The biological and behavioural effects of electroconvulsive stimulus in rodents: investigation and translational implications of a genetic animal model of depression

by

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Dedication

This thesis is dedicated to:

My dear husband and friend, Kwadwo Kyeremanteng, whose perfect blend of inspiration, balance, guidance and support empowered, prodded, and sometimes even carried me through the long marathon of this dissertation.

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It truly takes a village to get someone through a Doctoral Program in Clinical Psychology. At least this was my experience, and there are so many people to thank.

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List of Abbreviations

ACTH	adrenocorticotropic releasing hormone
APA	American Psychiatric Association
ANOVA	analysis of variance
AMI-SF	Autobiographical Memory Interview – Short Form
BSA	bovine serum albumen
BDNF	brain-derived neurotrophic factor
CREB	cAMP response element binding protein
CER	conditioned emotional response
CB	corrected to blood
CRF	corticotropin releasing factor
cAMP	cyclic-adenosine monophosphate
DSM-IV	Diagnostics and Statistics Manual, 4th Edition
ECS	electroconvulsive stimulus
ECT	electroconvulsive therapy
ELISA	enzyme-linked immunosorbent assay
EDTA	ethylenediaminetetraacetic acid
ERK	extracellular-regulated protein kinase
FRL	Flinder's Resistant Line
FSL	Flinder's Sensitive Line
FST	forced swim test
HPA	hypothalamic-pituitary-adrenal
ID	injected dose
LTP	long-term potentiation
MDD	major depressive disorder
MDE	major depressive episode
mBDNF	mature BDNF
MINI	Mini International Neuropsychiatric Interview
mRNA	mitochondrial ribonucleic acid
MAPK	mitogen activated protein kinase
MADRS	Montgomery-Asberg Depression Rating Scale
MWM	morris water maze

MANOVA	multiple ANOVA
NGF	nerve growth factor
OF	open field
PMSF	phenylmethylsulfonyl fluoride
PI3K	phosphatidyl-inositol 3 kinase
PDE4	phosphodiesterase 4
proBDNF	promoter BDNF
QIDS-SR	Quick Inventory of Depressive Symptomatology – Self-Rated
RIA	radioimmunoassay
RMANOVA	repeated measures ANOVA
RAVLT	Rey Auditory Verbal Learning Test
SSRI	selective serotonin reuptake inhibitor
trkB	tyrosine kinase B
WAIS-III	Wechsler Adult Intelligence Scale, 3rd Edition
WKY	Wistar Kyoto

Abstract

Electroconvulsive therapy (ECT) is one of the oldest and most effective treatments for depression; however, its biological underpinnings are poorly understood. Brain-derived neurotrophic factor (BDNF) and the hypothalamic-pituitary-adrenal (HPA) axis are two chemical messenger systems implicated in the antidepressant action and cognitive side effects of ECT. The Wistar-Kyoto (WKY) strain is a genetic model of depression that shows biological, cognitive, behavioural, and treatment-response abnormalities, making it potentially a useful model in which to investigate the underpinnings of the action of electroconvulsive stimulus (ECS: the amimal model of ECT). In addition, the WKY presents a potentially useful model for translational research on depression. The WKY strain is particularly valuable for the measurement of serum BDNF protein, for which the association with antidepressant treatments is much less clear (mostly stemming from investigations in humans) than that between brain BDNF and antidepressant treatments in rodent studies.

The three studies presented add insight into the biological and behavioural effects of ECS. The first study (chapter 2) found no evidence of increased (*R*)-[11C]rolipram binding (an indirect marker of cyclic-adenosine monophosphate, cAMP) in the brain, despite significant increases of brain BDNF protein expression after repeated ECS. The second study (chapter 3) demonstrated the validity of the WKY strain in the investigation of ECS. Relative to Wistar controls, WKY showed similar antidepressant and cognitive effects (despite some abnormal behavioural responses), immediate but not sustained increases in brain BDNF protein, and a novel finding of increased extra-hypothalamic CRF after 5 daily ECS. The final study (chapter 4) demonstrated baseline strain differences in serum (WKY < Wistar) but not brain BDNF and, in both strains, no change in serum BDNF despite significant changes in brain BDNF after repeated ECS treatment.

Preliminary results from a human pilot study investigating similar measures in a small group of people receiving ECT for depression are also presented.

The results of this body of work advance our understanding of the activation and role of brain and serum measures of BDNF and the HPA axis in ECS/ECT, and raise important issues in the translation of research from basic science to the human condition of depression.

Chapter 1: General Introduction

Electroconvulsive therapy (ECT) is a widely used and effective treatment for depression (Greenberg & Kellner, 2005); however, the biological underpinnings of its therapeutic effectiveness and its negative side effects, particularly on cognition, are poorly understood. Brain-derived neurotrophic factor (BDNF; Duman, 1998) and the hypothalamic-pituitary-adrenal (HPA) axis (Arborelius, Owens, Plotsky, & Nemeroff, 1999) have been suggested as potential common mechanisms of action of multiple antidepressant treatments, including ECT. Although BDNF and products of the HPA axis can be measured in human blood and cerebrospinal fluid, the technology does not yet exist to measure these in the human brain *in vivo*. For this reason, animal research is indispensible in the research of the physiological and behavioural effects of depression and its treatments. Animal research in depression has also been improved by: 1) the development of rodent behavioural tasks that assess depression- and anxiety-like behaviours and specific cognitive functions, such as subtypes of memory that are affected in human depression; and 2) the development of etiological, behavioural, and particularly novel genetic models of depression (such as the Flinders Sensitive Line [FSL: Braw et al., 2006] and Wistar-Kyoto strain [WKY: Malkesman et al., 2005]). Considering the continuing advancements in animal research, the validation of animal models of depression is required to facilitate the translation of physiological and behavioural findings in animal research to the human condition of depression.

In a series of three studies, the physiological mechanism of ECT and its effects on depression and cognition is investigated. These studies use the animal model of ECT, electroconvulsive stimulus (ECS) and an inbred genetic animal model of depression, the WKY strain compared with its healthy outbred control, the Wistar strain. Given the findings in these rodent strains, the relationship between physiological measures commonly used in human studies

of depression and those that we are capable of using in animal studies is investigated. The challenges in translating the findings from animal models to the human condition are highlighted in a pilot study involving 5 people who underwent ECT for the treatment of depression, presented in the appendix.

In this introduction, the human condition and treatment of depression will be described, followed by a description of how these can be modelled and studied in rodents. Then, the evidence supporting the investigation of BDNF and the HPA axis (corticotrophin releasing factor [CRF] and cortisol) in depression and ECT/ECS will be discussed.

Depression & ECT

Major depressive disorder (MDD) is a mental illness characterized by feelings of sadness, guilt, anhedonia, abnormal sleep patterns, abnormal appetite, and cognitive features such as memory loss and poor concentration (American Psychiatric Association, 1994). Major depressive disorder affects approximately 17% of the population at some point in their lives, and affects women at twice the rate of men. It is one of the leading causes of disease burden in the world, with widespread health and socioeconomic costs, including increased use of the health care system, worsened outcomes in medical conditions such as heart disease and cancer, and increased absenteeism from work (Greden, 2001).

Despite the high prevalence and severity of this illness, depression is notably under recognized and under treated. For those who do obtain treatment, up to 50% do not respond to initial pharmacotherapy, and after failing several types of pharmacotherapy, many become labelled as treatment resistant (Souery, Papakostas, & Trivedi, 2006). ECT is a common treatment of choice for those at the final stages of treatment resistance (Souery et al., 2006). ECT is also an effective treatment for patients with depression in need of immediate intervention, such

as those at high risk for suicide or with catatonic or psychotic features that may preclude basic self-care (Greenberg et al., 2005).

Cognitive function

Depression

There is substantial evidence for cognitive impairment in depression, and cognitive dysfunction is used in diagnostic criteria (American Psychiatric Association, 1994) and symptom inventories of depression (Beck, Steer, & Brown, 1996). One of the most common cognitive impairments associated with depression involves memory, summarized in several comprehensive reviews and meta-analyses (e.g., Burt, Zembar, & Niederehe, 1995). The impairment of memory in depression is linked with biological factors associated with depression (Frodl et al., 2006), including disruptions of certain chemical messengers investigated in the proposed studies (including brain-derived neurotrophic factor, cortisol, and corticotropin releasing factor, as discussed in greater detail below).

Memory impairment in depression primarily involves deficits in anterograde memory, demonstrated by deficits on immediate and delayed free recall and recognition tests (Burt et al., 1995). Immediate recall is more impaired than delayed recall, verbal memory is more impaired than visual memory, and inpatients are more impaired than outpatients, suggesting that depression severity may influence the severity of memory deficits. Working memory, a form of short-term or immediate memory that involves holding information for easy access, manipulation, and retrieval, is also affected in depression (Fossati, Amar, Raoux, Ergis, & Allilaire, 1999).

ECT

Similar to depression, the most common and salient cognitive disturbances associated with ECT involve memory (Sackeim, 2000), supported by several comprehensive reviews of memory impairment after ECT (Datto, 2000; Rami-Gonzalez et al., 2001). Memory deficits after ECT primarily involve declarative memory, of which retrograde amnesia for autobiographical events is the most salient and persistent cognitive deficit experienced (Rami-Gonzalez et al., 2001) and possibly the most important cognitive factor contributing to subjective memory complaints of patients receiving ECT (Coleman et al., 1996). Retrograde amnesia can occur for events up to two years prior to treatment, and is more severe for more recent events (Squire, Slater, & Miller, 1981; Weiner, Rogers, Davidson, & Squire, 1986). For some patients this effect is transient (a few weeks to 6 months), whereas for others it can be more persistent (Rami-Gonzalez et al., 2001).

Pre-treatment cognitive impairment (caused by depression: Sobin et al., 1995) predicts increased cognitive side-effects of ECT (Sobin et al., 1995). Although the cognitive functions affected by ECT are somewhat different (i.e., retrograde memory vs. new learning and immediate recall), their link suggests that there may be a common mechanism, possibly a biological factor such as alterations in chemical systems, influencing cognitive dysfunction (especially memory) in these two conditions. Other factors introducing variability into the findings of post-ECT cognitive impairment include remaining depressive symptoms after treatment (Coleman et al., 1996; Sackeim et al., 1993), and treatment-related factors such as placement of the electrodes and electrical stimulus parameters (Datto, 2000; Rami-Gonzalez et al., 2001). To illustrate the complexities in the relationship between clinical response to ECT and its cognitive side effects, bilateral temporal electrode placement is still considered the gold

standard of ECT treatment, producing the best clinical response and remission of depressive symptoms. However, it is coupled with a much higher rate of memory side effects when compared with other electrode placements, such as unilateral or bifrontal placement (American Psychiatric Association: Task Force on Electroconvulsive Therapy, 2001). Because of the overwhelming effects of ECT on retrograde memory, retrograde memory is the primary cognitive outcome variable of these studies.

Symptoms of anterograde amnesia, including immediate memory deficits, are also common after ECT (Datto, 2000; Rami-Gonzalez et al., 2001). In contrast with symptoms of retrograde amnesia, symptoms of anterograde amnesia appear more transient, peaking during a course of ECT (Steif, Sackeim, Portnoy, Decina, & Malitz, 1986) and normally recovering within a few weeks after the last treatment (Frith et al., 1983; Fujita et al., 2006; Steif et al., 1986; Zervas & Jandorf, 1993).

Animal Models

Depression

Several models have been developed to simulate the behavioural and physiological characteristics of human depression in animals (rodents) (Pryce et al., 2005; Yadid et al., 2000; Will, Aird, & Redei, 2003; Willner, 1984; Willner, 1990). Many of these models have been developed on the basis of the behavioural and environmental factors involved in human depression (Willner, 1990; Pryce et al., 2005). More recently, genetic animal models of depression that are based on physiological susceptibility to environmental factors (stress), depression-like behavioural responses (i.e., learned helplessness), and neurochemical changes (stress hormones, monoamines, and others), have been developed (Will et al., 2003; Yadid et al., 2000).

The WKY rat strain is one example of a genetic animal model of depression. This strain was initially bred as the normotensive control line for a spontaneous hypertensive rat. Discovery that this line demonstrated behavioural and neurochemical changes associated with depression, led to its investigation as a genetic rodent model of human depression. The WKY line's natural outbred control is the Wistar rat strain, although some studies have used the Sprague-Dawley strain as a control. When compared with their natural controls, the WKY strain demonstrates numerous depression-like behaviours, including: decreased escape behaviour in the learned helplessness (foot shock) paradigm (Pare, 1994); increased immobility in the forced swim test (Malkesman et al., 2005; Pare, 1989; Rittenhouse, Lopez-Rubalcava, Stanwood, & Lucki, 2002; Tejani-Butt, Pare, & Yang, 1994; Will et al., 2003); lower levels of social play (Malkesman et al., 2006); decreased consumption of saccharin solution (Malkesman et al., 2005), and others (Pare, 2000; Pare, 1993).

Anxiety-like behavioural findings in the WKY strain have also been striking and include: increased immobility and freezing in the open-field test (Braw et al., 2006); increased immobility and freezing in the elevated plus maze (Braw et al., 2006); and decreased departure from home cage in the emergence test (a variant on the open-field test: Pare, Tejani-Butt, & Kluczynski, 2001). The difference between models of depression- and anxiety-like behaviours in animals is a source of ongoing debate, and even in humans these two syndromes are difficult to delineate (Kaplan & Sadock, 1998). The WKY strain also shows increased alcohol consumption, which can be attenuated by treatment with the tricyclic antidepressant, imipramine (Pare, Pare, & Kluczynski, 1999).

The WKY strain shows physiological abnormalities that are ethologically related to human depression, which can be generally divided into two categories: monoamine and neuroendocrine.

Monoamine abnormalities include alterations in genes related to monoamine transporter sites, post-synaptic receptors, binding, and turnover rates (De La Garza & Mahoney, III, 2004; Pare & Tejani-Butt, 1996; Pearson, Stephen, Beck, & Valentino, 2006; Tejani-Butt et al., 1994). Neuroendocrine abnormalities include: increased HPA axis responsiveness to stress including sustained high corticosterone levels in response to stressors, and reduced CRF binding and its synthesis (as reflected by reduced mRNA levels) in the anterior pituitary (indicating dysfunctional regulation of the HPA axis: De La Garza et al., 2004; Hauger, Shelat, & Redei, 2002; Rittenhouse et al., 2002). Although hyper-responsiveness of the HPA axis is a key feature of the WKY strain, exposure to stressors may exacerbate but is not required to observe the depressive-like behavioural and physiological changes seen in the WKY strain (Pare et al., 2001; Tejani-Butt et al., 1994).

Most interesting is the response of the WKY strain to antidepressant treatment. Pharmacotherapy targeting dopamine and noradrenaline, but not specifically serotonin (i.e., not selective serotonin reuptake inhibitors [SSRIs]) has shown effective normalization of the depression- and anxiety-like behaviours seen in the WKY strain (Tejani-Butt, Kluczynski, & Pare, 2003; Will et al., 2003). In contrast, other studies have found a decrease in responsiveness of the WKY strain to both acute and chronic antidepressant treatment, which could not be explained by differences in pharmacokinetics or the monoamine system (Lahmame & Armario, 1996; Lahmame, Grigoriadis, De Souza, & Armario, 1997). Based on these findings, Lahmame and colleagues (1997) have suggested that the WKY strain could represent a model of treatment-resistant depression.

Finally, the cognitive functions of the WKY strain have not received much attention. A handful of studies have shown clear impairments in memory function in the WKY strain

(compared with SD and Wistar strains), exemplified by decreased learning in water maze paradigms (Clements & Wainwright, 2006; Grauer & Kapon, 1993; Robertson, Clements, & Wainwright, 2008), and also in the T-maze (Low, Whitehorn, & Hendley, 1984). Though not fully substantiated, treatment-resistance and impaired memory function are the key features of the WKY strain that make it of particular interest in studies of ECT/ECS, giving face validity to this model and potentially increasing the ability to translate animal research findings to human depression.

ECT

A strong line of evidence supports ECS as a valid and effective model of ECT that reduces depression- and anxiety-like behaviour in healthy animals and animal models of depression. ECS increases locomotor activity/mobility in the open-field in chronically and acutely stressed rodents (Katz & Schmaltz, 1980) and decreases immobility in the forced swim test in healthy animals (Porsolt, Anton, Blavet, & Jalfre, 1978; Sattin, Pekary, & Lloyd, 1994; Zyss, Gorka, Kowalska, & Vetulani, 1997) and more recently in two established genetic animal models of depression, the FSL (Flinders Sensitive Line: Jimenez-Vasquez et al., 2007), and the WKY (Krahl, Senanayake, Pekary, & Sattin, 2004).

It has also been shown that ECS reproduces the cognitive features associated with ECT, especially deficits in memory. Repeated ECS affects both retrograde and anterograde memory, primarily in rodents, but also in other species including rhesus macaques (Moscrip, Terrace, Sackeim, & Lisanby, 2004; Moscrip, Terrace, Sackeim, & Lisanby, 2006; Spellman et al., 2008). The most important finding in rodent studies is the effect of ECS on retrograde memory, which is the most common memory complaint of humans who have received ECT. Impairments in retention of tasks learned prior to ECS treatment have been observed in spatial mazes (Morris

Water Maze: Bohbot, Otahal, Liu, Nadel, & Bures, 1996), passive-avoidance tasks (Andrade et al., 2002; Lerer, Stanley, Keegan, & Altman, 1986), and conditioned emotional response (Brady, Hunt, & Stebbins, 1953; Brady, Hunt, & Geller, 1954; Geller, Sidman, & Brady, 1955; Hunt, Jernberg, & Brady, 1952; Misanin, Miller, & Lewis, 1968). Similar to human research, animal studies have also demonstrated that some ECS parameters affect performance on memory tasks. For example, increased frequency and number of ECS administrations positively correlate with level of memory impairment (Andrade et al., 2002; Andrade, Rao, Sekhar, Rani, & Venkataraman, 1994). In addition, the time lapse between learning trials and a single ECS administration has an impact on learning, with shorter time lapses between learning and ECS leading to increased memory impairment (Bohbot et al., 1996; Holzhauer & Bures, 1986), which corresponds with human findings of retrograde memory impairment after ECT.

The most robust finding of anterograde memory impairments after ECS is on spatial learning (a form of new learning, or anterograde memory), using paradigms such as the Morris Water Maze. Indeed, repeated ECS consistently impairs spatial learning on the Morris Water Maze task in rodents (Lukoyanov, Sa, Madeira, & Paula-Barbosa, 2004; Reid & Stewart, 1997; Stewart & Reid, 2000). Other anterograde memory paradigms in which rodents demonstrated impairments after repeated ECS include other spatial navigation tasks (Andrade et al., 1994; Andrade, Joseph, Chandra, Vankataraman, & Rani, 1994), and passive avoidance learning tasks (Andrade, Kurinji, Sudha, & Chandra, 2002; Lerer et al., 1986).

The biological mechanism of these memory impairments is not clear and several hypotheses have been suggested. The two chemical messenger systems investigated in this study, BDNF and the HPA axis, have been implicated in depression, ECT/ECS, and memory.

Biological factors

The chemical messenger system most commonly associated with depression is the monoaminergic system (Lopez-Munoz & Alamo, 2009). However, depression is now understood to involve several other chemical messengers and neuronal pathways outside of the monoamine system (Alamo & Lopez-Munoz, 2009). Two chemical messenger systems implicated in depression and as potential common targets of antidepressant treatments, the HPA axis and BDNF, have been linked to the affective and cognitive features of depression and antidepressant treatments.

HPA axis

Depression and treatment. The HPA axis organizes the body's physiological response to stressors. Cells in the paraventricular nucleus of the hypothalamus secrete CRF and arginine vasopressin, which stimulate release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH travels through the blood to stimulate the release of corticosteroids (i.e., cortisol in humans and corticosterone in rodents) from the adrenal cortex. Corticosteroids provide a negative feedback onto this system at the level of the pituitary and the hypothalamus which can be divided into fast feedback and delayed feedback, and are differentially regulated by glucocorticoid and mineralocorticoid receptors (Checkley, 1996). The mineralocorticoid/ glucocorticoid receptor occupation ratio regulates negative feedback onto the HPA axis, whereby mineralocorticoid receptor occupation produces inhibitory tone and glucocorticoid receptor occupation produces excitatory tone on the HPA axis. Prolonged expression of high concentrations of glucocorticoids creates an imbalance in this regulatory system that results in chronic hyperactivity of the HPA axis (Holsboer, 2000).

Hyperactivity of the HPA axis has been widely implicated in depression, and the evidence for this dysfunction has been summarized in several comprehensive reviews (Arborelius et al., 1999; Bao, Meynen, & Swaab, 2008; Plotsky, Owens, & Nemeroff, 1998). The primary observed HPA axis dysregulations include heightened basal hormone secretions and abnormal responses to hormone challenge tests. Heightened basal levels of CRF in cerebrospinal fluid, ACTH in plasma, and cortisol in cerebrospinal fluid, plasma, saliva, and urine, have been observed in depressed patients compared with healthy controls (Arborelius et al., 1999; Bao et al., 2008; Plotsky et al., 1998). These findings implicate all three major HPA axis modulators (cortisol, ACTH, and CRF) in HPA axis dysregulation in depression. However, cortisol has been a particular target of many of the hypotheses on the biological mechanisms of depression. The corticosteroid hypothesis of depression stems from research on glucocorticoid hormones and their related receptors. As discussed above, there is substantial evidence for hypercortisolemia in depression. In addition, there are three other lines of evidence implicating glucocorticoids in depression (reviewed in: Plotsky et al., 1998): 1) exogenous administration of glucocorticoids is associated with psychiatric and cognitive side effects (Wolkowitz, 1994); 2) treatment with drugs that interfere with the synthesis of glucocorticoids have antidepressant effects (Murphy, 1997); and 3) antidepressant treatment (including ECT) and remission of depressive symptoms is associated with normalization of HPA axis function (Holsboer & Barden, 1996). The findings in rodents have been similar. Acute ECS is associated with increases in plasma corticosterone and ACTH, which normalize to levels of sham-controls after 10 treatments (Thiagarajan, Gleiter, Mefford, Eskay, & Nutt, 1989). Chronic ECS attenuates stressor-induced decreases in glucocorticoid receptor mRNA expression, which correlates with decreased depression-like behaviour (Hageman, Nielsen, Wortwein, Diemer, & Jorgensen, 2009). Acute and chronic ECS

also counteract glucocorticoid-induced inhibition of neuronal and glial proliferation in the hippocampus, which correlates with volumetric changes (Hellsten et al., 2002; Wennstrom, Hellsten, Ekstrand, Lindgren, & Tingstrom, 2006). These findings suggest that in depressed patients who show dysregulation of the HPA axis, changes in HPA axis function may be associated with response to ECT.

The evidence linking CRF to depression and ECS/ECT is less pronounced. Elevated CRF levels are typically associated with heightened depression- and anxiety-like behaviours in animals (Rotzinger, Lovejoy, & Tan, 2010), and CRF_1 antagonists are associated with antidepressant and anxiolytic effects (Holsboer & Ising, 2008). Several studies have shown an increase in CRF mRNA expression after single (Garcia-Garcia, Llewellyn-Jones, Fernandez, I, Fuentes, & Manzanares, 1998) and repeated ECS in the hypothalamus (Brady, Lynn, Glowa, Le, & Herkenham, 1994; Herman et al., 1989), implicating it in the activation and regulation of the HPA axis after ECS. However, no studies have investigated CRF in extra-hypothalamic brain regions after ECS.

Cognitive function. HPA axis activity has also been widely linked with cognitive function. Endogenous and exogenously administered glucocorticoids are implicated in hippocampal-dependent memory functions (i.e., declarative memory; reviewed in: Het, Ramlow, & Wolf, 2005; Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). After exogenous administration of glucocorticoids, people demonstrate deficits in the retrieval of previously learned information, including word lists learned just prior to glucocorticoid administration and personal events that have occurred across the lifespan (i.e., autobiographical memory; Buss, Wolf, Witt, & Hellhammer, 2004). Glucocorticoids have also been implicated in prefrontal-cortex regulated memory functions, as high doses of exogenously administered glucocorticoids negatively impact

prefrontal cortex- regulated working memory, whereas moderate doses negatively impact hippocampal-regulated declarative memory (Lupien et al., 2007). These associations between glucocorticoids and cognitive function demonstrate a possible common biological mechanism for the memory deficits seen in depression and those seen as a result of ECT. In a compelling study, Neylan and colleagues found that higher baseline plasma cortisol levels (particularly afternoon levels) in people with depression predict greater cognitive dysfunction (especially in executive function) after ECT (Neylan et al., 2001). In rodents, interruption of glucocorticoid function during ECS via the administration of the glucocorticoid receptor antagonist mifepristone eliminated the retrograde amnesia associated with 5 daily ECS (Nagaraja et al., 2007).

CRF is also expected to play a role in memory. High levels of hippocampal CRF after stress and activation of the CRF_1 receptor interfere with memory (Chen et al., 2010; Heinrichs et al., 1996; Ivy et al., 2010) which can be prevented by administration of CRF_1 antagonists (Brunson, Grigoriadis, Lorang, & Baram, 2002; Gallagher, Orozco-Cabal, Liu, & Shinnick-Gallagher, 2008). However, the effects of CRF on memory are also linked to arousal, likely due to the role of CRF in the activation of the sympathetic nervous system, where low levels of CRF interfere with memory formation, particularly for fear learning (Gulpinar & Yegen, 2004; Sousa, Cerqueira, & Almeida, 2008). This data suggests that the role of CRF in memory could occur in an inverted U-shaped curve, where low and high levels of CRF are detrimental to memory and moderate levels are optimal.

Brain-derived neurotrophic factor

Depression and treatment. BDNF is a neurotrophin that plays an important role in neuronal proliferation and survival in the brain (Huang & Reichardt, 2001). In animals and humans, lower

brain and blood levels of BDNF have been linked to depression and its treatments (Bocchio-Chiavetto et al., 2010; Warner-Schmidt & Duman, 2006).

Animal research has offered some insight into the relationship between BDNF, depression and its treatments. Rodents exposed to prolonged stress (an animal model of depression; Willner, 1984) show lower levels of BDNF protein and mRNA in regions of the brain implicated in the pathophysiology of depression in humans (e.g., hippocampus, frontal cortex, striatum; Smith, Makino, Kvetnansky, & Post, 1995b; Warner-Schmidt et al., 2006). Interestingly, two major genetic rodent models of depression have not been widely investigated in this regard; however, one study demonstrated that the FSL strain shows lower BDNF protein in hippocampus despite higher BDNF protein in serum (Elfving et al., 2010), whereas BDNF has not been investigated in the WKY strain.

Studies of ECS in rodents have produced consistent and strong evidence that BDNF protein and mRNA levels are altered after ECS (Nibuya, Morinobu, & Duman, 1995; Altar, Whitehead, Chen, Wortwein, & Madsen, 2003). There are multiple methods for the administration of ECS, with treatments varying in frequency, number, placement of electrodes, and electrical stimulus parameters. In addition, there are two measures of BDNF that are used: mRNA expression and protein levels. The most common method (and that used in this series of studies) involves administering ECS once daily via ear-clip electrodes for either 1 day (single ECS) or multiple days (repeated ECS). BDNF protein is considered a better indicator of functional BDNF in the brain than its mRNA expression (Altar et al., 1997) and the measurement method is nearly identical in rodents and humans, as well as in brain tissue and blood, making it useful for translational research. Using similar treatment parameters, studies have shown that single and repeated ECS have different effects on BDNF, and that its effects

also differ based on measurement of mRNA expression or protein levels. BDNF mRNA expression increased rapidly (within 2 h) following a single ECS, in various depression-relevant brain regions (e.g., frontal cortex, hippocampus), but such elevations were not sustained in these regions (Nibuya et al., 1995). However, studies reveal that when ECS is administered repeatedly (commonly 10 daily ECS) the BDNF mRNA expression increases tend to persist for up to 18 hours after the final treatment (Nibuya et al., 1995). In contrast to mRNA changes, brain BDNF protein levels are not significantly increased after a single ECS, but do increase widely and significantly after 10 daily treatments of ECS. However, Altar and colleagues have shown that 10 treatments are not necessary, as BDNF protein increased significantly even after 4 daily ECS treatments, and does not increase any further over the next 6 daily treatments (Altar et al., 2003). Altar and colleagues also showed a time course of BDNF protein changes after 4 daily ECS treatments. In their study, BDNF protein was increased at 6 hours after the final ECS, peaks around 15 hours, and then returns to baseline levels by 36 hours. Interestingly however, in the FSL model of depression, repeated ECS did not lead to an increase in brain BDNF protein, despite an increase in nerve growth factor in the striatum (Angelucci, Aloe, Jimenez-Vasquez, & Mathe, 2003). In healthy animals, repeated but not single treatment of antidepressant drugs also increases BDNF, though to a lesser extent than ECS (Altar et al., 2003; Nibuya et al., 1995). This effect is also larger and more widespread in the case of BDNF mRNA expression rather than protein levels (Altar et al., 2003; Nibuya et al., 1995). Lower BDNF levels in stressed animals (animal models of depression) and humans with depression recover to normal range with a variety of antidepressant treatments, including ECT/ECS, antidepressant drugs, and transcranial magnetic stimulation (Castren, Voikar, & Rantamaki, 2007). In rodents, these antidepressant-induced increases in BDNF are shown to induce structural changes in the brain (Malberg, Eisch,

Nestler, & Duman, 2000), which may be required for the behavioural effectiveness of antidepressants (Santarelli et al., 2003). The normalization of BDNF protein after ECS in rodents is associated with improved performance on measures of depression-like behaviours, such as the forced swim test (Li, Suemaru, Cui, & Araki, 2007). Also, when administered intracerebrally, BDNF has antidepressant-like effects in rodent models of depression, such as learned helplessness (Eisch et al., 2003; Shirayama et al., 2002; Siuciak et al., 1997).

The findings in animal studies are grossly supported by those in human studies. However, currently no method exists for the measurement of BDNF in any form in the human brain *in vivo*, and thus BDNF is measured in the blood (serum, plasma, or platelets). There is strong evidence that people with depression show lower levels of BDNF protein in the blood, primarily in serum (Aydemir et al., 2006; Aydemir, Deveci, Taskin, Taneli, & Esen-Danaci, 2007; Bocchio-Chiavetto et al., 2010; Cattaneo et al., 2010; Deveci et al., 2007; Gervasoni et al., 2005; Gonul et al., 2005; Gorgulu & Caliyurt, 2009; Hu et al., 2010; Karege et al., 2002a; Karege et al., 2005; Piccinni et al., 2008; Umene-Nakano et al., 2009; but see: Basterzi et al., 2009). Findings in plasma and platelets suggest a similar trend as those seen in serum BDNF, but studies are fewer and thus results are more variable (plasma: Karege et al., 2005; Lee, Kim, Park, & Kim, 2007; Piccinni et al., 2008; Yoshimura et al., 2010; but see: Bocchio-Chiavetto et al., 2010; Kim et al., 2007; Serra-Millas et al., 2011; platelets: Pandey et al., 2010; Serra-Millas et al., 2011). The evidence of lower serum BDNF in depression is additionally supported by findings of lower levels of BDNF mRNA expression in the post-mortem brains of people with depression (Chen, Dowlatshahi, MacQueen, Wang, & Young, 2001). Serum BDNF is also correlated with objective and subjective measures of depression severity in some (Bus et al., 2011; Gervasoni et al., 2005;

Gorgulu et al., 2009; Hu et al., 2010; Karege et al., 2002a; Yoshimura et al., 2011), but not all studies (Bocchio-Chiavetto et al., 2006; Lang, Bajbouj, Gallinat, & Hellweg, 2006).

In people with depression, serum BDNF increases after treatment with antidepressant drugs in most studies (Bocchio-Chiavetto et al., 2006; Cattaneo et al., 2010; Gervasoni et al., 2005; Gonul et al., 2005; Hu et al., 2010; Matrisciano et al., 2009; Molendijk et al., 2011; Okamoto et al., 2008; Sen, Duman, & Sanacora, 2008; Shimizu et al., 2003), but not all (Basterzi et al., 2009; Monteleone, Serritella, Martiadis, & Maj, 2008; Piccinni et al., 2008). After ECT, findings have been even more variable (Bocchio-Chiavetto et al., 2006; Fernandes et al., 2009; Gronli, Stensland, Wynn, & Olstad, 2009; Hu et al., 2010; Okamoto et al., 2008). Several studies found no changes in serum BDNF at an early time point (within 1-7 days) following completion of ECT (Bocchio-Chiavetto et al., 2006; Fernandes et al., 2009; Gronli et al., 2009). However, two studies suggest that two factors may be involved in increases in serum BDNF protein levels after ECT: 1) lower baseline serum BDNF protein, and 2) length of time after ECT. These two studies found that only a subset of participants that showed or at least trended toward lower baseline serum BDNF protein levels showed increases in serum BDNF protein immediately (days to 1 week) after completion of ECT (Bocchio-Chiavetto et al., 2006; Okamoto et al., 2008). Bocchio-Chiavetto and colleagues (2006) also demonstrated that serum BDNF protein was significantly increased in their whole sample at 1 month post-ECT, regardless of baseline levels. One discrepancy between these two studies is that Okamoto and colleagues (2008) correlated the significant change in serum BDNF protein to treatment response, while Bocchio-Chiavetto and colleagues (2006) found no correlation between these two factors. Several studies in the antidepressant drug literature have also debated whether significant changes in serum BDNF protein are related to the effectiveness of treatment (Basterzi et al., 2009; Huang et al.,

2001; but see: Tadic et al., 2011). The findings from these studies raise the following questions: 1) are changes in serum BDNF protein levels related to the action of antidepressant treatments, specifically ECT?; 2) are these related to state of depression and thus treatment effectiveness?; and 3) are these changes only seen in a subset of patients with lower baseline serum BDNF protein, and perhaps a biological predisposition to this neurochemical abnormality? Indeed, the relationship between serum BDNF and depression is influenced by several factors in addition to those mentioned above, including baseline antidepressant treatment exposure (Diniz et al., 2010; Molendijk et al., 2011; Ozan et al., 2010; Shimizu et al., 2003), type of antidepressant treatment such as some types of antidepressant drugs (Matrisciano et al., 2009; Molendijk et al., 2011), ECT (Bocchio-Chiavetto et al., 2006), sleep deprivation (Gorgulu et al., 2009), but not vagus nerve stimulation or transcranial magnetic stimulation (Lang et al., 2006), and gender (females < males: Bus et al., 2011; Gervasoni et al., 2005; Huang et al., 2001; Ozan et al., 2010; Karege et al., 2002a; but see: Bocchio-Chiavetto et al., 2010).

The inability to measure BDNF in the human brain *in vivo* introduces many barriers to the translation of the results from animal studies to the human condition of depression. The relationship between serum and brain BDNF protein is not yet clear, and greater variability in human participant groups due to individual differences, environmental factors and the complex nature of the human condition of depression and its treatments lead to greater variability in human compared with highly controlled animal studies. However, despite this variability, strong evidence exists to suggest some role for BDNF in depression and ECT.

Cognitive function. Another piece of evidence supporting a role for BDNF in depression and ECT is its link with memory. BDNF protein and mRNA levels are associated with memory functions regulated by the hippocampus, amygdala, and parietal cortex through multiple

mechanisms, including contributing to alterations in synapses involved in long-term potentiation and activation of protein transcription by cyclic-adenosine monophosphate (cAMP) response element binding protein (CREB) via extracellular-regulated protein kinase (Erk) and phosphatidyl-inositol 3 kinase (PI3K) pathways (Bekinschtein, Cammarota, Izquierdo, & Medina, 2008; Lu, Christian, & Lu, 2008). No studies to date have assessed the association between BDNF protein and cognitive function in humans, although the functional BDNF-precursor polymorphism (val66met) has been implicated in cognitive function in healthy adults and people with depression and other mental illnesses (e.g., schizophrenia and bipolar disorder; Egan et al., 2003; Hariri et al., 2003; Rybakowski et al., 2006; Tsai, Cheng, Yu, Chen, & Hong, 2003).

Interactions

In addition to the evidence implicating these chemical messenger systems individually in depression, cognitive function, and ECT, there is evidence that BDNF and the HPA axis interact. These interactions (compared with the independent action of these chemical messenger systems) may also influence risk for depression, treatment response, and cognitive function. The relationship between BDNF and the HPA axis has been well established in animal studies. BDNF mRNA is increased in the hippocampus by acute stress and adrenalectomy, and decreased by prolonged stress or exogenously administered glucocorticoids (reviewed in: Givalois et al., 2004). When applied exogenously (into the ventricles), BDNF leads to an increase in CRF mRNA expression and a decrease in CRF protein levels in the hypothalamus, indicating recruitment of CRF neurons after exogenous BDNF administration and thus a possible role for BDNF in activation of the HPA axis (Givalois et al., 2004). In addition, the multiple roles that glucocorticoids have been shown to play in neuroplasticity (Abraham, Harkany, Horvath, &

Luiten, 2001) may be related to the distinct effects of glucocorticoid and mineralocorticoid receptors on neurotrophic factors, such as BDNF (Sousa et al., 2008). Finally, though not investigated in this study, neuroactive steroids, which show a differential response to acute and chronic activation of the HPA axis, have shown a role in antidepressant treatments, memory, and neuroprotection (MacKenzie, Odontiadis, Le Melledo, Prior, & Baker, 2007), suggesting a further connection between neuroprotective factors and the stress response system. These findings are in line with the widely accepted notion that the biological basis of depression (and therefore also its treatments) is multidimensional, involving complex interactions between many neurological structures and chemical messenger systems. Although there are several studies that investigate these two chemical messenger systems independently, there is a lack of research that allows the investigation of the interactions between these two important systems in depression and its treatment, especially with ECT.

Summary

Considering the magnitude of research on the biological basis of depression and pharmacological antidepressant treatments, ECT has received comparatively little attention. However, its undeniable effectiveness in people who demonstrate resistance to antidepressant drug treatment and its unique clinical and cognitive effects make it an important target of research and a possible gateway to understanding the biological basis of depression and antidepressant treatments. Substantial research in both animals and humans has unveiled biological factors that appear to be related to features of depression and ECT, particularly affective and cognitive features. The following studies were designed to investigate the role of two chemical messenger systems (BDNF and the HPA axis) in affective symptoms and cognitive function related to ECT/ECS and depression.

The first study investigated in the Wistar strain the mechanism of release of BDNF. Based on the increases in BDNF after diverse antidepressant treatments, it has been suggested that BDNF may represent an end product of a common mechanism of action of antidepressant treatments (Duman, 1998), and Duman suggested in his seminal paper that this may occur through activation of CREB, possibly by the cAMP second messenger pathway. Indeed there is converging evidence by one of our collaborators implicating the cAMP pathway in CREB-induced BDNF activation after antidepressant drug treatment (Lourenco, Kenk, Beanlands, & DaSilva, 2006). Therefore, with the hypothesis that activation of the cAMP second messenger pathway may represent a common mechanism of action of antidepressant drugs and ECT, we measured BDNF protein and the activation of cAMP via the binding of (*R*)-[^{11}C]rolipram to phosphodiesterase 4 (PDE4, the enzyme involved in breakdown of cAMP: Houslay & Milligan, 1997) in brain tissue after 1, 5, and 10 daily ECS.

In review of the findings from the first study, we began to uncover questions about the functional role of BDNF in depression. BDNF has been widely implicated in depression in humans and animals, but its association with treatment for people with depression is less clear. Most of the research in animals has used healthy animal strains and investigations in animal models of depression had been more variable. Therefore, we sought to investigate some of the established animal findings in an animal model of depression that has received little attention in the ECS research field. The second study assessed the validity of the WKY strain (compared with the Wistar strain) as an animal model of depression in which to investigate the biological (BDNF and HPA axis) and behavioural (cognitive and affective) effects of ECS. The findings in this model gave insight into the relationship between the biological and behavioural effects of

ECS, with novel findings in the involvement of HPA axis-related neurochemicals and again bringing question to the functional role of BDNF in depression and ECS.

The questions arising from the second study led us to human studies which have found variable associations between serum BDNF protein, depression and its treatments. The value of the WKY model in translational research is tested in the investigation of the relationship between BDNF protein in serum and brain tissue in the Wistar and WKY strains exposed to 5 daily ECS or sham treatments. A final appendix is attached in which data from a pilot study of 5 people with depression undergoing treatment with ECT and the translational value of this research are discussed. These studies provide insight into the investigation of the biological underpinnings of depression and ECS, particularly in relation to BDNF and the HPA axis, the validity of the WKY strain in this area of research, and the translation of findings in animal studies in ECS to the human condition of depression and its treatment with ECT.

Preface to Chapter 2

BDNF: a common mechanism of action of antidepressant treatments?

ECT and antidepressant drugs may share a similar underlying mechanism of action involving CREB (Duman, 1998). BDNF is transcribed by CREB, and several types of antidepressant treatment lead to the increased expression of CREB mRNA (Nibuya et al., 1995; Nibuya, Nestler, & Duman, 1996). BDNF transcription can be induced by phosphorylation of CREB through multiple second messenger pathways, including cellular activation and Ca2+/Calmodulin second messenger pathways (Murray, Hayes, Gall, & Isackson, 1998; Zafra, Lindholm, Castren, Hartikka, & Thoenen, 1992), and the cAMP second messenger pathway (Itoh, Tokumura, & Abe, 2004; Nakagawa et al., 2002).

There is converging evidence implicating the cAMP pathway in CREB-induced BDNF activation after antidepressant drug treatment. Substantial support for the theory of cAMP involvement in depression and its treatments comes from studies on rolipram, a drug that inhibits cAMP breakdown by PDE4 (Krause & Kuhne, 1988). Rolipram has antidepressant properties in rodents (Itoh et al., 2003; Itoh et al., 2004) and humans (e.g., Fleischhacker et al., 1992), although with a severe side effect of nausea in humans (Hebenstreit et al., 1989). The radiolabelled enantiomer of rolipram, (*R*)-[^{11}C]rolipram, is a useful marker of PDE4, demonstrating binding without interfering with PDE4 productivity (Fujita et al., 2005; Lourenco, DaSilva, Warsh, Wilson, & Houle, 1999; Lourenco, Houle, Wilson, & DaSilva, 2001). Increased (*R*)-[^{11}C]rolipram binding in brain suggests increased activity of PDE4 in response to increased cAMP, and thus is thought to represent an indirect measure of cAMP activity. Supporting a role of the cAMP second messenger pathway in the activity of antidepressant treatments, (*R*)-[^{11}C]rolipram binding increases after acute antidepressant drug administration affecting

noradrenaline and serotonin, but not dopamine (Lourenco et al., 2006). Given the evidence of changes in (*R*)-[^{11}C]rolipram binding after antidepressant drug administration, the following study investigated the binding of (*R*)-[^{11}C]rolipram after ECS.

Several individuals were involved in the design and implementation of this work and/or the preparation of this manuscript. Those with significant contributions in one or more of these areas are listed on the title page. The author of this thesis is the first author on this manuscript, and was involved in every step of the design and implementation of this work and the preparation of this manuscript.

Chapter 2: Increased brain-derived neurotrophic factor with no change in (*R*)-[^{11}C]rolipram binding to phosphodiesterase-4 after electroconvulsive stimulus in the rat

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Abstract

Aims: A single administration of antidepressant drug can increase (*R*)-[^{11}C]rolipram binding to phosphodiesterase-4 (PDE4), an indirect measure of second messenger cyclic-adenosine 3',5'-monophosphate (cAMP) activity in the rat brain. In accordance with the hypothesis that pharmacotherapy and electroconvulsive therapy (ECT) may share a common mechanism of action involving cAMP (as they both increase cAMP response-element binding protein transcription of brain-derived neutrotophic factor), we attempt to extend our previous findings to electroconvulsive stimulus (ECS), a model of ECT in animals.

Main Methods: Sixty male Wistar rats were administered 1, 5, or 10 daily ECS or sham treatments. (*R*)-[^{11}C]Rolipram was injected 3 hours after the last treatment and animals were euthanized 45 minutes later. Seven brain regions were isolated (frontal cortex, rest of cortex, hippocampus, thalamus, hypothalamus, cerebellum, and brainstem) and analyzed for BDNF protein expression and (*R*)-[^{11}C]rolipram retention.

Key Findings: Relative to sham-treated controls, BDNF protein density was significantly increased after 1 ECS only in the hippocampus. After 5 ECS, however, increases were also observed in the frontal cortex, and other cortical tissue. Finally, after 10 ECS increases were noted in the hippocampus, frontal cortex, and thalamus. In contrast, (*R*)-[^{11}C]rolipram binding remained unchanged in any of the brain regions examined after 1, 5, or 10 ECS treatments .

Significance: The lack of change in (*R*)-[^{11}C]rolipram binding after single or repeated ECS, despite increases in BDNF protein, suggests that ECS may activate CREB via cAMP in a different time window or via a different second messenger pathway than antidepressant drug treatment.

Introduction

Electroconvulsive therapy (ECT) is a longstanding and effective treatment for depression. However, the mechanism of action of ECT (or electroconvulsive stimulus in animals: ECS) remains poorly understood. Since antidepressant drugs as well as ECT appear to up-regulate BDNF (Altar et al., 2003; Nibuya et al., 1995), it has been suggested that these two treatment modalities may share a common underlying mechanism of action (Duman, 1998). BDNF transcription is regulated by the interactions of the CREB transcription factor interactions with the CRE modulator DNA region. Antidepressant drugs and ECT/ECS increase the expression of CREB mRNA in the brain (Jeon et al., 1997; Nibuya et al., 1995).

CREB is stimulated by several intracellular second messenger pathways involving: cAMP, activity dependent Ca^{2+}/calmodulin, mitogen-activated protein kinase and extracellular signal-regulated kinase (MAPK/ERK), and others (Mayr & Montminy, 2001). Two of these signalling cascades that are also implicated in BDNF transcription include Ca^{2+}/calmodulin (Murray et al., 1998; Zafra et al., 1992) and cAMP (Itoh et al., 2004; Nakagawa et al., 2002).

Increased cAMP production has been proposed as a common element in the molecular mechanism of antidepressant action of pharmacotherapy and ECT/ECS (Duman, 1998). cAMP is formed following stimulation of a number of G protein-coupled receptors, including serotonin and norepinephrine receptors. cAMP elicits intracellular effects by the activation of protein kinase A and is broken down by the PDE4 family of isozymes (Houslay et al., 1997). Support for the theory of cAMP involvement in depression and its treatments comes from the convergence of serotonergic and adrenergic antidepressant treatments on this second messenger, as well as studies using rolipram, a drug that inhibits cAMP breakdown by PDE4 (Krause et al., 1988). Rolipram has antidepressant properties in rodents (Itoh et al., 2003; Itoh et al., 2004) and humans

(Fleischhacker et al., 1992), although its use in the treatment of depression was limited by its side effect of nausea (Hebenstreit et al., 1989). The ^{11}C-radiolabelled active (*R*)- stereoisomer of rolipram, (*R*)-[^{11}C]rolipram, is a useful marker of PDE4, demonstrating PDE4-specific binding in brain regions that is sensitive to treatments affecting cAMP signalling (Fujita et al., 2005; Lourenco et al., 1999; Lourenco et al., 2001). Since PDE4 phosphorylation increases (*R*)-[^{11}C]rolipram binding, increased tracer retention in the brain suggests increased activity and/or expression of PDE4 in response to increased cAMP, and thus (*R*)-[^{11}C]rolipram binding functions as an indirect measure of cAMP activity. Supporting a role of the cAMP second messenger pathway in the activity of antidepressant treatments, we have previously shown that (*R*)-[^{11}C]rolipram binding increases after acute antidepressant drug administration affecting noradrenaline and serotonin (which regulate the cAMP second messenger pathway via g protein-coupled receptors [Duman, 1998]), but not dopamine neurotransmission (Lourenco et al., 2006).

In an effort to investigate the role of cAMP second messenger pathway in BDNF release after ECS, this study investigated (*R*)-[^{11}C]rolipram binding and BDNF protein levels in seven rodent brain regions after 1, 5, and 10 daily ECS sessions.

Method

Subjects

Male Wistar rats (350 – 400g) purchased from Charles River (St. Constant, Quebec) were housed in pairs (with one ECS-treated and one sham-treated rat per cage) and kept on a 12-hour dark/light cycle with lights on at 07:00. Animals had *ad libitum* access to food and water. All experiments were conducted in accordance with the Canadian Council of Animal Care guidelines and were approved by the animal care committee of the University of Ottawa Institute of Mental Health Research.

Groups

Animals were randomly assigned into groups based on treatment type (ECS or sham) and duration (1, 5, or 10 daily treatments), with n = 10 per group, for a total N = 60. In the 10 ECS group, 3 ECS-treated animals and their shams were excluded due to physical side effects of the ECS treatment (most notably, musculoskeletal damage), resulting in n = 7 for the 10 ECS and matched sham groups.

Electroconvulsive stimulus

The ECS was administered daily via ear clip electrodes: (55-65 mA, 0.8 sec, 60 Hz square wave pulse). Typical seizures lasted between 45 to 60 sec, with tonic and clonic activity identified by full extension of the hind limbs and repeated flexion-extension of the forelimbs, respectively. The sham-treated groups received the same treatment, except the electrical stimulus was not applied.

Tissue preparation

Animals were euthanized by decapitation at 3.75 hours after the last ECS (0.75 hours after (*R*)-[^{11}C]rolipram injection). This time point was chosen based on past studies with acute antidepressant administration (Lourenco et al., 2006). Trunk blood was collected into tubes containing EDTA. Brains were immediately removed and dissected on ice into 7 brain regions: hippocampus, frontal cortex, neocortex, striatum, thalamus, cerebellum, and brainstem. For BDNF analysis, tissue was immediately frozen on dry ice and stored at -80 °C. Prior to analysis, brain tissue was thawed on ice, weighed and homogenized in PIPES buffer, pH 7.3, containing 500 mM NaCl, 2% BSA, 0.2% Triton X-100, 0.1% NaN3, 2 mM EDTA, 200 μM PMSF, 0.4% protease inhibitor cocktail (Sigma-Aldrich, Canada) (Pollock et al., 2001). For (*R*)-[^{11}C]rolipram analysis, brain tissue and blood were analyzed immediately.

BDNF ELISA

BDNF protein was analyzed using enzyme-linked immunosorbent assay (ELISA) according to manufacturer instructions (R&D Systems, Minneapolis, MN). Homogenized tissue was acidified to a pH of 3 for 15 min using 8M HCl, neutralized to a pH of 7 using 6M NaOH, then centrifuged at 12000 rpm for 5 minutes. ELISA was performed according to manufacturer's specifications, with PIPES buffer substituted for all dilutions of samples and standards.

(R)-[^{11}C]Rolipram

The methods used for (*R*)-[^{11}C]rolipram production, injection, and detection have been previously described (Lourenco et al., 2001; Lourenco et al., 2006). Rats were immobilized using a rodent restraining device (Harvard apparatus, Canada). Tails were warmed with a heating lamp to induce vasodilation. (*R*)-[^{11}C]rolipram was synthesized as previously described (Kenk et al., 2007; Kenk et al., 2008) with high radiochemical purity (>95%) and specific activity (10.7–58.5 GBq/μmol; 290–1580 mCi/μmol) at the time of first injection. Rats were injected via tail vein with (*R*)-[^{11}C]rolipram, 1.2-2.0 mCi in 0.3 mL of vehicle at 3 hours after the last ECS- or sham-treatment. Radioactivity retained in individual brain regions and in trunk blood was corrected for decay and radioactivity remaining in tails and syringes. Measured radioactivity expressed as % injected dose per gram of tissue (%ID/g) was divided by the level of radioactivity in the blood (corrected to blood: %ID/g CB).

Statistical Analysis

Due to variability in baseline (sham) BDNF levels between assays, all data are expressed as percent of sham. T-tests were used to assess differences in BDNF protein and (*R*)-

[^{11}C]rolipram binding between ECS- and sham-treated animals in each of the 7 brain regions evaluated. One-way analysis of variance (ANOVA) was used to assess differences in relative changes in BDNF protein and (*R*)-[^{11}C]rolipram binding between the three treatment durations. Tukey post-hoc analysis was used to evaluate specific group differences for each brain region.

Results

BDNF

After a single ECS treatment, BDNF levels were significantly elevated in the hippocampus ($t(18) = 2.57$, $p = 0.02$) in ECS- relative to sham-treated animals. The other six regions evaluated showed no significant differences after ECS-treatment. After 5 daily treatments, BDNF levels were significantly elevated in the hippocampus ($t(18) = 3.28$, $p = 0.004$), frontal cortex ($t(18) = 4.81$, $p = 0.001$), striatum ($t(18) = 2.98$, $p = 0.013$), and neocortex ($t(18) = 5.49$, $p < 0.001$), whereas the thalamus, cerebellum, and brainstem showed no significant differences. After 10 daily treatments, BDNF levels were significantly elevated in the hippocampus ($t(12) = 2.24$, $p = 0.045$), frontal cortex ($t(9) = 2.91$, $p = 0.017$), and thalamus ($t(12) = 2.37$, $p = 0.043$). Differences in the other four regions analyzed did not reach significance after 10 ECS, although trends towards increased BDNF expression in the neocortex and cerebellum at this time point likely evaded statistical significance due to a reduction in statistical power due to a lower n in this group coupled with the high variability associated with the ELISA assay. BDNF protein measurement was not affected by (*R*)-[^{11}C]rolipram administraton (data not presented).

With ANOVA, significant differences in relative increases in BDNF protein (ECS relative to sham) between 1, 5, and 10 ECS were seen in the frontal cortex ($F(2, 23) = 14.43$, $p < 0.001$), striatum ($F(2, 26) = 6.39$, $p = 0.006$), brainstem ($F(2, 26) = 4.04$, $p = 0.03$), and neocortex ($F(2, 25) = 20.57$, $p < 0.001$), and approached significance in the thalamus ($F(2, 26) = 3.39$, $p = 0.05$).

There were no significant changes observed in the hippocampus (where all 3 groups exhibited similar elevations post-ECS) and cerebellum (where all 3 groups showed no significant elevations post-ECS). Post-hoc analysis revealed that in the frontal cortex and neocortex, 5 and 10 ECS induced greater elevations in BDNF compared with 1 ECS ($p < 0.01$). In the brainstem, 5 ECS induced greater elevations in BDNF compared with 10 ECS ($p < 0.05$), and in the thalamus, 10 ECS induced greater elevations in BDNF compared with 1 ECS ($p < 0.05$). In the striatum, 5 ECS induced greater elevations in BDNF compared with 10 ECS ($p < 0.01$) and 1 ECS ($p < 0.05$) (figure 1).

(R)-[^{11}C]Rolipram

There were no significant changes in (*R*)-[^{11}C]rolipram binding after 1, 5, or 10 daily ECS treatments (figure 2). In comparison of relative increases in (*R*)-[^{11}C]rolipram binding in ECS-treated animals at the three time points, one-way ANOVA was only significant for cerebellum ($F(2, 26) = 4.54$, $p = 0.02$), where post-hoc analysis revealed that relative (*R*)-[^{11}C]rolipram binding was significantly lower after 1 compared with 10 daily ECS treatments ($p < 0.02$). Relative changes in ECS-treated animals did not differ at any of the time points in the other six regions evaluated.

Discussion

In light of the observations that 1) both antidepressant drugs and ECS up-regulate the expression of BDNF and its receptor (Altar et al., 2003; Nibuya et al., 1995), 2) BDNF (via CREB) is a potential downstream target of the cAMP signalling pathway (Duman, 1998), and 3) a single antidepressant drug administration induces increases in (*R*)-[^{11}C]rolipram binding to PDE4, suggesting cAMP signalling activation (Lourenco et al., 2006), the present study sought to determine if the ECS-induced increases in BDNF levels are related to increases in cAMP. The

findings of this study showed that while a relatively incremental enhancement (in size and distribution) of brain BDNF protein levels was observed after 1, 5, or 10 daily ECS treatments, (*R*)-[^{11}C]rolipram binding was not significantly changed at the same time points. Despite the evidence for involvement of the cAMP second messenger system in the phosphorylation of CREB and elevations in BDNF protein after antidepressant drug administration (Altar et al., 2003; Lourenco et al., 2006), the findings of this study suggests that the cAMP second messenger pathway may not be directly linked to the elevations in brain BDNF protein after ECS.

Distinct intracellular processes may be involved in the phosphorylation of CREB after antidepressant drug and ECS treatments, even if the hypothesis that the common mechanism of action of antidepressant drugs and ECS involves CREB transcription of BDNF holds true. One group found that activity-induced increases in BDNF protein in the hippocampus were not significantly enhanced by the stimulation of cAMP (Fukuchi, Tabuchi, & Tsuda, 2005). Another major pathway involved in the activation of CREB is the activity-dependent Ca^{2+}/calmodulin second messenger pathway (Shieh, Hu, Bobb, Timmusk, & Ghosh, 1998; Tao, Finkbeiner, Arnold, Shaywitz, & Greenberg, 1998), which has been suggested as a potential mechanism of elevations in brain BDNF protein after ECS (Popoli et al., 2001). However, to our knowledge, no evidence directly implicates the involvement of this second messenger pathway following ECS.

Other explanations for the lack of alterations in (*R*)-[^{11}C]rolipram retention must also be considered. The primary alternative explanation involves the time window in which (*R*)-[^{11}C]rolipram binding was assessed. There is evidence supporting different time points for the alterations in the cAMP-CREB cascade following ECS. A single ECS leads to a maximal increase in the cerebral cortex in phosphorylated CREB within 10 minutes (Jeon et al., 1997) and

in cAMP (analyzed using a protein analysis method) within 45 seconds (Lust, Goldberg, & Passonneau, 1976b; Lust, Dye, Deaton, & Passonneau, 1976a). But longer-term treatments (10 daily ECS and up to 21 days of antidepressant drug administration) were associated with increases in CREB mRNA and CRE binding (as well as in BDNF and tyrosine kinase B [trkB] receptor mRNA) in the hippocampus at 18 hours after treatment (Nibuya et al., 1995). It was also previously shown that following a single antidepressant drug treatment, brain (*R*)-[^{11}C]rolipram binding peaks between 3 and 4 hours post-treatment (Lourenco et al., 2006). Based on the hypothesis that the activation of cAMP second messenger system and release of BDNF after repeated ECS activation is due to a similar mechanism of action as antidepressant drugs, we hypothesized that (*R*)-[^{11}C]rolipram binding would occur at a similar time point after ECS as seen after a single administration of antidepressant drugs. However, the current results do not support this finding, and pilot investigations with (*R*)-[^{11}C]rolipram injection at earlier time points (1 and 2 hours post-ECS) revealed no change in (*R*)-[^{11}C]rolipram retention (unpublished data).

In addition to the time-dependent issues, future studies should also consider that repeated or chronic administration of antidepressant treatments could produce effects on cAMP activation that might be quite distinct from those of acute or single treatment. Repeated administration of antidepressant drugs leads to desensitization of the monoamine receptors (Blier & De Montigny, 1994), which alters, and may be modulated through, the intracellular mechanisms related to the cAMP and the Ca^{2+}/calmodulin second messenger pathways (Popoli et al., 2001). Alterations in the monoamine receptors and their associated second messenger pathways may down-regulate cAMP, causing a non-significant (*R*)-[^{11}C]rolipram binding similar to that seen in the current study after repeated ECS. Indeed, repeated, long-term administration of antidepressant drugs has

led to findings of no increases (Reierson, Mastronardi, Licinio, & Wong, 2009) or even down-regulation of cAMP (Reierson et al., 2009). This may be related to the presence of multiple PDE4 isoforms (PDE4A, B, C and D, each with multiple variants), which may be differentially regulated following antidepressant treatment (Reierson et al., 2009).

Conclusion

The current study found no change in (*R*)-[^{11}C]rolipram binding in the rat brain after a single or repeated ECS, despite incremental and widespread increases in BDNF protein. If the stimulation of the cAMP second messenger system cascade is involved in a common outcome of CREB activation and resulting increase in brain BDNF protein after diverse antidepressant treatments, measurement of cAMP regulation of this process using (*R*)-[^{11}C]rolipram requires consideration of different time windows after ECS, compared with antidepressant drug treatment.

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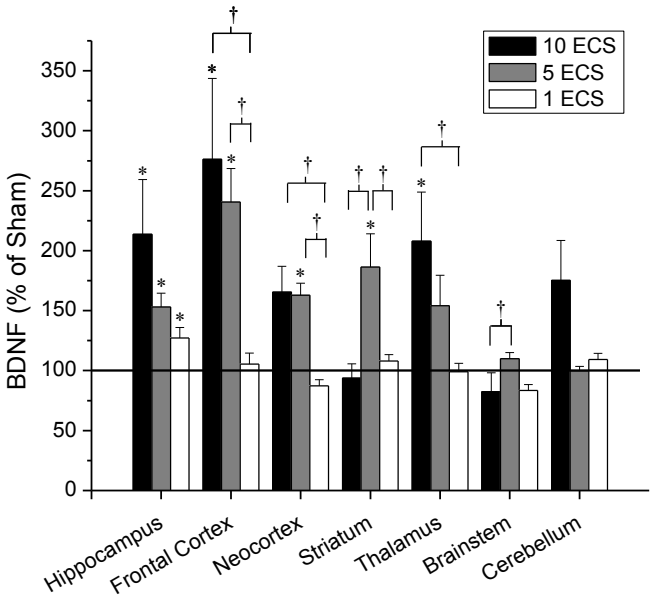

Figure 1. BDNF protein expressed as percent of sham. Bar represents 100% or sham. * $p < 0.05$ relative to sham; † $p < 0.05$ relative to 1, 5, or 10 ECS.

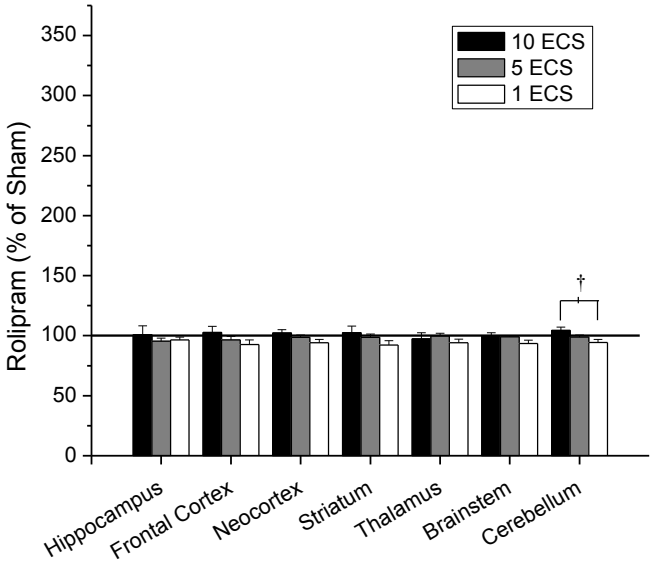

Figure 2. (*R*)-[^{11}C]rolipram binding expressed as percent of sham. Bar represents 100% or sham. † $p < 0.05$ relative to 1, 5, or 10 ECS.

Preface to Chapter 3

Will an animal model of depression elucidate the role of antidepressant-induced changes in brain BDNF and facilitate the translation of these findings to the human condition of depression?

The previous study offers some contradiction to the argument that there is a common intracellular mechanism such as the cAMP second messenger pathway underlying antidepressant drug treatment and ECT. However, the common finding of increases in brain BDNF protein and mRNA expression in rodents after various antidepressant treatments still leaves open the possibility that brain BDNF may be involved in the common action of these treatments, even if in fact treatment-(antidepressant vs ECT) induced increases in BNDF are achieved through different intracellular mechanisms.

That being said, we still have little evidence supporting a direct role for BDNF in the antidepressant effect of antidepressant drugs and ECT. Although BDNF has been directly tied to changes in "depressive-like behaviours" in animals (Shirayama et al., 2002), many of these studies have been performed in "normal" outbred rat strains, such as the Sprague-Dawley and Wistar strains, and studies in animal models of depression, particularly the novel genetic models, are few and do not necessarily demonstrate the same findings as in the outbred strains (Angelucci et al., 2003). In addition, BDNF has been implicated in many other functional roles, most notably in memory (Egan et al., 2003), suggesting that BDNF may play a role in the cognitive aspects of depression and its treatments. Given these questions, the second series of studies investigated the biological and behavioural effects of ECS in the WKY strain. The WKY strain is also known for its hyperresponsiveness to stress and abnormalities in the HPA axis (De La Garza et al., 2004). Therefore, given the implication of the HPA axis in depression

(Arborelius et al., 1999) and ECT (Holsboer et al., 1996), and the interactions between the HPA axis and BDNF (Givalois et al., 2004), cortisol and CRF were also measured as outcome factors in this study.

Several individuals were involved in the design and implementation of this work and/or the preparation of this manuscript. Those with significant contributions in one or more of these areas are listed on the title page. The author of this thesis is the first author on this manuscript, and was involved in every step of the design and implementation of this work and the preparation of this manuscript.

Chapter 3: The effects of repeated ECS on depression-like behaviour and memory performance in the Wistar and Wistar-Kyoto rat: Relationship to extra-hypothalamic corticotrophin-releasing factor and brain-derived neurotrophic factor

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Abstract

Background: Investigations in healthy outbred rat strains has shown a potential role for BDNF and the HPA axis in the antidepressant and memory side effects of ECT (or ECS in animals). Animal models of depression must be validated for use in the investigation of the neurobiological underpinnings of ECT/ECS. The WKY rat strain is assessed as a potential genetic model of depression useful in research on ECT/ECS.

Methods: Wistar and WKY rats received 5 days of repeated ECS or sham treatment and were assessed 1 and 7 days later in three separate studies for 1) depression-like behaviour and mobility; 2) retrograde memory; and 3) brain BDNF protein, brain corticotropin-releasing factor and plasma corticosterone levels.

Results: In study one, despite WKY rats showing initially elevated depression-like behaviour relative to the Wistar rats, both strains showed the expected antidepressant response at 1 day following ECS, which was sustained at 7 days. In study two, expected retrograde memory impairments were evident in both strains at both time points. In study three, baseline BDNF, CRF, and corticosterone profiles were similar in the two strains. At 1 day after ECS, Wistar and WKY rats showed similar elevations in brain BDNF and extra-hypothalamic CRF and no change in plasma corticosterone. At 7 days after ECS, Wistar rats showed sustained elevations of brain BDNF and CRF, whereas WKY rats showed a normalization of brain BDNF, despite sustained elevations of brain CRF. No strain or treatment effects on plasma corticosterone were seen.

Limitations: The application of a stressor pre-ECS may produce different physiological responses to ECS in the WKY strain. The measurement of BDNF and CRF used does not allow for analysis of utilization and synthesis.

Conclusions: The WKY strain is validated as a useful genetic model of depression with which to investigate ECS/ECT. Extra-hypothalamic CRF elevations and possible early changes in BDNF may contribute to memory deficits after ECS. However, brain BDNF and CRF do not appear to contribute to depression-like behaviour in either strain.

Introduction

Electroconvulsive therapy (ECT) is an effective treatment for pharmacotherapy-resistant depression (Greenberg et al., 2005). However, it causes negative cognitive side-effects, particularly impacting retrograde autobiographical memory (Datto, 2000; Rami-Gonzalez et al., 2001). Despite its clinical use to treat depression for over 70 years (Greenberg et al., 2005), the biological underpinnings of the antidepressant and cognitive properties of ECT remain poorly understood.

Research in rodents has led to several insights into the behavioral and biological components of ECT. In 'healthy' outbred rat strains, electroconvulsive stimulus (ECS, the animal model of ECT) has been widely shown to decrease immobility in the forced swim test (FST), a behavioral marker of effective antidepressant treatments (Porsolt et al., 1978; Zyss et al., 1997; Sattin et al., 1994). ECS also interferes with retrograde memory in spatial mazes (e.g., Morris Water Maze) (Bohbot et al., 1996), passive-avoidance tasks (Andrade et al., 2002; Lerer et al., 1986), and conditioned emotional response (CER) paradigms (Brady et al., 1954; Brady et al., 1953; Geller et al., 1955; Hunt et al., 1952; Misanin et al., 1968).

Neurochemical systems involving BDNFand the HPA axis have also been implicated in depression, memory, and ECS in outbred rat strains. Large increases of BDNF mRNA and protein are elicited in the brains of healthy outbred rats by antidepressant drug administration (Altar et al., 2003; Nibuya et al., 1995) and repeated ECS (4-10 daily administrations) (Altar, 1999; Altar et al., 2003; Angelucci et al., 2003; Li et al., 2006; Li et al., 2007; Nibuya et al., 1995). However, in the FSL, a genetic model of depression, the hallmark elevations in brain BDNF protein after ECS were not evident, despite increased brain nerve growth factor (NGF)

(Angelucci et al., 2003) and changes in depression-like behaviour in the FST (Jimenez-Vasquez et al., 2007).

Hyperactivity of the HPA axis has been widely implicated in depression (Arborelius et al., 1999; Bao et al., 2008; Plotsky et al., 1998). In healthy outbred rats, elevated glucocorticoid levels are attenuated by repeated ECS (Hageman et al., 2009) and are linked with retrograde memory impairments after ECS (Nagaraja et al., 2007). CRF has been implicated in the stress-related features of depression (Heinrichs & Koob, 2004; Nemeroff & Vale, 2005), and CRF receptor antagonists have antidepressant properties (Holsboer et al., 2008). More recently, CRF has been implicated in memory as blockade of CRF_1 receptors reverses both the hippocampal dendritic spine loss and memory deficits observed after prolonged stressor exposure (Chen et al., 2010). Yet in several inbred rat strains, including animal models of depression, it has been reported that there was no correlation between baseline performance in the FST and brain CRF mRNA (Lahmame et al., 1997).

The replication of findings from healthy outbred rodents to inbred rodent models of depression assists in the translation of findings from rodent studies to the human condition of depression. The WKY strain of rat has been used as a genetic model of depression as it demonstrates depression- and anxiety-like behaviours (Braw et al., 2006; Malkesman et al., 2005; Pare, 1994; Will et al., 2003), working memory deficits (Grauer et al., 1993; Robertson et al., 2008), abnormalities in monoamine (De La Garza et al., 2004; Pare et al., 1996; Pearson et al., 2006; Tejani-Butt et al., 1994) and neuroendocrine activity (De La Garza et al., 2004; Hauger et al., 2002; Redei, Solberg, Kluczynski, & Pare, 2001), and possible resistance to pharmacological treatments for depression (Lahmame et al., 1996; Lahmame et al., 1997).

Moreover, in one study, repeated ECS reduced depression-like behaviour in WKY rats in the FST (Krahl et al., 2004).

Three studies were completed to examine the WKY strain for behavioral and neurochemical changes immediately (1 day) and at a longer delay (7 days) after repeated ECS (5 daily administrations), compared to its natural outbred control, the Wistar strain. First, the effectiveness of repeated ECS in eliciting the expected antidepressant effect in the FST was established in the WKY and Wistar strains. In a second study, the effect of repeated ECS on retrograde memory was assessed. And in the final study, neurochemical changes related to BDNF and the HPA axis after repeated ECS were assessed at selected brain regions that may be relevant to the effects of ECT in humans. With these results, we can discuss the validity of the WKY strain as a genetic model of depression that can be useful in ECS research.

Method

Subjects

Male Wistar (200-450g) and WKY (150-300g) rats (Charles River, Quebec, Canada) between 7 to 10 weeks of age were housed in pairs by strain (with one ECS-treated and one sham-treated per cage) and kept on a 12 hour dark/12 hour light cycle with lights on at 07:00. Animals had free access to food and water. All experiments were conducted in accordance with the Canadian Council of Animal Care guidelines and were approved by the animal care committee of the University of Ottawa Institute of Mental Health Research.

Electroconvulsive Stimulus

The ECS was administered once daily for 5 days via ear clip electrodes: (55-65 mA, 0.8 sec, 60 Hz square wave pulse). Typical seizures lasted between 45 to 60 sec, with tonic and clonic activity identified by full extension of the hindlimbs and repeated flexion-extension of the

forelimbs, respectively. The sham-treated groups received the same treatment, except the stimulus was not applied.

Study One

Animals were randomly assigned to eight groups (one ECS-treated and one sham-treated group for each strain [Wistar and WKY] at each time point [1 and 7 days post-ECS]; n = 9-10 per group), and were assessed for depression-like behavior using the FST and mobility using the open field (OF).

Forced Swim Test

The FST is a behavioral despair paradigm widely used to evaluate the effectiveness of antidepressant drugs (Porsolt, Le Pichon, & Jalfre, 1977; Schiller, Pucilowski, Wienicke, & Overstreet, 1992). The forced swim arena consisted of a clear Plexiglas cylinder (20 x 45 cm, water height 20 cm, temperature 25 °C; Stoelting Co., Wood Dale, Illinois). A habituation session (15 min) was performed at 1 or 6 days post-ECS and a test session (5 min) was performed one day after habituation, at 2 or 7 days post-ECS. The time spent immobile (no movement of the limbs, trunk, or tail) was recorded during the test session using ODlog software (Macropod Software) by trained experimenters blinded to condition.

Open Field Test

The OF was used to assess locomotor activity (Courvoisier, Moisan, Sarrieau, Hendley, & Mormede, 1996; Matto & Allikmets, 1999). Rats were placed in the center of the square arena (60 cm) and video recorded for 5 minutes. The total number of squares crossed was assessed, which provided an index for general locomotor activity (Correa, Arizzi, Betz, Mingote, & Salamone, 2003).

Study Two

Animals were randomly assigned to 12 groups. Eight groups (one ECS-treated and one sham-treated group for each strain [Wistar and WKY] at each time point [1 and 7 days post-ECS]; n = 9-10 per group) were assessed for retrograde memory in a Conditioned Emotional Response (CER) paradigm (one ECS-treated and one sham-treated group for each strain [Wistar and WKY] at each time point [1 and 7 days post-ECS]). Four groups were assessed in the Morris Water Maze (MWM) paradigm (one ECS-treated and one sham-treated group for each strain [Wistar and WKY], with the same groups assessed at both time points [1 and 7 days post-ECS] in a repeated measures paradigm).

Conditioned Emotional Response

CER was used to assess retrograde memory for conditioned contextual fear. This paradigm has been described previously (Mountney, Sillberg, Kent, Anisman, & Merali, 2006). Training (20 min) was performed on the last day of ECS habituation (prior to ECS treatment). Briefly, animals were placed in a conditioning chamber, with a floor comprised of 16 stainless steel rods (4 mm diameter, 1.4 cm apart) connected to a shock generator (Coulbourn Instruments, model H13-16, Whitehall, PA, USA), where they received six foot shocks (1.0 mA, 1 sec) on a random schedule with an average inter-trial interval (ITI) of 1 min. Testing (20 min) was performed at 1 or 7 days post-ECS. Contextual fear was assessed by placing rats in the same conditioning chamber as in training. Duration of freezing behavior was recorded using ODlog software (Macropod Software) by trained experimenters blinded to condition.

Morris Water Maze

MWM was used to assess retrograde spatial memory, and this paradigm was guided by previously published methods (Chen, Zhang, Yang, Dong, & Zhang, 2009; Vorhees & Williams,

2006). A MWM tub (176.5 cm diameter) was filled with water (50 cm deep) made opaque with black non-toxic paint and placed in a room with substantial visual cues. For training, a black platform (10 cm diameter) was placed 1.5 cm below the water's surface in the centre of the northeast (NE) quadrant of the maze. Rats were placed in the maze facing the wall at one of four starting points (south, west, northwest, southeast), with order randomized each day. Timing of trials was started when the rat turned and began swimming away from the wall. This modification greatly minimized the confound of freezing/floating behavior often seen in the WKY strain upon entering the maze (Grauer et al., 1993). If the rat did not find the platform in 120 sec, it was led to the platform by the experimenter. Once the rat reached the platform, it was allowed to remain on the platform for 30 sec, after which it was removed and placed back in the water at the next starting point or, after the final trial, removed from the maze, towel-dried, and placed under a heat lamp for 15 min. In training, time to reach the platform was measured and a value of 120 sec given to trials in which the animal failed to find the platform in the allotted time. Reference memory for the location of the platform was assessed using probe trials (60 sec) in which the platform was removed from the maze and the animal was started in the southwest quadrant. Training was performed for six days in a block of six consecutive training trials per day. After five training days, animals had reached the recommended asymptotic performance (data not shown) (Vorhees et al., 2006). The sixth training day was added after the baseline probe trial to mitigate any extinction effects. Thus, probe trials were performed in a repeated-measures design at three time points: (1) baseline, after 5 days of training and 1 day prior to the start of ECS, (2) 1 day after completion of ECS, and (3) 7 days after completion of ECS. On probe trials, time spent in each quadrant and swimming speed was recorded using the tracking software EthoVision 3 (Noldus Information Technology, Virginia, US).

Study Three

Animals were randomly assigned to eight groups (one ECS-treated and one sham-treated group for each strain [Wistar and WKY] at each time point [1 and 7 days post-ECS]; n = 9-10 per group) and were assessed for the following biological markers: plasma levels of corticosterone and brain tissue levels of BDNF and CRF.

Tissue preparation

Animals intended for neurochemical analysis were euthanized by decapitation at 1 or 7 days post-ECS. Trunk blood was immediately collected in tubes containing spell out! EDTA (70 μM), placed on ice and spun in a refrigerated centrifuge (4°C, 3000 rpm, 15 min), after which plasma was isolated and frozen at -80 °C until analysis. Brains were immediately removed and dissected on ice into 8 regions: hippocampus, striatum, frontal cortex, neocortex, hypothalamus, thalamus, cerebellum, and brainstem. Once removed, brain tissue was immediately frozen on dry ice and stored at -80 °C until analysis. Prior to analysis, brain tissue was weighed and homogenized in 100 mM PIPES buffer, pH 7.3, containing 500 mM NaCl, 2% BSA, 0.2% Triton X-100, 0.1% NaN_3, 2 mM EDTA, 200 μM PMSF, 0.4% protease inhibitor cocktail (Sigma-Aldrich, Canada). (Pollock et al., 2001)

BDNF ELISA

BDNF protein was analyzed using ELISA according to manufacturer instructions (R&D Systems, Minneapolis, MN). Homogenized tissue was acidified to a pH of 3 for 15 min using 8M HCl, neutralized to a pH of 7 using 6M NaOH, then centrifuged at 12000 rpm for 5 minutes. ELISA was performed according to manufacturer's specifications, with PIPES buffer substituted for all dilutions of samples and standards.

CRF and corticosterone radioimmunoassay

The detection and quantification of CRF was achieved through a solid-phase high-sensitivity adaptation or modification (Maidment & Evans, 1991) of the double-antibody liquid phase radioimmunoassay (RIA) originally described by Vale and colleagues (Vale et al., 1983). Corticosterone levels were measured using a commercial RIA kit (MPbiomedicals, Solon, OH) according to manufacturer instructions. These methods have been detailed previously (Merali et al., 2009).

Statistical Analysis

2 (Treatment: ECS, Sham) x 2 (Strain: Wistar, WKY) Multiple ANOVA (MANOVA) was used to assess effects and interactions of treatment and strain at 1 and 7 days post-ECS for neurochemistry outcome factors, BDNF and CRF. 2 x 2 ANOVA was used to identify group differences with respect to plasma corticosterone and behaviour changes (FST, OF, and CER). As each time point had its own sham group, separate analyses were completed for the different groups at 1 and 7 days. Repeated measures ANOVA (RMANOVA) was used to identify group differences in the MWM. Significant main effects and interactions were followed up with t-tests. A bonferonni correction was applied to the alpha account for multiple comparisons. Square root transformations were applied to the dependent variables BDNF, CRF, and FST time spent immobile to correct for mild skew and achieve homogeneity of variances. As groups assessed at 1 and 7 days had their own sham control groups, comparisons between time points were not systematically completed. Therefore, point comparisons of interest (identified post hoc) between any of the outcome factors at 1 and 7 day time points were completed using relative (% of sham) changes in the outcome factors (e.g., did hippocampal BDNF levels of ECS relative to shams

decrease significantly from 1 to 7 days post-ECS in the Wistar and WKY strains?). Statistics for these point comparisons were calculated using t-tests ($p < 0.05$).

Results

Study One

Forced Swim Test

At 1 day post-ECS, ANOVA of time spent immobile in the FST showed significant main effects for treatment ($F(1, 31) = 27.3$, $p < 0.001$) and strain ($F(1, 32) = 41.8$, $p < 0.001$). Post hoc analysis revealed that ECS significantly reduced time spent immobile compared with sham treatment in both Wistar ($t(16) = -3.90$, $p = 0.001$) and WKY rats ($t(16) = -3.53$, $p = 0.003$). In addition, WKY rats spent more time immobile compared with Wistar rats, and this was true in both ECS- ($t(16) = -5.38$, $p < 0.001$) and sham-treated groups ($t(16) = -4.27$, $p = 0.001$) (figure 1A). ECS-treated WKY rats performed at the same level as Wistar shams ($t(16) = 0.89$, $p = 0.39$).

At 7 days post-ECS, ANOVA showed significant main effects for treatment ($F(1, 32) = 20.8$, $p < 0.001$) and strain ($F(1, 32) = 44.6$, $p < 0.001$). Post-hoc analysis revealed that the effect of treatment (ECS reduced immobility relative to sham treatment) was significant for Wistar ($t(16) = -3.66$, $p = 0.002$) and WKY rats ($t(16) = -3.12$, $p = 0.007$). Strain differences (WKY were more immobile than Wistar) were also maintained at this later time point in ECS- ($t(16) = -4.16$, $p = 0.001$) and sham-treated groups ($t(16) = -5.30$, $p < 0.001$). ECS-treated WKY rats continued to perform at the same level as Wistar shams ($t(16) = 1.69$, $p = 0.11$) (figure 1B).

Open Field

At 1 day post-ECS, ANOVA of total squares crossed showed a main effect of strain ($F(1, 32) = 121.6$, $p < 0.001$), but not treatment ($F(1, 32) = 2.3$, $p = 0.14$). On post-hoc analysis, WKY

rats showed significantly lower total squares crossed (i.e., lower locomotor activity) relative to Wistar rats, which was true of both ECS- and sham-treated groups (ECS: $t(16) = 10.06$, $p < 0.001$; Sham: $t(16) = 6.50$, $p < 0.001$) (figure 2A).

At 7 days post-ECS, ANOVA showed a main effect of treatment ($F(1, 32) = 19.5$, $p < 0.001$) and strain ($F(1, 32) = 217.3$, $p < 0.001$). Post-hoc analysis revealed that ECS decreased mobility in both strains (Wistar: $t(16) = -2.98$, $p = 0.009$; WKY: $t(16) = -4.62$, $p < 0.001$). WKY rats remained less mobile than WKY rats, which was true of both ECS- and sham-treated groups (ECS: $t(16) = 8.32$, $p < 0.001$; sham: $t(16) = 13.76$, $p < 0.001$) (figure 2B).

Study Two

Conditioned emotional response

At 1 day post-ECS, ANOVA of total freezing time in the CER showed a significant strain x treatment interaction ($F(1, 31) = 26.6$, $p < 0.001$). Post hoc analysis revealed that ECS-treated WKY rats froze more than matched shams ($t(16) = 4.63$, $p < 0.001$). A trend in the opposite (and expected) direction did not reach significance in Wistar rats with the applied bonferroni correction ($t(16) = 2.57$, $p = 0.021$), likely due to high variability in the data (figure 3A). Although there were no differences between the strains in their sham-treated controls, ECS-treated WKY rats froze more than ECS-treated Wistar rats ($t(16) = -6.65$, $p < 0.001$).

At 7 days post-ECS, the interaction between strain x treatment remained significant ($F(1, 32) = 6.6$, $p < 0.02$). Post hoc analysis revealed no significant treatment effects. A trend toward an increase in freezing behaviour in sham-treated Wistars relative to WKY did not reach significance with the applied bonferroni correction ($t(16) = 2.40$, $p = 0.029$), but suggests better retention of this task over the long delay in the untreated animals. The non-significant visual

trend for an effect of treatment in reducing freezing time continued to be observed in the Wistar rats (figure 3B).

Morris Water Maze

Initial analysis revealed a significant difference in baseline performance between ECS and sham groups in the Wistar strain. To account for this initial discrepancy, data at all testing time points (baseline, 1 day, and 7 days post-treatment) were transformed by taking the percentage of the average baseline performance within each group. RMANOVA showed a significant within-subjects effect of time point ($F(2, 72) = 25.92$, $p < 0.001$) and a significant interaction between time point and treatment ($F(2, 72) = 8.68$, $p < 0.001$). Post-hoc analysis revealed that ECS-treatment reduced time spent in the goal quadrant in the Wistar and WKY strains at both time points, although the visually identified effect in the WKY did not reach significance with the high variability and the applied bonferonni correction at the later time point (Wistar 1d: $t(16) = -5.47$, $p < 0.001$; 7d: $t(16) = -4.03$, $p = 0.001$; WKY 1d: $t(16) = -2.79$, $p = 0.012$; 7d: $t(16) = -2.50$, $p = 0.022$) (figure 4). The observed reduction in the time that sham-treated WKY rats spent in the goal quadrant between 1 and 7 days suggested a loss of memory over time in this group; however, this effect did not reach significance ($t(16) = 2.13$, $p = 0.062$) (Figure 4).

Study Three

BDNF

At 1 day post-ECS, MANOVA showed significant multivariate effects for treatment (Pillai's Trace = 0.872, $F(8, 25) = 25.91$, $p < 0.001$) and strain (Pillai's Trace = 0.573, $F(8, 25) = 4.20$, $p = 0.003$). Only significant main effects on univariate analysis were investigated with post-hoc analysis, which revealed that ECS increased BDNF protein levels relative to matched shams in the Wistar hippocampus ($t(16) = 5.78$, $p < 0.001$), frontal cortex ($t(16) = 5.64$, $p <$

0.001), neocortex (t(16) = 3.92, p = 0.001), hypothalamus (t(16) = 3.30, p = 0.009), and brainstem (t(16) = 4.00, p = 0.001), and approached significance in the thalamus (t(16) = 2.87, p = 0.011). In the WKY, ECS increased BDNF in the hippocampus (t(16) = 5.65, p < 0.001), frontal cortex (t(16) = 6.87, p < 0.001), neocortex (t(16) = 8.14, p < 0.001), striatum (t(16) = 3.82, p = 0.002), thalamus (t(16) = 4.08, p = 0.001), and hypothalamus (t(16) = 3.98, p = 0.001), and approached significance in the brainstem (t(16) = 2.26, p = 0.038). There were no strain differences in BDNF protein, although differences did approach significance in the cerebellum (Wistar > WKY) and frontal cortex (WKY > Wistar) (table 1 and figure 5A-B).

At 7 days post-ECS, MANOVA showed significant multivariate effects for treatment (Pillai's Trace = 0.572, F(8, 24) = 4.01, p = 0.004) and strain (Pillai's Trace = 0.457, F(8, 24) = 2.52, p = 0.038). ECS increased BDNF protein levels relative to matched shams in the Wistar hippocampus (t(16) = 3.44, p = 0.007), frontal cortex (t(16) = 3.43, p = 0.003), and neocortex (t(16) = 2.86, p = 0.011), hypothalamus (t(16) = 3.30, p = 0.009), and in the WKY brainstem (t(16) = 3.98, p = 0.001). Again, strain differences were not clearly evident, although Wistar rats showed slightly higher levels of BDNF in the hypothalamus relative to WKY that approached significance (ECS: t(16) = 2.67, p = 0.022; sham: t(16) = 2.34, p = 0.033) (table 1 and figure 5C-D).

Independent t-tests confirmed the observed reductions (data analyzed as percent of sham) in BDNF in ECS-treated groups in both strains between 1 and 7 days post-ECS in the WKY hippocampus (t(16) = 3.84, p = 0.001), frontal cortex (t(16) = 4.54, p < 0.001), neocortex (t(16) = 5.39, p < 0.001), striatum (t(16) = 3.28, p = 0.005), and thalamus (t(16) = 3.18, p = 0.006), with Wistar rats approaching significance in the frontal cortex (t(16) = 2.26, p = 0.038).

CRF

At 1 day post-ECS, MANOVA showed significant multivariate effects for treatment (Pillai's Trace = 0.86, $F(8, 25) = 19.19$, $p < 0.001$) and strain (Pillai's Trace = 0.586, $F(8, 25) = 4.14$, $p = 0.002$). Post-hoc follow up to significant main effects on univariate analysis revealed that ECS increased CRF protein levels relative to matched shams in the Wistar hippocampus ($t(16) = 4.09$, $p = 0.001$), frontal cortex ($t(16) = 6.24$, $p < 0.001$), and neocortex ($t(16) = 4.13$, $p = 0.001$), and in the WKY in the same regions: hippocampus ($t(16) = 5.95$, $p < 0.001$), frontal cortex ($t(16) = 8.34$, $p < 0.001$), and neocortex ($t(16) = 12.18$, $p < 0.001$). Strains differed in CRF levels where ECS-treated Wistar rats showed lower CRF in the frontal cortex ($t(16) = -3.46$, $p = 0.003$) and sham-treated Wistars showed lower levels of CRF in the striatum ($t(16) = -3.23$, $p = 0.005$) and the brainstem ($t(16) = -7.56$, $p < 0.001$) (table 2 and figure 6 A-B).

At 7 days post-ECS, MANOVA showed a significant multivariate effect for treatment (Pillai's Trace = 0.718, $F(8, 25) = 7.97$, $p < 0.001$) but not strain (Pillai's Trace = 0.209, $F(8, 25) = 0.823$, $p = 0.59$). Post-hoc follow up to significant main effects on univariate analysis showed that CRF remained significantly elevated in ECS-treated Wistar hippocampus ($t(16) = 3.32$, $p = 0.004$), frontal cortex ($t(16) = 4.03$, $p = 0.001$), and neocortex ($t(16) = 4.73$, $p < 0.001$), and in ECS-treated WKY hippocampus ($t(16) = 5.49$, $p < 0.001$), frontal cortex ($t(16) = 5.83$, $p < 0.001$), and neocortex ($t(16) = 3.74$, $p = 0.002$) (table 2 and figure 2C-D).

Independent t-tests indicated that observed relative reductions (data analyzed as percent of sham) in CRF between 1 and 7 days post-ECS was significant only in the WKY neocortex ($t(16) = 3.62$, $p = 0.002$).

Corticosterone

Despite a visual trend to increased plasma corticosterone in ECS-treated animals at 1 day post-ECS, there were no significant differences between strains or treatment groups in corticosterone levels at either 1 or 7 days post-ECS.

Discussion

The primary objective of the present investigation was to assess the usefulness of a genetic model of depression, the WKY strain, in investigations of ECS. To accomplish this task, we assessed the short- and longer-term impact of repeated ECS on behaviour (related to depression and memory) and on neurochemical changes (related to BDNF and the HPA axis) in WKY rats and their outbred natural control strain, Wistar rats. In the first study, the well-known effectiveness of repeated ECS to elicit an antidepressant-like effect (Krahl et al., 2004; Li et al., 2007; Porsolt et al., 1977) was demonstrated in the WKY and Wistar strains. This was reflected by a significant decrease in percent of time spent immobile in the FST in ECS- compared to sham-treated rats. The antidepressant effect was evident at both 1 and 7 days post-ECS in both strains, demonstrating a sustained antidepressant effect of 5 daily ECS. WKY rats spent overall markedly more time immobile than Wistar rats, regardless of treatment. Following ECS treatment however, levels of immobility in the WKY rats became comparable to those of Wistar sham-treated controls at both the 1 and 7 day post-ECS time points, suggesting that repeated ECS normalizes this depressive-like behaviour in the WKY strain to levels typically observed in an outbred control strain, and that this effect is persistent. The marked immobility in the WKY relative to the Wistar strains is exemplified in the OF test, even after an ECS-induced reduction of mobility was seen in both strains. Similar baseline behavioural differences in FST and OF have been shown between WKY rats and Sprague-Dawley rats (O'Mahony, Clarke, Gibney, Dinan, & Cryan, 2011).

In the second study, it was demonstrated that ECS also caused the expected retrograde memory impairment in both strains as indicated by significantly less time spent in the target quadrant (that previously contained the escape platform) of the MWM in ECS- compared with sham-treated rats at 1 day after treatment, and this effect was sustained for at least 7 days. The sustained retrograde memory impairment in this study replicates this important negative side effect of ECT seen in people with depression (Rami-Gonzalez et al., 2001), and extends previous findings of retrograde memory impairments shown at 1 day after treatment (Andrade et al., 2002). The performance of Wistar rats in the CER paradigm, a contextual and fear-based memory task, appeared to support the MWM findings of impaired retrograde memory in the ECS-treated Wistar rats, which, at least at the 1 day time point, appeared to freeze less than matched shams when placed back into the same environment where they had previously been shocked (data did trend in this direction, but did not reach significance with the strict bonferroni correction). In contrast, this effect was reversed in the ECS-treated WKY rats, which froze more than matched shams when exposed to the contextual cues associated with the footshock. Although this finding seems counterintuitive, it is noteworthy that the WKY strain is hyperresponsive to anxiogenic situations (Braw et al., 2006), which might be manifested behaviorally as reduced mobility/enhanced freezing. Indeed our OF results of an overall increased immobility/freezing behaviour in the WKY strain support this contention. Similarly, in both the FST and MWM, increased levels of immobility/freezing were observed in the WKY strain, a finding consistent with other studies (Grauer et al., 1993). In fact, the freezing observed in the MWM was so profound in our pilot studies that we modified the testing procedure (by starting timing the trials when the rat turned and began swimming away from the wall rather than immediately upon being placed in the MWM) to minimize the impact of this potential

confounding variable. Therefore, perhaps not surprisingly, exposure to ECS had the anticipated behavioral outcome in the WKY rats in the water-based tasks measuring depression-like behavior (FST: where immobility is the primary outcome variable assessed) and memory (MWM: where modifications of the task compensated for this confounding behavior), but appeared to exacerbate the freezing/immobility response which lead to interference with expected behavioral responses in two of the other tasks, which were in dry environments and have known anxiety/fear components (OF and CER). Attention to and possibly adjustment for anxiety/fear components of behavioural tests appears essential in the investigation of other behaviours in the WKY strain. In addition, the dry vs. wet environments of the different testing paradigms cannot be ignored, as rodents have shown different performance in dry land and water-based memory tasks in other studies (Ormerod & Beninger, 2002).

In the third study, Wistar and WKY strains showed the expected widespread elevations in BDNF protein at 1 day after ECS, in regions implicated in depression and memory (i.e., consistently in the hippocampus, frontal cortex, and neocortex), but not in regions typically excluded from neurobiological theories of depression, such as the cerebellum. When assessed 7 days after ECS, the effects of ECS on BDNF were still evident in many depression-relevant regions in the Wistar strain, but only in the brainstem of WKY rats. This could point to physiological disturbances in this strain in addition to known monoamine and HPA axis abnormalities (De La Garza et al., 2004; Hauger et al., 2002; Pearson et al., 2006) that could be relevant to the abnormal behavioral profile characteristic of these rats (Grauer et al., 1993; Pare, 1994). The prolonged maintenance of BDNF levels for several days after repeated ECS (seen in the current study in the Wistar strain) has been reported after 14 daily ECS (Li et al., 2007), but not 4 daily ECS sessions (Altar, 1999). Although differences in the ECS stimulus (i.e., current,

stimulus duration, and number of ECS) or other factors, such as animal strain and age, may contribute to the discrepant findings between the above-mentioned and current studies, another influential factor on BDNF protein quantification is the method employed for its measurement. In the current study, the use of PIPES buffer and the acidification step in the ELISA method allowed for additional lysis of cells and the measurement of total intra- and extra-cellular BDNF. ELISA does not differentiate between the two forms of the BDNF protein: proBDNF and mature(m)BDNF, which exist primarily in the intracellular and extracellular space, respectively (Greenberg, Xu, Lu, & Hempstead, 2009). Discrepancies in the induction of pro- and mBDNF after different classes of antidepressant treatments or at different time points after treatment could potentially account for differences in reported findings on BDNF protein.

An interesting neurochemical finding of this third study was the widespread and sustained elevations of extra-hypothalamic CRF after ECS in both strains, including at the hippocampus, frontal cortex, and neocortex. While interpretation of this finding is difficult without concomitant assessment of peptide synthesis and/or utilization, there are several reports showing increased CRF gene expression within the hypothalamus (Brady et al., 1994; Garcia-Garcia et al., 1998; Herman et al., 1989) as well as at one extrahypothalamic site (dentate hilus) (Smith, Makino, Kim, & Kvetnansky, 1995) following repeated exposure to ECS. Based on these findings, it is likely that the observed ECS-induced increases in CRF levels are indicative of increased peptide synthesis/utilization.

In looking across the three studies, the findings do not suggest a temporal association between the BDNF and behavioral changes at baseline and at 7 days after ECS, offering no support to the hypothesis that BDNF is related to depression or memory-related behaviours. At baseline, there were no differences in brain BDNF between the two strains, despite markedly

higher depression-like behaviour in the WKY strain. At 1 day after ECS, both strains exhibited sharp increases in brain BDNF and decreases in depression-like behaviour. However, at 7 days after ECS, the neurochemical and behavioural dissociations returned; brain BDNF levels stayed elevated in depression-relevant brain regions in Wistar, but not WKY rats, while depression-like behaviour remained significantly attenuated in the WKY but not the Wistar rats. Interestingly, retrograde memory deficits were present in both strains at both post-ECS time points, despite the different temporal profiles of BDNF protein expression post-ECS. Although an association between BDNF levels and the antidepressant effects of ECT/ECS has long been hypothesized, several studies have struggled to correlate BDNF protein (at baseline or after treatment) (Bocchio-Chiavetto et al., 2008; Li et al., 2007; Shimizu, Hashimoto, & Iyo, 2004) with measures of depression or depression-like symptoms (Gomez, Lahmame, de Kloet, & Armario, 1996; Lahmame et al., 1997). For example, as previously mentioned, the hallmark elevations in brain BDNF protein after ECS were not evident in the FSL, despite increased brain NGF (Angelucci et al., 2003) and changes in depressive-like behaviour in the FST (Jimenez-Vasquez et al., 2007).

A similar lack of temporal association between extra-hypothalamic CRF and depression-like behaviours was observed. However, the findings of the current study do suggest a temporal association between CRF and memory-related behaviours, as elevated brain CRF and retrograde memory deficits were present in both strains at both post-ECS time points. Recent studies suggested that high levels of hippocampal CRF after stress and activation of the CRF_1 receptor interfere with memory (Chen et al., 2010; Heinrichs et al., 1996; Ivy et al., 2010). These functional and cellular effects are prevented by CRF_1 antagonists given before or after the stressor exposure (Brunson et al., 2002; Gallagher et al., 2008).

Elevated CRF levels may also be contributing to the anxiogenic response and driving the increased freezing behaviour observed in the OF and CER paradigm in the WKY strain (Rotzinger et al., 2010). In this regard, the WKY strain shows abnormalities in CRF mRNA (Shepard & Myers, 2008) and binding (Lahmame et al., 1997), and HPA axis hyper-responsiveness (Hauger et al., 2002; Solberg, Olson, Turek, & Redei, 2001). Perhaps an abnormal responsiveness to elevations in CRF in WKY relative to Wistar rats might explain the increased mobility disturbance seen in the OF and CER in this study. The lack of changes in plasma corticosterone noted in the present investigation are surprising, considering that the HPA axis has been shown to be hyperresponsive in WKY rats (De La Garza et al., 2004; Rittenhouse et al., 2002) although Thiagarajan et al. (1989) demonstrated that repeated ECS treatments can cause a normalization of HPA axis function.

Limitations

Although the analysis of temporal associations can be suggestive of relationships between physiological and behavioural variables, more specific analysis of the function of BDNF and CRF in the brain is necessary. This could include assessment of the utilization and synthesis of BDNF and CRF, differential analysis of pro- and mBDNF, and introduction of trkB and CRF_1 receptor antagonists in conjunction with repeated ECS and behavioural measurement. In addition, the WKY strain's hyperresponsiveness to stress (Braw et al., 2006), although not necessary to see its depression-like behavioural and physiological abnormalities (Pare et al., 2001; Tejani-Butt et al., 1994), is an important factor in this strain's value as a potential translational model of depression. A stressor was not introduced in this study, and therefore the effect of stress on BDNF and extra-hypothalamic CRF expression could not be examined.

Conclusion

The model of 5 daily ECS proved effective at eliciting behavioral (depression-like and memory effects) and neurochemical (BDNF and CRF) changes in both strains, verifying its effectiveness in a genetic model of depression and possible treatment resistance, the WKY strain. The WKY strain may prove useful in uncovering the biological underpinnings of the behavioral outcomes of ECS/ECT. Future investigations into the neurobiological underpinnings of the behavioral effects of ECS/ECT may target CRF as a potential important contributor to the memory impairments after repeated ECS/ECT.

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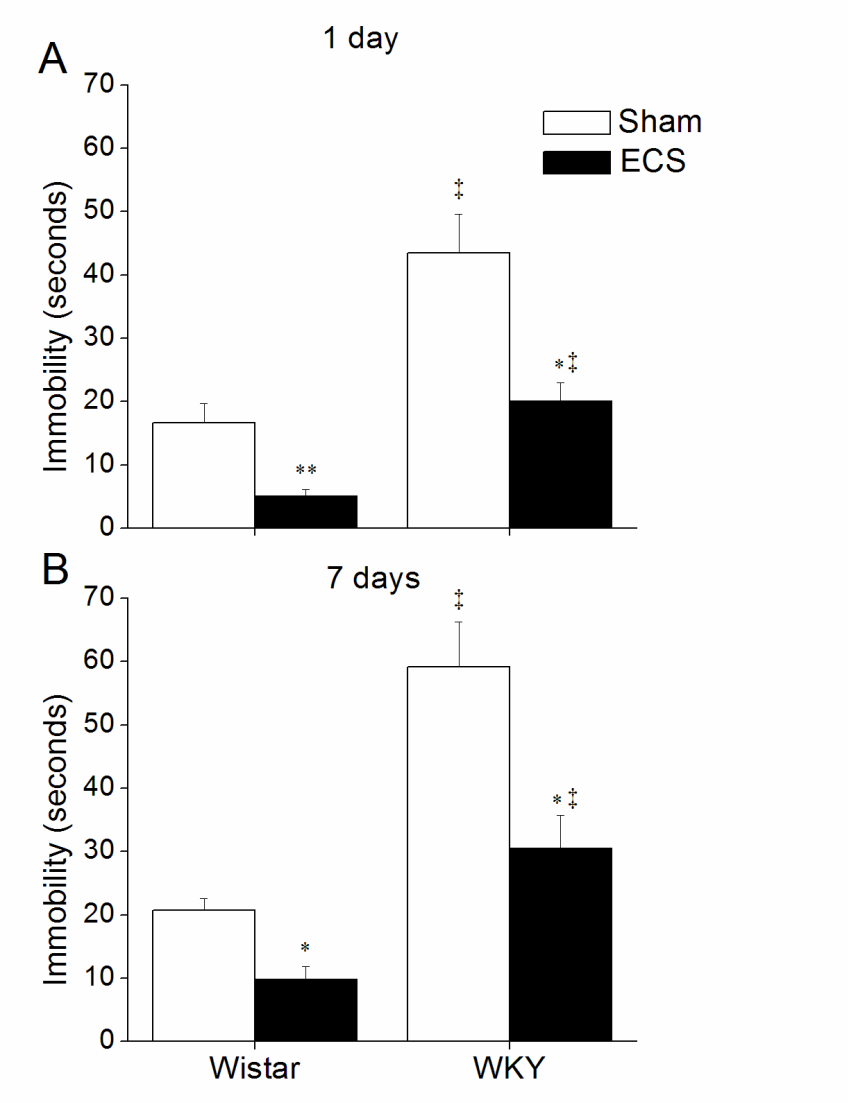

Figure 1. Time spent immobile in the FST at 1 day (A) and 7 days (B) after ECS. At 1 and 7 days post-ECS, a significant reduction in immobility time in both Wistar and WKY strains was observed relative to shams. Strains differed at baseline (sham groups) and after treatment (ECS groups) at both time points. * $p < 0.01$, ** $p < 0.001$ relative to sham; † $p < 0.01$, ‡ $p < 0.001$ relative to Wistar.

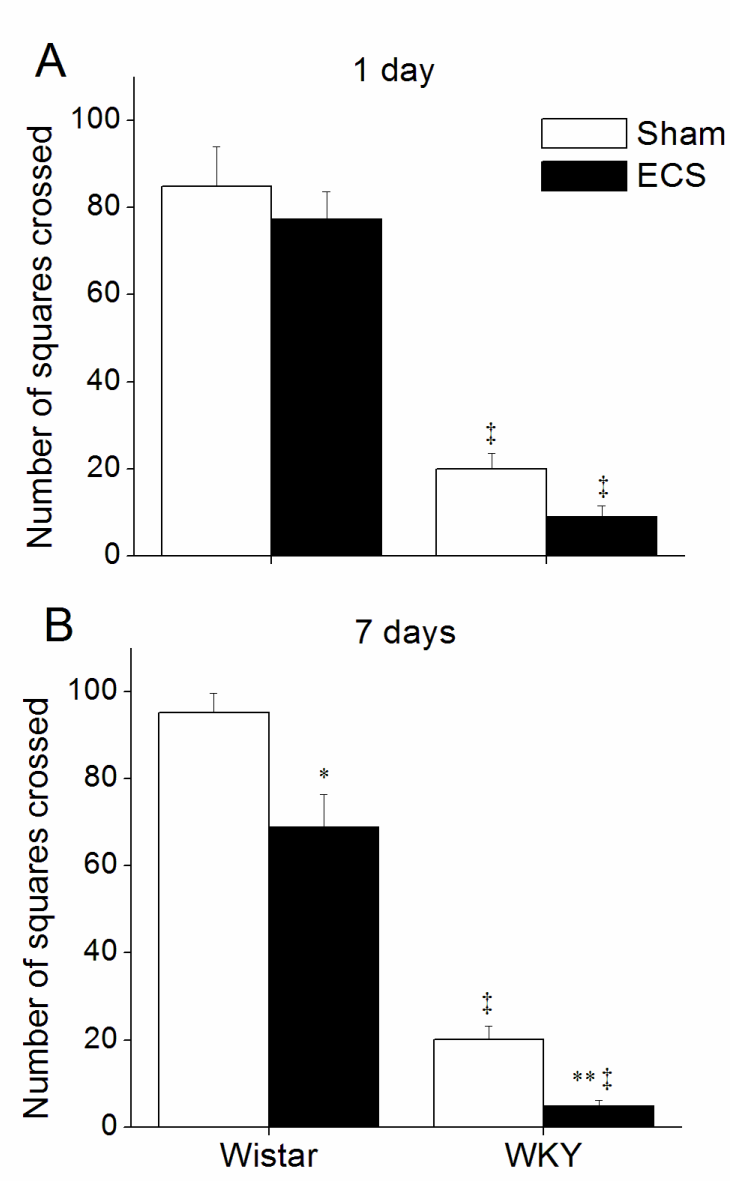

Figure 2. Total number of squares crossed in the OF at 1 day (A) and 7 days (B) after ECS. The WKY strain showed significantly less mobility in the OF than the Wistar strain at both time points. ECS did not significantly affect mobility at 1 day in either strain. However, at 7 days ECS-treated animals were significantly less mobile than shams in both strains. * $p < 0.01$, ** $p < 0.001$ relative to sham; † $p < 0.01$, ‡ $p < 0.001$ relative to Wistar.

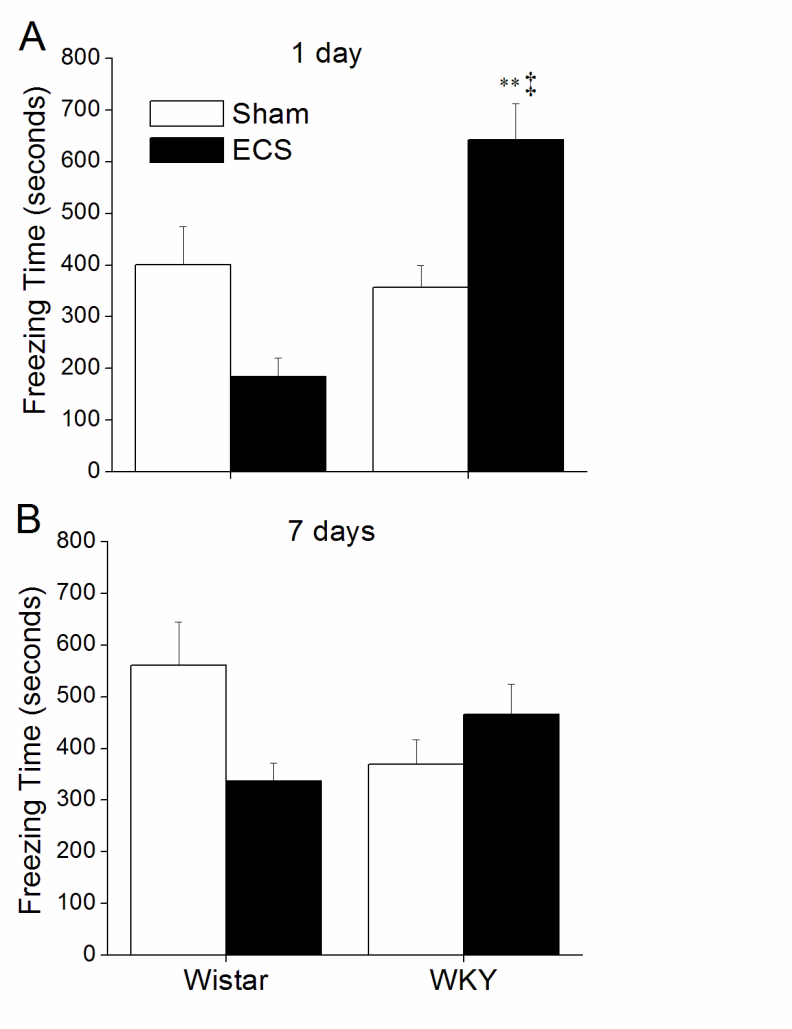

Figure 3. Total freezing time in CER (20 minute testing session) at 1 day (A) and 7 days (B) after ECS. At 1 day, ECS significantly increased freezing time in the WKY strain. The trend to reduced freezing time in ECS-treated Wistar rats was not significant at 1 or 7 days. Mobility differences between the strains (see figure 2) appeared to affect performance on the CER. At 1 day, ECS-treated WKY rats froze significantly more than ECS-treated Wistar rats; however, this effect did not remain significant at 7 days. * $p < 0.01$, ** $p < 0.001$ relative to sham; † $p < 0.01$, ‡ $p < 0.001$ relative to Wistar.

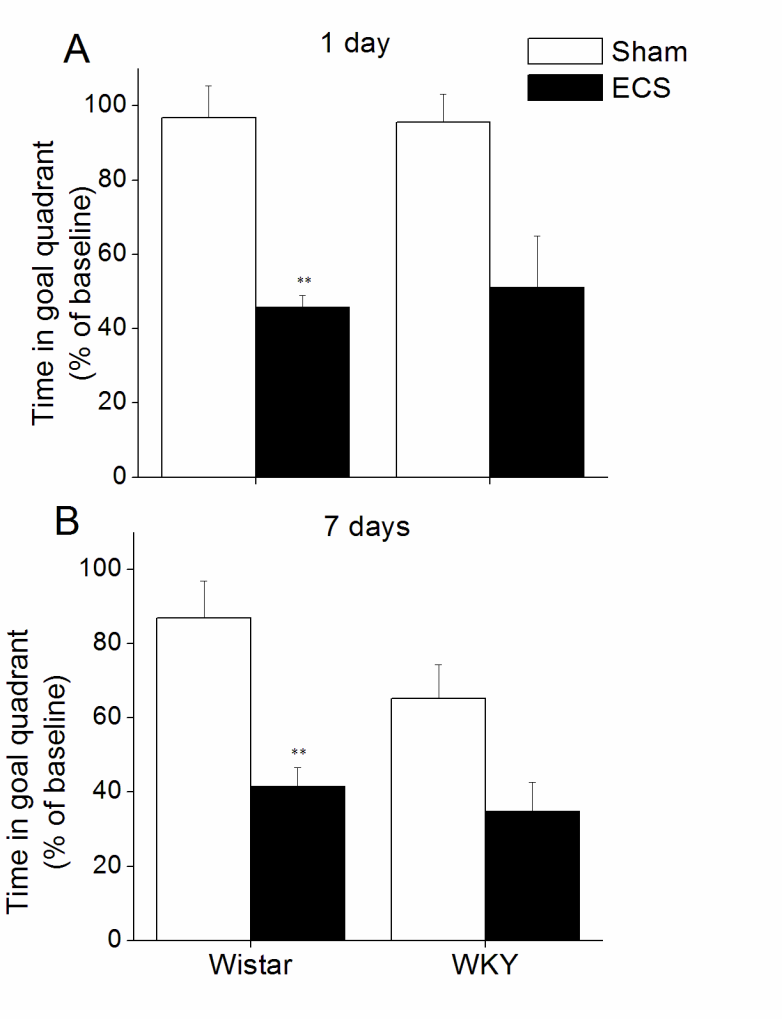

Figure 4. Time spent in the NE goal quadrant in MWM at 1 day (A) and 7 days (B) after ECS (expressed as % of baseline pre-ECS performance). At 1 and 7 days, ECS significantly reduced time spent in the goal quadrant in the Wistar strain, indicating a persistent deficit in retrograde spatial memory for this task. The same effect was observed in the WKY strain, although it did not reach significance on post-hoc analysis with the bonferonni correction (1d: $p = 0.012$; 7d: $p = 0.022$). The observed effect of reduced time spent in the goal quadrant in sham-treated WKY between 1 and 7 days did not reach significance ($p = 0.062$). * $p < 0.01$, ** $p < 0.001$ relative to sham; † $p < 0.01$, ‡ $p < 0.001$ relative to Wistar.

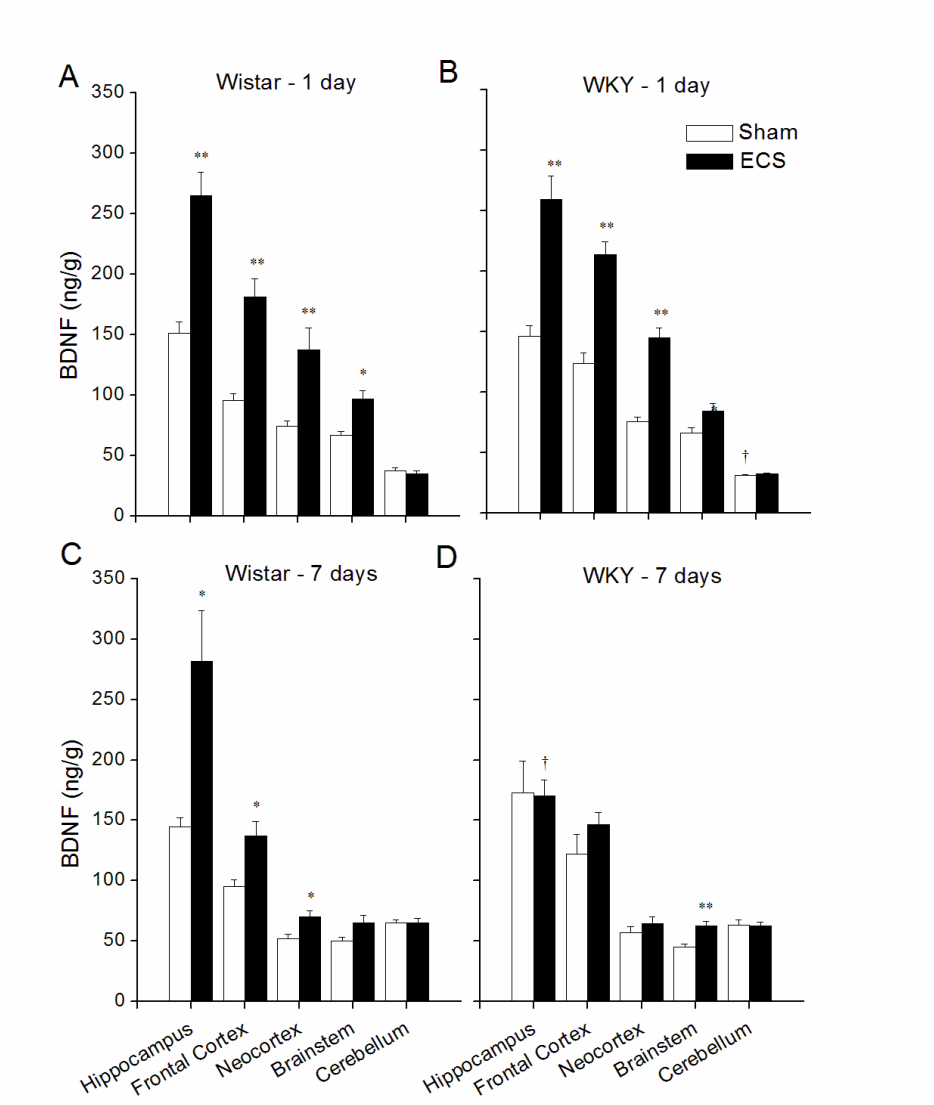

Figure 5. BDNF protein levels in 5 brain regions at 1 day (A, B) and 7 days (C, D) after ECS. At 1 day, BDNF elevations were significant in multiple brain regions in the (A) Wistar and (B) WKY strains (e.g., hippocampus, frontal cortex and neocortex), but not in the cerebellum in either strain. At 7 days, results were significant in these same regions in the (C) Wistar strain, but not in the (D) WKY strain, in which only brainstem levels reached significance. * $p < 0.01$, ** $p < 0.001$ relative to sham; † $p < 0.01$, ‡ $p < 0.001$ relative to Wistar.

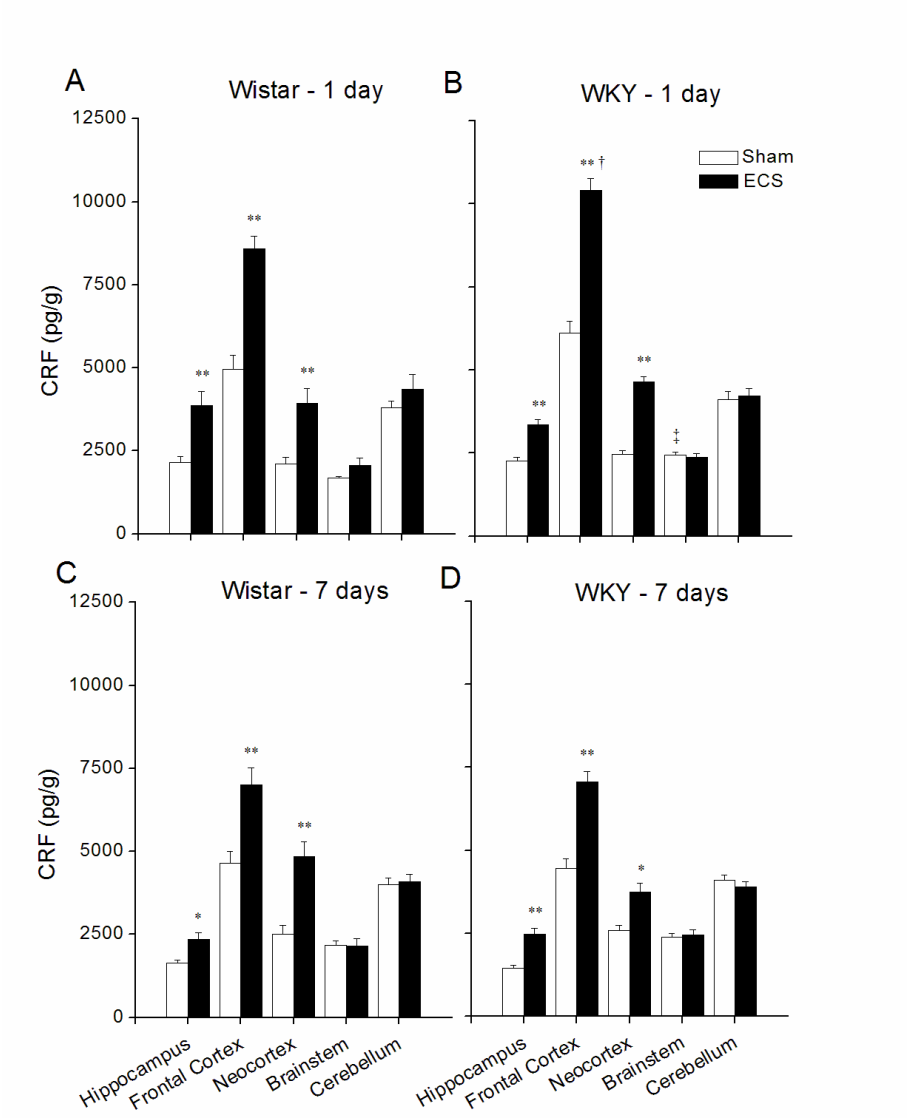

Figure 6. CRF levels in 5 brain regions at 1 day (A, B) and 7 days (C, D) after ECS. At 1 day, CRF elevations were significant in (A) Wistar and (B) WKY strains in several areas similar to that seen in BDNF (Figure 5) including the hippocampus, frontal cortex, and neocortex but not in the brainstem or cerebellum in either strain. At 7 days, results were significant in the same regions in (C) Wistar and (D) WKY strains. * $p < 0.01$, ** $p < 0.001$ relative to sham; † $p < 0.01$, ‡ $p < 0.001$ relative to Wistar.

Table 1. BDNF (expressed as mean % of sham ± SEM).

	1 day		7 days	
Brain Region	Wistar	WKY	Wistar	WKY
Hippocampus	175 ± 13**	177 ± 13**	176 ± 26*	116 ± 9
Frontal Cortex	190 ± 16**	173 ± 9**	144 ± 12*	120 ± 8
Neocortex	186 ± 24**	191 ± 11**	136 ± 9*	113 ± 10
Striatum	117 ± 7	140 ± 8*	139 ± 18	105 ± 6
Thalamus	167 ± 22	171 ± 17**	119 ± 5	103 ± 13
Hypothalamus	138 ± 11*	150 ± 11**	138 ± 18*	127 ± 9
Brainstem	146 ± 10*	127 ± 9	131 ± 12	138 ± 8**
Cerebellum	93 ± 5	104 ± 3	100 ± 6	99 ± 5

* $p \leq 0.01$ ** $p \leq 0.001$, relative to sham.

Table 2. CRF (expressed as mean % of sham ± SEM).

	1 day		7 days	
Brain Region	Wistar	WKY	Wistar	WKY
Hippocampus	180 ± 20**	148 ± 7**	144 ± 12*	170 ± 12**
Frontal Cortex	173 ± 7**	170 ± 6**	150 ± 11**	158 ± 7**
Neocortex	186 ± 21**	188 ± 6**	194 ± 17**	144 ± 10*
Striatum	135 ± 14	111 ± 6	133 ± 12	107 ± 9
Thalamus	116 ± 15	119 ± 13	112 ± 20	118 ± 10
Hypothalamus	102 ± 19	86 ± 10	68 ± 13	108 ± 15
Brainstem	123 ± 14	97 ± 5	99 ± 10	103 ± 6
Cerebellum	114 ± 12	103 ± 5	102 ± 5	95 ± 4

* $p \leq 0.01$ ** $p \leq 0.001$, relative to sham.

Preface to Chapter 4

What is the value of serum relative to brain BDNF protein measurement?

The WKY strain does appear to represent a useful model for the investigation of ECS. Features of the WKY strain also suggest high translatability to human research in ECT, including resistance to pharmacological treatment (Lahmame et al., 1997) and altered physiological response to ECT/ECS. The findings from the study presented in chapter 3 raised numerous questions. In our line of research, we were particularly interested in the findings of the dissociations seen between alterations in behaviour and alterations in physiological measures. This again led us to question the functional role of changes in brain BDNF protein after ECT. The research in people receiving ECT for the treatment of depression suggests that alterations in serum BDNF protein are more difficult to detect following ECT compared with antidepressant drug treatment (e.g., Bocchio-Chiavetto et al., 2010; Bocchio-Chiavetto et al., 2006). If the peripheral BDNF protein is thought to be reflective of central BDNF protein, we would expect the reverse to be true, as in animals, increases in brain BDNF protein are larger and more widespread after ECT compared with antidepressant drug administration (Altar et al., 2003). Therefore, in addition to the question regarding the functional role of antidepressant-induced changes in brain BDNF protein, we questioned the functional role of serum BDNF protein and the ability of these measures to reflect what is happening in the brain. This was particularly relevant as we were planning a pilot study to translate the findings in the animal studies to people receiving ECT for the treatment of depression, in which serum BDNF protein was one of our primary outcome factors. Therefore in the following study, we assessed serum and brain BDNF protein in the Wistar and WKY strains using a similar experimental design as employed in chapter 3 (at 1 and 7 days after 5 sham- or ECS-treatments), which was also the design mimicked

in the human pilot study. Strain and treatment differences in serum BDNF protein as well as brain-serum correlations were investigated.

Several individuals were involved in the design and implementation of this work and/or the preparation of this manuscript. Those with significant contributions in one or more of these areas are listed on the title page. The author of this thesis is the first author on this manuscript, and was involved in every step of the design and implementation of this work and the preparation of this manuscript.

Chapter 4: No correlation between brain tissue and serum brain derived neurotrophic factor protein in the Wistar and Wistar-Kyoto rat strains receiving repeated electroconvulsive stimulus.

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Abstract

Background: Serum BDNF protein has been related to depression and less consistently to its treatments in human studies. However, animal studies have failed to demonstrate a clear link between BDNF protein in serum and brain tissue.

Methods: Serum and brain tissue levels of BDNF protein are measured with ELISA in the WKYand Wistar strains at one and seven days after 5 daily electroconvulsive stimulus or sham treatments.

Results: The WKY strain showed lower baseline serum BDNF protein relative to Wistar controls. After 5 electroconvulsive stimuli, BDNF protein density was significantly increased in hippocampus and cortical regions, but not cerebellum or serum BDNF. A clear correlation between brain and serum BDNF was not observed in either strain or treatment group.

Discussion: Despite lower baseline serum BDNF protein in the WKY strain, a lack of change in serum BDNF after electroconvulsive stimulus and a lack of correlation between brain and serum BDNF protein calls into question the relevance of serum BDNF as a measure of depression and treatment response.

Introduction

BDNF has been associated with depression symptoms in humans and depression-like behaviours in animals, as well as a variety of antidepressant treatments. Rodents exposed to prolonged stress (an animal model of depression; Willner, 1990) show lower levels of BDNF protein and mRNA in regions of the brain implicated in the pathophysiology of depression in humans (e.g., hippocampus and prefrontal cortex; Smith et al., 1995b; Warner-Schmidt et al., 2006), and these levels increase with repeated antidepressant treatments, including ECS (an animal model of ECT) and, to a lesser extent, antidepressant drugs (Altar et al., 2003; Nibuya et al., 1995).

Although there is no method for the measurement of BDNF in brain tissue *in vivo*, people with depression show lower levels of BDNF protein in serum relative to healthy controls (Aydemir, Deveci, & Taneli, 2005; Aydemir et al., 2007; Bocchio-Chiavetto et al., 2010; Cattaneo et al., 2010; Deveci et al., 2007; Gervasoni et al., 2005; Karege et al., 2002a; Karege et al., 2005; Gonul et al., 2005; Gorgulu et al., 2009; Matrisciano et al., 2009; Piccinni et al., 2008; Umene-Nakano et al., 2009), although not all studies have found this (Basterzi et al., 2009). As with brain BDNF protein in animals, serum BDNF protein in humans has been correlated with measures of depression severity in most studies (Bus et al., 2011; Gervasoni et al., 2005; Gorgulu et al., 2009; Hu et al., 2010; Karege et al., 2002a; Yoshimura et al., 2011) but not all (Bocchio-Chiavetto et al., 2006; Lang et al., 2006), and has been shown to increase after treatment with antidepressant drugs in many studies (Bocchio-Chiavetto et al., 2006; Cattaneo et al., 2010; Gervasoni et al., 2005; Matrisciano et al., 2009; Molendijk et al., 2011; Shimizu et al., 2003) but not all (Basterzi et al., 2009; Monteleone et al., 2008; Piccinni et al., 2008) and in

some studies after ECT (Bocchio-Chiavetto et al., 2006; Fernandes et al., 2009; Gronli et al., 2009; Hu et al., 2010; Okamoto et al., 2008).

Although some studies have found a relationship between post-treatment increases in serum BDNF protein and changes in the severity of depression (i.e., the effectiveness of treatments for depression; Okamoto et al., 2008; Tadic et al., 2011), many have failed to find this correlation (Basterzi et al., 2009; Bocchio-Chiavetto et al., 2006; Huang et al., 2001), calling into question the meaning of depression-related decreases and treatment-related increases in serum BDNF. Further to the question of the function of serum BDNF in depression, is whether BDNF protein in the serum is actually reflective of BDNF protein in brain tissue.

In order to expand this line of research, we assessed serum and brain tissue BDNF protein in two rat strains, the WKY strain, a genetic model of depression (Malkesman et al., 2005) and its natural outbred control, the Wistar strain at two time points: 1 day and 1 week after repeated ECS or sham treatments.

Method

Subjects

Male Wistar (250 – 350g) and WKY (150 – 250g) rats (Charles River, Quebec, Canada) between 7 to 8 weeks of age were housed in pairs by strain (with one ECS-treated and one sham-treated per cage) and kept on a 12 hour dark/12 hour light cycle with lights on at 07:00. Animals had free access to food and water. All experiments were conducted in accordance with the Canadian Council of Animal Care guidelines and were approved by the animal care committee of the University of Ottawa Institute of Mental Health Research. Wistar and WKY rats were randomly assigned to groups (n = 10) based on treatment (ECS or sham) and time of euthanasia (1 day or 7 days post-treatment) (N = 80).

Electroconvulsive Stimulus

The ECS was administered once daily for 5 days via ear clip electrodes: (55-65 mA, 0.8 sec, 60 Hz square wave pulse). Typical seizures lasted between 45 to 60 sec, with tonic and clonic activity identified by full extension of the hindlimbs and repeated flexion-extension of the forelimbs, respectively. The sham-treated groups received the same treatment, except the stimulus was not applied.

Tissue preparation

Animals were euthanized by decapitation at 1 day or 7 days after treatment. Trunk blood was immediately collected into tubes (70 μM), and stored at room temperature for 1 hour, then at 4°C for 1 hour to allow blood to clot. Serum was separated by centrifugation (4°C, 3000 rpm, 15 min), isolated and frozen at -80 °C until analysis.

Brains were immediately removed and 4 brain regions were dissected on ice: hippocampus, frontal cortex, neocortex, and cerebellum, which were immediately frozen on dry ice and stored at -80 °C. Prior to analysis, brain tissue was thawed on ice, weighed and homogenized in PIPES buffer, pH 7.3, containing 500 mM NaCl, 2% BSA, 0.2% Triton X-100, 0.1% NaN3, 2 mM EDTA, 200 μM PMSF, 0.4% protease inhibitor cocktail (Sigma-Aldrich, Canada) (Pollock et al., 2001).

BDNF ELISA

BDNF protein was analyzed using enzyme-linked immunosorbent assay (ELISA) according to manufacturer instructions (R&D Systems, Minneapolis, USA). Homogenized tissue was acidified to a pH of 3 for 15 min using 8M HCl, neutralized to a pH of 7 using 6M NaOH, then centrifuged at 12000 rpm for 5 minutes from which the supernatant was collected for

analysis. ELISA was performed according to manufacturer's specifications, with PIPES buffer substituted for all dilutions of samples and standards.

Statistical Analysis

Cases that demonstrated outliers (≥ 2 standard deviations from the mean) in any of the dependent variables were removed prior to analysis, resulting in n = 7-9 for the groups. Square root transformations were applied to the dependent variables: hippocampus, frontal cortex, and cerebellum to correct for mild skew and kurtosis and achieve homogeneity of variances and normal distributions. 2 (Treatment: ECS, Sham) x 2 (Strain: Wistar, WKY) MANOVA was used to assess effects and interactions of treatment and strain at 1 and 7 days post-ECS, with the 4 brain regions and serum BDNF protein levels entered as the dependent variables. Spearman correlations were computed for BDNF protein in the brain tissue (divided by region and a composite average) and serum in each group. Alpha was set at $p < 0.05$ for all analyses.

Results

Brain tissue

At 1 day following treatment (figure 1 A-B), ECS induced an increase in BDNF protein levels in both strains in the hippocampus ($F(1, 33) = 29.51$, $p < 0.001$), frontal cortex ($F(1, 33) = 73.11$, $p < 0.001$), and neocortex ($F(1, 33) = 25.85$, $p < 0.001$), but not in the cerebellum ($F(1, 33) = 0.35$, $p = 0.556$). Wistar rats showed higher levels of BDNF protein relative to WKY in cerebellum ($F(1, 33) = 9.53$, $p = 0.004$).

At 7 days following treatment (figure 1 C-D), the ECS-induced increases in BDNF protein levels remained significant in the frontal cortex ($F(1, 33) = 5.93$, $p = 0.021$), and neocortex ($F(1, 33) = 4.36$, $p = 0.046$), but not in the hippocampus ($F(1, 33) = 2.56$, $p = 0.121$), which was due to a reduction in BDNF protein levels in the ECS-treated WKY group as well as high variability in

both strains at this time point. BDNF protein in the cerebellum also remained unchanged by ECS treatment and BDNF protein levels did not differ between strains in brain tissue at this time point.

Serum

Serum BDNF protein was not affected by treatment at either 1 or 7 days post-ECS (1 day: $F(1, 33) = 0.94$, $p = 0.341$; 7 days: $F(1,32) = 0.51$, $p = 0.483$). However, a significant difference between strains was observed at both time points, where Wistar rats showed higher serum BDNF protein levels relative to WKY rats (1 day: ($F(1, 33) = 19.68$, $p < 0.001$; 7 days: $F(1, 33) = 4.58$, $p = 0.041$) (figure 1).

Correlations

Serum BDNF protein levels were not correlated with any individual brain tissue levels or a composite score of all tissue levels of BDNF protein regardless of strain, treatment condition, or time point (table 1). In an attempt to replicate the findings from Sartorius and colleagues, a composite score for hippocampus and frontal cortex tissue BDNF protein levels was calculated by averaging across these regions (Sartorius et al., 2009). Interestingly, a significant correlation between serum BDNF protein and this hippocampus-frontal cortex composite score was revealed in the ECS-treated Wistar rats at 1 day post-ECS ($r = 0.823$, $p = 0.023$). This correlation was not significant in any of the other groups. Although no serum correlations were significant, it is interesting to note that correlations between brain tissue and serum BDNF protein levels trended in the positive direction in Wistar rats, but in the negative direction in WKY rats (see table 1).

Discussion

Levels of BDNF in the blood (particularly in serum) have been proposed as a potential physiological correlate of symptoms of depression and treatment response. However, although

the findings in human studies suggest a lower level of serum BDNF in depression (Bocchio-Chiavetto et al., 2006), they have not consistently linked changes in serum BDNF protein to antidepressant treatment response (Basterzi et al., 2009; Bocchio-Chiavetto et al., 2006; Huang et al., 2001; but see: Okamoto et al., 2008; Tadic et al., 2011). Results from the current study showed that basal levels of serum BDNF were significantly lower in the WKY strain, a genetic rodent model of depression relative to its healthy outbred control, the Wistar strain, supporting the notion of decreased baseline serum levels of BDNF in depression.

Despite strain differences in serum BDNF protein, no strain differences were seen in BDNF protein in four brain regions: hippocampus, frontal cortex, neocortex, and cerebellum. In addition, despite a large treatment effect of 5 repeated ECS, which increased BDNF protein levels in depression-relevant brain tissues (hippocampus and cortical regions) in both strains, there was no effect of treatment on serum BDNF in either strain. It has been suggested by human and rodent research that changes in serum BDNF protein after a course of ECT/ECS may not occur immediately (as seen after antidepressant treatment in humans), and may take up to one month post-ECT in humans (Bocchio-Chiavetto et al., 2006; Fernandes et al., 2009; Gronli et al., 2009; Hu et al., 2010; Okamoto et al., 2008) and one week post-ECS in rodents (Sartorius et al., 2009) to be detectable. However, in the current study no change was detected in serum BDNF protein at one day or one week post-ECS.

Overall, a correlation between brain tissue and serum BDNF protein was not supported. Of the multiple correlations tested in this study, only one significant correlation was found between serum BDNF protein and an average of BDNF protein levels in the frontal cortex and hippocampus in the Wistar strain at one day post-ECS. Indeed, this lack of clear correlation is supported by two other studies that have found only single and subgroup-specific small to

moderate correlations between serum and brain tissue BDNF in adult rats, despite testing correlations between multiple groups and outcome factors (Elfving et al., 2010; Sartorius et al., 2009). The average of frontal cortex and hippocampus BDNF protein levels was an attempt to replicate the findings of Sartorius and colleagues (2009), who demonstrated a small (roughly 10%) but significant increase in serum BDNF protein at 7 days, but not at five other time points ranging from 3 hours to 14 days after five ECS in Sprague-Dawley rats (Sartorius et al., 2009). Contrary to the findings of the current study, a small but significant correlation (Spearman's rho = 0.40) was shown between brain tissue (an average of hippocampus and frontal cortex levels) and serum BDNF in sham-treated animals, but not after ECS (Sartorius et al., 2009).

The findings of the current study are also not consistent with findings in the Flinders Sensitive Line, another genetic rat model of depression (Elfving et al., 2010). Contrary to the findings in human studies of serum BDNF protein, Elfving and colleagues (2010) found higher baseline BDNF protein in serum and whole blood, lower BDNF protein in the hippocampus and no difference in the frontal cortex and cerebral spinal fluid in untreated FSL relative to its control, the Flinder's Resistant Line (FRL). Only when the FSL and FRL rats were combined to increase power of the sample, a moderate negative correlation ($r = -0.65$) between hippocampus and serum BDNF was shown, but no correlation was found between frontal cortex and serum BDNF or in these groups individually (Elfving et al., 2010).

It is possible that ECT/ECS simply does not produce the same effects on peripheral BDNF protein as antidepressant drugs, and this could be due to the mode of administration, with antidepressant drugs being administered systemically and ECT/ECS being administered locally. However, ECT/ECS is known and in this study again demonstrated to produce large changes in BDNF protein in depression-relevant brain regions, an effect that is larger and more robust than

that induced by antidepressant drugs (Altar et al., 2003; Nibuya et al., 1995), and thus we would expect to see some change in serum BDNF protein if it is in fact reflective of brain BDNF protein. Not seeing this effect suggests that clearer changes in serum BDNF protein after antidepressant drug administration may reflect a change in peripheral BDNF that is not reflective of central BDNF. Indeed, little is known about the source and functional role of peripheral BDNF. Although there is evidence that BDNF does cross the blood brain barrier in adult mice (Pan, Banks, Fasold, Bluth, & Kastin, 1998), Karege and colleagues showed that the correlation between brain and serum BDNF protein in the Wistar rat was clearer during early stages of development (newborn to juvenile rats) and stabilized in adulthood, which the authors suggest could represent a decline in the interaction between peripheral and central BDNF in adulthood (Karege, Schwald, & Cisse, 2002b). BDNF may play different roles and circulate differently between peripheral and central systems at different stages of development.

The WKY strain would seem an ideal translational model in which to investigate BDNF in depression and ECS. The WKY has shown several behavioural (Braw et al., 2006; De La Garza et al., 2004; Malkesman et al., 2005) and neurochemical features of depression, mostly involving monoamines (De La Garza et al., 2004; Lahmame et al., 1997; Redei et al., 2001) and glucocorticoids (De La Garza et al., 2004), as well as the "depression-like" phenotype of reduced mobility in the forced swim test (see Chapter 3; Krahl et al., 2004). We have also shown that low mobility in the forced swim test is responsive to five daily repeated ECS, returning to levels of the untreated Wistar strain (see Chapter 3), and that WKY show the expected treatment-related increases in brain BDNF protein. In addition, the conditions of the study and the characteristics of the WKY strain are similar to the factors that carry an impact on the relationship between serum BDNF and depression and its treatments in humans, such as lower baseline serum BDNF

levels (Bocchio-Chiavetto et al., 2006; Gervasoni et al., 2005; Shimizu et al., 2003) and a lack of baseline antidepressant treatment exposure (Diniz et al., 2010; Molendijk et al., 2011; Ozan et al., 2010; Shimizu et al., 2003).

In this study, one could argue that the findings in human studies of serum BDNF in depression and ECT are in fact mirrored: a clear demonstration of lower serum BDNF protein in untreated depression and a lack of clear correlation between serum BDNF protein and the antidepressant effectiveness of ECT/ECS. Although we did not perform behavioural testing in this study, we can see the same trend in the WKY as in human studies. Lower serum BDNF levels may appear to correspond with the known depressive-like behaviour (immobility in the forced swim test) in untreated WKY, but this correlation would become invalidated after ECS, when serum BDNF does not change despite decreased immobility in the forced swim test (see Chapter 3; Krahl et al., 2004).

In conclusion, the findings of this study continue to call into question the correlation and relationship between serum and brain BDNF protein. The value of serum BDNF protein as a reflection of depressive symptomatology, severity, and in particular treatment response continues to be unconfirmed.

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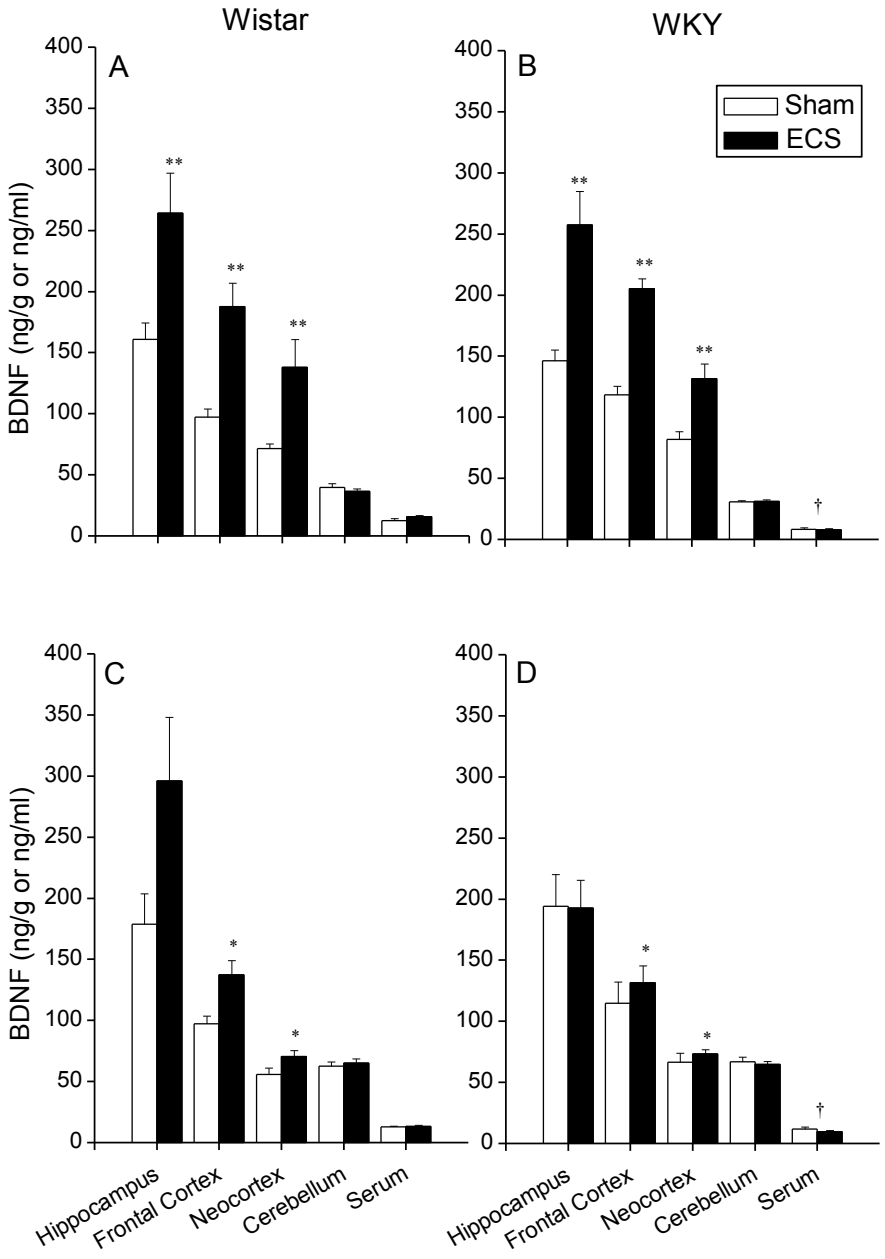

Figure 1. BDNF protein levels in brain tissue (ng/mg) and serum (ng/ml) in ECS- and sham-treated animals at 1 day (A and B) and 7 days (C and D) post-ECS. ECS vs. Sham: * $p < 0.05$, ** $p < 0.005$. Wistar vs. WKY: † $p < 0.05$, ‡ $p < 0.005$.

Table 1. Spearman's correlation coefficients between serum and brain tissue BDNF protein.

	Hippocampus	Frontal Cortex	Neocortex	Cerebellum	Hippocampus + Frontal Cortex
1 day					
Sham					
Wistar	0.03	0.13	0.08	-0.65	0.08
WKY	-0.32	-0.31	0.20	0.15	-0.63
ECS					
Wistar	0.07	0.71	0.43	0.50	0.82*
WKY	-0.45	0.00	-0.35	0.25	-0.52
7 days					
Sham					
Wistar	-0.58	-0.05	0.25	0.10	-0.45
WKY	-0.66	-0.71	-0.14	-0.26	-0.77
ECS					
Wistar	0.23	0.20	0.45	0.03	0.23
WKY	-0.33	0.33	-0.29	-0.10	-0.19

* $p < 0.05$.

Chapter 5: General Discussion

The three animal studies performed present novel findings on the physiological mechanism of action of ECS and the physiological and behavioural effects of ECS in an animal model of depression relative to healthy controls. In combination with a pilot study in people who underwent ECT for the treatment of depression, they also shed light on the challenges facing the translation of research findings from animal to human studies.

In the first study, the cAMP second messenger system (measured indirectly via (*R*)-[^{11}C]rolipram binding to PDE4 and shown to be increased after antidepressant drug administration [Lourenco et al., 2006]) was demonstrated to not likely be the mechanism underlying the action of repeated ECS to elevate BDNF protein levels in the brain. The chief explanations for the negative findings in this study are a time window that missed the effects of cAMP (which could have occurred in a much shorter time span after ECS compared with antidepressant drugs; Jeon et al., 1997; Lust et al., 1976b; Lust et al., 1976a), or that a different second messenger pathway is activated after ECS compared with antidepressant drugs, which is supported by the finding that activity-induced increases in BDNF protein in the hippocampus are not significantly enhanced by the addition of cAMP stimulation (Fukuchi et al., 2005).

The physiological measures were evaluated at 1 to 4 hours (1 to 3 hours not presented), as it has been shown that (*R*)-[^{11}C]rolipram is maximally increased at 4 hours after a single antidepressant drug administration (Lourenco et al., 2006). However, it is possible that after a single ECS the activation of cAMP is rapid (within minutes; Lust et al., 1976a; Lust et al., 1976b) and that chronic antidepressant treatment (whether ECS or drug) could produce different effects on cAMP activation. Chronic administration of antidepressant drugs leads to desensitization of the monoamine receptors (Blier et al., 1994) which likely occurs due to

modification of intracellular mechanisms related to the cAMP and the Ca2+/Calmodulin second messenger pathways (Popoli et al., 2001). Alterations to the second messenger pathways with repeated treatments could create a similar profile in (*R*)-[^{11}C]rolipram binding to that seen in the current study after repeated ECS. However, unlike the studies with antidepressant drugs, a single ECS treatment caused no change in (*R*)-[^{11}C]rolipram binding, despite increases in BDNF protein in the hippocampus, at least at 4 hours post-ECS. In addition, other groups have repeatedly shown that a single antidepressant drug administration does not lead to elevations in brain BDNF protein (Nibuya et al., 1995), despite the known substantial increases in (*R*)-[^{11}C]rolipram binding after a single antidepressant drug treatment. These lines of evidence call into question the importance of cAMP in the elevation of BDNF protein after any antidepressant treatment and suggest the involvement of other possible pathways to the phosphorylation of CREB. These could include other serine/threonine-specific kinases, including MAPK/ERK and protein kinase B, the activity-dependent Ca2+/Calmodulin second messenger pathway, and others (Mayr et al., 2001; Tao et al., 1998; Shieh et al., 1998; Shieh & Ghosh, 1999), many of which have been implicated in cell survival and proliferation (Popoli et al., 2001). Improved understanding of the specificity in regulation of CREB phosphorylation and transcription of target genes, such as BDNF, may lead to a better understanding of the molecular basis for the antidepressant and other (e.g., cognitive) effects of antidepressant treatments.

The review of the negative findings of the first study led to questions about the functional role of increases in BDNF in the antidepressant and cognitive effects of ECS. We saw that examining these questions in the context of the abnormal physiological and behavioural environment of depression, as in animal models of depression, could offer unique insights into the role of BDNF in depression and the behavioural (treatment response and cognitive) effects of

ECS. An animal model of depression that was theoretically useful in ECS research, the WKY strain, was investigated. As the WKY rat is also known for its hyperresponsiveness to stress and hyperactivity of the HPA axis (De La Garza et al., 2004; Hauger et al., 2002; Redei et al., 2001), and the HPA axis is also of interest in relation to the action of antidepressant treatments and cognition (Arborelius et al., 1999; Bao et al., 2008; Plotsky et al., 1998), this chemical messenger system was evaluated in addition to BDNF.

In the second study, the WKY strain demonstrated some interesting physiological and behavioural differences from the Wistar strain. Physiologically, the WKY showed similar baseline levels of brain BDNF protein and CRF, and plasma corticosterone. Repeated ECS had the expected effects on brain BDNF protein, which showed large and widespread increases in depression-relevant brain regions, such as the hippocampus, frontal cortex, and neocortex. The regionally corresponding increases in CRF were a novel finding that will be discussed in detail below. But first, in comparison of strains, similar elevations (in magnitude and location) in brain BDNF protein and CRF were seen at 1 day after repeated ECS. However, these elevations were not as persistent in the WKY compared with the Wistar strain, mostly decreasing by nearly half or returning to baseline by 7 days post-ECS. Surprisingly, there was no change in corticosterone after repeated ECS, which may be explained by a normalization of corticosterone with repeated compared with single ECS (Thiagarajan et al., 1989). (Indeed we have also seen elevations in corticosterone at 4 and 24 hours after a single ECS in the Wistar strain: Ogilvie et al., 2007). The more rapid return to baseline of ECS-induced elevations in brain BDNF protein and CRF in the WKY strain are suggestive of a physiological abnormality in this strain in the BDNF and extra-hypothalamic CRF systems, in addition to the known monoamine and HPA axis abnormalities in this strain, which warrants further investigation.

The WKY strain is also behaviourally distinct from the Wistar strain, showing greater depression-like behaviours (greater immobility in the FST) and possibly greater anxiety-like behaviours (lower mobility/activity in the open field test). Repeated ECS did effectively reduce the depression-like behaviour in the FST, but appeared to increase anxiety-like immobility or freezing behaviour in other tasks (such as the open field, MWM, and CER). Repeated ECS also affected the retrograde memory of both strains, as expected. Although this pattern of performance in the CER was clouded by freezing behaviour in the WKY strain, a reduction in recall for this task was apparent in the Wistar strain, particularly at 1 day post-ECS. Performance in the MWM showed a clearer negative effect of ECS on retrograde memory that was similar in both strains.

Although not the primary intent of this study, it is interesting to note that the strain and treatment effects on physiological and behavioural outcome factors did not temporally correspond. Most notably, the distinct profile of greater baseline depression- and anxiety-like behaviour in the WKY compared with the Wistar strain was not mirrored by a difference in physiological outcome factors measured (BDNF, CRF, or corticosterone). In addition, post-ECS changes in these behaviours also did not temporally correspond with the physiological changes.

The co-occurrence of elevations of CRF and BDNF after ECS in similar brain regions (e.g., hippocampus, frontal cortex, and neocortex) is interesting, although not surprising given that the genes for CRF and BDNF are transcribed by CREB. The mechanisms through which BDNF and CRF elevations elicited by ECS may come to influence behavior have yet to be elucidated. BDNF has been widely implicated in depression (Castren et al., 2007) and memory (Egan et al., 2003); however, the findings of this study did not suggest a clear relationship between the changes in BDNF and either of these behaviours, which has been noted by another

group in their studies on the FSL and FRL strains (Angelucci et al., 2003; Jimenez-Vasquez et al., 2007).

It is of particular interest that CRF changes were seen in extra-hypothalamic brain regions, as the role of CRF after ECT/ECS has focused almost exclusively on the hypothalamus (Brady et al., 1994; Garcia-Garcia et al., 1998). Although the CRF_1 receptor is located throughout the brain (Hauger et al., 2002; Nemeroff et al., 2005), the role of CRF in the hippocampus, frontal cortex, and neocortex is not well understood. The majority of research on extra-hypothalamic CRF has focused on the amygdala and the locus coeruleus (Koob & Heinrichs, 1999; Weiss et al., 1994), although findings from studies investigating the prefrontal cortex have suggested that extra-hypothalamic CRF systems may function independently from the HPA axis (Merali, Anisman, James, Kent, & Schulkin, 2008). In the brains of suicide victims, overexpression of CRF and underexpression of CRF_1 receptors is seen in some regions of the frontal cortex and overexpression of CRF is seen in the locus coeruleus (Merali et al., 2004; Merali et al., 2006), although this effect has not been seen in all studies (e.g., Hucks, Lowther, Crompton, Katona, & Horton, 1997).

Although the function of CRF in extra-hypothalamic brain regions is not yet elucidated, its simple presence suggests some functional role in frontal- and hippocampus-related behaviours, such as emotional regulation, including depression and anxiety (see Hauger et al., 2002), and cognition, including learning and memory (see Croiset, Nijsen, & Kamphuis, 2000). It seems unlikely that elevated CRF in cortical brain regions and the hippocampus would be related to an antidepressant effect after ECS. Elevated CRF levels are typically associated with heightened depression- and anxiety-like behaviours in animals (Rotzinger et al., 2010), and CRF_1 antagonists are associated with antidepressant and anxiolytic effects (Holsboer et al., 2008). In

addition, it has been reported that there was no correlation between baseline performance in the FST and extra-hypothalamic CRF mRNA in several inbred strains, including WKY (Lahmame et al., 1997).

Elevations in CRF could be related to the anxiogenic effects of ECS, though only seen in the WKY strain (Lahmame et al., 1997; Rotzinger et al., 2010; Shepard et al., 2008), as well as to the memory effects in both strains. High levels of hippocampal CRF after stress and activation of the CRF_1 receptor interfere with memory (Chen et al., 2010; Heinrichs et al., 1996; Ivy et al., 2010), likely by altering dendritic arborisation (Chen et al., 2004) and spine integrity in CA3 and disrupting long-term potentiation (LTP) formation (Chen et al., 2010). These functional and cellular effects are prevented by CRF_1 antagonists given before or after the stressor exposure (Brunson et al., 2002; Gallagher et al., 2008). The effects of CRF in the hippocampus are likely related to its effects on cellular activation through CREB phosphorylation (Chen, Fenoglio, Dube, Grigoriadis, & Baram, 2006; Gallagher et al., 2008). One caveat in studies in this area is that the stressors leading to increased CRF were introduced in early-life. Although some of these effects were seen in middle-aged rats (Ivy et al., 2010), the induction of increased CRF (e.g., stressor or transgenic mouse model) still occurred in early life. Thus, more conclusive evidence is required to determine if a similar CRF-mediated mechanism contributes to memory impairments observed following an induction of increased extra-hypothalamic CRF, such as that seen after repeated ECS, in adulthood.

Acute changes in BDNF and CRF may be sufficient (i.e., it may not be necessary for them to persist) to induce the persistent behavioural changes seen in the WKY strain. It is also likely that the widespread neuronal activation after ECS stimulates several interacting neurochemical systems, and thus it is highly likely that these chemical messengers are not acting alone if they

do indeed have an impact on behaviour. Much further study in these areas would be needed to wean out the specific effects in particular of these post-ECS physiological changes on behaviour. A valuable subsequent study would block these ECS-induced increases in BDNF and CRF and investigate any corresponding changes in behaviour, such as the effect of a trkB or CRF_1 antagonist on ECS-induced affective and memory effects in adult rats.

For this series of studies, however, with a particular interest in the translational value of these findings in BDNF, the line of inquiry led to questions about the findings of serum BDNF protein measurement in people with depression and in response to ECT, as in human studies, decreased serum BDNF protein has been shown in depression, with variable results in changes in serum BDNF protein after treatment. Therefore, in the third study we questioned the utility of the measurement of serum BDNF protein in human studies and sought to use the WKY strain and ECS as a translational model to further investigate the relationship between serum and brain BDNF protein. Studies in rodents have had difficulty correlating serum and brain BDNF protein (Elfving et al., 2010; Sartorius et al., 2009). Significant correlations in these studies were found only under very specific circumstances and alphas were never corrected for the multiple comparisons performed (Elfving et al., 2010; Sartorius et al., 2009). Karege and colleagues found that in Wistar rats the interaction between peripheral and central BDNF may be related to age, and may be stronger in early developmental stages and decline in adulthood (Karege et al., 2002b).

Indeed we had similar difficulty correlating serum and brain BDNF protein in adult WKY and Wistar rats. Despite the finding of lower serum BDNF protein in the WKY relative to Wistar strain, which nicely mirrors the strong evidence of lower serum BDNF protein in people with depression relative to healthy controls, we did not find these strain differences in brain BDNF

protein. The lack of correlation at baseline was further supported by our investigations into the treatment effects of repeated ECS on serum and brain BDNF protein. In brain, we see a sharp immediate increase in BDNF, even after one ECS in hippocampus (as seen in Chapter 2 in the Wistar strain) and more widely after five ECS (as seen in both strains in Chapter 3). Given these highly significant findings, if brain BDNF changes are mirrored by serum BDNF changes to any degree, we would expect to see some evidence of change in serum BDNF protein after ECS. However, we did not find any change in serum BDNF after repeated ECS in either strain.

Findings of changes in serum BDNF protein in humans after ECT are variable (Bocchio-Chiavetto et al., 2006; Fernandes et al., 2009; Gronli et al., 2009; Hu et al., 2010; Okamoto et al., 2008), more so than after antidepressant drugs (Bocchio-Chiavetto et al., 2010). This could be explained by the mechanism of administration of the treatments, with antidepressant drugs administered systemically and ECT more directly to the brain. The source of BDNF in the blood is still unknown and may be different than that in the brain. Also, although BDNF does cross the blood brain barrier (Pan et al., 1998) it does not appear that changes in the brain would explain changes in the blood, as we show that immediate and large increases in the brain after ECT are not represented in the blood, at least not immediately. This does suggest that BDNF protein levels in the serum are not directly linked with those in the brain, and may have a different source.

Like in most human conditions, several factors (essentially more tightly controlling the participant pool) are necessary to see the changes in serum BDNF protein after antidepressant treatment. Seemingly the factor showing the greatest importance in this regard is the requirement of lower basal serum BDNF protein levels. Some studies have found lower serum BDNF only in untreated (Diniz et al., 2010; Molendijk et al., 2011; Ozan et al., 2010) or treatment naive

(Shimizu et al., 2003) patients with depression. Indeed, Shimizu and colleagues (2003) found that only those patients who were treatment naive and showed pre-treatment or baseline BDNF levels one or more standard deviations below the mean of healthy controls showed a significant increase in serum BDNF with antidepressant drug treatment. The necessity of lower baseline serum BDNF protein in order to see the post-treatment increases was also seen in two other studies using antidepressant drugs (Gervasoni et al., 2005) and ECT (Bocchio-Chiavetto et al., 2006). Although the animal models of depression clearly cannot perfectly mimic the complex human condition of depression, the features of the WKY strain and some of the parameters of this study should make an ideal match for these conditions. The WKY rats used in our study were treatment naive and had lower baseline serum BDNF levels relative to their healthy controls. Both strains displayed a clear physiological treatment effect of increased brain BDNF protein levels, and in the previous study have been shown to also display a clear behavioural treatment effect of ECS in the forced swim test. Finally, physiological outcomes were assessed at an immediate and a delayed time point after ECS, potentially an important factor in the detection of serum BDNF changes in human studies (Bocchio-Chiavetto et al., 2006). However, despite these seemingly ideal circumstances, serum BDNF did not clearly correlate with brain BDNF after numerous correlations were tested. These discrepancies in the findings of human and animal studies, despite similarities between the conditions of both groups, clearly highlight the difficulties in translational research. Although one could argue that the findings of a clear lower baseline serum BDNF protein level in the "depressed" WKY strain, and difficulty showing changes in serum BDNF protein post-ECS do in fact translate well to the findings in human studies. It is clear however that the findings of no correlation between brain and serum BDNF

protein in this animal study challenge the assumption that serum BDNF protein measurements reflect brain BDNF protein and hold any functional utility in depression and its treatments.

In our human pilot study, we expected to find some similar behavioural and physiological findings as in the second animal study, such as decreases in depression, possible increases in anxiety (especially for those with baseline high anxiety), and increases in serum BDNF after ECT. However, these findings were not clear in the small group of participants involved. Mostly this was due to a high rate of variability in the data. Overall, ECT did seem to decrease depression slightly at the immediate time point post-ECT, although this change was very small and inconsistent between subjects, with only one patient coming into remission. The other behavioural aspects measured were marked by even greater inconsistency. As one might expect with so much variability in the behavioural results, the physiological outcome factors were also variable. Plasma cortisol measurements showed no discernable pattern and were highly inconsistent between participants. Serum BDNF protein was also inconsistent and if anything showed a trend to a positive association with depression severity, as opposed to the expected negative correlation. CRF was not measurable in humans, again pointing to some of the continued limitations in translational research. Perhaps similar to the general finding of the second animal study, we did not see any strong trends to reasonably suggest alterations in the physiological outcome factors may be associated with behavioural outcome factors. However, it would be useful in a future study to have a control group to assess normal changes in these variables over time.

In the end, given the findings of the third animal study, the inconsistent changes in serum BDNF and their lack of correspondence with any of the behavioural outcome measures in the human pilot study, make interpretation difficult with respect to what is happening with BDNF in

the human brain post-ECS. These findings add to the question of the functional validity of serum BDNF measurements in depression and after antidepressant treatments, particularly with the differences in findings after systemically administered pharmaceuticals compared with locally administered ECT.

Another question remaining regarding serum BDNF protein in depression is whether changes in serum BDNF represent changes in mBDNF or proBDNF. This is important because the two forms of BDNF have different roles: mBDNF binds to the trkB receptor and is associated with a cascade of events promoting cell survival, whereas its precursor, proBDNF, which is cleaved to form mBDNF, binds to the p75 receptor and is associated with a cascade of events promoting cell death (Martinowich, Manji, & Lu, 2007). One study suggested that BDNF protein measured in the blood is represented by a higher proportion of mBDNF compared with proBDNF (Bocchio-Chiavetto et al., 2010). However, these authors argued that this finding was not conclusive as it was based on an inability to measure a clear signal from proBDNF (Bocchio-Chiavetto et al., 2010), which is difficult to detect in serum due to its low presence relative to mBDNF (Katoh-Semba et al., 2007).

The question consistently raised across these three animal studies and the human pilot study spoke to the functional role of BDNF in depression. In fact, recent arguments have been formed against the hypothesized role for BDNF in depression (e.g., Groves, 2007). Initial suggestions that the structural changes occurring in rodent brain after antidepressant drug administration (thought to be related to changes in BDNF and other neurotrophins) are necessary for the antidepressant-like behavioural effects (Santarelli et al., 2003), have been disputed (Bessa et al., 2009). Several studies have additionally struggled to find correlations between polymorphisms in the val66met allele and depressive symptoms in humans (Bocchio-Chiavetto

et al., 2006; Hong et al., 2003; Li et al., 2007; Shimizu et al., 2003; Tsai et al., 2003), as well as baseline BDNF protein levels and depression-like behaviours in animals (Chapters 3 and 4; Duman & Monteggia, 2006). Although it is impossible to ignore the substantial support for some role of BDNF in depression (Castren et al., 2007; Duman et al., 2006), these findings highlight the lack of understanding about the precise roles of BDNF in depression and antidepressant treatment response. Lower levels of blood BDNF protein have been found in several other mental illnesses (Ikeda et al., 2008; Monteleone et al., 2008), dementia (Leyhe, Stransky, Eschweiler, Buchkremer, & Laske, 2008), as well as healthy adults exposed to stress (Mitoma et al., 2008; Okuno et al., 2011), and may represent a common feature of multiple conditions involving the brain. One common underlying feature of many of these disorders is stress. The stress-hypothesis of depression is well-validated (Plotsky et al., 1998), with changes in the HPA axis one of the hallmark features of depression and many other mental health and neurological conditions (Nemeroff et al., 2005; Plotsky et al., 1998). As discussed in the introduction, BDNF and the HPA axis do interact in the brain (Givalois et al., 2004) and may interact in the blood as well. Decreased brain or serum BDNF protein may represent another common effect of the experience of stress. Certainly, decreased brain BDNF protein has been shown more consistently in behavioural animal models of depression involving stress induction (Smith, Makino, Kvetnansky, & Post, 1995a) than in the novel genetic models of depression (Chapters 3 and 4; Elfving et al., 2010). This hypothesis is also supported by the findings of decreased serum BDNF in the WKY strain, which is known to be hyperresponsive to stress (De La Garza et al., 2004), although this hypothesis remains challenged by the discrepancy in the finding of "normal" levels of brain BDNF relative to the Wistar strain.

BDNF is also highly linked with cognition, and particularly memory (Egan et al., 2003). Although the val66met allele has not been consistently associated with depression, it has been associaterd with cognitive and memory functions (Egan et al., 2003). Cognition is affected in many stress-related conditions including mental illnesses and dementias, and even in the healthy brain exposed to stress. The findings of chapter 3 do not suggest a clear association between the large increases in BDNF protein and changes in memory after ECS, although we cannot rule out the possibility that large initial increases in BDNF protein post-ECS may be sufficient to interfere with the long term consolidation of memory, causing a loss of recent experiential memories that would extend beyond any subsequent normalization of brain BDNF protein levels. The effect of intra-cerebral administration of BDNF protein or an agonist on memory function would be an interesting future investigation.

If the phosphorylation of CREB is a common mechanism of antidepressant treatments, there is a possibility that some of the resulting by-products of increased gene transcription by CREB are unrelated to the antidepressant effect, and rather are involved in side effects of treatment on, for example, memory, appetite, weight gain, libido, and others. Further investigation is needed into the role of BDNF in the other side effects of antidepressant treatment.

Summary

Although the precise mechanism and functional role of changes in BDNF protein and HPA axis targets in the brain after ECT are not yet elucidated, this series of studies offer insight into this area of research. CREB may represent a common downstream target of antidepressant treatments; however, the data suggest that the cAMP second messenger pathway is likely not a common target of antidepressant drug and ECT treatments.

The usefulness of the WKY strain in the investigation of the physiological mechanisms of the effects of ECS on depression and cognition is validated. The WKY rat certainly shows some physiological and behavioural abnormalities beyond those well-established, including low serum BDNF and a shorter sustained increase in BDNF after ECS. This study also demonstrated the potential role for extra-hypothalamic CRF and potential correspondence with BDNF in ECS, which has not previously been shown. These findings also suggest that corticosterone may not play an important role in treatment response or cognitive side effects.

Finally, the lack of correlation between serum and brain BDNF protein, despite seemingly ideal translational circumstances (an animal model, treatment model, and experimental design with good face validity to the human condition) challenges the validity of serum BDNF findings in human studies of depression and its treatments.

Translation of research findings from animal to human studies is of increasing demand in the research community. However, this process is not easy or straight forward, and developing studies based on the ease of ability to translate the data from animal to human may limit the possibilities of animal studies that allow us to make discoveries beyond what we can do with human research. Translation of results from animal studies may come in the future with advancements in technologies in human research, but let us be careful not to let a focus on translational value of research hold back the advancement of animal and basic science research.

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Appendix

A human pilot study: the role of BDNF in cognitive and affective effects of ECT

The human pilot study was designed to translate the findings of the rodent investigations to humans. Thus we attempted to investigate the same neurochemical and behavioural targets at biologically relevant time points after a course of ECT in humans suffering from depression. This research was intended to demonstrate the association between serum BDNF and plasma cortisol and the cognitive and antidepressant effects of ECT in people with depression. Based on the literature, we expected to see a negative correlations between serum BDNF, but no correlations between plasma cortisol (as we did not expect this measure to change over the course of ECT), and performance on measures of memory and depression severity. However, after completing our third animal study, in which serum BDNF was not correlated with ECS treatment or with brain BDNF, we may have expected to see no change in these measures. In either case, we hoped to translate the findings from the animal studies to this human study.

Method

Five participants were recruited, three through the psychiatry department at the General campus of the Ottawa Hospital and two through the Mood Disorders Unit at the Royal Ottawa Hospital. Participants met criteria for primary unipolar MDD (Diagnositcs and Statistics Manual, 4th Edition [DSM-IV]: American Psychiatric Association, 1994). Bilateral temporal ECT was administered according to American Psychiatric Association (APA) guidelines (American Psychiatric Association: Task Force on Electroconvulsive Therapy, 2001). Demographics and treatment parameters are presented in Table 1.

Participants attended 3 visits, immediately (1-5 days) pre-ECT, immediately (3-7 days) post-ECT, and later (25-35 days) post-ECT. Diagnostic and psychological assessment included

Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998), Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979), Quick Inventory of Depressive Symptomatology-self-rated (QIDS-SR) (Rush et al., 2003). Cognitive assessment included: Columbia University Autobiographical Memory Interview-Short Form (AMI-SF) (McElhiney, Moody, & Sackeim, 1997), Rey Auditory-Verbal Learning Task (RAVLT) (Strauss, Sherman, & Spreen, 2006), and Wechsler Adult Intelligence Scale, 3rd Edition (WAIS-III): Digit Span subtest (The Psychological Corporation, 1997). Alternate forms were used for all cognitive tests to control for practice effects.

Venous blood samples were drawn from which plasma and serum were separated and kept refrigerated at 4°C for immediate analysis or frozen at -80°C for later analysis of serum BDNF protein by ELISA using the human BDNF Quantikine Kit (R&D systems, Minneapolis) and plasma cortisol by RIA using the ImmuChem Coated Tube Cortisol ^{125}I RIA kit (MPbio, Costa Mesa, CA) according to the manufacturer's instructions.

Results and Discussion

The goal of this study was to analyze individual within-subject factors related to changes in memory, mood, BDNF, and cortisol. However, with few participants and high variability, particularly with BDNF and cortisol measures, we were unable to perform within-subject (regression or correlation) analysis. However, there are some interesting findings to note. With a high level of variability, normally median scores would be taken to reflect the action of the group; however, because of the tendency of individual members to stay within a range of scores on most measures, in this case, median scores generally reflected only one individual in the group.Therefore, averages were used as an overall reflection of the group results. Level of depression did decrease slightly after ECT in experimenter-rated depression scores (MADRS)

from an average (standard deviation) of pre-ECT 34.6 (8.4) to post-ECT 1: 28.6 (13.1) and 30 (7.5), a 6-point change from baseline at visit 2, moving participants from severe to moderate depression (1 week post-ECT) and a 4-point change from baseline at visit 3 moving participants back to borderline-severe depression (1 month post-ECT). Changes in self-rated depression and examiner-rated anxiety scores followed a similar pattern, though less pronounced (figure 1). This moderate 6-point drop on the MADRS between visits 1 and 2 was driven by only two individuals, and otherwise there was such variability in the mood response to ECT that it is impossible to draw any conclusions about the relationship between cognitive and physiological measures and the antidepressant action of ECT.

In terms of cognition, all participants showed a decline in recall of information on the AMI-SF (to an average of 75% of baseline). However, it is unclear as to whether this decline is related to ECT treatment because this test does not supply norms for performance in a healthy population. It would be useful to have a healthy control group as a comparison in this study. It is interesting to note that in two participants, AMI-SF scores increased slightly (5 to 10% increase) between visits 2 and 3, which may suggest a return of some autobiographical declarative memory that was disrupted immediately after ECT. However, this was not the case in three other participants who either showed a slight decline or no change in performance from visit 2 to 3. Also, these changes in AMI-SF performance did not show any visible relationship to cortisol, BDNF, or depression severity. Measures of working memory (digit span) and new learning (RAVLT) did not suggest any significant effect of ECT.

Cortisol showed no discernable pattern of changes after ECT. Overall, the averages were slightly increased at both post-ECT time points (visit 1: 12; visit 2: 20; visit 3: 18 μg/dl). However, in individual participants the plasma cortisol levels were quite variable, with baseline

levels ranging from 3 to 22 μg/dl, and unique patterns of changes from baseline at the post-ECT time points that were not visibly related to changes in BDNF or the cognitive or affective measures.

Average serum BDNF protein did not show the expected increases at either post-ECT time point, overall staying relatively stable across time points. Interestingly, most participants showed a premorbid serum BDNF protein level between approximately 60-75 ng/ml; however, one participant showed a baseline serum BDNF protein level just below 50 ng/ml. In this participant, serum BDNF protein increased to 62 ng/ml at 1 month post-ECT, despite staying stable around 50 ng/ml within 1 week post-ECT (figure 1). This finding is in line with the suggestion that a lower baseline BDNF protein level may be required to see post-treatment changes in serum BDNF, and that these changes may only become apparent at a later time point after ECT (Bocchio-Chiavetto et al., 2006; Fernandes et al., 2009; Gronli et al., 2009; Hu et al., 2010; Okamoto et al., 2008; Sartorius et al., 2009).

Some studies have found that serum BDNF protein is negatively correlated with depression severity (Basterzi et al., 2009; Bocchio-Chiavetto et al., 2006; Huang et al., 2001), while others have not (Okamoto et al., 2008; Tadic et al., 2011). In this study, when serum BDNF protein and MADRS depression scores were compared, it appeared that they were mostly positively associated (figure 1). Given the findings from the third animal study that suggests that serum and brain BDNF protein levels are not easily correlated before, immediately after, or at 1 week after repeated ECS in an animal model of depression and its healthy control, it is difficult to draw any conclusions from the trend of positive association between BDNF and depression scores in this human pilot study.

Translational research is becoming increasingly encouraged and supported in the research community. The reason for this became clear in this study, which faced many barriers to translating our animal research design to the human condition of depression. The conditions and tools available in human and animal research differ greatly. Animal studies allow for rigid study design characteristics, including, but not limited to, the genetic make-up of the subjects, the treatment protocol (e.g., ECS stimulus strength, frequency, and duration), and the testing conditions. Animal research also allows for the investigation of neurochemicals *in-vitro*, for which there have not yet been comparable measures developed *in-vivo* (e.g., brain levels of BDNF and CRF). Indeed, the findings from this study exemplify the difficulties in translating animal research findings to the human condition, highlighting the variability in human condition that will always be nearly impossible to mirror in the animal models and the need to develop better technology for the investigation of neurochemicals in the human brain *in vivo*.

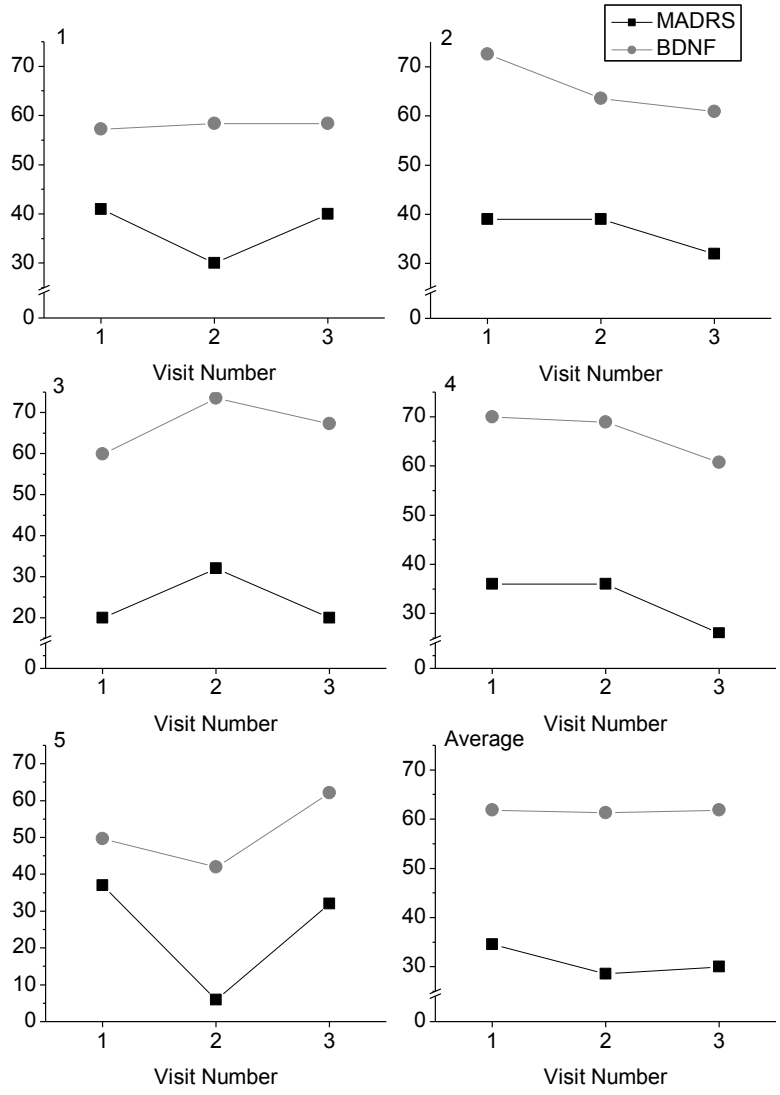

Figure 1. BDNF (ng/ml) and MADRS raw scores in five individual participants and overall average.

Table 1. Demographics and Treatment Parameters.

Demographics	**median (range)**
Age	46 y (41-61)
Gender	4 m, 1 f
Education	13 y (12-21)
Income	$50-75,000 ($10,000 - >100,000)
Ethnicity	5 Caucasian
BMI	28 (21-32)
Duration current MDE	12 months (2-60)
# past MDEs	> 10 (4 - >10)
Years since first MDE	35 y (30-50)
Family MDD	3 yes, 2 no
Treatment	**median (range)**
Past ECT	3 yes, 2 no
Medication	1 SSRI alone, 3 SSRI + 1 agent, 1 Other AD + 1 agent
# ECT sessions	12 (10-15)

AD: antidepressant drug; ECT: Electroconvulsive Therapy; MDD: Major Depressive Disorder;

MDE: Major Depressive Episode; SSRI: selective serotonin reuptake inhibitor;.