuropharmacology, 52*(1), 244-252. doi: S0028-3908(06)00212-7 [pii] 10.1016/j.neuropharm.2006.07.007

Vuletic, V., Sapina, L., Lozert, M., Lezaic, Z., & Morovic, S. (2012). Anxiety and depressive symptoms in acute ischemic stroke. *Acta Clin Croat, 51*(2), 243-246.

Vyas, A., Jadhav, S., & Chattarji, S. (2006). Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. *Neuroscience, 143*(2), 387-393. doi: 10.1016/j.neuroscience.2006.08.003

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci, 22*(15), 6810-6818. doi: 20026655

Vyas, A., Pillai, A. G., & Chattarji, S. (2004). Recovery after chronic stress fails to reverse amygdaloid neuronal hypertrophy and enhanced anxiety-like behavior. *Neuroscience, 128*(4), 667-673. doi: S0306-4522(04)00613-X [pii] 10.1016/j.neuroscience.2004.07.013

Wachelder, E. M., Moulaert, V. R., van Heugten, C., Verbunt, J. A., Bekkers, S. C., & Wade, D. T. (2009). Life after survival: long-term daily functioning and quality of life after an out-of-hospital cardiac arrest. *Resuscitation, 80*(5), 517-522. doi: 10.1016/j.resuscitation.2009.01.020

Wall, A. M., Corcoran, A. E., O'Halloran, K. D., & O'Connor, J. J. (2014). Effects of prolyl-hydroxylase inhibition and chronic intermittent hypoxia on synaptic transmission and plasticity in the rat CA1 and dentate gyrus. *Neurobiol Dis, 62*, 8-17. doi: 10.1016/j.nbd.2013.08.016

Wall, V. L., Fischer, E. K., & Bland, S. T. (2012). Isolation rearing attenuates social interaction-induced expression of immediate early gene protein products in the medial prefrontal cortex of male and female rats. *Physiol Behav, 107*(3), 440-450. doi: 10.1016/j.physbeh.2012.09.002 S0031-9384(12)00298-3 [pii]

Walsh, J. J., Friedman, A. K., Sun, H., Heller, E. A., Ku, S. M., Juarez, B., . . . Han, M. H. (2014). Stress and CRF gate neural activation of BDNF in the mesolimbic reward pathway. *Nat Neurosci, 17*(1), 27-29. doi: 10.1038/nn.3591

Wan, Q., Gao, K., Rong, H., Wu, M., Wang, H., Wang, X., . . . Liu, Z. (2014). Histone modifications of the Crhr1 gene in a rat model of depression following chronic stress. *Behav Brain Res, 271*, 1-6. doi: 10.1016/j.bbr.2014.05.031

Wang, D., & Corbett, D. (1990). Cerebral ischemia, locomotor activity and spatial mapping. *Brain Res, 533*(1), 78-82. doi: 0006-8993(90)91798-L [pii]

Wang, H., Spiess, J., Wong, P. T., & Zhu, Y. Z. (2011). Blockade of CRF1 and CCK2 receptors attenuated the elevated anxiety-like behavior induced by immobilization stress. *Pharmacol Biochem Behav, 98*(3), 362-368. doi: 10.1016/j.pbb.2011.01.022

Wang, H. D., Fukuda, T., Suzuki, T., Hashimoto, K., Liou, S. Y., Momoi, T., . . . Nakanishi, H. (1999). Differential effects of Bcl-2 overexpression on hippocampal CA1 neurons and dentate granule cells following hypoxic ischemia in adult mice. *Journal of Neuroscience Research, 57*(1), 1-12.

Wang, H. L., Tsai, L. Y., & Lee, E. H. (2000). Corticotropin-releasing factor produces a protein synthesis--dependent long-lasting potentiation in dentate gyrus neurons. *J Neurophysiol, 83*(1), 343-349.

Wang, H. L., Wayner, M. J., Chai, C. Y., & Lee, E. H. (1998). Corticotrophin-releasing factor produces a long-lasting enhancement of synaptic efficacy in the hippocampus. *European Journal of Neuroscience, 10*(11), 3428-3437.

Wang, M. J., Lin, S. Z., Kuo, J. S., Huang, H. Y., Tzeng, S. F., Liao, C. H., . . . Chen, W. F. (2007). Urocortin modulates inflammatory response and neurotoxicity induced by microglial activation. *J Immunol, 179*(9), 6204-6214.

Wang, S., Yuan, Y., Xia, W., Li, F., Huang, Y., Zhou, Y., & Guo, Y. (2012). Neuronal apoptosis and synaptic density in the dentate gyrus of ischemic rats' response to chronic mild stress and the effects of Notch signaling. *Plos One, 7*(8), e42828. doi: 10.1371/journal.pone.0042828

Wang, S. H., Zhang, Z. J., Guo, Y. J., Teng, G. J., & Chen, B. A. (2008). Hippocampal neurogenesis and behavioural studies on adult ischemic rat response to chronic mild stress. *Behav Brain Res, 189*(1), 9-16. doi: 10.1016/j.bbr.2007.11.028

Wang, S. H., Zhang, Z. J., Guo, Y. J., Zhou, H., Teng, G. J., & Chen, B. A. (2009). Anhedonia and activity deficits in rats: impact of post-stroke depression. *J Psychopharmacol, 23*(3), 295-304. doi: 0269881108089814 [pii] 10.1177/0269881108089814

Wang, S. S., Kamphuis, W., Huitinga, I., Zhou, J. N., & Swaab, D. F. (2008). Gene expression analysis in the human hypothalamus in depression by laser microdissection and real-time PCR: the presence of multiple receptor imbalances. *Mol Psychiatry, 13*(8), 786-799, 741. doi: 10.1038/mp.2008.38

Wang, S. S., Wang, Y. G., Chen, H. Y., Wu, Z. P., & Xie, H. G. (2013). Expression of genes encoding cytokines and corticotropin releasing factor are altered by citalopram in the hypothalamus of post-stroke depression rats. *Neuro Endocrinol Lett, 34*(8), 773-779. doi: NEL340813A05 [pii]

Wang, T. Y., Chen, X. Q., Du, J. Z., Xu, N. Y., Wei, C. B., & Vale, W. W. (2004). Corticotropin-releasing factor receptor type 1 and 2 mRNA expression in the rat anterior pituitary is modulated by intermittent hypoxia, cold and restraint. *Neuroscience, 128*(1), 111-119. doi: 10.1016/j.neuroscience.2004.06.023

S0306-4522(04)00534-2 [pii]

Wang, W., Ji, P., & Dow, K. E. (2003). Corticotropin-releasing hormone induces proliferation and TNF-alpha release in cultured rat microglia via MAP kinase signalling pathways. *J Neurochem, 84*(1), 189-195.

Wang, W., Wang, T., Feng, W. Y., Wang, Z. Y., Cheng, M. S., & Wang, Y. J. (2014). Ecdysterone protects gerbil brain from temporal global cerebral ischemia/reperfusion injury via preventing neuron apoptosis and deactivating astrocytes and microglia cells. *Neurosci Res, 81-82*, 21-29. doi: 10.1016/j.neures.2014.01.005 S0168-0102(14)00006-6 [pii]

Wang, X. D., Chen, Y., Wolf, M., Wagner, K. V., Liebl, C., Scharf, S. H., . . . Schmidt, M. V. (2011a). Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic remodeling. *Neurobiol Dis, 42*(3), 300-310. doi: 10.1016/j.nbd.2011.01.020

Wang, X. D., Chen, Y., Wolf, M., Wagner, K. V., Liebl, C., Scharf, S. H., . . . Schmidt, M. V. (2011b). Forebrain CRHR1 deficiency attenuates chronic stress-induced cognitive deficits and dendritic remodeling. *Neurobiol Dis*. doi: S0969-9961(11)00032-5 [pii] 10.1016/j.nbd.2011.01.020

Wang, X. D., Labermaier, C., Holsboer, F., Wurst, W., Deussing, J. M., Muller, M. B., & Schmidt, M. V. (2012). Early-life stress-induced anxiety-related behavior in adult mice partially requires forebrain corticotropin-releasing hormone receptor 1. *Eur J Neurosci, 36*(3), 2360-2367. doi: 10.1111/j.1460-9568.2012.08148.x

Wang, X. D., Rammes, G., Kraev, I., Wolf, M., Liebl, C., Scharf, S. H., . . . Schmidt, M. V. (2011). Forebrain CRF(1) modulates early-life stress-programmed cognitive deficits. *J Neurosci, 31*(38), 13625-13634. doi: 10.1523/JNEUROSCI.2259-11.2011

Wang, X. D., Su, Y. A., Wagner, K. V., Avrabos, C., Scharf, S. H., Hartmann, J., . . . Schmidt, M. V. (2013). Nectin-3 links CRHR1 signaling to stress-induced memory deficits and spine loss. *Nat Neurosci, 16*(6), 706-713. doi: 10.1038/nn.3395

Wann, B. P., Boucher, M., Kaloustian, S., Nim, S., Godbout, R., & Rousseau, G. (2006). Apoptosis detected in the amygdala following myocardial infarction in the rat. *Biol Psychiatry, 59*(5), 430-433. doi: S0006-3223(05)00906-6 [pii] 10.1016/j.biopsych.2005.07.018

Wasserman, D., Sokolowski, M., Rozanov, V., & Wasserman, J. (2008). The CRHR1 gene: a marker for suicidality in depressed males exposed to low stress. *Genes Brain Behav, 7*(1), 14-19. doi: 10.1111/j.1601-183X.2007.00310.x

Wasserman, D., Wasserman, J., Rozanov, V., & Sokolowski, M. (2009). Depression in suicidal males: genetic risk variants in the CRHR1 gene. *Genes, Brain and Behavior, 8*(1), 72-79. doi: 10.1111/j.1601-183X.2008.00446.x

Watanabe, Y., Gould, E., & McEwen, B. S. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Res, 588*(2), 341-345. doi: 0006-8993(92)91597-8 [pii]

Watson, D. J., Loiseau, F., Ingallinesi, M., Millan, M. J., Marsden, C. A., & Fone, K. C. (2012). Selective blockade of dopamine D3 receptors enhances while D2 receptor antagonism impairs social novelty discrimination and novel object recognition in rats: a key role for the prefrontal cortex. *Neuropsychopharmacology, 37*(3), 770-786. doi: 10.1038/npp.2011.254

Watters, O., & O'Connor, J. J. (2011). A role for tumor necrosis factor-alpha in ischemia and ischemic preconditioning. *J Neuroinflammation, 8*, 87. doi: 10.1186/1742-2094-8-87

Weathington, J. M., Arnold, A. R., & Cooke, B. M. (2012). Juvenile social subjugation induces a sex-specific pattern of anxiety and depression-like behaviors in adult rats. *Horm Behav, 61*(1), 91-99. doi: 10.1016/j.yhbeh.2011.10.008 S0018-506X(11)00264-9 [pii]

Webster, E. L., Barrientos, R. M., Contoreggi, C., Isaac, M. G., Ligier, S., Gabry, K. E., . . . Sternberg, E. M. (2002). Corticotropin releasing hormone (CRH) antagonist attenuates adjuvant induced arthritis: role of CRH in peripheral inflammation. *J Rheumatol, 29*(6), 1252-1261.

Wei, R., Phillips, T. M., & Sternberg, E. M. (2002). Specific up-regulation of CRH or AVP secretion by acetylcholine or lipopolysaccharide in inflammatory susceptible Lewis rat fetal hypothalamic cells. *J Neuroimmunol, 131*(1-2), 31-40.

Weidenfeld, J., Leker, R. R., Gai, N., Teichner, A., Bener, D., & Ovadia, H. (2011). The function of the adrenocortical axis in permanent middle cerebral artery occlusion: effect of glucocorticoids on the neurological outcome. *Brain Res, 1407*, 90-96. doi: 10.1016/j.brainres.2011.06.035

Weidenfeld, J., Siegal, T., & Ovadia, H. (2013). Delayed effects of brain irradiation--part 1: adrenocortical axis dysfunction and hippocampal damage in an adult rat model. *Neuroimmunomodulation, 20*(1), 57-64. doi: 10.1159/000342522

Weil, Z. M., Norman, G. J., Barker, J. M., Su, A. J., Nelson, R. J., & Devries, A. C. (2008). Social isolation potentiates cell death and inflammatory responses after global ischemia. *Mol Psychiatry, 13*(10), 913-915. doi: 10.1038/mp.2008.70 mp200870 [pii]

Weinstock, M., Razin, M., Schorer-Apelbaum, D., Men, D. S., & McCarty, R. (1998). Gender differences in sympathoadrenal activity in rats at rest and in response to footshock stress. *International Journal of Developmental Neuroscience, 16*(3-4), 289-295. doi: Doi 10.1016/S0736-5748(98)00021-5

Weiser, M. J., Osterlund, C., & Spencer, R. L. (2011). Inhibitory effects of corticosterone in the hypothalamic paraventricular nucleus (PVN) on stress-induced adrenocorticotrophic hormone secretion and gene expression in the PVN and anterior pituitary. *J Neuroendocrinol, 23*(12), 1231-1240. doi: 10.1111/j.1365-2826.2011.02217.x

Welling, L. C., & Figueiredo, E. G. (2010). Microglia, corticotropin-releasing hormone, and spinal cord injury. *World Neurosurg, 74*(4-5), 388-389. doi: 10.1016/j.wneu.2010.10.010

Wellman, C. L. (2001). Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *J Neurobiol, 49*(3), 245-253. doi: 10.1002/neu.1079 [pii]

Wexler, B. C. (1970). Metabolic changes in response to acute cerebral ischemia following bilateral carotid artery ligation in arteriosclerotic versus nonarteriosclerotic rats. *Stroke, 1*(2), 112-121.

Whitlock, J. R., Heynen, A. J., Shuler, M. G., & Bear, M. F. (2006). Learning induces long-term potentiation in the hippocampus. *Science, 313*(5790), 1093-1097. doi: 313/5790/1093 [pii] 10.1126/science.1128134

Whyte, E. M., & Mulsant, B. H. (2002). Post stroke depression: epidemiology, pathophysiology, and biological treatment. *Biol Psychiatry, 52*(3), 253-264.

Williams, T. J., & Milner, T. A. (2011). Delta opioid receptors colocalize with corticotropin releasing factor in hippocampal interneurons. *Neuroscience, 179*, 9-22. doi: 10.1016/j.neuroscience.2011.01.034

Wlk, M., Wang, C. C., Venihaki, M., Liu, J., Zhao, D., Anton, P. M., . . . Pothoulakis, C. (2002). Corticotropin-releasing hormone antagonists possess anti-inflammatory effects in the mouse ileum. *Gastroenterology, 123*(2), 505-515.

Wolf, O. T., Convit, A., de Leon, M. J., Caraos, C., & Qadri, S. F. (2002). Basal hypothalamo-pituitary-adrenal axis activity and corticotropin feedback in young and older men: relationships to magnetic resonance imaging-derived hippocampus and cingulate gyrus volumes. *Neuroendocrinology, 75*(4), 241-249. doi: 54715

Wong, M. L., Loddick, S. A., Bongiorno, P. B., Gold, P. W., Rothwell, N. J., & Licinio, J. (1995). Focal cerebral ischemia induces CRH mRNA in rat cerebral cortex and amygdala. *Neuroreport, 6*(13), 1785-1788.

Wood, G. E., & Shors, T. J. (1998). Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones. *Proc Natl Acad Sci U S A, 95*(7), 4066-4071.

Wood, S. K., McFadden, K. V., Grigoriadis, D., Bhatnagar, S., & Valentino, R. J. (2012). Depressive and cardiovascular disease comorbidity in a rat model of social stress: a putative role for corticotropin-releasing factor. *Psychopharmacology (Berl), 222*(2), 325-336. doi: 10.1007/s00213-012-2648-6

Woody, M. L., Kudinova, A. Y., McGeary, J. E., Knopik, V. S., Palmer, R. H., & Gibb, B. E. (2015). Influence of maternal depression on children's brooding rumination: Moderation by CRHR1 TAT haplotype. *Cogn Emot*, 1-13. doi: 10.1080/02699931.2014.998631

Woolley, C. S., Gould, E., & McEwen, B. S. (1990a). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res, 531*(1-2), 225-231. doi: 0006-8993(90)90778-A [pii]

Woolley, C. S., Gould, E., & Mcewen, B. S. (1990b). Exposure to Excess Glucocorticoids Alters Dendritic Morphology of Adult Hippocampal Pyramidal Neurons. *Brain Research, 531*(1-2), 225-231. doi: Doi 10.1016/0006-8993(90)90778-A

Wu, H. C., Chen, K. Y., Lee, W. Y., & Lee, E. H. (1997). Antisense oligonucleotides to corticotropin-releasing factor impair memory retention and increase exploration in rats. *Neuroscience, 78*(1), 147-153.

Wu, J., Ma, D. L., Ling, E. A., & Tang, F. R. (2012). Corticotropin releasing factor (CRF) in the hippocampus of the mouse pilocarpine model of status epilepticus. *Neurosci Lett, 512*(2), 83-88. doi: 10.1016/j.neulet.2012.01.057

Wu, T. C., & Grotta, J. C. (2013). Hypothermia for acute ischaemic stroke. *Lancet Neurol, 12*(3), 275-284. doi: 10.1016/S1474-4422(13)70013-9

Xiao, Z. M., Liu, W. H., Gao, K., Wan, Q. R., Yang, C., Wang, H. L., . . . Liu, Z. C. (2011). Interaction between CRHR1 and BDNF Genes Increases the Risk of Recurrent Major Depressive Disorder in Chinese Population. *Plos One, 6*(12). doi: ARTN e28733 DOI 10.1371/journal.pone.0028733

Xu, J. F., Chen, X. Q., Du, J. Z., & Wang, T. Y. (2005). CRF receptor type 1 mediates continual hypoxia-induced CRF peptide and CRF mRNA expression increase in hypothalamic PVN of rats. *Peptides, 26*(4), 639-646. doi: S0196-9781(04)00512-1 [pii]
10.1016/j.peptides.2004.11.014

Yamashita, K., Vogel, P., Fritze, K., Back, T., Hossmann, K. A., & Wiessner, C. (1996). Monitoring the temporal and spatial activation pattern of astrocytes in focal cerebral ischemia using in situ hybridization to GFAP mRNA: Comparison with sgp-2 and hsp70 mRNA and the effect of glutamate receptor antagonists. *Brain Res, 735*(2), 285-297. doi: Doi 10.1016/0006-8993(96)00578-1

Yamashita, K., Wiessner, C., Lindholm, D., Thoenen, H., & Hossmann, K. A. (1997). Post-occlusion treatment with BDNF reduces infarct size in a model of permanent occlusion of the middle cerebral artery in rat. *Metab Brain Dis, 12*(4), 271-280.

Yamashita, T., Ninomiya, M., Acosta, P. H., Garcia-Verdugo, J. M., Sunabori, T., Sakaguchi, M., . . . Sawamoto, K. (2006). Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. *Journal of Neuroscience, 26*(24), 6627-6636. doi: Doi 10.1523/Jneurosci.0149-06.2006

Yamori, Y., Horie, R., Handa, H., Sato, M., & Fukase, M. (1976). Pathogenetic similarity of strokes in stroke-prone spontaneously hypertensive rats and humans. *Stroke, 7*(1), 46-53.

Yan, B., He, J., Xu, H., Zhang, Y., Bi, X., Thakur, S., . . . Li, X. M. (2007). Quetiapine attenuates the depressive and anxiolytic-like behavioural changes induced by global cerebral ischemia in mice. *Behav Brain Res, 182*(1), 36-41. doi: S0166-4328(07)00234-3 [pii]
10.1016/j.bbr.2007.05.002

Yan, B., Wang, D. Y., Xing, D. M., Ding, Y., Wang, R. F., Lei, F., & Du, L. J. (2004). The antidepressant effect of ethanol extract of radix puerariae in mice exposed to cerebral ischemia reperfusion. *Pharmacol Biochem Behav, 78*(2), 319-325. doi: 10.1016/j.pbb.2004.04.010S0091305704001157 [pii]

Yan, Q., Rosenfeld, R. D., Matheson, C. R., Hawkins, N., Lopez, O. T., Bennett, L., & Welcher, A. A. (1997). Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. *Neuroscience, 78*(2), 431-448. doi: S0306452296006136 [pii]

Yan, X. B., Wang, S. S., Hou, H. L., Ji, R., & Zhou, J. N. (2007). Lithium improves the behavioral disorder in rats subjected to transient global cerebral ischemia. *Behav Brain Res, 177*(2), 282-289. doi: S0166-4328(06)00636-X [pii]
10.1016/j.bbr.2006.11.021

Yan, X. X., Baram, T. Z., Gerth, A., Schultz, L., & Ribak, C. E. (1998). Co-localization of corticotropin-releasing hormone with glutamate decarboxylase and calcium-binding proteins in infant rat neocortical interneurons. *Exp Brain Res, 123*(3), 334-340.

Yang, C., Shirayama, Y., Zhang, J. C., Ren, Q., & Hashimoto, K. (2015). Regional differences in brain-derived neurotrophic factor levels and dendritic spine density confer resilience to inescapable stress. *Int J Neuropsychopharmacol, 18*(7), pyu121. doi: 10.1093/ijnp/pyu121

Yang, F. C., & Liang, K. C. (2014). Interactions of the dorsal hippocampus, medial prefrontal cortex and nucleus accumbens in formation of fear memory: difference in inhibitory avoidance learning and contextual fear conditioning. *Neurobiol Learn Mem, 112*, 186-194. doi: 10.1016/j.nlm.2013.07.017

Yang, S. J., Yu, H. Y., Kang, D. Y., Ma, Z. Q., Qu, R., Fu, Q., & Ma, S. P. (2014). Antidepressant-like effects of salidroside on olfactory bulbectomy-induced pro-inflammatory cytokine production and hyperactivity of HPA axis in rats. *Pharmacol Biochem Behav, 124*, 451-457. doi: 10.1016/j.pbb.2014.07.015

Yang, X. D., Liao, X. M., Uribe-Marino, A., Liu, R., Xie, X. M., Jia, J., . . . Si, T. M. (2015). Stress during a critical postnatal period induces region-specific structural abnormalities and dysfunction of the prefrontal cortex via CRF1. *Neuropsychopharmacology, 40*(5), 1203-1215. doi: 10.1038/npp.2014.304

Yi, L. T., Li, J., Liu, B. B., Luo, L., Liu, Q., & Geng, D. (2014). BDNF-ERK-CREB signalling mediates the role of miR-132 in the regulation of the effects of oleanolic acid in male mice. *J Psychiatry Neurosci, 39*(5), 348-359.

Yilmaz, G., & Granger, D. N. (2008). Cell adhesion molecules and ischemic stroke. *Neurol Res, 30*(8), 783-793. doi: 10.1179/174313208X341085

Yin, Y. Q., Luo, A. L., Guo, X. Y., Li, L. H., & Huang, Y. G. (2007). Postoperative neuropsychological change and its underlying mechanism in patients undergoing coronary artery bypass grafting. *Chin Med J (Engl), 120*(22), 1951-1957.

Yoo, K. Y., Lee, C. H., Choi, J. H., Sohn, Y., Cho, J. H., Hwang, I. K., . . . Won, M. H. (2011). Changes in corticosteroid hormone receptors in the ischemic gerbil hippocampal CA1 region following repeated restraint stress. *Neurochem Res, 36*(5), 701-712. doi: 10.1007/s11064-010-0384-z

Yoo, K. Y., Lee, C. H., Park, J. H., Hwang, I. K., Park, O. K., Kwon, S. H., . . . Won, M. H. (2011). Antioxidant enzymes are differently changed in experimental ischemic hippocampal CA1 region following repeated restraint stress. *J Neurol Sci, 302*(1-2), 33-42. doi: 10.1016/j.jns.2010.12.006

Yousuf, S., Atif, F., Sayeed, I., Tang, H., Wang, J., & Stein, D. G. (2015). Long-term behavioral deficits and recovery after transient ischemia in middle-aged rats: Effects of behavioral testing. *Restor Neurol Neurosci, 33*(3), 251-261. doi: 10.3233/RNN-140450

Yrjanheikki, J., Keinanen, R., Pellikka, M., Hokfelt, T., & Koistinaho, J. (1998). Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci U S A, 95*(26), 15769-15774.

Yu, D. K., Yoo, K. Y., Shin, B. N., Kim, I. H., Park, J. H., Lee, C. H., . . . Won, M. H. (2012). Neuronal damage in hippocampal subregions induced by various durations of transient cerebral ischemia in gerbils using Fluoro-Jade B histofluorescence. *Brain Res, 1437*, 50-57. doi: 10.1016/j.brainres.2011.12.029

S0006-8993(11)02230-X [pii]

Yu, H., Wang, D. D., Wang, Y., Liu, T., Lee, F. S., & Chen, Z. Y. (2012). Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. *J Neurosci, 32*(12), 4092-4101. doi: 10.1523/JNEUROSCI.5048-11.2012

Yuan, H., Xu, S., Wang, Y., Xu, H., Wang, C., Zhu, Q., . . . Shi, X. (2010). Corticotrophin-releasing hormone (CRH) facilitates axon outgrowth. *Spinal Cord, 48*(12), 850-856. doi: 10.1038/sc.2010.47

Yuhas, Y., Weizman, A., Chrousos, G. P., Ovadia, H., & Ashkenazi, S. (2004). Involvement of the neuropeptide corticotropin-releasing hormone in an animal model of Shigella-related seizures. *J Neuroimmunol, 153*(1-2), 36-39. doi: 10.1016/j.jneuroim.2004.04.009

Zafra, F., Hengerer, B., Leibrock, J., Thoenen, H., & Lindholm, D. (1990). Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. *EMBO J, 9*(11), 3545-3550.

Zhang, J., Geula, C., Lu, C., Koziel, H., Hatcher, L. M., & Roisen, F. J. (2003). Neurotrophins regulate proliferation and survival of two microglial cell lines in vitro. *Exp Neurol, 183*(2), 469-481. doi: S001448860300222X [pii]

Zhang, J. C., Wu, J., Fujita, Y., Yao, W., Ren, Q., Yang, C., . . . Hashimoto, K. (2015). Antidepressant effects of TrkB ligands on depression-like behavior and dendritic changes in mice after inflammation. *Int J Neuropsychopharmacol, 18*(4). doi: 10.1093/ijnp/pyu077

Zhang, J. C., Yao, W., Dong, C., Yang, C., Ren, Q., Ma, M., . . . Hashimoto, K. (2015). Comparison of ketamine, 7,8-dihydroxyflavone, and ANA-12 antidepressant effects in the social defeat stress model of depression. *Psychopharmacology (Berl), 232*(23), 4325-4335. doi: 10.1007/s00213-015-4062-3

Zhang, R., Zhang, Z., Zhang, C., Zhang, L., Robin, A., Wang, Y., . . . Chopp, M. (2004). Stroke transiently increases subventricular zone cell division from asymmetric to symmetric and increases neuronal differentiation in the adult rat. *J Neurosci, 24*(25), 5810-5815. doi: 10.1523/JNEUROSCI.1109-04.200424/25/5810 [pii]

Zhang, Y., Liu, W., Ma, C., Geng, J., Li, Y., Li, S., . . . Cong, B. (2012). Endoplasmic reticulum stress contributes to CRH-induced hippocampal neuron apoptosis. *Exp Cell Res, 318*(6), 732-740. doi: 10.1016/j.yexcr.2012.01.006

Zhang, Z. J., Peng, D. T., Zhu, H. Y., & Wang, X. M. (2012). Experimental evidence of Ginkgo biloba extract EGB as a neuroprotective agent in ischemia stroke rats. *Brain Research Bulletin, 87*(2-3), 193-198. doi: DOI 10.1016/j.brainresbull.2011.11.002

Zhao, B., Zhao, C. Z., Zhang, X. Y., Huang, X. Q., Shi, W. Z., Fang, S. H., . . . Wei, E. Q. (2012). The new P2Y-like receptor G protein-coupled receptor 17 mediates acute neuronal injury and late microgliosis after focal cerebral ischemia in rats. *Neuroscience, 202*, 42-57. doi: 10.1016/j.neuroscience.2011.11.066

S0306-4522(11)01372-8 [pii]

Zhao, J., Qi, X. R., Gao, S. F., Lu, J., van Wamelen, D. J., Kamphuis, W., . . . Swaab, D. F. (2015). Different stress-related gene expression in depression and suicide. *J Psychiatr Res, 68*, 176-185. doi: 10.1016/j.jpsychires.2015.06.010

Zhao, L. R., Mattsson, B., & Johansson, B. B. (2000). Environmental influence on brain-derived neurotrophic factor messenger RNA expression after middle cerebral artery occlusion in spontaneously hypertensive rats. *Neuroscience, 97*(1), 177-184.

Zhao, Y., Valdez, G. R., Fekete, E. M., Rivier, J. E., Vale, W. W., Rice, K. C., . . . Zorrilla, E. P. (2007). Subtype-selective corticotropin-releasing factor receptor Agonists exert contrasting, but not opposite, effects on anxiety-related Behavior in rats. *Journal of Pharmacology and Experimental Therapeutics, 323*(3), 846-854. doi: DOI 10.1124/jpet.107.123208

Zhao, Y., Xiao, M., He, W., & Cai, Z. (2015). Minocycline upregulates cyclic AMP response element binding protein and brain-derived neurotrophic factor in the hippocampus of cerebral ischemia rats and improves behavioral deficits. *Neuropsychiatr Dis Treat, 11*, 507-516. doi: 10.2147/NDT.S73836

Zhao, Y. D., Ou, S., Cheng, S. Y., Xiao, Z., He, W. J., Zhang, J. H., & Ruan, H. Z. (2013). Dendritic development of hippocampal CA1 pyramidal cells in a neonatal hypoxia-ischemia injury model. *Journal of Neuroscience Research, 91*(9), 1165-1173. doi: 10.1002/jnr.23247

Zheng, Z., Zhao, H., Steinberg, G. K., & Yenari, M. A. (2003). Cellular and molecular events underlying ischemia-induced neuronal apoptosis. *Drug News Perspect, 16*(8), 497-503. doi: 420 [pii]

Zhou, Z. M., Lu, T. T., Xu, G. L., Yue, X. Y., Zhu, W. S., Ma, M. M., . . . Liu, X. F. (2011). Decreased serum brain-derived neurotrophic factor (BDNF) is associated with post-stroke depression but not with BDNF gene Val66Met polymorphism. *Clinical Chemistry and Laboratory Medicine, 49*(2), 185-189. doi: Doi 10.1515/Cclm.2011.039

Zhu, D. Y., Liu, S. H., Sun, H. S., & Lu, Y. M. (2003). Expression of inducible nitric oxide synthase after focal cerebral ischemia stimulates neurogenesis in the adult rodent dentate gyrus. *J Neurosci, 23*(1), 223-229. doi: 23/1/223 [pii]

Zi, W. J., & Shuai, J. (2013). Cortisol as a Prognostic Marker of Short-Term Outcome in Chinese Patients with Acute Ischemic Stroke. *PLoS One, 8*(9). doi: ARTN e7275810.1371/journal.pone.0072758

Zitman, F. M., & Richter-Levin, G. (2013). Age and sex-dependent differences in activity, plasticity and response to stress in the dentate gyrus. *Neuroscience, 249*, 21-30. doi: 10.1016/j.neuroscience.2013.05.030

Ziv, Y., Ron, N., Butovsky, O., Landa, G., Sudai, E., Greenberg, N., . . . Schwartz, M. (2006). Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat Neurosci, 9*(2), 268-275. doi: 10.1038/nn1629

Zobel, A., Wellmer, J., Schulze-Rauschenbach, S., Pfeiffer, U., Schnell, S., Elger, C., & Maier, W. (2004). Impairment of inhibitory control of the hypothalamic pituitary adrenocortical system in epilepsy. *Eur Arch Psychiatry Clin Neurosci, 254*(5), 303-311. doi: 10.1007/s00406-004-0499-9

Zobel, A. W., Nickel, T., Kunzel, H. E., Ackl, N., Sonntag, A., Ising, M., & Holsboer, F. (2000). Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J Psychiatr Res, 34*(3), 171-181.

Zoladz, P. R., Park, C. R., Halonen, J. D., Salim, S., Alzoubi, K. H., Srivareerat, M., . . . Diamond, D. M. (2012). Differential expression of molecular markers of synaptic plasticity in the hippocampus, prefrontal cortex, and amygdala in response to spatial learning, predator exposure, and stress-induced amnesia. *Hippocampus, 22*(3), 577-589. doi: 10.1002/hipo.20922

Zorrilla, E. P., & Koob, G. F. (2004). The therapeutic potential of CRF1 antagonists for anxiety. *Expert Opin Investig Drugs, 13*(7), 799-828. doi: EID130706 [pii]10.1517/13543784.13.7.799

Zorrilla, E. P., Logrip, M. L., & Koob, G. F. (2014). Corticotropin releasing factor: a key role in the neurobiology of addiction. *Front Neuroendocrinol, 35*(2), 234-244. doi: 10.1016/j.yfrne.2014.01.001

Zorrilla, E. P., Schulteis, G., Ormsby, A., Klaassen, A., Ling, N., McCarthy, J. R., . . . De Souza, E. B. (2002). Urocortin shares the memory modulating effects of corticotropin-releasing factor (CRF): mediation by CRF1 receptors. *Brain Res, 952*(2), 200-210.

Zorrilla, E. P., Valdez, G. R., Nozulak, J., Koob, G. F., & Markou, A. (2002). Effects of antalarmin, a CRF type 1 receptor antagonist, on anxiety-like behavior and motor activation in the rat. *Brain Res, 952*(2), 188-199. doi: S000689930203189X [pii]