Hyperarousal symptoms of PTSD in veterans correlate to neuromelanin-sensitive MRI signal in the locus coeruleus, a putative measure of norepinephrine system function

Adelina McCall

Thesis submitted in partial fulfillment of the requirements for the degree of Masters in Cellular & Molecular Medicine

Department of Cellular and Molecular Medicine

Faculty of Medicine

ABSTRACT

Post-traumatic stress disorder (PTSD) is a heterogenous psychiatric condition that affects thousands of individuals each year. Of those who experience this condition, military members including members of the Canadian Armed Forces (CAF) are particularly vulnerable, demonstrating high prevalence rates of PTSD-related symptoms. Moreover, individuals with PTSD are at increased risk for comorbid conditions and are at greater risk for suicide due to the overwhelming, debilitating nature of PTSD symptoms. In previous research, hyperarousal symptoms associated with PTSD have been linked to dysregulation in the locus coeruleus norepinephrine (LC-NE) system, a vast neuromodulatory system responsible for regulating arousal, attention, autonomic and memory-related functions. Advancements in neuroimaging methods have advanced our ability to study connectivity in vivo such that small structures like the LC can be further studied in human samples. Specifically, neuromelanin-sensitive MRI (NM-MRI), a novel, non-invasive neuroimaging method has been shown to detect changes in neuromelanin (NM)-related signal in both the LC and substantia nigra (SN). NM is a dark pigment that accumulates over the lifespan in catecholamine-dominant centers such as the LC and SN and is the by-product of catecholamine oxidation. NM-MRI can be used to image these centers in vivo due to the paramagnetic properties offered by NM. Furthermore, when excess cytosolic catecholamine levels are present in select neurons, NM production is thought to be increased, resulting in increased NM signal from the LC. This could potentially be a marker for dysregulation as many conditions have been associated with variability of this system. Previously, NM-MRI has been used in other clinical settings such as in Parkinson's disease (PD), Alzheimer's disease (AD), schizophrenia and depression; however, this current investigation is the first to utilize this imaging modality in the context of PTSD. Specifically, we hypothesized that increased NM-MRI signal in the LC would correlate with increasing severity of hyperarousal symptoms in individuals with PTSD. We also predicted that the opposite would be true for comorbid depression symptom severity, as reduced LC signal has been previously correlated with clinical measures of comorbid depression using NM-MRI. As per our primary hypothesis, we observed a significant positive correlation between NM-MRI signals in the caudal elements of the LC with hyperarousal symptom severity in 22 PTSD subjects (r= 0.54, p= 0.017; partial correlation controlling for depression symptom severity, age, and sex). In contrast, we did not find any evidence to support our secondary hypothesis, because a non-significant trend correlating LC NM-MRI signal and depression symptom severity was obtained (r= -0.30, p=0.22; partial correlation controlling for hyperarousal severity, age, and sex). Based on these results, we were able to build on previously conducted work to further investigate the utility of NM-MRI in the detection of variability in LC-NE system as it pertains to psychiatric conditions known to show dysregulation of this system such as PTSD. In addition, this thesis provides further evidence to support the automation of NM-MRI analytical methods, thus supporting their potential utility for future clinical research. Our findings also provide support for the use of NM-MRI as a potential measure of NE activity; further, this work provided preliminary evidence supporting the use of NM-MRI in a clinical, psychiatric setting, where the technique may serve as a biomarker of PTSD pathology. With these findings in mind, additional validation studies can be conducted to verify the use of NM-MRI as a biomarker for NE system dysregulation. This would potentially allow for advancements in targeted treatment options for PTSD, particularly those targeting the LC-NE system, thus potentially increasing patient stratification and treatment efficacy.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor Dr. Clifford Cassidy for his continuous guidance and support throughout this entire journey. Thank you for acknowledging my strengths, supporting my weaknesses and overall, for your surreal mentorship over the past two years. Furthermore, thank you for providing me countless opportunities to participate and learn. Your love for your work has driven me to be a better researcher and has given me perspective for what is to come in my future endeavours.

Secondly, I would like to thank my team for their continuous support over the past two years. Thank you to Rami Hamati, Tram Nguyen, and Krysta Boutin-Miller for making me feel welcome in the early months of my arrival, as well as your on-going support in admin-related, research-related and day-to-day activities. You all have made my journey and transition into clinical research so much easier and for that I am forever thankful. I also want to thank Rami Al Haddad for his contribution to this body of work and ample support during the countless hours we spent trying to run the analysis for this project. Thank you for your patience, dedication, and time during this process.

Furthermore, I would also like to thank my thesis advisory committee members (Dr. Natalia Jaworska, Dr. Georg Northoff and my co-supervisor Dr. Michael Schlossmacher) for taking the time to be on my committee, attend my annual meetings and offer your most honest feedback for the work I have presented. Your recommendations and support have contributed greatly to who I have become as a researcher, and I am so very grateful to have such an amazing team of researchers in my corner during this process. Thank you.

Finally, I would like to offer my greatest thanks to my support network. Thank you to my mom, Angela (better known as my chief editor) and my dad, Gary for their endless love, support, and compassion. Thank you for always believing in me and always supporting my dreams, no matter how long it may take for me to get there. I owe my success to you and for that I am forever grateful. In addition, thank you to my sister Stephanie for your everlasting support. You rock little sis and I hope you accomplish everything you have ever dreamed of. And finally, thank you to my partner Marcus for being my biggest cheerleader, my rock and for being an important disconnect/distraction from the science world. Thank you for your energy, unlimited optimism, and endless support. You bring out the best of me each and every day, some of which I hope can be seen throughout in this body of work. Thank you for always being my person.

TABLE OF CONTENTS

ABBREVIATIONS

2D GRE-MT = 2D gradient response echo sequence with magnetization transfer contrast

AD = Alzheimer’s disease

ADHD = Attention-deficit/hyperactivity disorder

ANS = Autonomic nervous system

BDI-II = Beck Depression Inventory-II

CAF = Canadian Armed Forces

CAPS-5 = Clinician Administered PTSD Scale for DSM-5

CNR = Contrast-to-noise ratio

CRF = Corticotropin-releasing factor

DA = Dopamine

DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

HPA axis = Hypothalamic-pituitary-adrenal axis

LC = Locus coeruleus

LC-NE system = Locus coeruleus norepinephrine system

LEC-5 = Life Events Checklist for DSM-5

MAPS = Multidimensional Assessment of PTSD Subtypes

M.I.N.I = Mini-International Neuropsychiatric Interview

MT = Magnetization transfer

NDRI = Norepinephrine-dopamine reuptake inhibitor

NE = Norepinephrine

NM = Neuromelanin

NM-MRI = Neuromelanin-sensitive magnetic resonance imaging

OSI Clinic = Operational Stress Injury Clinic

PFC = Prefrontal cortex

PCL-5 = PTSD Checklist for DSM-5

PD = Parkinson’s disease

PTSD = Post-traumatic stress disorder

REB = Review ethics board

RBD = REM sleep behaviour disorder

RDOC= Research Domain Criteria Initiative

SN = Substantia nigra

SNRI = Serotonin-norepinephrine reuptake inhibitor

SSRI = Selective serotonin reuptake inhibitor

TH-IR = Tyrosine hydroxylase immunoreactivity

LIST OF TABLES

LIST OF FIGURES:

CHAPTER 1: INTRODUCTION (LITERATURE REVIEW)

1.1.1 Locus Coeruleus – Norepinephrine System

In this chapter I will be discussing the locus coeruleus norepinephrine (LC-NE) system. This system modulates numerous central and peripheral functions and variability in the function of this system can be observed in numerous neuropsychiatric conditions such as depression, anxiety, Alzheimer's disease (AD) and post-traumatic stress disorder (PTSD), the latter being most relevant to the work presented here.

More specifically, this chapter will include information about the anatomy and physiology of the locus coeruleus (LC), the function of this system, its different projections (both efferent and afferent) and will conclude by considering the clinical significance of the LC-NE system including specific disorders in which LC-NE dysregulation can be observed. The ultimate purpose of this chapter will be to introduce this system and how it is relevant to our current work, thus providing rationale for our hypotheses discussed later.

1.1.2 – Anatomy

The human LC is a small, bilateral brainstem structure located in the rostral pontine tegmentum (Sharma et al., 2010) adjacent to the floor of the fourth ventricle (Keren et al., 2009). This structure also occupies part of the midbrain as the rostral end reaches close to the level of the inferior colliculus (Keren et al., 2009; Sharma et al., 2010). In addition, the LC is thought to have structural divisions in that, the rostral and caudal parts of the LC may be implicated in different functions (see below in Section 1.1.4). This indicates that alongside structural divisions there may also be functional divisions of the LC, thereby, implicating the LC in a wide range of functions (Samuels and Szabadi, 2008; Keren et al., 2009).

In terms of the cytoarchitecture of the LC, the vast majority of neurons in the LC are norepinephrine (NE)-containing (Dahlström and Fuxe, 1964). This makes the LC the central nucleus for NE projections, thus contributing the vast majority of NE innervation across the brain (Euler, 1946; Dahlström and Fuxe, 1964). There are two notable cell types within the LC, larger multipolar cells, as well as small fusiform cells (Swanson, 1976; Grzanna and Molliver, 1980; Schwarz and Luo, 2015) and this identification was conducted in rat models as limited cytoarchitecture research has been conducted in humans. With that being said, one human study conducted by German and colleagues confirmed that many of the cells were indeed "oval to fusiform in shape" (German et al., 1988), a finding that was similar to early animal research (Swanson, 1976; Groves and Wilson, 1980; Grzanna and Molliver, 1980; Gerfen and Sawchenko, 2016). Furthermore, there is a difference in the distribution of cells with respect to their rostral-caudal orientation within the LC, thus providing evidence for the proposed topographical and functional organization of this structure (Waterhouse et al., 1983)[1]. Finally, many of these cells are pigmented and this pigment was later confirmed to be neuromelanin (NM), a dark pigment that accumulates in the catecholaminergic cells within the LC and substania nigra (SN) (German et al., 1988). This feature is also relevant for our current work, and this will become relevant in Section 1.2.1

Together, this early work has established the association of the LC with a major neurotransmitter system, the NE system. This work also provided some indication of topographical and functional organization of the LC. Furthermore, these early reports confirmed that many of the cells in the LC contained NM, a fundamental observation work presented here.

---

[1] This notion of topographical organization will be re-introduced in Section 1.1.4 where I will discuss how different sub-populations of neurons in the rostral versus caudal portions of the LC are hypothesized to project to different targets, thus implicating different regions of the LC in different functions. This is an important consideration for the work presented here as we chose to explore the proposed functional organization of this system in our analysis.

To better understand the size of the LC, several quantification studies have been conducted to determine the number of cells that make up the LC. Here, such methods utilize the presence of NM, since, as previously mentioned, the vast majority of LC neurons contain this pigment, thus differentiating LC cells from cells of surrounding structures (German et al., 1988). Specifically, by utilizing a neuron-specific stain, pigmented neurons of the LC can be easily identified and then quantified by a series of serial counts (Brody and Vijayashankar, 1979). On average, 18,000 pigmented cells could be identified in the left LC (Brody and Vijayashankar, 1979; German et al., 1988). This research was conducted on post-mortem human brain samples and demonstrated the small size of this structure ex vivo. In addition, researchers from this study also observed that as the subjects aged, the number of pigmented cells began to decline indicating an age-related loss in LC neurons (Brody and Vijayashankar, 1979)[2]. This is important from a clinical perspective since it has been well documented that LC cell loss is a known pathology in diseases such as AD and Parkinson's Disease (PD).

Quantification studies using immunohistochemical methods (monoamine-specific fluorescence) found that the majority of neurons in the lower brain stem region were catecholaminergic in nature (Dahlström and Fuxe, 1964). In addition, Baker and colleagues expanded on this finding utilizing tyrosine hydroxylase immunoreactivity (TH-IR) to confirm not only that the cells in the LC were catecholaminergic, but specifically they were NE-containing (Baker et al., 1989). From this observation, the average number of TH-positive cells in the human LC samples were found to be 53,900 and this number was comparable to the number of NM-containing cells (n= 54,896) which were also found to be TH-positive (Baker et al., 1989). This result confirms that pigmented neurons

---

[2] This decline of pigmented neurons in the LC may indicate early signs of pathology since it is generally accepted that NM-concentration increases as individuals age. This steady incline of NM occurs until approximately age sixty before leveling out (Mann and Yates, 1979; Halliday et al., 2006).

of the LC also contained NE, thus linking the LC to the NE neurotransmitter system as well as to NM (Dahlström and Fuxe, 1964; Baker et al., 1989). In addition, TH-IR has also been applied across species to compare the relative size of the LC between humans and non-human primate groups (Sharma et al., 2010). This research provided evidence to suggest significant phylogenetic variation between species thereby supporting the notion that an increased volume of neurons may contribute to human-specific behavioural changes during evolution and how dysregulation of the LC-NE system plays a role in human-specific illnesses (i.e. psychiatric conditions) (Sharma et al., 2010). This study also supports the reliability of TH-IR methods across various species and therefore reinforces its validity in related studies[3] (Sharma et al., 2010).

In summary, the above quantification studies have demonstrated the size of the LC relative to other non-human species. This has allowed us to speculate about the role of the LC in human-related conditions and ultimately has demonstrated how small this structure is within the human brain by establishing the true number of cells within this structure. In addition, this research provides support for the challenges faced by neuroimaging research for investigating this system in-vivo, thus emphasizing the need for novel imaging modalities such as neuromelanin-sensitive magnetic resonance imaging (NM-MRI) where structures such as the SN and LC can be independently studied based on their chemical properties (see Chapter 2 for details).

To conclude, the LC is a small, bilateral midbrain structure that is made up of mostly NM and NE-containing cells. Using classic staining methods and immunoreactivity, early research was able to

---

[3] TH-IR has also been conducted in rodent models to isolate LC neurons based on their TH contents. The following references are studies in which this methodology was utilized: (Nestler et al., 1990; Melia et al., 1992; Verma et al., 2006). The research studies described above and noted previously validates this quantification method across numerous species thus giving an accurate representation of the true size of the human LC.

quantify the approximate number of cells in the LC, thus allowing us to better understand the size of this structure. In addition, this early research suggests a strong relationship between the LC and the NE neurotransmitter system, thus making up a major modulatory system in the human nervous system. This suggests that alterations in LC function may be linked with dysregulation or imbalance in the central and/or peripheral NE neurotransmitter system, a fundamental aspect of our current work. Finally, these studies also provided insight into the different efferent and afferent projections associated with the LC-NE system and thus can give insights into the possible functions the LC-NE system may be associated with, including how this system may be implicated in human-related conditions such as neurodegeneration and psychiatric illness.

1.1.3 – Physiology

In this section, I will discuss the physiology of the LC-NE system. I will examine how the LC has been shown to modulate different patterns of physiological activity and how these patterns may be relevant to psychopathology.

The LC is thought to have two patterns of physiological activity: tonic and phasic. Both of these physiological states can fluctuate from being low or high in activity (Aston-Jones and Bloom, 1981; Berridge and Waterhouse, 2003) and are synergistically dependent on one another (Howells et al., 2012). Furthermore, each physiological state can be defined based on its discharge rate and how much NE is released during their corresponding active states (Aston-Jones and Bloom, 1981; Devilbiss and Waterhouse, 2011), thus defining when each state is occurring in the brain.

To begin, the tonic physiological state of the LC is defined as the “background” activity level experienced by the LC. This rate of activity is characterized by slow discharge rates that range from 0.1 to 5.0 Hz (Aston-Jones and Bloom, 1981; Devilbiss and Waterhouse, 2011) and results in a slow release of NE to various targets (see Section 1.1.4 for details on efferent and afferent projections). In contrast, heighted tonic activity can be observed during states of increased arousal and from a pathophysiological perspective, high tonic LC activity has been associated with stress and increasing levels of anxiety and/or hyperarousal, characteristic features in panic disorders, generalized anxiety disorder and trauma-related conditions such as PTSD (Berridge and Waterhouse, 2003; Atzori et al., 2016; McCall et al., 2017). Furthermore, heighted tonic activity of the LC has be suggested to be directly associated with cortical arousal in that when cortical arousal is heighted, tonic activity increases, thus causing the release of additional NE as a response (Howells et al., 2010). This is important since this indicates that anxiety-related and/or hyperarousal symptomology may be associated with increases in NE release, thus contributing to pathological features observed in various psychiatric conditions[4] (Southwick et al., 1999; Sherin and Nemeroff, 2011; Hendrickson and Raskind, 2016). Additionally, NE receptors have also been shown to have specific binding affinity with respect to NE release and it has been shown that even receptors with low binding affinity become activated in periods of distress, providing additional evidence to support increased NE release at LC targets in times of heighted anxiety or stress (Atzori et al., 2016). Finally, tonic activity of the LC also seems to correlate with different wakefulness states, and during REM sleep, tonic activity disappears entirely consistent with the need to dampen cortical arousal during sleep (Aston-Jones and Bloom, 1981; Takahashi et al., 2010; Hayat et al., 2020). This has been described in several electrophysiological studies (see previous citation) but is beyond the scope of this thesis.

In contrast, the phasic activity level for the LC is described as a state of spontaneous and fast discharge rates (10-20Hz) (Devilbiss and Waterhouse, 2011). Specifically, phasic activation of LC

---

[4] Many studies investigating NE concentrations in PTSD have also found increased amounts of NE in the cerebrospinal fluid of individuals with PTSD, specifically military PTSD (Geracioti et al., 2001; Hendrickson et al., 2018). This suggests that increased tonic activity of the LC may lead to increase NE release thus contributing to these altered states of arousal (Howells et al., 2012).

neurons facilitates attentional processes such as in the presence of novel stimuli (Howells et al., 2012). When this occurs, there is a physiological shift from tonic to phasic firing (Howells et al., 2012). This allows individuals to 1) bring their attention to the novel stimuli (alert), and 2) focus their attention on the stimuli (orient) (Posner et al., 2006; Howells et al., 2012) In addition, it has been found that phasic activation can synergistically depend on the basal tonic activity of the LC, suggesting that when tonic activity is elevated, as seen with increased arousal, patterns of phasic activity occur outside their optimal range producing attentional impairments[5] (Howells et al., 2012). This has been suggested to lead to increased vigilance, activation of broad scanning attentional processes and reduced performance on cognitive/behavioural tasks (Aston-Jones and Cohen, 2005; Howells et al., 2012). These features, alongside hyperarousal are yet other characteristics of psychiatric conditions such as PTSD, suggesting that altered LC physiology, and consequently increased NE release may contribute to symptoms of this condition.

1.1.4 – Projections & Functions

Next, I will discuss the different efferent and afferent pathways associated with the LC. The LC projects widely across the brain, implicating it in numerous biological functions. When these pathways become dysregulated, this may contribute to the pathogenic features observed in some neuropsychiatric conditions. As such, by understanding the targets of the LC and the structures that feedback to the LC, we may begin to understand what happens at a systems level in conditions such as PTSD where the LC-NE is suggested to be affected.

---

[5] During states of distress or anxiety, tonic activity occurs outside its optimal range. As phasic firing also depends on this optimal range, phasic firing is also affected during time leading to increased NE release (Howells et al., 2012). This increases cortical arousal, reducing optimal performance in cognitive and/or behavioural tasks contributing to impairment of this system. Impairment here is only defined as lack of optimal performance on cognitive tasks as suboptimal arousal (hyperarousal in states of anxiety or hypoarousal in conditions such as attention deficit hyperactivity disorder) (Howells et al., 2012).

1.1.4 (a): Efferent Projections

Despite its small size, the LC has widespread connections throughout the brain (see Figure 1 for diagram of LC targets). Using anterograde and retrograde tracing methods, many studies have delineated the projections to and from the LC. Delineating these pathways helps to understand the functions of the LC-NE system both centrally and peripherally (Cedarbaum and Aghajanian, 1978; Waterhouse et al., 1983; Simpson et al., 1997; McCall et al., 2015, 2017; Gerfen and Sawchenko, 2016). In addition, topographical organization may indicate that different subpopulations of neurons in the LC may project to different targets, implicating different regions of the LC in different functions (Waterhouse et al., 1983). However, due to the small size of the LC, it has been difficult to characterize the functional organization of the LC for instance along its rostra-caudal axis, emphasizing the need for more advanced methodology (i.e. in-vivo projection tracing) (Keren et al., 2009). Summarized below are the general projections for the LC including both the efferent and afferent pathways. Also summarized below are the general functional modalities associated with these pathways, emphasizing those most relevant for the current project.

With respect to the efferent projections of the LC, a study by Szabadi and colleagues in 2013 suggests there are three primary efferent pathways leading out of the LC (Szabadi, 2013; Bari et al., 2020). First, there is the ascending pathway which contains projections to the cortex, subcortical limbic structures as well as the thalamus and the other midbrain nuclei (Szabadi, 2013; Bari et al., 2020; Morris et al., 2020a). This efferent pathway is implicated in emotional memory (van Stegeren, 2008; Tully and Bolshakov, 2010; Jacobs et al., 2020), the stress response (Berridge and Waterhouse, 2003; McCall et al., 2015), autonomic nervous system (ANS) regulation (i.e. both central parasympathetic and sympathetic functions) (Samuels and Szabadi, 2008), as well as functions related to the sleep-wake cycle (Berridge and Waterhouse, 2003; Samuels and Szabadi, 2008; Berridge et al., 2012) and other executive functions (Aston-Jones and Cohen, 2005; Bari et al., 2020).

Due to the vast innervation of this pathway within the prefrontal cortex (PFC), limbic structures and ANS centers, brain regions critical in regulation of emotion, behavior and stress response (Samuels and Szabadi, 2008; Szabadi, 2013) dysregulation in this pathway (e.g. enhanced NE release at these targets), may further contribute to symptoms of psychiatric illness (e.g. hyperarousal in PTSD) (Geracioti et al., 2001; Morey et al., 2015; Ronzoni et al., 2016; Naegeli et al., 2018).

The other two major pathways projecting from the LC are the descending pathway and the cerebellar pathway (Szabadi, 2013). Of these two additional pathways, the descending pathway is better understood (Bari et al., 2020). The descending projections innervate the spinal cord and contribute to motor outputs (Samuels and Szabadi, 2008; Szabadi, 2013). It is also hypothesized that these descending projections play a vital role in pain modulation, specifically pain desensitization, as the NE receptors here have analgesic properties (Westlund and Dan Coulter, 1980; Clark and Proudfit, 1991; Taylor and Westlund, 2017; Bari et al., 2020). In addition, the descending projections are also thought to mediate peripheral autonomic centers, however, this is less well understood (Samuels and Szabadi, 2008). In particular, it is suggested that there is a complex relationship between LC activation and modulation of autonomic physiological responses (i.e. changes in respiration, heart rate and blood pressure), though this requires further investigation in human samples (Fritschy and Grzanna, 1990; Bruinstroop et al., 2012). Altogether, based on the number of efferent projections, the LC has vast neuromodulatory capabilities and when dysregulation of this system occurs, as observed in numerous disorders, dysfunction of these target areas could account for the altered circuit function and maladaptive behavior.

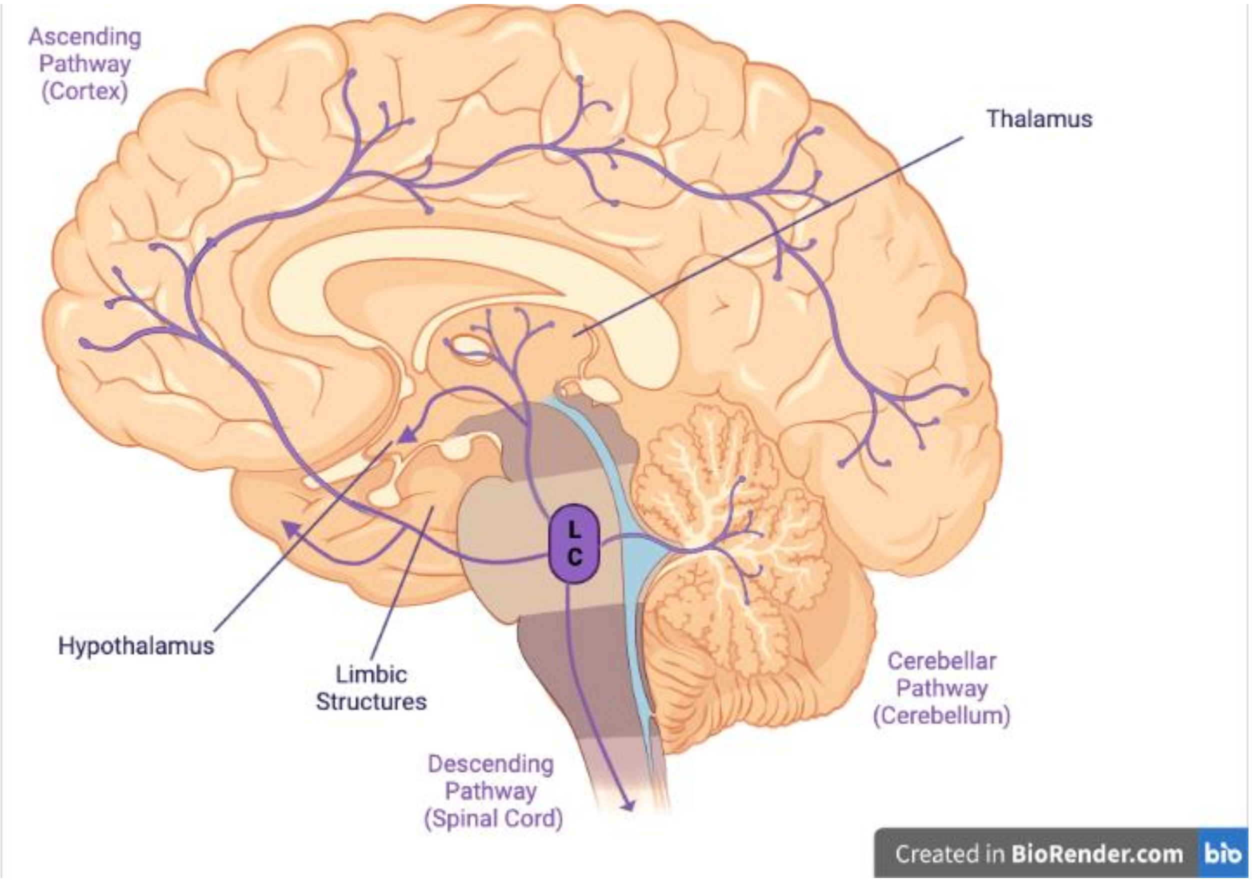

Figure 1: Visual of LC targets. The LC projections are widespread across the brain. Highlighted here are the three established LC pathways: the ascending pathway, the descending pathway, and the cerebellar pathway. Other targets of interest include structures of the limbic system, the thalamus, and hypothalamus. Image was created by the author AM using open access medical illustration website BioRender.com.

1.1.4 (b): Afferent Projections:

Finally, in terms of afferent projections, many of the innervated centers described above also feedback to the LC, thereby modulating its physiological activity and altering the release capacity of NE to LC targets (Aston-Jones et al., 1991; Valentino and Van Bockstaele, 2008). This feedback network allows for regulation of the LC from other centers which can have both adaptative and maladaptive effects. For instance, the LC receives vast innervation from the amygdala, a major limbic structure involved in regulation of emotions, memory and the stress response (i.e. regulation of the "fight or flight" response) (Wallace et al., 1989; Charney et al., 1998; McCall et al., 2015). Specifically, in recent research it has been suggested that upon stress-induced activation, the amygdala releases corticotropin-releasing factor (CRF) along efferent projections targeting the LC (Aston-Jones et al., 1991; Weiss et al., 1994; Valentino and Van Bockstaele, 2008)[6]. This suggests, that upon interaction with LC CRF receptors, the LC shifts from low tonic activity to heighted tonic activity, linked to heighted levels of anxiety (Valentino and Van Bockstaele, 2008; Kovács, 2013; McCall et al., 2015). In addition, animal research investigating the role of the LC-amygdala connection in PTSD showed that abnormal increases in NE at the level of the amygdala may underlie hyperarousal symptomology in PTSD, providing additional evidence to support how interconnected systems and dysregulation of these systems may contribute to disease pathology (Ronzoni et al., 2016).

In addition, other structures such as the PFC, regions of the hypothalamus, brainstem, and indirect afferents from the spinal cord have been observed to project to the LC (Szabadi, 2013). Here, the PFC is thought to help regulate the tonic activity of the LC, whilst the hypothalamic structures help coordinate the LC's role in sleep-wake functions (Szabadi, 2013). Furthermore, brainstem structures such as the medulla oblongota are thought to feedback to the LC to help regulate autonomic functions (Kumagai et al., 2012), while populations of neurons in the spinal cord (i.e. the dorsal horn) send afferents to the LC containing information about pain and other sensory signals (Cedarbaum and Aghajanian, 1978; Szabadi, 2013).

---

[6] CRF is a stress hormone that is released during periods of increased anxiety/stress. It has an important role in regulating the hypothalamic-pituitary-adrenal (HPA) axis (i.e. initiates the release of cortisol, another key regulator of the stress response) (Kovács, 2013).

In conclusion, this summary further illustrates the complexity of the LC-NE system and how its numerous projections implicate it in a multitude of brain circuits and reveals the inherent complexity of downstream effects throughout the brain should the LC-NE system become dysregulated. Therefore, when investigating neurodegenerative or psychiatric disorders where LC-NE dysfunction is implicated, interactions with numerous brain regions should be considered.

1.1.5 Clinical Implications

Over the years, several neurodegenerative and psychiatric conditions have been associated with dysregulation in the LC-NE system. The purpose of this section will be to briefly discuss the clinical implications of the LC-NE system in neurodegenerative conditions such as AD and PD, with a more thorough discussion being dedicated to the role of LC-NE dysregulation in psychiatric conditions such as depression, anxiety, and PTSD.

1.1.5 (a): Neurodegenerative Conditions: Alzheimer's Disease & Parkinson's Disease

Alzheimer's Disease (AD)

AD is a chronic neurological condition characterized by neuronal death, neurofibrillary tangles and the accumulation of amyloid plaque leading to dementia-like symptoms. Briefly, one hallmark feature of AD is the loss of neurons, specifically in the LC (Mann, 1983; German et al., 1992; Manaye et al., 1995; Weinshenker, 2008; Kelly et al., 2017). In fact, research conducted utilizing post-mortem analyses have found that individuals with greater LC degeneration performed worse on neuropsychiatric and neurocognitive testing prior to death compared to those with less degeneration (Kelly et al., 2017). This research provides evidence for the role of the LC-NE system in several cortical processes including different forms of memory (i.e. working memory, episodic memory and semantic memory), arousal and attention, thereby implicating this system in the pathogenesis of neurodegenerative conditions such as AD (Kelly et al., 2017). While AD is not the focus of this thesis, it is notable that the methods utilized here (i.e. NM-MRI in the LC) have repeatedly been used to study this condition[7], thus, providing the foundation for the methods utilized in our current work.

Parkinson's Disease (PD)

In addition, to further illustrate the role of the LC in neurodegeneration, it has also shown that LC degeneration also occurs in PD, a neurodegenerative condition characterized by motor deficits and the accumulation of α-synuclein protein in several brain regions (Paredes-Rodriguez et al., 2020). In this context, degeneration of the LC is thought to occur in the early, asymptomatic stages of the disease (Braak stage 2) and has been observed in PD-related prodromal conditions such as REM sleep behaviour disorder (RBD) (Boeve et al., 2007) and Lewy body dementia (Carballo-Carbajal et al., 2019). This further implicates the LC in the underlying pathology of neurodegenerative conditions such as AD and PD and as such should be the target of investigation for further study in these areas of research.

1.1.5 (b): Psychiatric Conditions: Depression, Anxiety, and PTSD

Due to the extensive innervation with limbic structures such as the hippocampus, amygdala, hypothalamus and the PFC, the LC plays an important role in regulation of the stress response (Berridge and Waterhouse, 2003; McCall et al., 2015). The LC also innervates brain centers involved in modulation of the sympathetic and parasympathetic nervous systems, centers that play a vital role in the "fight-or-flight" response, linked to specific physiological responses (i.e. pupillometric responses, skin conductance, heart rate, blood pressure, etc.) which can be used as markers for increased arousal in both the central and peripheral nervous systems (Gutner et al., 2000; Orr and Roth, 2000; Felmingham et al., 2011; Murphy et al., 2011; Minassian et al., 2014; Cascardi et al.,

---

[7] A summary of the use of NM-MRI in AD research can be found in Section 1.2.4.

2015). Together, this suggests that when the stress response is dysregulated (i.e. chronic stress), as commonly seen in psychiatric conditions such as depression, anxiety and PTSD, the LC-NE system is also implicated in the pathology underlying these conditions and thus should be considered when establishing biomarkers of these diseases (Morris et al., 2020a)[8]. In addition, consistent with the involvement of the LC-NE system in psychiatric illness, numerous studies have investigated pharmaceutical remedies targeting this system for neuropsychiatric conditions such as depression, anxiety, and PTSD. To summarize, it has been observed that many of these NE-targeting drugs are moderately successful at treating symptoms of the above conditions thereby implicating this system in disease-related pathology (Connor et al., 1999; Southwick et al., 1999; Davidson et al., 2006; Strawn and Geracioti, 2008; Alexander, 2012; Raskind et al., 2013, 2018; Khachatryan et al., 2016; Grasser and Javanbakht, 2019; Hendrickson et al., 2021). Below is additional evidence to support the role of the LC-NE system in both depression, anxiety, as well as PTSD.

In the case of depression, post-mortem research found that individuals with depression had a marked reduction in NE transporters compared to healthy controls (Klimek et al., 1997). This finding was found to be consistent with existing pharmacological evidence (i.e. the use of serotonin-norepinephrine reuptake inhibitors (SNRIs) and norepinephrine-dopamine reuptake inhibitors (NDRIs) as the first-line agents for the treatment of depression) (Stahl, 1998; Gorman and Kent, 1999; Dionisie et al., 2021), as well as the monoamine hypothesis for depression which states that marked reduction of monoamine neurotransmitters including NE contribute to depression-related pathology (Stahl, 1998). Consequently, it should be noted that there are many inconsistencies with

---

[8] Under periods of chronic stress vast cellular and molecular changes occur at the level of the LC (i.e. increases in NE precursors and NE receptors/transporters at LC targets (Fan et al., 2014), overall increase in NE release and LC sensitivity to stress (Jedema et al., 2001) etc.) thereby leading to dysregulation of this system in condition such as anxiety, depression and PTSD where chronic, pathological stress are key characteristic features (Morris et al., 2020a).

the monoamine hypothesis of depression that cannot be explained simply by deficiencies in neurotransmitter systems such as the NE system (Racagni and Popoli, 2008). This therefore supports why NE-targeting drugs are only somewhat effective for treating depression-related symptoms and suggests that dysregulation of the LC-NE system may only be one of the many systems involved in in depression and anxiety pathology. As such, due to the many complexities associated with this condition, for the purposes of this current thesis, the relationship between NE and depression symptoms will only be explored secondary to primary analysis regarding PTSD.

Regarding anxiety, recent high resolution 7T NM-MRI has been utilized to delineate the LC in both healthy controls and in those with pathological anxiety (Morris et al., 2020b). Here, Morris and colleagues observed an increase in LC volumes in those with diagnosed anxiety disorders and were able to correlate these volumetric measures to clinical measures of anxiety (Morris et al., 2020b). They concluded that this was the first study to investigate the relationship between in-vivo LC measures and the manifestation of anxiety-related symptoms, thus providing further evidence to support the role of the LC-NE system in stress-related pathologies observed in psychiatric illnesses (Morris et al., 2020b). In addition, this research was also fundamental to our work in PTSD, a condition featuring prominent symptoms of anxiety.

Regarding the role of the NE system in PTSD, the disorder central to the current investigation, several studies have been published hypothesizing that imbalances in the NE system may lead to PTSD-related symptoms including those related to mood alterations, hyperarousal and disruption in sleep-wake cycles, including one of the most commonly reported symptoms, nightmares (Strawn and Geracioti, 2008; Hendrickson and Raskind, 2016; Betts et al., 2019). Specifically, numerous clinical trials have been conducted investigating the use of Prazosin, an NE alpha-1 antagonist for the treatment of PTSD symptoms (Strawn and Geracioti, 2008; Khachatryan et al., 2016; Raskind et al., 2018). The results from this body of research has been mixed, but researchers have speculated that the LC-NE system may be implicated in only a subset of symptoms (i.e. hyperarousal and sleep-wake cycle dysregulation) versus all PTSD symptom clusters (Hendrickson et al., 2021). Furthermore, other research has showed that greater startle responses (demonstrated by increased eye-blinks, skin conductance and heart rate response) were observed amongst individuals with PTSD in response to loud sounds and that these responses were significantly correlated with greater LC activity measured with BOLD fMRI (Naegeli et al., 2018). This finding makes sense since elevated startle response falls within the hyperarousal subdomain of PTSD (American Psychiatric Association, 2013). In addition, several studies have reported increased NE levels in the cerebrospinal fluid of individuals with PTSD indicating marked changes in the NE system in those with this condition (Geracioti et al., 2001; Hendrickson et al., 2018). These researchers also demonstrated that these elevated NE levels correlated strongly with severity of PTSD symptoms (particularly hyperarousal symptoms and sleep symptoms), further implicating the role of NE in PTSD pathology (Hendrickson et al., 2018). Furthermore, Morey and colleagues demonstrated increased LC activation in response to a functional MRI fear conditioning task in military veterans with PTSD (Morey et al., 2015). This further implicates dysregulation of the LC-NE system in PTSD pathology, specifically fear generalization and dysregulation in fear-related neurocircuitry that may give rise to physiological responses characteristic of hyperarousal (i.e. increased heart rate, blood pressure, pupil diameter, skin conductance response etc.) (Berridge, 2009; Morey et al., 2015; Hendrickson and Raskind, 2016). Together, these studies provides evidence to support the hypothesis that symptoms of PTSD are associated with a dysregulated LC-NE system, specifically that hyperactivity of this system may contribute to clinical symptoms and physiological manifestations of hyperarousal in PTSD (Gutner et al., 2000; Orr and Roth, 2000; Cascardi et al., 2015; Hendrickson and Raskind, 2016; Norrholm et al., 2016).

1.2.1 Neuromelanin & Neuromelanin-Sensitive MRI

In this section I will be discussing what NM is, the role of NM in the human brain, NM-MRI and how NM-MRI has been utilized in a clinical setting with regards to both neurodegenerative and psychiatric conditions. The purpose of this chapter is to understand what NM is and how a novel, non-invasive method known as NM-MRI was developed and has been useful in developing hypotheses for the current work.

1.2.2 Properties of Neuromelanin

NM is a dark brown pigment that accumulates in the cells of catecholaminergic neurons. The build-up of NM can be observed in the cell body of neurons in the LC and the substantia nigra (SN). The accumulation of NM in these regions is due to oxidative processes of dopamine (DA), NE and other catecholamines (Zecca et al., 2008). This occurs within the cytosol of these cells during a process known as neuromelanogenesis (Bazelon et al., 1967). In addition, NM is a unique type of pigment belonging to the melanin family and contains properties of eumelanins and pheomelanins (Odh et al., 1994) thereby, sharing resemblance with cutaneous melanin found in our skin and hair (Odh et al., 1994; Fedorow et al., 2005). Moreover, NM accumulates in autophagic organelles (Sulzer et al., 2000; Zucca et al., 2018) within the cells and over the lifespan, there is a significant increase in NM concentration in both the LC and SN structures (Mann, 1983; Manaye et al., 1995; Zucca et al., 2006; Zecca et al., 2008; Wakamatsu et al., 2012). Thus while NM accumulates continuously in catecholaminergic cells throughout the lifespan, clearance of NM, and thus, reduction in NM concentration only occurs as a result of cellular death, as in neurodegenerative conditions such as AD and PD (Kelly et al., 2017; Betts et al., 2019; Bari et al., 2020). Some researchers have theorized that the consistent increase in NM across the lifespan can ultimately create such an abundance of NM in the cells in late life that pathogenic effects may ensue and contribute to cell dysregulation (Carballo-Carbajal et al., 2019; Vila, 2019). This has been observed in cases of Lewy body dementia (i.e. pre-

PD) in which individuals demonstrated abnormally high levels of NM in comparison to healthy matched controls suggesting that NM accumulation in these individuals may be abnormally increased (Carballo-Carbajal et al., 2019). Conversely, it has also been speculated that abnormally high levels of NM may in part be due to a compensatory mechanism of this system in-vivo suggesting that degeneration still occurs, but remaining cells become hyperactive (Gannon et al., 2015; Liu et al., 2018; Sulzer et al., 2018; Weinshenker, 2018). This hyperactivity thus causes an increased accumulation of NM to compensate the loss of NM-containing cells due to diseases such as AD, PD, and prodromal syndromes such as Lewy body dementia (Gannon et al., 2015; Liu et al., 2018; Sulzer et al., 2018; Weinshenker, 2018). Together, the above research demonstrates the important relationship between NM and neurodegenerative conditions such as AD and PD and provides a unique perspective about possible future therapeutic targets for these conditions which could also be applied to other human-related conditions where NM-containing structures are implicated.

As for the role of NM in the brain, conflicting studies have suggested that NM may offer a protective function whist contained in autophagic organelles, but may accelerate degenerative processes once released, thereby promoting apoptosis (Zecca et al., 2008; Zhang et al., 2011). For example, in chemical analyses of NM synthesis, the formation of NM was found to be associated with the sequestering of free radical molecules known to be damaging to the surrounding cells (Enochs et al., 1994). These molecules included cytotoxic organic molecules (i.e. reactive oxygen species) (Zhang et al., 2011) and cytotoxic metals (i.e. iron) (Zecca et al., 2008), many of which lead to damaging redox activity in the cells. As a result, without the removal of these molecules, free-floating radical compounds have continuously been found to be the cause of cellular apoptosis, thus accelerating neurodegenerative processes in the human brain (Enochs et al., 1994).

In contrast, studies by Zecca and colleagues have demonstrated that free-floating NM can induce microglia activation thereby contributing to inflammation of surrounding SN neurons and inevitably leading to further cell death (Zecca et al., 2002, 2008; Beach et al., 2007; Zhang et al., 2011; Carballo-Carbajal et al., 2019). In addition, it has also been hypothesized that once NM is released extracellularly, previously sequestered cytotoxic molecules such as reactive iron can remain in the extracellular space which could also promote additional cell death and contribute to clinical pathological characteristics seen in PD (Gerlach et al., 2006; Zucca et al., 2017). As such, it appears that NM may have both protective and degenerative properties in the human brain and may contribute to neuropathology characteristic in several neurodegenerative conditions.

1.2.3 Neuromelanin-Sensitive MRI

NM-MRI is a novel, non-invasive neuroimaging method developed by Sasaki and colleagues in 2006 to visualize NM-containing regions such as the LC and SN (Sasaki et al., 2006a). This technique is possible due to the paramagnetic properties of NM which exist due to the presence of iron bound to NM (Sasaki et al., 2006a). As a result, NM causes T1 shortening effects which thereby allows NM-containing centers to be observed on MRI images using specific MRI sequences (i.e. those including magnetization transfer (MT) contrast) (Enochs et al., 1997; Sasaki et al., 2006a; Trujillo et al., 2017). In addition, to further distinguish NM-MRI imaging from standard structural imaging, Figure 2 demonstrates the differences between a normal structural MRI image (left) and a NM-MRI image (right). Here the NM-MRI contrast allows the LC to be visualized as hyperintense regions within the pons (black arrows point to the location of the LC in both images).

Moreover, NM-MRI signal can be extracted using different analytical processes including the manual tracing approach conducted in many early studies including the initial one by Sasaki and colleagues (Sasaki et al., 2006a). Since the original publication, several advances have been made regarding the analysis of NM-MRI data including a new semi-automated technique developed by our lab (see Chapter 3 for details on the methods applied for this body of work). This automation allows for a reduction in unconscious biases that can occur during manual processing of neuroimaging data, and allows for a fast, reliable streamlined approach for analyzing this type of data. For the purpose of this work, a great deal of time was invested in automating this process such that we could reduce the number of manual steps typically required. As such, advancing the methodology of extracting and analyzing LC NM-MRI data became one of our objectives for this thesis.

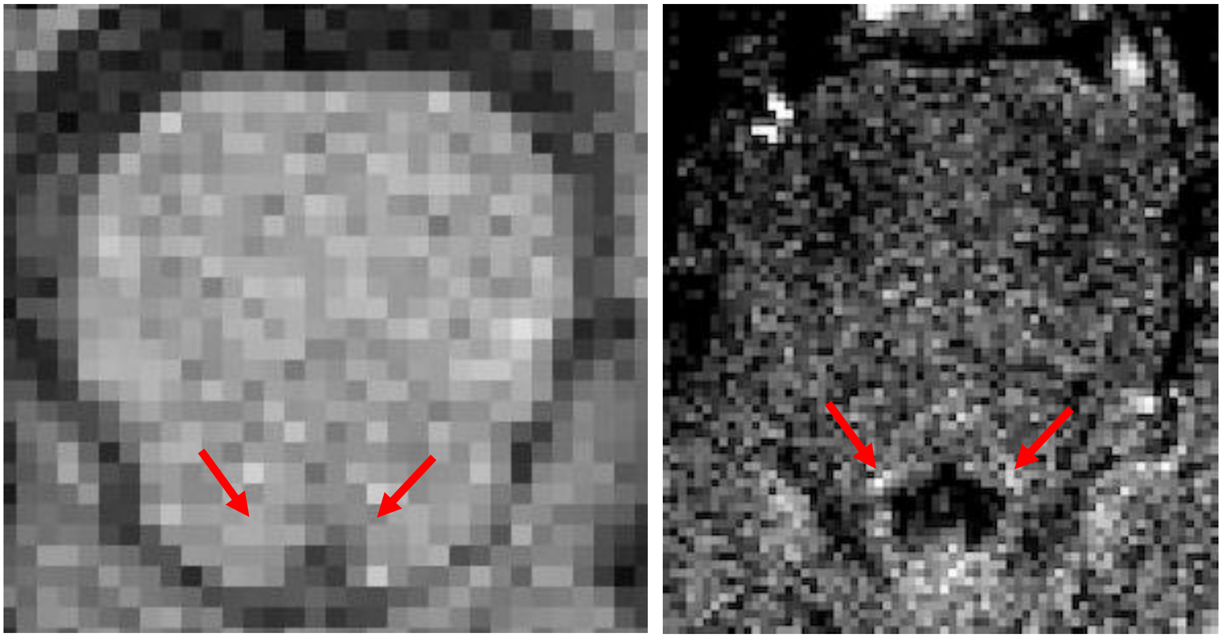

Figure 2: Unprocessed MRI images of the pons highlighting LC location. Left: Structural T1 scan of the pons (red arrows demonstrate where the bilateral LC would be anatomically). Right: NM-MRI scan of the pons in which the bilateral LC be identified as hyperintense voxels at the posterior end (red arrows indicate relative location of LC).

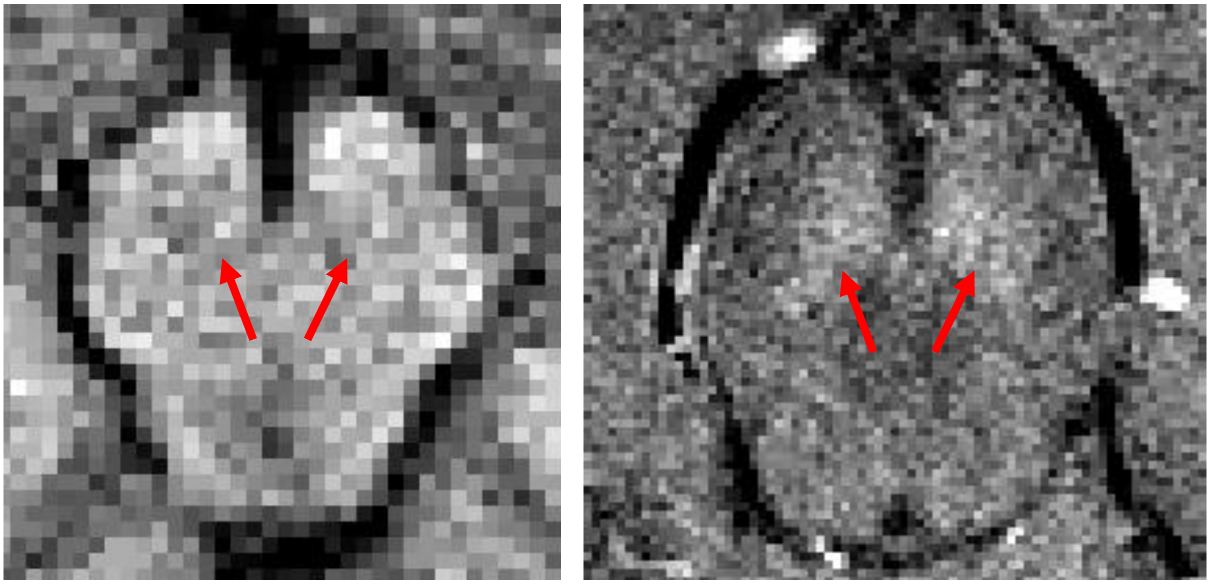

Figure 3: Unprocessed MRI images of the midbrain highlighting SN location. Left: Structural T1 scan of the midbrain (red arrows demonstrate where the bilateral SN would be anatomically). Right: NM-MRI scan of the midbrain in which the bilateral SN can be identified as hyperintense voxels at the superior end (red arrows indicate relative location of SN).

1.2.4 Applications of Neuromelanin-Sensitive MRI: Clinical Significance

As discussed in Section 1.1.5, a characteristic feature of both AD and PD is the selective loss of NM-containing neurons in the LC. As a result, decreased NM concentration is observed in individuals with these conditions thereby leading to many of the characteristic symptoms associated with these conditions (i.e. loss of NM-containing cells in the DA rich SN has been associated with the classical motor symptoms of PD) (Lees et al., 2008; Vila, 2019). The loss of pigmented cells in relation to these conditions was first shown in post-mortem studies in which researchers were able to quantify the loss of NM-containing cells in either the LC and/or the SN (Mann and Yates, 1979; German et al., 1988, 1992; Baker et al., 1989). Specifically, in an early study by Mann and Yates, researchers conducted a post-mortem analysis amongst individuals with PD (Mann and Yates, 1979). Here, they were able to manually count the pigmented neurons of the SN and when compared to healthy subjects, were able to determine that there was a significant decrease in NM concentration in the SN of individuals with this condition (Mann and Yates, 1979). Similarly, research conducted in AD populations found loss of pigmentation in the LC[9] (Mann, 1983; German et al., 1992; Manaye et al., 1995; Weinshenker, 2008; Kelly et al., 2017), thus implicating NM-containing regions in the neurodegenerative characteristics of these conditions.

Due to the link between NM and neurodegeneration, NM-MRI was developed to further study populations of individuals with such conditions in vivo. Much of this work has compared NM-MRI signal in healthy individuals and those diagnosed with AD and PD. For example, in the original clinical study applying NM-MRI, a significant decrease in NM-MRI signal was observed in both the LC and SN in individuals with PD (Sasaki et al., 2006a). This reduction in NM-containing cells in both regions was not observed in the comparative healthy control group, indicating that this new neuroimaging method could be used not only to visualize NE and DA containing structures such as the LC and SN, but also to detect changes in these structures in relation to PD pathology (Sasaki et al., 2006a). Subsequently, the method has been used to investigate a wide range of neuropsychiatric conditions (see Cassidy et al., 2019, 2020; Biondetti et al., 2020; Gaurav et al., 2021; Wengler et al., 2021 for recent examples of clinical NM-MRI research). And while many of the early NM-MRI studies focused on the SN signal, the LC has been of particular relevance to studies in AD. For example, individuals with AD showed a 22% reduction in NM-MRI signal in the LC compared to matched controls (Hou et al., 2021). This research emphasizes the role of the LC-NE system in degenerative conditions such as AD and the relevance of NM-MRI to detect such alterations. This research has also helped to develop NM-MRI methods for the LC, as a marker of progression of neurodegenerative conditions. NM-MRI may be used not only as a marker of loss of catecholamine

---

[9] See Section 1.1.5 for additional details.

cells, but also as a measure of the function of these neurotransmitter systems in non-neurodegenerative conditions such as psychiatric disorders. With these pioneering studies developing NM-MRI methods for the LC in neurodegenerative conditions, the potential arises for more exploratory work in more novel applications, including psychiatric disorders where the norepinephrine system is implicated, thus providing the foundation for our current work.

Indeed, NM-MRI has been applied to several psychiatric conditions including psychosis, depression, addiction and now PTSD. For example, previous research from our group has suggested that NM-MRI could be used as a proxy measure for DA function in the SN and be utilized in the context of psychosis, a syndrome characterised by hyperactivity of the DA system (Cassidy et al., 2019). In addition, this study also acted as a validation study for NM-MRI methods in that it was used to validate both the imaging methodology and analysis method for measuring NM-MRI signal at high resolution (Cassidy et al., 2019). This validation consisted of comparing NM-MRI data to gold-standard PET data (Endres et al., 1997; Laruelle et al., 1997; Ito et al., 2017) and post-mortem NM analyses (Zecca et al., 2002; Keran et al., 2015), methods that have been previously utilized to detect changes in the DA system (PET-imaging) as well as to measure NM concentration (post-mortem analyses) (Cassidy et al., 2019). Based on this, this work supported the use of NM-MRI to measure changes in the DA-SN system even in the absence of degeneration (Cassidy et al., 2019). Finally, this work provided the fundamental support for the current investigation, that NM-MRI signal may be sensitive to disorders of catecholamine function, presumably due to increased release or turnover of these neurotransmitters, leading to elevated NM formation. Based on this, we speculated that a similar approach could now be conducted for the LC-NE system such that we could further validate the use NM-MRI method for applications to this system, for instance in patients with PTSD, a condition where the LC-NE system may be hyperactive.

1.3.1 Post-Traumatic Stress Disorder

In the previous section, I briefly discussed the role of the LC-NE system in psychiatric illnesses including PTSD. Specifically, I described how the LC-NE may contribute to PTSD-related symptoms such as hyperarousal and how modulation of the system can contribute to increased treatment efficacy in those affected. The purpose of this section will be to describe PTSD, who it affects, epidemiological findings, diagnostic criteria, pathophysiological information, risk and protective factors, and therapeutics.

PTSD is a mental health condition that can develop in response to a traumatic event. Those affected report directly experiencing or witnessing events such as war, sexual or physical assault, car accidents, fire, natural disaster, and/or childhood abuse (American Psychiatric Association, 2013). As a result, debilitating symptoms related to these experiences can become present and can severely affect different aspects of an individual's life. Common symptoms reported by individuals with PTSD include hypervigilance, dissociation, avoidance behaviours, sleep disturbances (i.e. nightmares), flashbacks and overall alterations in mood and cognition. In short, these symptoms are associated with an overall decrease in the quality-of-life of affected individuals and has been associated with high rates of disability (Sareen et al., 2007) including an increased risk for suicidality (Chou et al., 2020). As a result, there is a need for PTSD-related research and clinical treatment development for individuals affected by this condition.

1.3.2 Epidemiology

PTSD is a heterogeneous condition that affects numerous demographics within our society. In Canada, it is suggested that 9.2% of individuals from the general public will meet diagnostic criteria for PTSD during their lifetime (Van Ameringen et al., 2008), while 2.4% will meet diagnostic criteria in the last month, suggesting a relatively high prevalence rate of this condition in our country (Van Ameringen et al., 2008).

Amongst the demographics affected by this condition, military members are at a greater risk for trauma exposure due to the nature of their experiences. For the purpose of our current work, military PTSD will remain the focus since the individuals who participated in our study were veterans of the Canadian Armed Forces (CAF). Epidemiological research in military populations found that amongst 8441 CAF members, approximately 86% reported having experienced one or more traumatic events during their time in the military[10] (Brunet et al., 2015). Of these individuals, the conditional rate for PTSD development was 7.7% and the lifetime prevalence rate of PTSD was 6.6% (Brunet et al., 2015). Based on this data, it is clear that there is a high prevalence of PTSD diagnosis amongst military populations, thus emphasizing the need for current PTSD research in these populations.

Furthermore, individuals with military PTSD have been shown to also have a higher incidence for comorbidities including mental health conditions such as mood disorders (i.e. depression) and substance abuse (Jacobsen et al., 2001; Van Ameringen et al., 2008; Gros et al., 2010, 2015; Pompili et al., 2013; Flory and Yehuda, 2015; Klaric et al., 2017; Albott et al., 2021). For instance, amongst 154 veterans with military PTSD, the vast majority (~ 98%) met criteria for other mental illnesses (Klaric et al., 2017). In addition, the majority of these individuals either reported being in a current depressive episode (~ 42%), or had a history of depression (~ 36%) (Klaric et al., 2017). Other reported conditions included general anxiety disorder (~ 83%), panic disorder with features of agoraphobia (~ 11%) and substance abuse, specifically alcohol abuse (approx. 34%) (Klaric et al.,

---

[10] This is compared to the amount of trauma exposure experienced by the general population which was recorded to be approximately 76.1% (Van Ameringen et al., 2008), indicating greater trauma exposure is prevalent amongst military members, including CAF members.

2017). Some individuals from this sample also reported suicidal ideation (26%) which is consistent with other evidence (Klaric et al., 2017). In conclusion, this study is one of many that demonstrate the presence of comorbidities in PTSD[11]. This adds complexity not only to research efforts investigating PTSD, but also from a clinical perspective with respect to treatment options for individuals with these conditions (i.e. due to the overlapping nature of PTSD symptoms with other psychiatric conditions, several augmentation approaches may be needed when addressing an individual's treatment needs) (Gros et al., 2012; Flory and Yehuda, 2015).

With regards to observed gender differences seen in PTSD, it appears that females on average are twice as likely to report symptoms associated with this condition in comparison to men (Pearson et al., 2014). This is also true for other mental illnesses such as depression and anxiety (McLean et al., 2011; Hyde and Mezulis, 2020). In fact, one pre-trauma risk factor for developing PTSD is gender in that being a woman may make an individual more susceptible to developing PTSD after experiencing a traumatic event (Sayed et al., 2015).[12]

In addition, from a military perspective, there is an uneven distribution between the number of men and women serving in the army such that this skewed distribution may affect the gender differences with respect to the prevalence rate of military PTSD[13] (Crum-Cianflone and Jacobson, 2014). For example, in a recent study that looked at gender differences amongst refugees with PTSD, researchers found that similar to many war veterans, or active duty members, refugees also witnessed

---

[11] High comorbidity rates are also observed across community samples with PTSD and is not unique to military populations (see Kessler et al., 1995; Sareen et al., 2007).

[12] It has been reported that females may also be overrepresented in this population since women are more likely to experience sexual assault, a common traumatic event that can lead to the development of PTSD-related symptoms (Breslau et al., 1999; Kelley et al., 2009; Freedy et al., 2010; Maguen et al., 2012; Crum-Cianflone and Jacobson, 2014).

[13] Because of this we also anticipated an uneven distribution amongst participants. This prediction was accurate as we had more male than female recruits for our study.

traumatic war-related events and as such also suffered from military PTSD (Ainamani et al., 2020). In this study, they found that when they looked at the dose-response effect between men and women, women were more likely to report increased severity of their PTSD-related symptoms in response to experiencing a less extreme form of trauma compared to men (Ainamani et al., 2020). Women refugees were also found to have a higher prevalence of PTSD (94% of 182 women in this study) compared to men (84% of 143 men in this study) (Ainamani et al., 2020). Furthermore, a review found that in 7 of the 18 studies reviewed, women were more likely to develop PTSD symptoms post-deployment compared to men (Crum-Cianflone and Jacobson, 2014). The authors suggested that this may be due to women having a long history of serving as aids in the war and thus being exposed to more human remains or gruesome injuries compared to men (Hoge et al., 2007; Crum-Cianflone and Jacobson, 2014). Other hypotheses to support the observed PTSD-related gender differences, suggest that women are more likely to experience childhood abuse, sexual assault or domestic violence that may predispose them and as such sensitize them to future traumatic events (Freedy et al., 2010; Maguen et al., 2012; Crum-Cianflone and Jacobson, 2014). It is also theorized that due to the gender differences in the stress response, women may manifest stress/anxiety differently compared to men and as such be more susceptible for PTSD development (Rosenfield, 2000; Crum-Cianflone and Jacobson, 2014). Finally, if we look specifically at the sex differences amongst CAF PTSD-related symptom reporting, we see that woman are more likely to report symptoms of PTSD (8.8.%) compared to men (4.7%), indicating that females may be more affected by traumatic events compared to their male counterparts (Pearson et al., 2014). Altogether, this research emphasizes the gender differences observed in the epidemiology of PTSD, particularly military PTSD. In addition, this research provides rationale for the study of this condition in the female population while also emphasizing the disproportionate ratio between male and female populations within the military such that woman appear to be at greater risk for experiencing PTSD-related symptoms after exposure to trauma. As such, for the purpose of this current work, we made sure to include females in our sample to account for the gender differences observed in the literature[14].

1.3.3 Diagnostic Criteria

PTSD is characterized by a distinct set of diagnostic criteria. Individuals who are diagnosed with PTSD often experience a wide range of symptoms, many of which relate to hyperarousal, dissociation, intrusion, and other symptoms that are often considered comorbid with other mental illnesses such as depression and anxiety (i.e. alterations in mood and/or cognition and sleep disturbances) (American Psychiatric Association, 2013). Furthermore, any individual (i.e. any age, sex, gender, culture or ethnicity) can experience PTSD and like in many other mental health illnesses, each individual can experience heterogeneity with respect to the symptoms that manifest as a result of this condition. This emphasizes the need for clinical guidelines when assessing and/or treating this condition.

Diagnostic criteria for a PTSD are defined in The Diagnostic and Statistical Manual for Mental Disorders, 5th Edition (DSM-5). (American Psychiatric Association, 2013). The DSM-5 is considered the gold-standard assessment tool for diagnosing mental health conditions and is one of the most clinically used sources amongst practicing psychiatrists[15]. In regards to this condition, there is a particular need for structured diagnostic guidelines as individuals with this condition report a heterogeneous combination of symptoms. One fundamental requirement for a PTSD diagnosis is

---

[14] Although we included women participants in our study, the ratio of men and women participants in our sample disproportionally favoured men. This was not our intentions as recruitment for this study is still on-going and was forced to stop due to the on-going pandemic.

[15] It should be noted here that many assessment tools (i.e. interviews or self-report assessments) have been developed based on the DSM's diagnostic criteria and many of these tools are used concurrently with the DSM-5 criteria in clinical settings. Below, assessment tools such as the CAPS-5 and PCL-5 have been developed for assessing PTSD symptoms and are commonly used in research settings.

trauma exposure, which is defined as the direct experience of or witness to a traumatic event (Criterion A of the DSM-5) (American Psychiatric Association, 2013). Fulfilling Criterion A establishes that a traumatic event has occurred and that an individual's symptoms may be occurring as a result of this event. Commonly reported traumatic events that have been reported to meet Criterion A include experiencing war, sexual or physical assault, car accidents, fire, natural disaster, childhood abuse etc. (American Psychiatric Association, 2013). Furthermore, in addition to Criterion A, individuals must experience specific symptoms to meet criteria for a PTSD diagnosis. These PTSD-related symptoms are subdivided into clusters and a certain number of symptoms from each criterion must be endorsed to establish a PTSD diagnosis. A visual summary of the DSM-5 diagnostic criteria can be found in Figure 4 (American Psychiatric Association, 2013).

For the purpose of our current work, "Criterion E: Marked alterations in arousal and reactivity" (American Psychiatric Association, 2013) is the criterion of interest since hyperarousal symptoms of PTSD have been previously correlated with marked alterations in the LC-NE system (Morey et al., 2015; Ronzoni et al., 2016; Naegeli et al., 2018). Figure 5 summarizes the symptoms associated with this cluster and from a diagnostic perspective, two out of the six must be presented in order for a diagnosis to be given (American Psychiatric Association, 2013).

Figure 4: Summary of DSM-5 diagnostic criteria for PTSD. There are eight diagnostic criteria that must be met in order for a PTSD diagnosis to be warranted. Of these eight criteria, there are four symptom groups (Criterion B, C, D, and E). According to the DSM-5, symptoms of each criterion must be apparent in order for a diagnosis to be given. In addition, symptoms must be apparent for more than a one-month period (Criterion F), be clinically significant (i.e. affect numerous aspects of one’s life) (Criterion G), and must not be due to any underlying conditions (i.e. substance use or medical illness) (Criterion H).

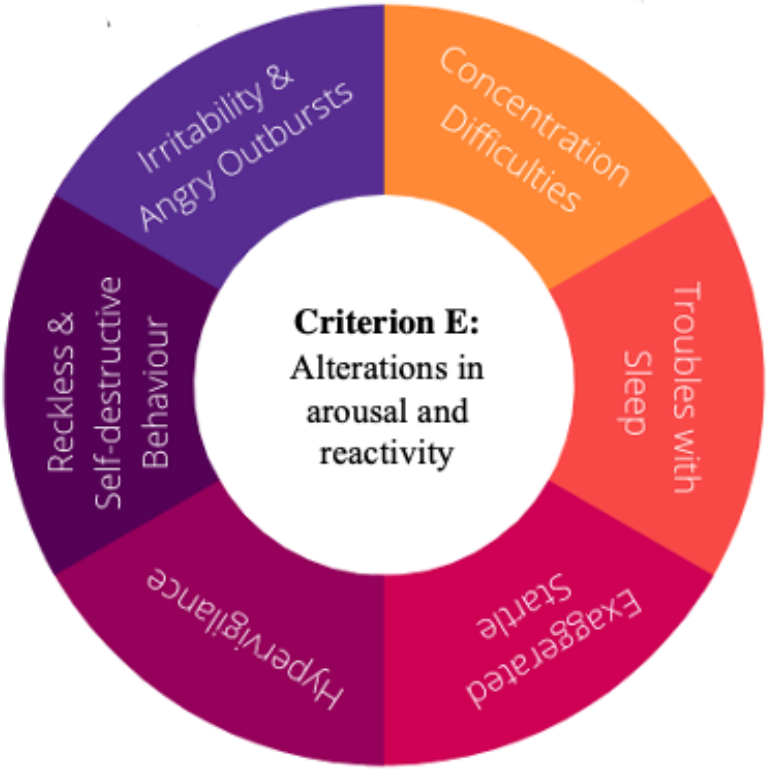

Figure 5: Summary of "Criterion E: Marked alterations in arousal and reactivity" from the DSM-5's PTSD diagnostic criteria. Of the six symptoms shown here, at least two must be apparent in order for a PTSD diagnosis to be given. These symptoms are relevant for our current work given the pre-established relationship between hyperarousal-related symptoms and the LC-NE system.

Furthermore, the DSM-5 also includes a "specify when" section where a distinction can be made between dissociative and non-dissociative PTSD diagnoses (American Psychiatric Association, 2013). These subtypes of PTSD are important since different subtypes may have different underlying symptoms that should be considered on an individual basis. For example, in the dissociative subtype, an individual may experience symptoms related to depersonalization or derealization in which an individual may feel detached from oneself or observe themselves in an alternate or dream-like reality (American Psychiatric Association, 2013). These symptoms may differ from the classical "non-dissociative” subtype of PTSD, however, should still be considered during the initial assessment since these symptoms may also be indicative of PTSD (American Psychiatric Association, 2013).

From a research perspective, two psychometric questionnaires have been developed based on the above DSM-5 diagnostic criteria for assessing PTSD in a research setting. The first is the Clinician Administered PTSD Scale for DSM-5 (CAPS-5) (Blake et al., 1995), and the second is the PTSD Checklist for DSM-5 (PCL-5) (Blanchard et al., 1996). For the focus of our current research, we used the CAPS-5 to assess symptoms associated with PTSD. Here, this tool allowed us to gain information about the individual’s trauma and the symptoms they experienced. It also allowed us to confirm if an individual met the criteria for PTSD according to the DSM-5. More importantly, this tool has shown good validity and reliability in military PTSD populations, thereby justifying our use of it in the work presented here (Weathers et al., 2018).

In terms of the psychometrics of this tool, the CAPS-5 is a thirty-question interview which assesses the symptom domains established by the DSM-5. It is considered the gold standard assessment tool for PTSD diagnosis and symptom recording, and is commonly used across clinical research (Griffin et al., 2004; Weathers et al., 2018; Gilmour et al., 2020). There are three versions of this questionnaire: the lifetime version which establishes the worst month for which PTSD symptoms were most prevalent and distressing, the past month version which looks at the PTSD symptoms over the course of the last consecutive month and the past week version which can be used to establish what symptoms an individual has experienced over the past week (Blake et al., 1995). Diagnoses can be made based on the responses from the lifetime and past month versions.

In addition, there is second tool, The Life Events Checklist for the DSM-5 (LEC-5) (Gray et al., 2004) that should be used alongside the CAPS-5 to establish Criterion A of the DSM-5 (trauma exposure) in a clinical research setting. This tool, is a self-report questionnaire based on the DSM-5 Criterion A and is used to assess the types and amount of trauma/trauma exposure individuals have experienced or witnessed, or if these events were a part of the individual's jobs (Gray et al., 2004). In addition, the LEC-5 can be used to help determine how many traumatic events an individual has experienced, how they experienced them (i.e. directly experienced, learned about or witnessed) and can also be used to establish which trauma (if more than one) has the greatest impact on their day-to-day life. This will then be the trauma that is assessed when administering the CAPS-5 interview.

1.3.4 Pathophysiology of PTSD

As with other mental illnesses, PTSD is a complex, multimodal disease that has numerous pathological features. For the purpose of this discussion, I will focus primarily on the neuroanatomical changes and neurotransmitter imbalances associated with this condition, including discussing functional and structural neuroimaging evidence and the role of the serotonin system in the manifestation of PTSD symptoms. In addition, numerous studies have also been conducted looking at both hormonal changes and genetic links to the disease which will not be discussed in detail here. Altogether, due to the complex nature of this condition, all of these factors should be considered when attempting to identify biomarkers of this condition and for the purpose of our current work, we will be focusing primarily on the relationship between PTSD symptoms and an imbalance in a specific neurotransmitter system, the NE system.

Neuroimaging studies have correlated PTSD symptoms to changes in brain structure and function. For instance, in a structural cross-sectional imaging study by both Gurvits and colleagues (Gurvits et al., 1996) and Bremner and colleagues (Bremner et al., 1995), researchers noted reduced hippocampus volume amongst individuals with PTSD in comparison to healthy controls. In addition, both above studies were conducted in populations with military PTSD, thus establishing structural differences in relation to this condition. Furthermore, abnormalities in hippocampal structure and function has been hypothesized to be linked to abnormal emotional regulation, dysregulated fear learning, and deficits in memory (i.e. emotional memory, declarative memory and long and short term memory circuits) (Harnett et al., 2020). Furthermore, additional work by Bremner and colleagues expanded on these deficits in declarative memory and short-term memory, demonstrating clear evidence that these systems are in fact dysregulated in individuals with PTSD (Bremner et al., 1993, 1997; Douglas Bremner et al., 1995; Douglas Bremner, 2001). Moreover, these deficits have also been linked to other subcortical limbic circuits such as the PFC and amygdala pathways, thus also implicating these systems in PTSD pathology (Bremner et al., 1995; Milad et al., 2009; Fani et al., 2015; Harnett et al., 2020). More importantly, the LC-NE system is also thought to play an important role in modulation of this pathway, thus providing additional support for the role of the LC-NE system in PTSD pathology. For instance, due to the vast innervation of the LC on the limbic system, the LC plays an important role in threat-based learning and modulates emotional memory in response to aversive conditions (Bremner et al., 1996; van Stegeren, 2008; Jacobs et al., 2020; Morris et al., 2020a). Dysregulation of these functions are hallmark features in stress-related conditions such as anxiety and PTSD thus supporting the role of the LC-NE system and its targets[16] in the pathophysiology of the disease (Harnett et al., 2020).

Regarding functional neuroimaging work, numerous studies have been conducted to investigate PTSD and how the functional activity of different brain regions may contribute to the underlying

---

[16] Though not discussed here, structural differences in LC targets have also been reported. Studies of this nature include structural investigation of the amygdala, PFC and white matter structures such as the cingulum bundle and stria terminalis/fornix (Harnett et al., 2020). Here, main findings indicate both morphometric and volumetric changes of these structures in those with PTSD (Harnett et al., 2020). To date, due to the small size of the LC, no structural imaging of the LC has been completed in PTSD populations. Structural analysis of the LC however has been conducted amongst individuals with pathological anxiety where the LC was observed to be enlarged in individuals with this condition (Morris et al., 2020b). This was then positively correlated with anxiety-related hyperarousal, further suggesting an association between the LC, and altered reactivity (i.e. hyperactivity of this system in relation to anxiety disorders).

pathology associated with this condition. For example, altered activity was observed in many limbic structures including the amygdala, PFC and the hippocampus (Hughes and Shin, 2011). Specifically, in a study by Shin and colleagues amongst veterans of the Vietnam war, individuals with PTSD demonstrated increased amygdala activity in response to trauma-related cues (Shin et al., 2005). Other functional imaging studies have utilized fear-conditioning paradigms where altered amygdala activity in individuals with PTSD has been observed. Specifically, during extinction learning, Milad and colleagues observed increased amygdala activity relative to the control group, indicating altered extinction learning in those with PTSD[17] (Milad et al., 2009). In addition, regarding the LC, few studies have been conducted investigating the functional activity of this structure in vivo. This is mainly due to the challenges of imaging the LC due to its small size thereby causing available studies to have notable limitations (i.e. large voxel size and/or poor resolution) (Morris et al., 2020b). Examples of published studies include work by Morey and colleagues (Morey et al., 2015), and Naegeli and colleagues (Naegeli et al., 2018), both of which used LC-targeted functional imaging methods and observed that the LC was hyperactive in individuals with PTSD. This research was fundamental for the development of our hypothesis concerning the correlation between increased LC activity and PTSD symptomology. With respect to NM-MRI imaging, no studies have been conducted using this methodology in a PTSD study, thus, making this the first study of its kind in this sample population.

Furthermore, changes in activity and structure can also mean changes in underlying molecular systems including neurotransmitter systems. Specifically, the catecholamine and serotonin systems have been implicated in PTSD pathology and this is evident through pharmacological and molecular

---

[17] Researchers from this study speculated that altered extinction learning may contribute to problems during the consolidation of extinction memory, a process known to be affected in those with PTSD.

research[18]. For example, evidence supporting the role of the serotonin system in PTSD pathology comes from the moderate success of serotonin-targeting pharmacological agents (i.e. selective serotonin reuptake inhibitors (SSRIs)) to treat PTSD-related symptoms, as well as the copious amount of animal research employing methods such as microdialysis. Specifically, researchers discovered that it was the serotonin receptors in the amygdala that facilitated these actions and as such may be compromised in conditions such as PTSD which is known to have pathological features such as abnormal fear and threat responses (Zanoveli et al., 2009). Furthermore, it has also been shown that agonists of these serotonin receptors may mediate symptoms commonly seen in both PTSD and other anxiety-related thus providing further evidence for the role of this system in PTSD pathology (Southwick et al., 1997). In contrast, clinical trials administering pharmacological agents such as SSRIs have shown some positive effects in treating symptoms related to PTSD (Connor et al., 1999; Stein et al., 2006; Papakostas et al., 2007), however, the results for these trials are mixed supporting the complexities associated with this condition and emphasizing the need for more accurate biomarkers which may increase treatment efficacy in those seeking treatment[19].

1.3.5 Risk & Protective Factors Associated with PTSD

Due to the complexities associated with PTSD, it has been well established that not everyone who experiences a traumatic event will go on to develop PTSD-related pathology. This suggests that like many other disease states, there may be numerous risks and/or protective factors that increases (or decrease) the likelihood of an individual developing this condition. For example, Brewin (Brewin et al., 2000) and Ozer (Ozer et al., 2003) both conducted meta-analyses on the topic identifying a list of

---

[18] In the above section on the LC-NE system I discussed in detail the role of the LC-NE system in PTSD pathology. As such, for the purpose of this discussion I will focus on the serotonin system thus providing a well-rounded overview of the neurotransmitter imbalances associated with PTSD.

[19] At the end of this section a more thorough discussion will be had regarding the clinical trials for SSRI use in PTSD treatment.

common factors implicated in the development/prevention of PTSD-related pathology. Here, the first set of described factors discussed in these analyses are pre-trauma factors or factors that predispose an individual to developing PTSD based on innate characteristics (Brewin et al., 2000; Ozer et al., 2003). Pre-trauma factors include things such as age, gender, socioeconomic status, lower education, IQ, age and family psychiatric history (Sareen, 2014; Sayed et al., 2015; Carlson et al., 2016) and of these pre-trauma factors, gender and IQ were found to more strongly predict PTSD vulnerability compared to other factors where only weak associations were observed (Brewin et al., 2000; Ozer et al., 2003; Sareen, 2014). Specifically in regards to gender, it was found that women were not only at greater risk for developing PTSD, but they were also more likely to experience more severe PTSD-related symptoms compared to their male counterparts (Carmassi et al., 2014; Hu et al., 2017)[20]. Furthermore, it has also been demonstrated that prior experience with mental illness (i.e. anxiety disorders, major depression and/or substance use disorder) (Cameron et al., 2006; Sareen, 2014), as well as previous exposure to traumatic experiences (Brewin et al., 2000; Ozer et al., 2003), specifically childhood abuse or maltreatment (Brewin et al., 2000; DiGangi et al., 2013; Breslau et al., 2014; Carlson et al., 2016) can significantly increase one's susceptibility to developing PTSD. In contrast, in the meta-analysis by Brewin and colleagues, higher levels of education were found to be protective against PTSD symptom development, suggesting that individuals who have completed higher education may be more resilient for the development of PTSD post-trauma (Brewin et al., 2000). Other pre-trauma factors may also include genetic predisposition, though genetic markers for PTSD vulnerability are not well understood (Shalev et al., 1996; Holbrook et al., 2001) and as such will not be discussed in detail here.

---

[20] A full discussion surrounding gender differences in PTSD epidemiology can be found in Section 1.3.2.

Furthermore, in addition to pre-trauma factors there are also factors that can occur during the traumatic event. These factors are called peri-trauma factors or trauma-related factors and include factors directly related to the traumatic experience (Brewin et al., 2000; Ozer et al., 2003). Moreover, these factors can include things such as type of trauma experienced, duration of trauma (i.e. was the experience an isolated incident or was it repeated throughout one's life?), as well as the severity of the traumatic event (Sareen, 2014; Sayed et al., 2015). Specifically, in dedicated studies on this topic, it was found that there as a strong association between type of trauma and elevated risk for PTSD diagnosis (O'Donnell et al., 2008; Zatzick et al., 2008a). Specifically, if individuals experienced trauma that threatened injury or life, individuals would be more likely to report PTSD-related symptoms compared to incidents that were not associated with harm (i.e. women experiencing sexual violence are more likely to report PTSD-related symptoms associated with this experience) (Zatzick et al., 2008a). Furthermore, other factors that predicted PTSD development included experiencing dissociation during the traumatic event (Birmes et al., 2003) or if the individual suffered a mild traumatic brain injury (i.e. many veterans report history of mild traumatic brain injury during their deployment and often this injury occurs during an event which is more often than not considered to be traumatic)[21] (Stein and McAllister, 2009; Greer et al., 2020).

Finally, there are also several risk factors after the traumatic event has occurred that could contribute to increased susceptibility for PTSD. These are called post-trauma risk factors and include factors pertaining to how the individual responds to the events they have experienced and/or have witnessed. These factors include things such as an individual's coping skills (Brewin et al., 2000; Tsai et al., 2012; Sayed et al., 2015), the quality of an individual's social support network (Ozer et al., 2003; Sayed et al., 2015) and an individual's exposure to medical services/therapy after a traumatic event

---

[21] It has been well established that there is a high comorbidity rate between mild traumatic brain injury and PTSD (Schneiderman et al., 2008; Halbauer et al., 2009; Stein and McAllister, 2009; Kontos et al., 2013; Yeo, 2021).

has occurred (i.e. formal therapy, psychological first aid post-trauma, hospital stays, degree of treatment for bodily injuries etc.) (Zatzick et al., 2008b; Forbes et al., 2011; Sayed et al., 2015). Specifically, it was found that individuals who displayed negative/avoidant coping behaviours post trauma, were more likely to report PTSD-related symptoms (Gil, 2005; Hooberman et al., 2010; Thompson et al., 2018). In contrast, when individuals reported positive/active coping mechanism, individuals were less likely to report PTSD-symptoms, suggesting that the type of coping style individuals display can increase the risk for developing PTSD or contribute to an individual's resiliency (Li and Nishikawa, 2012).

In conclusion, based on current literature, it is clear that there are many factors that can contribute to either risk or resiliency for PTSD development. However, it is unclear how these factors work together to mediate risk/resiliency adding further complexity to predicting who will develop PTSD after experiencing a traumatic event. This research provides further evidence to support the complex, heterogenous nature of PTSD, thus providing rationale for the continued study of this disorder.

1.3.6 Current Pharmacological Intervention Approaches

In closing this final chapter, this last subsection will be dedicated to discussing the current pharmacotherapies for PTSD and how with the advancement of biomarkers, more targeted treatment options may become available thus increasing treatment efficacy rates for those affected.

To date, many of the therapies available have been pharmacological agents that have been repurposed from other mental health conditions such as depression, anxiety and even psychosis. Drugs such antidepressants, anti-anxiety and second-generation anti-psychotic medications have shown moderate success in treating PTSD-related symptoms due to the overlapping symptoms between psychiatric conditions (i.e. alterations in mood, changes in sleep and appetite, irritability, anxiety etc.). However, clinical studies of such drugs have shown limited success for achieving full remission from PTSD, thus emphasising the need for more targeted approaches for treatment (Berger et al., 2009; Alexander, 2012). For example, SSRIs such as sertraline and paroxetine have received FDA approval for the treatment of PTSD in the United States (Berger et al., 2009). However, despite 60% of a clinical sample responding to these agents (i.e. demonstrating improvement in PTSD-related symptoms), only 20-30% of individuals in this study met full remission criteria (Zohar et al., 2002; Stein et al., 2006). In addition, other drugs in this category such as fluoxetine have also shown similar results, thus leading researchers and clinicians to search for other pharmacological agents which could be used to treat PTSD-related symptoms (Connor et al., 1999).

Furthermore, in more recent research, NE-targeting drugs have gauged the interest of researchers as these agents have also shown moderate effectiveness in treating PTSD symptoms. This line of therapeutics was suggested due to the speculated role of NE in symptoms such as hyperarousal and sleep disturbances (Southwick et al., 1999; Gören and Cabadak, 2015; Morey et al., 2015; Ronzoni et al., 2016; Naegeli et al., 2018). To be more specific, drugs such as venlafaxine (an SNRI), showed higher response rates compared to SSRIs (approx. 78%) and a slightly higher full remission rate (approx. 40%) when administered in clinical trials (Davidson et al., 2006). In addition, research investigating the use of an NE alpha-1 antagonist (Prazosin) to treat nightmares in military samples has also demonstrated variable results, thus providing additional evidence for the role of the NE system in PTSD pathology and as a potential treatment target (Strawn and Geracioti, 2008; Khachatryan et al., 2016; Raskind et al., 2018).

Together, this research indicates that like many other psychiatric conditions, symptoms associated with PTSD may in part be due to dysregulation in numerous neurotransmitter systems and thus may require an augmentation approach in order for full remission to be achieved (Metcalf et al., 2020).

This adds an additional level of complexity, since PTSD is a heterogeneous condition with a wide range of symptoms that are modulated by several neurobiological systems. As such, based on this research, there is a need for clinical biomarkers to assess for dysregulation in these biological systems, which therefore would allow for advanced, individualistic treatment plans for targeting these imbalances.

CHAPTER 2: RESEARCH HYPOTHESES & OBJECTIVES

In the previous chapter, I discussed the relationship between the LC-NE system and PTSD symptomology. Specifically, based on a review of the literature it is apparent that there is an association between LC-NE system dysregulation and PTSD hyperarousal symptoms. As a result, because of novel neuroimaging methods available to investigate the LC, this relationship can be further analyzed to gain additional knowledge about the role of the LC-NE system in PTSD pathology. As such, based on previously published work, we developed two hypotheses to further explore the relationship between the LC-NE system and PTSD symptoms.

2.1.1 Written Statements of Thesis Hypotheses:

Primary Hypothesis: We hypothesized that there would be a positive correlation between LC NM-MRI signal and hyperarousal symptom severity amongst participants with PTSD.

Secondary Hypothesis: We also hypothesized that there would be a negative correlation between LC NM-MRI signal and depression symptom severity amongst participants with PTSD.

2.2.1 Written Statements of Thesis Objectives:

Primary Objective: Expand on previous work conducted in the dopamine system (SN NM-MRI) by investigating the relationship between LC NM-MRI signal and symptom severity of hyperarousal and depressive symptoms in a sample of individuals with PTSD, a condition suspected to be associated with dysregulation of the LC-NE system.

Secondary Objective: Advance a novel neuroimaging methodology by improving methods of extracting and analyzing NM-MRI data, specifically by implementing a semi-automated approach.

Tertiary Objectives:

i) Provide preliminary evidence of LC NM-MRI signal as a measure of NE system function by relating it to symptoms known to be associated with elevated function of this system

ii) Advance the use of NM-MRI imaging in a clinical psychiatric setting perhaps as a potential biomarker useful for advancing PTSD diagnosis or treatment

CHAPTER 3: METHODS

3.1.1 Methological Approach

This data was obtained from a large, on-going study at the Royal's Institute for Mental Health Research known as A Multidimensional Assessment of PTSD Subtypes (MAPS). This is a multimodal study that incorporates neuroimaging, neurocognitive, epigenetic, sleep, social media, and psychological measures to assess and better understand subtypes of PTSD. From this study, neuroimaging and clinical data was selected with a focus of understanding the relationship between LC NM-MRI signal and hyperarousal symptom severity amongst individuals with PTSD.

3.1.2. Ethics

This study was approved by the review ethics board (REB) at The Royal Mental Health Center. Individuals who participated in the study provided both written and verbal consent and were told they could withdraw from the study at any time. This was repeated to them at each visit. Compensation was also provided to all participating individuals.

3.1.3 Study Protocol

As a part of the MAPS study, participants were subject to a pre-screening telephone interview to determine if they met the inclusion criteria for the study (see Section 3.1.3 (c)). Individuals who met the inclusion criteria were then invited to participate in the study protocol which would span over a two-year period and consist of six on-site visits. For the purpose of this work, neuroimaging procedures were performed on visit three. The clinical interviews/questionnaires were performed at various points throughout the study, however the questionnaires utilized in this body of research (i.e. the CAPS-5 and BDI-II) were both collected at visit one of the study prior to completion of the neuroimaging component[22]. Neuroimaging data was available from the baseline assessment time point (visit 3)[23].

3.1.3 (a) Participant Information & Demographics

From the overarching study, twenty-four individuals underwent neuroimaging procedures including NM-MRI and T1-structural imaging. Individuals were recruited either by word-of-mouth or through The Royal's Operational Stress Injury (OSI) Clinic. Of the twenty-four individuals who participated in the neuroimaging procedures, two individuals were removed (N=22). These two individuals were removed because they did not meet diagnostic criteria for PTSD and were considered controls in the

---

[22] The CAPS-5 was administered at the first visit for individuals meeting inclusion criteria for the PTSD group. The CAPS-5 was administered to ensure individuals in this group met diagnostic criteria for PTSD such that they could be included in this group for duration of the study. The BDI-II was also completed at the first visit and will be readministered at the 12- and 24-month follow-up visits. These visits had not yet occurred at the time of data analysis.

[23] No follow-up neuroimaging data was available at the time of data analysis but should be incorporated into this body of work at a later date.

MAPS study. Since there were only two individuals who did not meet PTSD criteria, no control group could be established. Thus, rather than a case-control design we pursued a dimensional study, focusing on a particular symptom domain, hyperarousal, amongst individuals with PTSD.

Clinical assessments including the LEC-5, CAPS-5 (past-month version) and Beck Depression Inventory (BDI-II) were conducted prior to neuroimaging (see below for the description of each psychometric measure). A summary of the CAPS-5 and BDI-II data can be found below in Table 3. A description of LEC-5 psychometrics and summary of the LEC-5 results is not shown here as the LEC-5 was not completed for every participant (i.e. only ten out of the total twenty-two participants completed this self-report measure). In addition, other assessments such as Positive and Negative Affect Schedule, Columbia Suicide Severity Rating Scale, Beck Anxiety Inventory, Dissociative Subtypes of PTSD Scale, Multiscale Dissociation Inventory, Pittsburgh Sleep Quality Index etc. were also administered, however, none of this data was used in our analysis and therefore will not be reported on/described.

3.1.3 (b) Psychometric Measures

Clinician Administered PTSD Scale for the DSM-5 (CAPS-5)

The CAPS-5 is a structured interview used as a diagnostic tool to establish the prevalence of PTSD-related symptoms that meet diagnostic criteria for PTSD according to the DSM-5 (Blake et al., 1995)[24]. This interview consists of thirty items that assesses different aspects of PTSD diagnostic criteria. These items are subdivided into the DSM-5 diagnostic criterion, with each section of the interview containing items related to a specific subgroup of symptoms. For example, Criterion E of

---

[24] Traditionally, the Life Events Checklist for the DSM-5 (LEC-5) is administered prior to the administration of the CAP-5 interview. For the study in question, only ten of the twenty-two included participants completed the LEC-5 assessment. This discouraged us from using it in our analysis and therefore a description of this measure is not included here.

the CAPS-5 corresponds to items related to hyperarousal. Here, questions relating to the symptoms of this subdomain are asked (i.e. **“**In the past month, have there been times when you felt especially irritable or angry and showed it in your behavior? “or “In the past month, have there been times when you were taking more risks or doing things that might have caused you harm?”) (American Psychiatric Association, 2013). Here, the series questions are asked to the individual where they are encouraged to expand on/give examples about their experience with each of the items in question. Furthermore, for each item a frequency and intensity rating can be given followed by determining if reported symptoms are directly related to the traumatic event in question. There are three versions of the CAPS-5; the lifetime, past month or past week version which are used to assess PTSD diagnostic criteria during these times[25]. In addition, this measure is also considered the gold-standard assessment tool for PTSD and has been widely used across numerous populations, showing good validity and reliability (Weathers et al., 2001). Here, two measures from the CAPS-5 past month version were included in our results: the average total hyperarousal symptom severity score and the average total CAPS-5 severity score for all symptom domains (see Table 3).

Beck Depression Inventory-II (BDI-II)

The BDI-II (Beck et al., 1996) is a self-report questionnaire that consists of twenty-one questions pertaining to depression symptom severity amongst individuals. This is not a tool used to diagnosis depression, but rather is used to assess severity of depressive symptoms. This psychometric measure has been validated across different populations and has been tested for reliability in establishing severity of depression-related symptoms in numerous peer-reviewed reports including samples which consisted of individuals with trauma exposure including PTSD samples (Beck et al., 1996; Buodo et

---

[25] The lifetime and past month versions are used to establish a PTSD diagnosis for these time points. The past week version can only be used to determine symptoms related to a potential future PTSD diagnosis since a PTSD diagnosis according to the DSM-5 requires the individual have experienced symptoms for more than one month. The past month version was used here.

al., 2012; Bryant, Moulds, Guthrie, & Nixon, 2005; Ehlers et al., 2005; Kubany et al., 2004). The total scores were used secondary to the CAPS-5 data to support our other hypothesis.

3.1.3 (c) Inclusion Criteria:

Individuals were included in the study if they:

i) Were between the ages of 18 and 65

ii) Had a current diagnosis of PTSD (based on MAPS clinical assessment using Primary Care PTSD screen and Mini-International Neuropsychiatric Interview's PTSD assessment)

iii) Member (past or present) of CAF

iv) Had a history of operational stress injury

v) Had history of experiencing deployment to conflict zone (post 2001)

vi) Were asymptomatic on M.I.N.I scales for mania/hypomania and/or psychotic disorder.

3.1.3 (d) Exclusion Criteria:

Individuals were excluded from the study if they:

i) Had a diagnosis of substance abuse disorder in the last six months or presented with significant symptoms of substance abuse disorder according to the M.I.N.I questionnaire.

ii) Recently travelled to a different time zone.

iii) Worked night or rotating shifts.

iv) Had a diagnosis of a major physical illness (i.e. neurological disorders, cancer etc.)

v) Experienced head trauma with a loss of consciousness for at least five minutes.

vi) Could not abstain from nicotine, caffeine, or alcohol for a twenty-four-hour period.

vii) Could not abstain from illicit drugs (not including cannabis) for a three-week period.

viii) Were prescribed and taking stimulant medications including Adderall, Concerta, Ritalin, Ephedrine Desoxyn, Dexedrine, Selegline, Dextrostat, Vyvans, Phentermine, Procentra, etc.

3.1.4 – MRI Acquisition & Parameters

All individuals that took part in the study underwent T1-wighted structural and NM-MRI imaging at The Royal's Institute of Mental Health Research's Brain Imaging Center in Ottawa, Ontario. Imaging procedures were performed on a Siemens 3T PET BIOGRAPH mMR scanner using a 12-channel head coil. NM-MRI images were collected using a 2D gradient response echo sequence with magnetization transfer contrast (2D GRE-MT) protocol and the details of the MRI parameters can be observed in Table 1**.** NM-MRI image acquisition time was approximately 7 minutes. This specific sequence allowed for delineation of the LC due to the high NM content in this region and thus allowed signal extraction of the LC to be feasible.

Table 1: Imaging parameters for NM-MRI scan.

NM-MRI Image Parameters	**Parameter Value**
Repetition Time (TR)	337 ms
Echo Time (TE)	3.97 ms
Flip Angle	50°
In-plane resolution	0.43×0.43 mm^2
FoV (partial coverage of midbrain)	165 × 220
Matrix	384 × 512
Number of Slices	10
Slice Thickness	3 mm

Slice Gap	0 mm
Magnetization Transfer Frequency Offset	1200 Hz
Number of Excitations (NEX)	6
Acquisition Time	7.24 minutes

High resolution structural T1-weighted images were also collected for each participant. Here, an MEMPRAGE sequence was utilized, and the applied MRI parameters can be observed in Table 2. T1-weighted image acquisition time was approximately 6 minutes. T1-wighted images were utilized in the preprocessing of NM-MRI data. Imaging procedures were repeated if necessary, and images were visually inspected for distortion upon acquisition.

Table 2: Imaging parameters for T1-weighted scan.

T1-Weighted Image Parameters	**Parameter Value**
Repetition Time (TR)	2500 ms
Echo Time (TE)	1.69 ms
Flip Angle	7°
Inversion Time	1050 ms
FoV (whole brain coverage)	162 x 192
Matrix	192 x 256
Number of Slices	256
Isotropic Voxel Size	1 mm
Acquisition Time	5.47 minutes

3.2.1 NM-MRI Analyses

3.2.2 Preprocessing of NM-MRI Data

SPM12 and custom MATLAB scripts were used to preprocess the NM-MRI images. First, NM-MRI images were coregistered to the T1-weighted structural images (Figure 6 (B)). A tissue segmentation procedure was then applied based on the T1-weighted images for all participants. T1-weighted images were then spatially normalized to standardized space (i.e. MNI space, 1 mm isotropic) thus allowing each person's structural image to be in a common space relative to one another (Figure 6 (C)). The normalization transforms were then applied to the NM-MRI image to bring these images also into standardized space. Spatial normalization was conducted using DARTEL procedures (Ashburner, 2007) A NM-MRI visualization template was created by taking an average of all participants normalized images (Figure 7 (A)). Next, intensity normalization procedures were conducted followed by spatial smoothing. Each stage of the preprocessing was quality checked to ensure accuracy of the applied procedures.

3.2.3 LC Signal Analysis & Extraction

To analyze the LC NM-MRI data, custom MATLAB scripts were developed to allow for automated processing of the LC NM-MRI data. An overinclusive mask was manually drawn on the visualization template. This mask was drawn to ensure coverage of the hyperintense voxels in the vicinity of the LC and would later act as search space to permit identification and signal extraction from the LC. The mask was drawn along the rostrocaudal axis of the brainstem with the rostrocaudal limits being assigned based on the location of the LC according to a brainstem atlas (Naidich et al., 2009). In addition, we also defined the boundaries of these limits based on cell counting studies of the LC where the LC was defined based on cells extending from the inferior colliculus to the posterior recess of the fourth ventricle (German et al., 1992). The posterior recess of the fourth ventricle represented the caudal landmark and the inferior colliculus represented the rostral landmark. In addition, another version of the mask was created by dividing the mask into three rostrocaudal sections (Figure 7 (B). Each segment was of equal length. Both the whole, and divided LC overinclusive masks were then converted back into native space using inverse normalization and co-registration procedures. The warped whole LC overinclusive mask was then used as a search space wherein to find the bilateral LC for everyone in native space. From here, a cluster-forming algorithm was applied on both the right and left sides to identify the LC as the six adjacent voxels with the brightest signal within this search space (Figure 7, (C, bottom right)). From here, we were able to compute the number of times this automated process failed by conducting visual inspection quality checks. These quality checks found that this novel, unique automated process functioned as expected for 93.5% of operations to correctly identify the LC. The remaining 6.5% of operations required manual correction to place six adjacent voxels to locate the LC.

Once the bilateral six voxels of the LC were identified for each individual, a contrast-to-noise ratio (CNR) was calculated for each voxel (*v*). Using the following formula (Equation #1), the CNR was calculated as the relative difference in signal intensity (*I)* between voxels in a reference region *(RR)* and the voxels in the LC. For the purpose of this calculation, the reference region was selected as a central pontine region anterior to the LC (Figure 7, (C. top). This region was selected because of the low NM concentration found here, thereby allowing it to be a suitable reference region for the purpose of calculating CNR values.

Equation #1:

$$\boxed{CNR_{\mathrm{v}} = (I_v - mode(I_{RR}))/mode(I_{RR})}$$

In addition, to add anatomical specificity to our analyses, the LC was divided into three segments to account for potential anatomical topography along the rostrocaudal axis of the LC. Here, every slice (where LC voxels were identified), were sorted into the rostral, central, or caudal segment respectively. Each slice was sorted based on which of the three divided overinclusive LC masks were apparent on the slice in native space (Figure 7 (B) shows the rostral, middle, and caudal overinclusive masks that may be selected for each slice). On the rare occasion, two divided masks would appear on the same slice and in this instance tie-breaking rules were applied in order to assign the slice to its correct segment. Specifically, the mask in which contained a greater number of LC voxels was favored (Figure 7 (C, bottom) shows that the caudal mask was specifically selected for this slice based on this specification). From here, a signal could be averaged from the CNR values of LC voxels from all slices belonging to each of the three segments thus providing NM information within the different anatomical divisions of the LC. The values were then stored and used for our statistical analyses seen below.

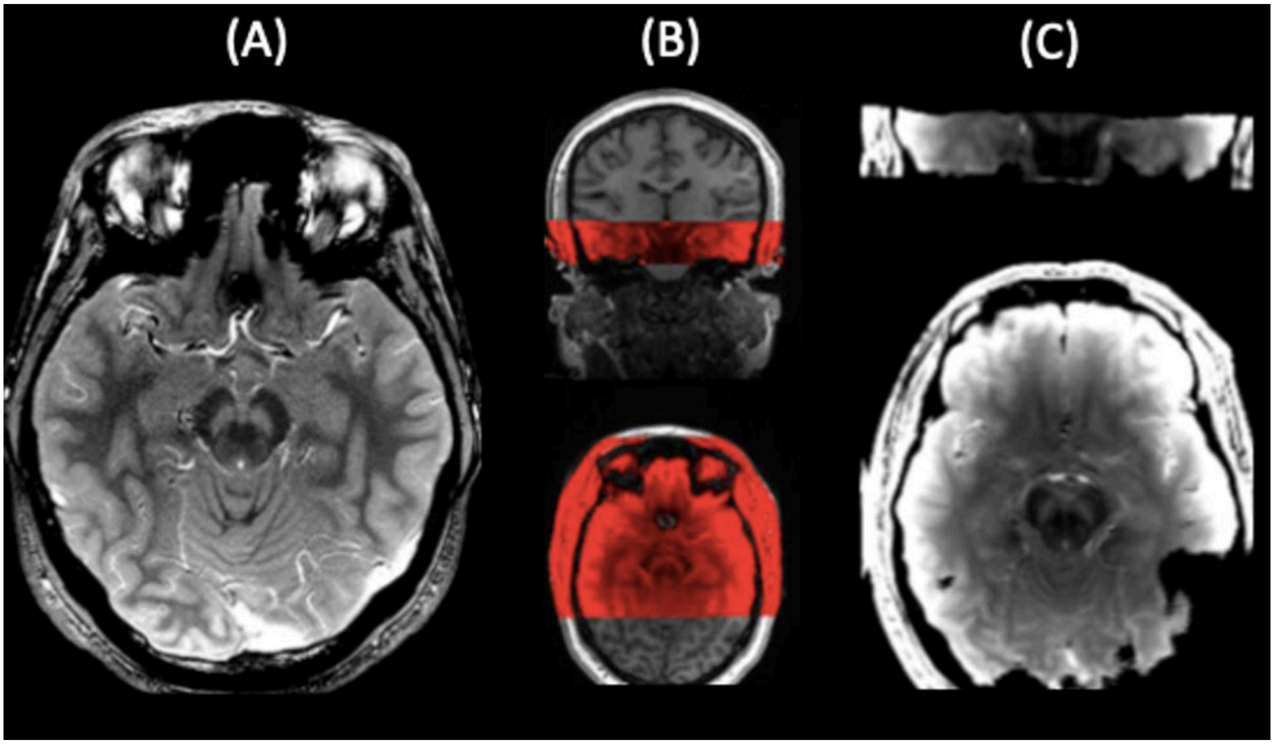

Figure 6: Summary of initial preprocessing steps used in the MRI image processing. (A): The raw NM-MRI image. (B): Co-registration of the NM-MRI image (red) to the structural T1 image (gray). (C): Spatial normalization of NM-MRI image. Figure 7 indicates subsequent steps in preprocessing and LC segmentation.

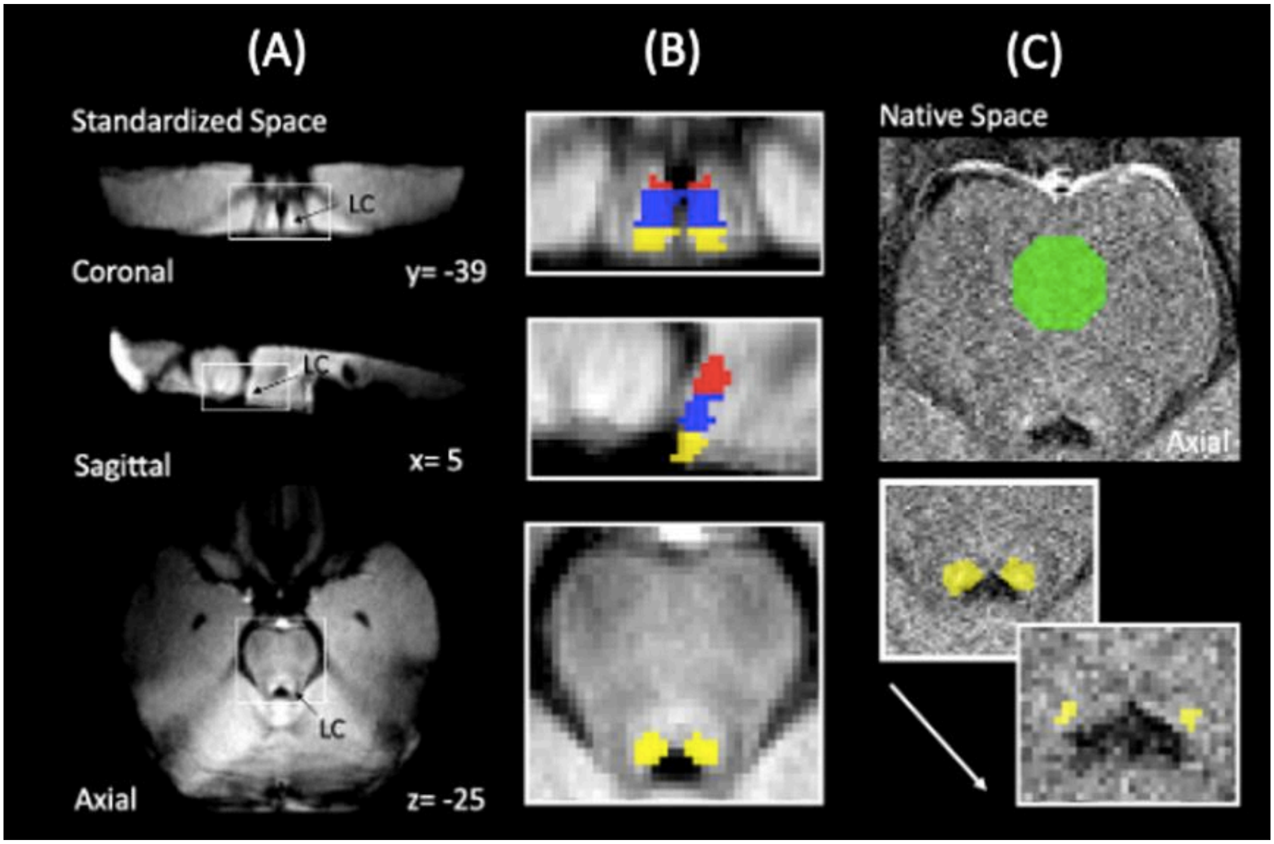

Figure 7: Application of overinclusive masks on visualization template and identification and extraction of bilateral LC signal. (A): Visualization template created by averaging NM-MRI images in standardized MNI space from all participants. LC location is indicated by black arrows. (B): Divided overinclusive masks of LC drawn on template. (C): Axial view of unprocessed NM-MRI image from a representative subject (central pons reference region (green)) (top). Close up of caudal overinclusive mask after warping from standardized space to native space (bottom left,

yellow) and the identification of six bilateral LC voxels within it for one participant on one slice (bottom right, yellow). LC signal is extracted from these voxels.

3.2.4 Statistical Analyses:

MATLAB with an SPM toolbox was used to perform the final statistical analyses. To determine the relationship between LC NM-MRI signal and clinical measures (i.e. CAPS-5 hyperarousal severity and BDI-II depression severity), parametric partial correlations were conducted. Here, the most important measures were LC NM-MRI signal for each of the three sections, hyperarousal severity, and comorbid depression severity, whilst the covariates used in the analysis were age, and sex. Parametric statistics were conducted based on the results from Lilliefors test indicating the measures were normally distributed in comparison to one another.

CHAPTER 4: RESULTS

4.1.1 Demographic and Clinical Characteristics

See Table 3 for a full summary of clinical and demographic information. Of the twenty-two individuals, 68% of the individuals were male. The average age of participants was 47.4 ± 8.6 years. Regarding the CAPS-5 scores, all individuals met Criterion A, thereby indicating all participating individuals had experienced a traumatic event according to the PTSD criteria of the DSM-5. Individual total hyperarousal cluster scores for the twenty-two participants ranged from 4 to 22 whilst individual’s total CAPS-5 severity scores ranged from 23-63. Furthermore, the average CAPS-5 hyperarousal severity score was calculated to be 12.1 ± 5.3, whilst the average total CAPS-5 severity score was found to be 40.6 ± 12.5.

Alongside PTSD symptomology, the BDI-II was administered to assess for the presence of comorbid depression symptoms and the severity of these symptoms in individuals with PTSD. The total BDI-II scores ranged from 8 to 52 whilst the average total BDI-II symptom severity score was calculated to be 27.2 ± 9.7. Based on the severity cut-off score guidelines established by the authors (Beck et al., 1996)[26] of the BDI-II, sixteen out of the twenty-two participants demonstrated moderate to severe depression symptom severity indicating that the majority of individuals demonstrated comorbid depression symptoms alongside their PTSD symptoms. From this analysis and prior research reporting evidence of reduced LC signal in individuals with depression (Sasaki et al., 2006b; Shibata et al., 2007, 2008), it was deemed important to control for the possible influence of comorbid depression symptoms on the LC NM-MRI signal.

Table 3: Summary of participant demographic and clinical data.

Measure	**CAF Members with Confirmed PTSD Diagnosis**
Sample size (n=)	22
Age (average, in years)	47.4 ± 8.6
Sex (males: females)	15:7 (32% female)
PTSD Diagnosis (n=)	22 (100%)
MDD Diagnosis (n=) (according to M.I.N.I.)	9 (41%)
Antidepressant use (n=) *	11 (50%)
Cannabis Use (n=)	6 (27%)

---

[26] The following are the BDI-II severity cut-off score guidelines: 0-13 for minimal depression symptom severity, 14-19 for mild severity, 20-28 for moderate severity and 29-63 for severe severity (Beck et al., 1996). These scores can be calculated by adding up the scores from all 21-items assessed using the BDI-II.

BDI-II (average total severity score)	27.2 ± 9.7
CAPS-5 (average total severity score)	40.6 ± 12.5
CAPS-5 (average hyperarousal severity score)	12.1 ± 5.3

*Antidepressant use was considered for anyone taking SSRIs, SNRIs, TCAs, NaSSAs, MAOIs, SARIs, NDRIs, and RIMA-class prescriptions.[27]

4.2.1 Imaging Results Summary

Overall, the study sample consisted of individuals who demonstrated a high prevalence of hyperarousal and comorbid depression symptoms. Given evidence that both symptom types may be associated with the LC NM-MRI signal, partial correlations were conducted to determine the relationship between LC NM-MRI signal and hyperarousal symptom severity, as well as comorbid depression symptom severity.

4.3.1 Hypothesis #1: LC NM-MRI Signal and CAPS-5 Hyperarousal Symptom Severity

As hypothesized, we observed a significant positive relationship between LC NM-MRI signal and hyperarousal symptom severity (r = 0.54, p = 0.017 partial correlation controlling for depression symptom severity, age, and sex; see Figure 8). This relationship was observed with respect to the caudal LC (Figure 8B) and was consistent with our initial hypothesis. In addition, we also examined the middle and rostral segments of the bilateral LC, although these segments were not found to be significant in relation to hyperarousal symptom severity (the CNR LC NM-MRI statistics for the rostral and middle segments were found to be r=-0.04, p=0.88 (middle segment) and r=-0.25, p=0.30 (rostral segment)).

---

[27] Table #3 Abbreviations: Selective serotonin reuptake inhibitors (SSRIs); serotonin-norepinephrine reuptake inhibitors (SNRIs); Tricyclic antidepressant (TCA); Noradrenergic and specific serotonergic antidepressants (NaSSAs); Monoamine oxidase inhibitors (MAOIs); Serotonin antagonist and reuptake inhibitors (SARIs); Norepinephrine and dopamine reuptake inhibitors (NDRIs); Reversible inhibitors of monoamine oxidase-A (RIMAs).

4.4.1 Hypothesis #2: LC NM-MRI Signal and BDI-II Depression Symptom Severity

To explore our secondary hypothesis, we conducted a parametric partial correlation between LC NM-MRI signal and comorbid depression symptom severity based on total BDI-II scores from each individual. This correlation was not found to be statistically significant (r= -0.30, p=0.22, partial correlation controlling for hyperarousal severity, age, and sex; see Figure 9) and suggests that despite our initial hypothesis, in this relatively small sample, LC NM-MRI signal and depression symptom severity were statistically unrelated. The CNR LC NM-MRI statistics for the middle and rostral segments were found to be r=-0.32, p=0.17 (middle segment) and r=-0.16, p=0.51 (rostral segment) and therefore were not statistically significant.

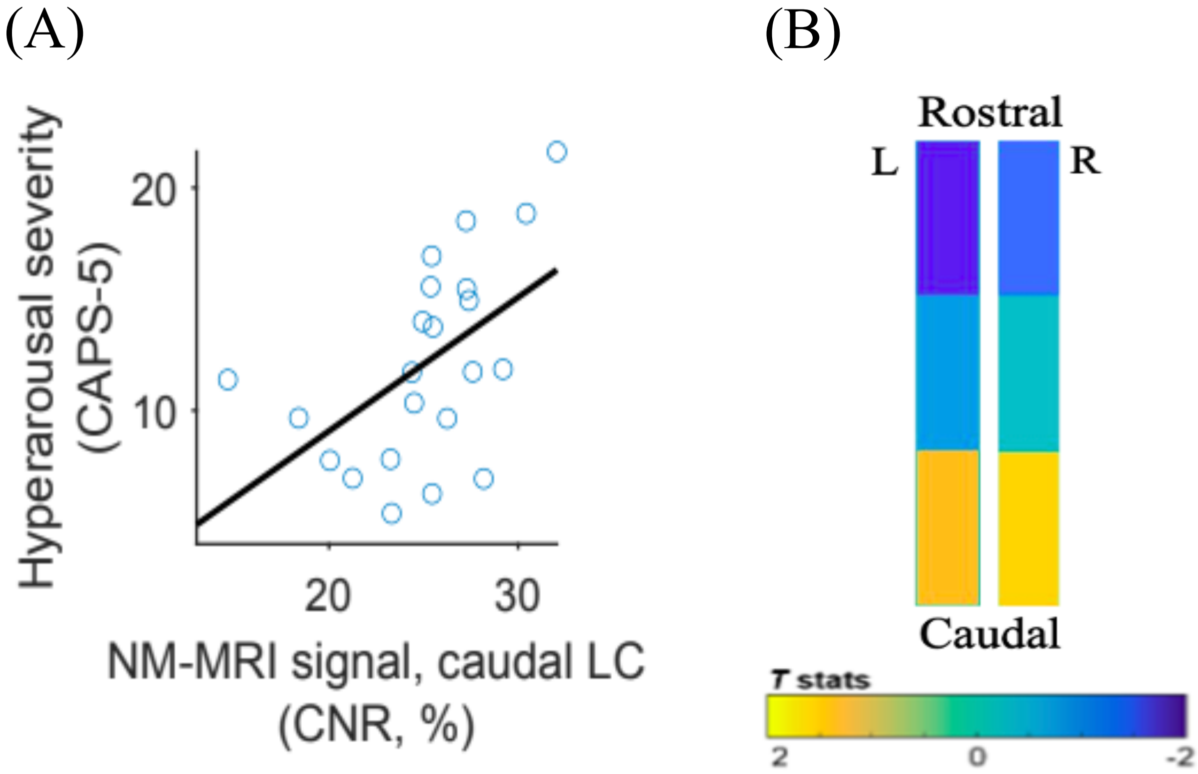

Figure 8: Relationship between LC NM-MRI signal and hyperarousal symptom severity. (A): Positive correlation between caudal LC NM-MRI signal and CAPS-5 hyperarousal symptom severity amongst individuals with military PTSD. (B): LC schematic representing the left and right rod-

shaped structure of the LC. The heat map below demonstrates the statistical relationship of LC signal to hyperarousal symptom severity for all LC segments bilaterally.

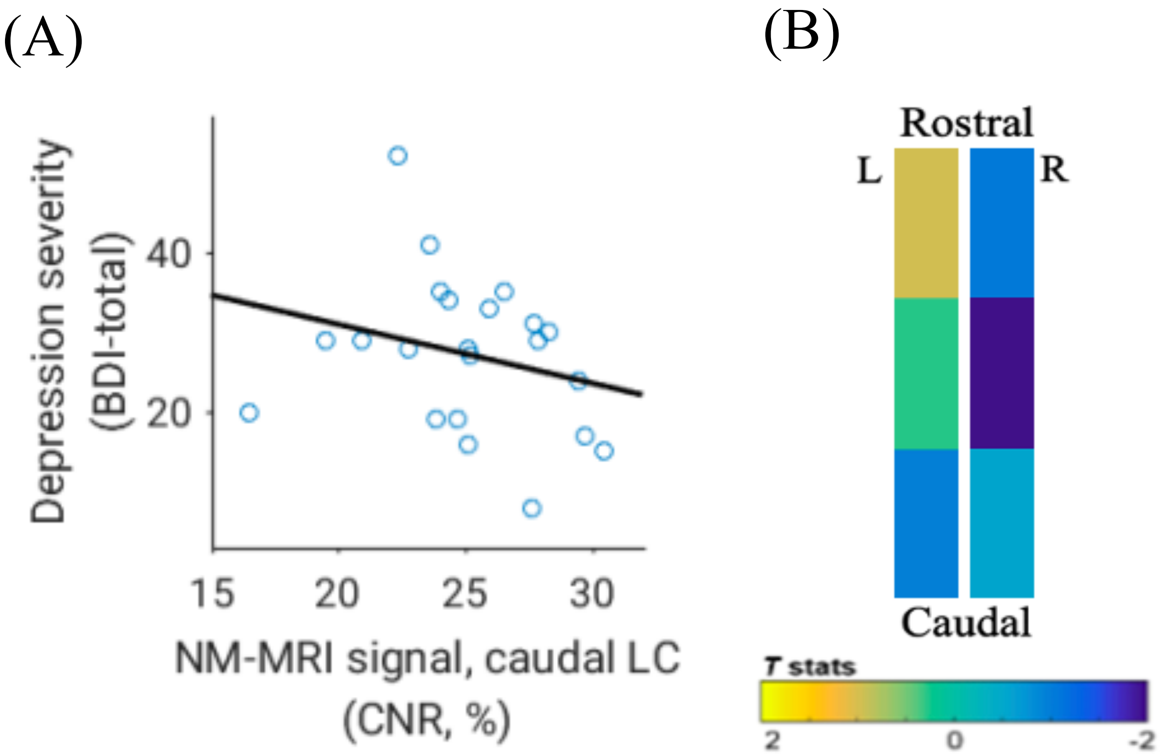

Figure 9: Relationship between LC NM-MRI signal and comorbid depression symptom severity. (A): Scatterplot demonstrating a non-significant correlation between LC NM-MRI signal and BDI-II total severity scores amongst individuals with military PTSD. (B): LC schematic representing the left and right rod-shaped structure of the LC. The heat map below demonstrates the statistical relationship of LC signal to comorbid depression symptom severity for all LC segments bilaterally.

CHAPTER 5: DISCUSSION

5.1.1 Summary of Primary Objective, Outcomes & Implications

The primary objective of this project was to build on previous work conducted utilizing NM-MRI in the dopamine system by investigating the relationship between LC NM-MRI signal and symptom severity of hyperarousal and comorbid depressive symptoms in a sample of individuals with PTSD, a condition suspected to be associated with dysregulation of the LC-NE system. With these objectives in mind, the following outcomes were observed.

5.1.2 LC NM-MRI and Hyperarousal Symptom Severity

First, we were able to demonstrate a positive significant relationship between caudal LC NM-MRI signal and hyperarousal symptom severity as established using the CAPS-5 clinical interview. This result was consistent with our initial hypothesis and converged with similar results relating LC-NE hyperactivity to the manifestation of PTSD symptoms (Southwick et al., 1999; Geracioti et al., 2001; Cascardi et al., 2015; Morey et al., 2015; Hendrickson and Raskind, 2016; Naegeli et al., 2018; Hendrickson et al., 2021). Specifically, Naegeli and colleagues reported that hyperactivity of the LC-NE system was correlated with hyperarousal symptomology such as exaggerated startle response established by the recording of increased eye-blinks, skin conductance and heart rate, all of which are also markers of enhanced NE activity (Naegeli et al., 2018). Furthermore, other researchers observed increases in circulating NE in the cerebrospinal fluid in those with PTSD, indicating elevated NE release from regions such as the LC could contribute to PTSD pathology (Geracioti et al., 2001; Hendrickson et al., 2018). Furthermore, these conclusions are in line with early physiological research which suggests increased physiological activity of the LC can contribute to abnormally elevated levels of cortical arousal which has been linked to stress-related conditions such as anxiety and PTSD (Florin-Lechner et al., 1996; Howells et al., 2012; McCall et al., 2015). With respect to the implications of this finding, if NM-MRI can be used as a direct measure of variable NE concentrations in the LC, NM-MRI could be used across disciplines to measure activity of the catecholamine system in vivo. This could extend beyond the field of psychiatry and be used to measure dysregulation in this system contributing to the establishment of biomarkers associated with specific disease profiles. In addition to expanding the use of this method across conditions, NM-MRI could also be combined with other imaging modalities (PET and functional MRI) to bridge gaps associated with understanding the neural correlates of different mental disorders. This would allow for a more multimodal approach to be taken thereby supporting frameworks such as Research Domain Criteria (RDoC) Initiative, a project whose goal is to take a dimensional approach to researching mental health disorders such that various underlying neurobiological factors are incorporated and considered (i.e. from dysregulation at a molecular level to dysregulation at a systems level) (National Institute of Mental Health, 2008; Insel et al., 2010). Likewise, such approaches may lead to the advancement of precision medicine for psychiatric conditions such as PTSD, thereby allowing targeted therapeutics to be developed for those suffering from these conditions.

5.1.3: LC NM-MRI and Comorbid Depression Symptom Severity

In addition, although not found to be significant statistically, we observed a small negative association between LC NM-MRI signal and comorbid depression symptom severity as determined using the BDI-II self-report measure. This result did not align with our predicted hypothesis suggesting no relationship could be established between LC NM-MRI signal and comorbid depression symptom severity in this study sample.

Furthermore, although this result was not found to be significant, the use of NM-MRI in this context has implications as NM-MRI has previously been used in the context of major depression and late life depression where significant results pertaining to LC signal and depression were attained (Sasaki et al., 2006b; Shibata et al., 2007, 2008; Wengler et al., 2021). Specifically, in work by Shibata and colleagues, the LC NM-MRI signal was found to be significantly reduced in individuals with depression compared to healthy controls (Shibata et al., 2007). Moreover, this finding was replicated one year later by the same group suggesting that reduced LC activity may contribute at least in part to depressive symptomology (Shibata et al., 2008). This research also aligns closely with pharmaceutical research suggesting the NE system could be a target for intervention for those with depression as some NE-targeting agents have been shown to be effective in treating depressive symptoms (i.e. SNRIs and NDRIs) (Golden et al., 1988; Gorman and Kent, 1999; Papakostas et al., 2006; Dionisie et al., 2021). This supports the association observed in this report and supports implications of this work in future research possibly by further testing this relationship in a greater sample size.

As to why no significant result was observed in our study pertaining to the relationship between LC NM MRI signal and comorbid depression symptoms, a few factors should be considered. For example, our study consisted of a small sample who exhibited predominant PTSD symptoms (i.e. all individuals had a confirmed diagnosis of PTSD). In this regard, depression symptoms were considered comorbid to these symptoms adding an additional level of complexity to the disease profile of participating individuals. This is consistent with what is found in the literature, suggesting that "…the co-occurrence of PTSD and MDD represents a trauma-related phenotype that is distinct from MDD and reflects a fundamental dimension of risk for psychopathology following trauma exposure" (Flory and Yehuda, 2015). This emphasizes that special considerations for this unique subtype should be given in future research, Furthermore, despite our controlling for hyperarousal symptoms severity, other PTSD symptoms may negate the effects of depression at the level of the brain (Nijdam et al., 2013; Albott et al., 2021). Based on these speculations, more research should be dedicated to elucidating the true effects of comorbid depression on the NM MRI signal, specifically in the context of PTSD.

5.2.1 Summary of Secondary Objective, Outcomes & Implications

In accordance with our secondary objective, this study also allowed us to advance and optimize our method for extracting and analyzing NM-MRI data, specifically from the LC. Here, we utilized a semi-automated approach in contrast to traditional manual approaches previously used in early LC NM-MRI research (Sasaki et al., 2006b; García-Lorenzo et al., 2013; Clewett et al., 2016; Dordevic et al., 2017; Liebe et al., 2020). This algorithm creates a streamlined process that requires minimal manual correction and is unique compared to other methods prevalent in the literature (Sasaki et al., 2006b; García-Lorenzo et al., 2013; Clewett et al., 2016; Dordevic et al., 2017; Liebe et al., 2020). Specifically, we found that by utilizing an automated algorithm, very minimal manual correction was required such that 93.5% of operations were completed successfully without manual intervention. This is consistent with other studies where an automated approach was taken to analyzing NM-MRI data (Chen et al., 2014; Liu et al., 2017; Cassidy et al., 2019). In addition, this approach also allowed us to explore the different subdivisions of the LC for which we were able to establish that it was the caudal segments of the LC that correlated with hyperarousal symptom severity in those with PTSD. This finding aligns with previous bodies of research which suggests the caudal regions of the LC may be important for autonomic regulation (Berridge and Waterhouse, 2003; Samuels and Szabadi, 2008; Jacobs et al., 2020), a system which is suggested n to be dysregulated in PTSD (Gutner et al., 2000; Orr and Roth, 2000; Felmingham et al., 2011; Norrholm et al., 2016; Dennis et al., 2017; Naegeli et al., 2018). This finding thereby also supports the continued use of an automated method in place of manual methodology as early manual methods would not be able to accurately delineate the above results (Chen et al., 2014; Liu et al., 2017).

Finally, by automating the analysis process of NM-MRI data, NM-MRI methods can be standardized across datasets, allowing for an efficient, streamlined approach to neuroimaging data analysis. This has major implications in the analysis of large data sets which normally would be extremely time consuming if done using manual methods (Chen et al., 2014; Liu et al., 2017). As such, continuing to utilize will increase the efficiency of neuroimaging analysis, reduce the number of errors that may occur and prevent the influence of biases that may arise using traditional, manual methods warranting its use in future research.

5.3.1 Summary of Tertiary Objectives, Outcomes & Implications

As per our tertiary objectives, we were also able to clearly demonstrate that NM-MRI could be used to measure variability within the LC-NE system, in the context of PTSD, a condition that previously has been shown to be associated with overactivity of this system in vivo (Southwick et al., 1999; Geracioti et al., 2001; Cascardi et al., 2015; Morey et al., 2015; Hendrickson and Raskind, 2016; Naegeli et al., 2018; Hendrickson et al., 2021). Both described imaging results supported this objective as LC NM-MRI was shown to detect variability of the LC-NE system in both directions[28] which aligned with previously published work(Shibata et al., 2007, 2008; Morey et al., 2015; Naegeli et al., 2018). Furthermore, by demonstrating the utility of NM-MRI to detect variability in this system we were able to successfully show that NM-MRI could be used in the context of psychiatric illness to capture in vivo system dysregulation of the catecholamine systems thereby allowing us to further explore LC-NE dysregulation as a possible biomarker for PTSD pathology. By identifying biomarkers in conditions such as PTSD, targeted therapeutics could be developed to improve dysregulation in brain systems such as the LC-NE system, increasing not only one’s efficacy to

---

[28] We recognize that our LC NM-MRI versus depression symptom severity correlation was not deemed to be statistically significant, however the effect that appears, according to the scatterplot, corresponds to what was previously published in the literature (Sasaki et al., 2006b; Shibata et al., 2007, 2008).

treatment (including increased likelihood for remission), but increasing an individual's quality of life leading to overall better health outcomes (Schmidt et al., 2013; Zoladz and Diamond, 2013; Lehrner and Yehuda, 2014; Michopoulos et al., 2015).

5.4.1 Strengths, Limitations & Future Directions:

5.4.2 Strengths

The purpose of this project was to conduct a dimensional study such that a novel neuroimaging method could be employed in a clinical setting, specifically, to examine its relationship to clinical measures in a sample of individuals with PTSD. This body of work is the first to employ NM-MRI imaging in a sample of individuals with PTSD.

This body of work and resulting findings were made possible due to the strengths implemented in the study design and methodology of our project. For example, despite our small sample size and the uneven distribution of men to women known within military populations, we made sure our sample was inclusive of female participants (i.e. the ratio between men and women included in our study was 15:7)[29]. This was an important consideration as sex-differences within PTSD have been previously suggested within the literature (Freedy et al., 2010; Maguen et al., 2012; Hu et al., 2017; Ainamani et al., 2020). Furthermore, by including female participants we aided in reducing the underrepresentation of women in military focused studies (Trego et al., 2010; Braun et al., 2015). In addition, our study controlled for use of psychostimulants in our inclusion and exclusion criteria as stimulants could potentially alter the pharmacology of the NE system (Fleckenstein et al., 2000; Rothman et al., 2001; Sofuoglu and Sewell, 2009; Schmidt and Weinshenker, 2014), causing indirect effects at the level of the NM system (Cassidy et al., 2020; Wang et al., 2021). This was previously

---

[29] Recruitment for this study remains active and the goal will be to have a representative sample such that men and women are evenly represented.

established in work by Wang and colleagues in 2021 who found that cocaine exposure may cause higher LC NM-MRI signal intensity (Wang et al., 2021). Furthermore, this work echoes previous work conducted by our lab looking at the effects of cocaine use disorder on the dopamine system using NM-MRI methods (Cassidy et al., 2020), thereby further emphasizing the importance of controlling these substances during our recruitment. Finally, our methods of extracting and analyzing LC NM-MRI in an automated way added considerable strength to our study as in doing so we were able to efficiently reduce the processing time of neuroimaging data, reduce the effects of internal biases and streamline the process such that the algorithm can easily be applied across data bases resulting in a standardized approach to novel neuroimaging analyses.

5.4.3 Limitations

The primary limitation of this project pertains to the size of the sample. Recruitment of participants is still on-going, however, has been stalled due to the on-going COVID-19 pandemic resulting in a smaller than expected sample size. As a result, the sample sized used in this body of work was limited (N=22). Furthermore, we were not able to obtain an even ratio between men and women in our study, which although was not unexpected, still skewed our distribution of men and women within our sample[30]. Moving forward, having a larger sample size and a more female-inclusive sample will allow the study to be more representative of the overarching population. In addition, we also hoped to include a control group of individuals who also had military related trauma exposure, however, did not develop PTSD. In this case, we would have hypothesized lower LC NM-MRI signal in this group compared to the PTSD group, as this would demonstrate increased LC NM-MRI signal would closely relate to PTSD-specific symptoms versus effects of experiencing trauma.

---

[30] We also listed this as a strength because we recognize there are challenges related to recruitment of female military members due to women being vastly underrepresented in military populations (The Government of Canada, 2020) and military-specific research (Trego et al., 2010; Braun et al., 2015).

Additionally, this would also allow us to explore an aspect of resiliency within this disease profile and is seen as a consideration for future research. In contrast, designing control groups in studies concerning military PTSD are difficult to accomplish due to the high prevalence of comorbid mental health issues prevalent in this population (Van Ameringen et al., 2008; Pompili et al., 2013; Gros et al., 2015; Klaric et al., 2017).. This makes it challenging to rule out other conditions such as mood disorders and substance use disorders where many of overlapping symptoms with PTSD can be observed (Jacobsen et al., 2001; Gros et al., 2010, 2012; Flory and Yehuda, 2015). This was also considered a limitation of our study as many participating individuals (72%) met the BDI-II cut-off for moderate to severe comorbid depression symptom severity, indicating a high prevalence of comorbid depressive symptoms in our sample.

5.4.4 Future Directions

As for the future directions of this research, we hope to resume research utilizing NM-MRI amongst individuals with PTSD. Here, we hope to conduct a study employing physiological measures to capture the hypothesized changes in ANS functioning. These measures would include things such as skin conductance, pupillometry, heart rate, blood pressure, and functional brain imaging during fear conditioning and would act as comparative measures for our NM-MRI data. This body of research would strengthen the work presented here, thus allowing further advances to be made in this field of research, including validation of the methods in the context of psychiatric illness.

In addition, as part of a longer-term vision we would also like to conduct a clinical trial to determine if NM-MRI could be used to predict treatment response following the administration of NE-targeted therapeutics (i.e. stellate ganglion block or NE-targeting drugs). This would provide further insight into the use of NM-MRI as a clinical biomarker and advance targeted treatment options for those with this condition, thus leading to increased treatment efficacy and better quality of life.

Finally, we also recognize that NM-MRI has many utilities in both neurodegenerative and psychiatric medicine and in the future, we hope to continue utilizing this methodology in a clinical setting. For instance, we hope to employ this methodology in a sample of children with Attention-deficit/hyperactivity disorder (ADHD)[31], as well as continue research amongst those with schizophrenia, PD and AD.

5.5.1 Conclusion:

In summary, this was the first study conducted utilizing NM-MRI in a clinical sample of veterans with PTSD. From this research, we were able to advance what is known about PTSD in veterans including insights into the role of the LC in relation to hyperarousal symptom severity. We were also able to demonstrate the utility of NM-MRI in tracking dysregulation of LC-NE system activity, specifically in a clinical sample where known dysregulation of this system has been previously established, thus further contributing to the validity of this method in a psychiatric setting. In addition, we were also able to optimize our methods for extracting and analyzing LC NM-MRI such that they can be accomplished semi-automatically. Finally, we were able to show that this novel neuroimaging method may be a potential biomarker for PTSD pathology, such that it was able to delineate changes in NM signal conveying possible information regarding changes in LC activity. Ultimately, this research has led to further advancement of PTSD research such that advanced targeted treatment may be a possible outlook in the near future based on the methods and results presented here.

---

[31] ADHD has also found to be associated with dysregulation of the catecholamine system (Biederman and Spencer, 1999; Prince, 2008; Wilens, 2008), therefore making it a population of interest for future NM-MRI work.

CHAPTER 6: BIBLIOGRAPHY

Ainamani HE, Elbert T, Olema DK, Hecker T (2020) Gender differences in response to war-related trauma and posttraumatic stress disorder - A study among the Congolese refugees in Uganda. BMC Psychiatry 20:1–9.

Albott S, Lee S, Cullen K, Thuras P, Lissek S, Wels J, Friedrich K, Krueger A, Lim K (2021) Characterization of Comorbid Posttraumatic Stress Disorder and Major Depressive Disorder Using Ketamine as an Experimental Medicine Probe. J Psychiatry Brain Sci.

Alexander W (2012) Pharmacotherapy for Post-traumatic stress disorder in combat veterans: Focus on antidepressants and atypical antipsychotic agents. P T 37:32–38.

American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders, 5th Edition: DSM-5.

Ashburner J (2007) A fast diffeomorphic image registration algorithm. Neuroimage 38:95–113.

Aston-Jones G, Bloom FE (1981) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1:876–886.

Aston-Jones G, Cohen JD (2005) Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. In: Journal of Comparative Neurology.

Aston-Jones G, Shipley MT, Chouvet G, Ennis M, van Bockstaele E, Pieribone V, Shiekhattar R, Akaoka H, Drolet G, Astier B, Charléty P, Valentino RJ, Williams JT (1991) Afferent regulation of locus coeruleus neurons: Anatomy, physiology and pharmacology. Prog Brain Res 88:47–75.

Atzori M, Cuevas-Olguin R, Flores G, Concetta Miniaci M, Esquivel-Rendon E, Garcia-Oscos F, Salgado-Delgado RC, Saderi N, Miranda-Morales M, Treviño M, Pineda JC, Salgado H (2016) Locus Ceruleus Norepinephrine Release: A Central Regulator of CNS Spatio-Temporal Activation? Front Synaptic Neurosci | www.frontiersin.org 1:25 Available at:

www.frontiersin.org.

Baker KG, Törk I, Hornung JP, Halasz P (1989) The human locus coeruleus complex: an immunohistochemical and three dimensional reconstruction study. Exp Brain Res 77:257–270.

Bari BA, Chokshi V, Schmidt K (2020) Locus coeruleus-norepinephrine: Basic functions and insights into Parkinson's disease. Neural Regen Res 15:1006–1013.

Bazelon M, Fenichel GM, Randall J (1967) Studies on neuromelanin: I. A melanin system in the human adult brainstem. Neurology 17:512–519.

Beach TG, Sue LI, Walker DG, Lue LF, Connor DJ, Caviness JN, Sabbagh MN, Adler CH (2007) Marked microglial reaction in normal aging human substantia nigra: Correlation with extraneuronal neuromelanin pigment deposits. Acta Neuropathol 114.

Berger W, Mendlowicz M V., Marques-Portella C, Kinrys G, Fontenelle LF, Marmar CR, Figueira I (2009) Pharmacologic alternatives to antidepressants in posttraumatic stress disorder: A systematic review. Prog Neuro-Psychopharmacology Biol Psychiatry 33.

Berridge C, Waterhouse B (2003) The locus coeruleus–noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Rev 42:33–84.

Berridge CW (2009) The locus coeruleus-noradrenergic system and stress: Implications for post-traumatic stress disorder. In: Post-Traumatic Stress Disorder: Basic Science and Clinical Practice.

Berridge CW, Schmeichel BE, España RA (2012) Noradrenergic modulation of wakefulness/arousal. Sleep Med Rev.

Betts MJ et al. (2019) Locus coeruleus imaging as a biomarker for noradrenergic dysfunction in neurodegenerative diseases. Brain 142:2558–2571.

Biederman J, Spencer T (1999) Attention-deficit/hyperactivity disorder (ADHD) as a noradrenergic disorder. Biol Psychiatry 46.

Biondetti E, Gaurav R, Yahia-Cherif L, Mangone G, Pyatigorskaya N, Valabrègue R, Ewenczyk C, Hutchison M, François C, Corvol JC, Vidailhet M, Lehéricy S (2020) Spatiotemporal changes in substantia nigra neuromelanin content in Parkinson's disease. Brain 143.

Birmes P, Brunet A, Carreras D, Ducassé JL, Charlet JP, Lauque D, Sztulman H, Schmitt L (2003) The predictive power of peritraumatic dissociation and acute stress symptoms for posttraumatic stress symptoms: A three-month prospective study. Am J Psychiatry 160.

Blake DD, Weathers FW, Nagy LM, Kaloupek DG, Gusman FD, Charney DS, Keane TM (1995) The development of a Clinician-Administered PTSD Scale. J Trauma Stress 8.

Blanchard EB, Jones-Alexander J, Buckley TC, Forneris CA (1996) Psychometric properties of the PTSD checklist (PCL). Behav Res Ther 34.

Boeve BF et al. (2007) Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease. Brain 130.

Braun LA, Kennedy HP, Sadler LS, Dixon J (2015) Research on U.S. military women: Recruitment and retention challenges and strategies. Mil Med 180.

Bremner JD, Krystal JH, Southwick SM, Charney DS (1996) Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. Synapse 23.

Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB (1995) MRI-based measurement of hippocampal volume in patients with combat- related posttraumatic stress disorder. Am J Psychiatry 152.

Bremner JD, Randall P, Vermetten E, Staib L, Bronen RA, Mazure C, Capelli S, McCarthy G, Innis RB, Charney DS (1997) Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse - A preliminary report. Biol Psychiatry 41.

Bremner JD, Scott TM, Delaney RC, Southwick SM, Mason JW, Johnson DR, Innis RB, McCarthy G, Charney DS (1993) Deficits in short-term memory in posttraumatic stress disorder. Am J Psychiatry 150.

Breslau N, Chilcoat HD, Kessler RC, Peterson EL, Lucia VC (1999) Vulnerability to assaultive violence: Further specification of the sex difference in post-traumatic stress disorder. Psychol Med 29.

Breslau N, Koenen KC, Luo Z, Agnew-Blais J, Swanson S, Houts RM, Poulton R, Moffitt TE (2014) Childhood maltreatment, juvenile disorders and adult post-traumatic stress disorder: A prospective investigation. Psychol Med 44.

Brewin CR, Andrews B, Valentine JD (2000) Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. J Consult Clin Psychol 68.

Brody H, Vijayashankar N (1979) A QUANTITATIVE STUDY OF THE PIGMENTED NEURONS IN THE NUCLEI LOCUS COERULEUS AND SUBCOERULEUS IN MAN AS RELATED TO AGING. J Neuropathol Exp Neurol 38:490–497.

Bruinstroop E, Cano G, Vanderhorst VGJM, Cavalcante JC, Wirth J, Sena-Esteves M, Saper CB (2012) Spinal projections of the A5, A6 (locus coeruleus), and A7 noradrenergic cell groups in rats. J Comp Neurol 520.

Brunet A, Monson E, Liu A, Fikretoglu D (2015) Trauma Exposure and Posttraumatic Stress Disorder in the Canadian Military. Can J Psychiatry 60:488–496.

Cameron CM, Purdie DM, Kliewer E V., McClure RJ (2006) Mental health: A cause or consequence of injury? A population-based matched cohort study. BMC Public Health 6.

Carballo-Carbajal I, Laguna A, Romero-Giménez J, Cuadros T, Bové J, Martinez-Vicente M, Parent A, Gonzalez-Sepulveda M, Peñuelas N, Torra A, Rodríguez-Galván B, Ballabio A, Hasegawa T, Bortolozzi A, Gelpi E, Vila M (2019) Brain tyrosinase overexpression implicates age-dependent neuromelanin production in Parkinson's disease pathogenesis. Nat Commun 10.

Carlson EB, Palmieri PA, Field NP, Dalenberg CJ, Macia KS, Spain DA (2016) Contributions of risk and protective factors to prediction of psychological symptoms after traumatic experiences. Compr Psychiatry 69.

Carmassi C, Akiskal HS, Bessonov D, Massimetti G, Calderani E, Stratta P, Rossi A, Dellosso L (2014) Gender differences in DSM-5 versus DSM-IV-TR PTSD prevalence and criteria comparison among 512 survivors to the LAquila earthquake. J Affect Disord 160.

Cascardi M, Chung L, Paré D (2015) Arousal-related pupil response to threat: A comparison of trauma-exposed individuals with and without PTSD. J Trauma Stress 28:370–374 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4743760/pdf/nihms755182.pdf.

Cassidy CM, Carpenter KM, Konova AB, Cheung V, Grassetti A, Zecca L, Abi-Dargham A, Martinez D, Horga G (2020) Evidence for Dopamine Abnormalities in the Substantia Nigra in Cocaine Addiction Revealed by Neuromelanin-Sensitive MRI. Am J Psychiatry.

Cassidy CM, Zucca FA, Girgis RR, Baker SC, Weinstein JJ, Sharp ME, Bellei C, Valmadre A, Vanegas N, Kegeles LS, Brucato G, Kang UJ, Sulzer D, Zecca L, Abi-Dargham A, Horga G (2019) Neuromelanin-sensitive MRI as a noninvasive proxy measure of dopamine function in the human brain. Proc Natl Acad Sci U S A 116:5108–5117.

Cedarbaum JM, Aghajanian GK (1978) Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. J Comp Neurol 178.

Charney DS, Grillon C, Bremner JD (1998) The neurobiological basis of anxiety and fear: Circuits, mechanisms, and neurochemical interactions (Part I). Neuroscientist 4.

Chen X, Huddleston DE, Langley J, Ahn S, Barnum CJ, Factor SA, Levey AI, Hu X (2014) Simultaneous imaging of locus coeruleus and substantia nigra with a quantitative neuromelanin MRI approach. Magn Reson Imaging 32.

Chou PH, Ito M, Horikoshi M (2020) Associations between PTSD symptoms and suicide risk: A comparison of 4-factor and 7-factor models. J Psychiatr Res 129.

Clark FM, Proudfit HK (1991) The projection of locus coeruleus neurons to the spinal cord in the rat determined by anterograde tracing combined with immunocytochemistry. Brain Res 538.

Clewett D V., Lee TH, Greening S, Ponzio A, Margalit E, Mather M (2016) Neuromelanin marks the spot: identifying a locus coeruleus biomarker of cognitive reserve in healthy aging. Neurobiol Aging 37:117–126 Available at: http://dx.doi.org/10.1016/j.neurobiolaging.2015.09.019.

Connor KM, Sutherland SM, Tupler LA, Malik ML, Davidson JRT (1999) Fluoxetine in post-traumatic stress disorder. Randomised, double-blind study. Br J Psychiatry 175.

Crum-Cianflone NF, Jacobson I (2014) Gender differences of postdeployment post-traumatic stress disorder among service members and veterans of the Iraq and Afghanistan conflicts. Epidemiol Rev 36:5–18.

Dahlström A, Fuxe K (1964) Localization of monoamines in the lower brain stem. Experientia 20:398–399.

Davidson J, Baldwin D, Stein DJ, Kuper E, Benattia I, Ahmed S, Pedersen R, Musgnung J (2006) Treatment of posttraumatic stress disorder with venlafaxine extended release: A 6-

month randomized controlled trial. Arch Gen Psychiatry 63.

Dennis PA, Kimbrel NA, Sherwood A, Calhoun PS, Watkins LL, Dennis MF, Beckham JC (2017) Trauma and autonomic dysregulation: Episodic-versus systemic-negative affect underlying cardiovascular risk in posttraumatic stress disorder. Psychosom Med 79.

Devilbiss DM, Waterhouse BD (2011) Phasic and tonic patterns of locus coeruleus output differentially modulate sensory network function in the awake rat. J Neurophysiol 105:69–87.

DiGangi JA, Gomez D, Mendoza L, Jason LA, Keys CB, Koenen KC (2013) Pretrauma risk factors for posttraumatic stress disorder: A systematic review of the literature. Clin Psychol Rev 33.

Dionisie V, Filip GA, Manea MC, Manea M, Riga S (2021) The anti-inflammatory role of SSRI and SNRI in the treatment of depression: a review of human and rodent research studies. Inflammopharmacology 29.

Dordevic M, Müller-Fotti A, Müller P, Schmicker M, Kaufmann J, Müller NG (2017) Optimal Cut-Off Value for Locus Coeruleus-to-Pons Intensity Ratio as Clinical Biomarker for Alzheimer’s Disease: A Pilot Study. J Alzheimer’s Dis Reports 1.

Douglas Bremner J (2001) Hypotheses and controversies related to effects of stress on the hippocampus: An argument for stress-induced damage to the hippocampus in patients with posttraumatic stress disorder. Hippocampus 11.

Douglas Bremner J, Randall P, Scott TM, Capelli S, Delaney R, McCarthy G, Charney DS (1995) Deficits in short-term memory in adult survivors of childhood abuse. Psychiatry Res 59.

Endres CJ, Kolachana BS, Saunders RC, Su T, Weinberger D, Breier A, Eckelman WC, Carson

RE (1997) Kinetic modeling of [11C]raclopride: Combined PET-microdialysis studies. J Cereb Blood Flow Metab 17.

Enochs WS, Petherick P, Bogdanova A, Mohr U, Weissleder R (1997) Paramagnetic metal scavenging by melanin: MR imaging. Radiology 204.

Enochs WS, Sarna T, Zecca L, Riley PA, Swartz HM (1994) The roles of neuromelanin, binding of metal ions, and oxidative cytotoxicity in the pathogenesis of Parkinson's disease: A hypothesis. J Neural Transm - Park Dis Dement Sect 7.

Euler US (1946) Sympathin in adrenergic nerve fibres. J Physiol 105.

Fan Y, Chen P, Li Y, Cui K, Noel DM, Cummins ED, Peterson DJ, Brown RW, Zhu MY (2014) Corticosterone administration up-regulated expression of norepinephrine transporter and dopamine β-hydroxylase in rat locus coeruleus and its terminal regions. J Neurochem 128:445–458 Available at: https://pubmed.ncbi.nlm.nih.gov/24111919/ [Accessed January 31, 2022].

Fani N, King TZ, Brewster R, Srivastava A, Stevens JS, Glover EM, Norrholm SD, Bradley B, Ressler KJ, Jovanovic T (2015) Fear-potentiated startle during extinction is associated with white matter microstructure and functional connectivity. Cortex 64.

Fedorow H, Tribl F, Halliday G, Gerlach M, Riederer P, Double KL (2005) Neuromelanin in human dopamine neurons: Comparison with peripheral melanins and relevance to Parkinson's disease. Prog Neurobiol 75.

Felmingham KL, Rennie C, Manor B, Bryant RA (2011) Eye tracking and physiological reactivity to threatening stimuli in posttraumatic stress disorder. J Anxiety Disord 25:668–673 Available at: http://dx.doi.org/10.1016/j.janxdis.2011.02.010.

Fleckenstein AE, Gibb JW, Hanson GR (2000) Differential effects of stimulants on monoaminergic transporters: Pharmacological consequences and implications for neurotoxicity. Eur J Pharmacol 406.

Florin-Lechner SM, Druhan JP, Aston-Jones G, Valentino RJ (1996) Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. Brain Res 742.

Flory JD, Yehuda R (2015) Comorbidity between post-traumatic stress disorder and major depressive disorder: Alternative explanations and treatment considerations. Dialogues Clin Neurosci 17.

Forbes D, Lewis V, Varker T, Phelps A, O'Donnell M, Wade DJ, Ruzek JI, Watson P, Bryant RA, Creamer M (2011) Psychological first aid following Trauma: Implementation and evaluation framework for high-risk organizations. Psychiatry 74.

Freedy JR, Magruder KM, Mainous AG, Frueh BC, Geesey ME, Carnemolla M (2010) Gender differences in traumatic event exposure and mental health among veteran primary care patients. Mil Med 175:750–758.

Fritschy J -M, Grzanna R (1990) Demonstration of two separate descending noradrenergic pathways to the rat spinal cord: Evidence for an intragriseal trajectory of locus coeruleus axons in the superficial layers of the dorsal horn. J Comp Neurol 291.

Gannon M, Che P, Chen Y, Jiao K, Roberson ED, Wang Q (2015) Noradrenergic dysfunction in Alzheimer's disease. Front Neurosci 9.

García-Lorenzo D, Longo-Dos Santos C, Ewenczyk C, Leu-Semenescu S, Gallea C, Quattrocchi G, Pita Lobo P, Poupon C, Benali H, Arnulf I, Vidailhet M, Lehericy S (2013) The coeruleus/subcoeruleus complex in rapid eye movement sleep behaviour disorders in Parkinson's disease. Brain 136.

Gaurav R, Yahia-Cherif L, Pyatigorskaya N, Mangone G, Biondetti E, Valabrègue R, Ewenczyk

C, Hutchison RM, Cedarbaum JM, Corvol JC, Vidailhet M, Lehéricy S (2021) Longitudinal Changes in Neuromelanin MRI Signal in Parkinson’s Disease: A Progression Marker. Mov Disord 36.

Geracioti TD, Baker DG, Ekhator NN, West SA, Hill KK, Bruce AB, Schmidt D, Rounds-Kugler B, Yehuda R, Keck J, Kasckow JW (2001) CSF norepinephrine concentrations in posttraumatic stress disorder. Am J Psychiatry 158.

Gerfen CR, Sawchenko PE (2016) An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: Immunohistochemical localization of an axonally transported plant lectin, Phaseolus vulgaris-leucoagglutinin (PHA-L). Brain Res 1645.

Gerlach M, Double K-L, Tribl F, Riederer P (2006) Neuromelanin and its Interaction with Iron as a Potential Risk Factor for Dopaminergic Neurodegeneration Underlying Parkinson´s Disease. Behav Pharmacol 17.

German DC, Manaye KF, White CL, Woodward DJ, McIntire DD, Smith WK, Kalaria RN, Mann DMA (1992) Disease-specific patterns of locus coeruleus cell loss. Ann Neurol 32.

German DC, Walker BS, Manaye K, Smith WK, Woodward DJ, North AJ (1988) The human locus coeruleus: Computer reconstruction of cellular distribution. J Neurosci 8:1776–1788.

Gil S (2005) Coping style in predicting posttraumatic stress disorder among Israeli students. Anxiety, Stress Coping 18.

Gilmour J, Romaniuk M, McLeay S, Harvey W, Romaniuk M, Crawford D, Colquhoun D, McD Young R, Dwyer M, Gibson J, O’Sullivan R, Cooksley G, Strakosch C, Thomson R, Voisey J, Lawford B (2020) Factor structure of posttraumatic stress disorder (PTSD) in Australian Vietnam Veterans: Confirmatory factor analysis of the Clinician-Administered PTSD Scale for DSM–5. J Mil Veteran Fam Heal 6.

Golden RN, Rudorfer M V., Sherer MA, Linnoila M, Potter WZ (1988) Bupropion in Depression: I. Biochemical Effects and Clinical Response. Arch Gen Psychiatry 45.

Gören MZ, Cabadak H (2015) Noradrenaline and Post-traumatic Stress Disorder. In: Comprehensive Guide to Post-Traumatic Stress Disorder.

Gorman JM, Kent JM (1999) SSRIS and SNRIs: Broad spectrum of efficacy beyond major depression. J Clin Psychiatry 60.

Grasser LR, Javanbakht A (2019) Treatments of Posttraumatic Stress Disorder in Civilian Populations. Curr Psychiatry Rep 21.

Gray MJ, Litz BT, Hsu JL, Lombardo TW (2004) Psychometric properties of the life events checklist. Assessment 11.

Greer N, Sayer NA, Spoont M, Taylor BC, Ackland PE, Macdonald R, McKenzie L, Rosebush C, Wilt TJ (2020) Prevalence and Severity of Psychiatric Disorders and Suicidal Behavior in Service Members and Veterans with and Without Traumatic Brain Injury: Systematic Review. J Head Trauma Rehabil 35.

Griffin MG, Uhlmansiek MH, Resick PA, Mechanic MB (2004) Comparison of the posttraumatic stress disorder scale versus the clinician-administered posttraumatic stress disorder scale in domestic violence survivors. J Trauma Stress 17.

Gros DF, Price M, Magruder KM, Frueh BC (2012) Symptom overlap in posttraumatic stress disorder and major depression. Psychiatry Res 196.

Gros DF, Simms LJ, Acierno R (2010) Specificity of posttraumatic stress disorder symptoms: An investigation of comorbidity between posttraumatic stress disorder symptoms and depression in treatment-seeking veterans. J Nerv Ment Dis 198.

Gros DF, Szafranski DD, Brady KT, Back SE (2015) Relations between pain, PTSD symptoms, and substance use in veterans. Psychiatry (New York) 78:277–287.

Groves PM, Wilson CJ (1980) Fine structure of rat locus coeruleus. J Comp Neurol 193.

Grzanna R, Molliver ME (1980) The locus coeruleus in the rat: An immunohistochemical delineation. Neuroscience 5:21–40.

Gurvits T V., Shenton ME, Hokama H, Ohta H, Lasko NB, Gilbertson MW, Orr SP, Kikinis R, Jolesz FA, McCarley RW, Pitman RK (1996) Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. Biol Psychiatry 40.

Gutner C, Pineles S, Griffin M, Bauer M, Weierich M, Resick P (2000) Physiological Predictors of Posttraumatic Stress Disorder. J Trauma Stress 23:775–784.

Halbauer JD, Ashford JW, Zeitzer JM, Adamson MM, Lew HL, Yesavage JA (2009) Neuropsychiatric diagnosis and management of chronic sequelae of war-related mild to moderate traumatic brain injury. J Rehabil Res Dev 46.

Halliday GM, Fedorow H, Rickert CH, Gerlach M, Riederer P, Double KL (2006) Evidence for specific phases in the development of human neuromelanin. J Neural Transm 113.

Harnett NG, Goodman AM, Knight DC (2020) PTSD-related neuroimaging abnormalities in brain function, structure, and biochemistry. Exp Neurol 330.

Hayat H, Regev N, Matosevich N, Sales A, Paredes-Rodriguez E, Krom AJ, Bergman L, Li Y, Lavigne M, Kremer EJ, Yizhar O, Pickering AE, Nir Y (2020) Locus coeruleus norepinephrine activity mediates sensory-evoked awakenings from sleep. Sci Adv 6.

Hendrickson RC, Millard SP, Pagulayan KF, Peskind ER, Raskind MA (2021) The Relative Effects of Prazosin on Individual PTSD Symptoms: Evidence for Pathophysiologically-

Related Clustering. Chronic Stress 5.

Hendrickson RC, Raskind MA (2016) Noradrenergic dysregulation in the pathophysiology of PTSD. Exp Neurol 284:181–195 Available at: http://dx.doi.org/10.1016/j.expneurol.2016.05.014.

Hendrickson RC, Raskind MA, Millard SP, Sikkema C, Terry GE, Pagulayan KF, Li G, Peskind ER (2018) Evidence for altered brain reactivity to norepinephrine in Veterans with a history of traumatic stress. Neurobiol Stress 8:103–111.

Hoge CW, Terhakopian A, Castro CA, Messer SC, Engel CC (2007) Association of posttraumatic stress disorder with somatic symptoms, health care visits, and absenteeism among Iraq war veterans. Am J Psychiatry 164:150–153.

Holbrook TL, Hoyt DB, Stein MB, Sieber WJ (2001) Perceived threat to life predicts posttraumatic stress disorder after major trauma: risk factors and functional outcome. J Trauma 51.

Hooberman J, Rosenfeld B, Rasmussen A, Keller A (2010) Resilience in trauma-exposed refugees: The moderating effect of coping style on resilience variables. Am J Orthopsychiatry 80.

Hou R, Beardmore R, Holmes C, Osmond C, Darekar A (2021) A case-control study of the locus coeruleus degeneration in Alzheimer's disease. Eur Neuropsychopharmacol 43.

Howells FM, Stein DJ, Russell VA (2010) Perceived mental effort correlates with changes in tonic arousal during attentional tasks. Behav Brain Funct 6.

Howells FM, Stein DJ, Russell VA (2012) Synergistic tonic and phasic activity of the locus coeruleus norepinephrine (LC-NE) arousal system is required for optimal attentional performance. Metab Brain Dis 27:267–274.

Hu J, Feng B, Zhu Y, Wang W, Xie J, Zheng X (2017) Gender Differences in PTSD: Susceptibility and Resilience. In: Gender Differences in Different Contexts.

Hughes KC, Shin LM (2011) Functional neuroimaging studies of post-traumatic stress disorder. Expert Rev Neurother 11.

Hyde JS, Mezulis AH (2020) Gender Differences in Depression: Biological, Affective, Cognitive, and Sociocultural Factors. Harv Rev Psychiatry 28.

Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K, Sanislow C, Wang P (2010) Research Domain Criteria (RDoC): Toward a new classification framework for research on mental disorders. Am J Psychiatry 167.

Ito H, Kawaguchi H, Kodaka F, Takuwa H, Ikoma Y, Shimada H, Kimura Y, Seki C, Kubo H, Ishii S, Takano H, Suhara T (2017) Normative data of dopaminergic neurotransmission functions in substantia nigra measured with MRI and PET: Neuromelanin, dopamine synthesis, dopamine transporters, and dopamine D2 receptors. Neuroimage 158:12–17 Available at: http://dx.doi.org/10.1016/j.neuroimage.2017.06.066.

Jacobs HIL, Priovoulos N, Poser BA, Pagen LHG, Ivanov D, Verhey FRJ, Uludağ K (2020) Dynamic behavior of the locus coeruleus during arousal-related memory processing in a multi-modal 7T fMRI paradigm. Elife 9.

Jacobsen LK, Southwick SM, Kosten TR (2001) Substance use disorders in patients with posttraumatic stress disorder: A review of the literature. Am J Psychiatry 158.

Jedema HP, Finlay JM, Sved AF, Grace AA (2001) Chronic cold exposure potentiates CRH-evoked increases in electrophysiologic activity of locus coeruleus neurons. Biol Psychiatry 49.

Kelley LP, Weathers FW, McDevitt-Murphy ME, Eakin DE, Flood AM (2009) A comparison of

PTSD symptom patterns in three types of civilian trauma. J Trauma Stress 22.

Kelly SC, He B, Perez SE, Ginsberg SD, Mufson EJ, Counts SE (2017) Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. Acta Neuropathol Commun 5:8.

Keran N, Taheri S, Vazey E, Morgan P, Granholm A-C, Aston-Jones G, Eckert M (2015) Histologic validation of locus coeruleus MRI contrast in post-mortem tissue. Neuroimage 113:235–245.

Keren N, Lozar C, Harris K, Morgan P, Eckert M (2009) In-Vivo Mapping of the Human Locus Coeruleus. Neuroimage 47:1261–1267.

Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB (1995) Posttraumatic Stress Disorder in the National Comorbidity Survey. Arch Gen Psychiatry 52.

Khachatryan D, Groll D, Booij L, Sepehry AA, Schütz CG (2016) Prazosin for treating sleep disturbances in adults with posttraumatic stress disorder: A systematic review and meta-analysis of randomized controlled trials. Gen Hosp Psychiatry 39:46–52 Available at: http://dx.doi.org/10.1016/j.genhosppsych.2015.10.007.

Klaric M, Lovric S, Kresic Coric M, Galic K, Coric S, Franciskovic T (2017) Psychiatric comorbidity and PTSD-related health problems in war veterans: Cross-sectional study. Eur J Psychiatry 31:151–157.

Klimek V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G, Ordway GA (1997) Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. J Neurosci.

Kontos AP, Kotwal RS, Elbin RJ, Lutz RH, Forsten RD, Benson PJ, Guskiewicz KM (2013) Residual effects of combat-related mild traumatic brain injury. J Neurotrauma 30.

Kovács KJ (2013) CRH: The link between hormonal-, metabolic- and behavioral responses to stress. J Chem Neuroanat 54.

Kumagai H, Oshima N, Matsuura T, Iigaya K, Imai M, Onimaru H, Sakata K, Osaka M, Onami T, Takimoto C, Kamayachi T, Itoh H, Saruta T (2012) Importance of rostral ventrolateral medulla neurons in determining efferent sympathetic nerve activity and blood pressure. Hypertens Res 35.

Laruelle M, Iyer RN, Al-Tikriti MS, Zea-Ponce Y, Malison R, Zoghbi SS, Baldwin RM, Kung HF, Charney DS, Hoffer PB, Innis RB, Bradberry CW (1997) Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. Synapse 25.

Lees AJ, Selikhova M, Andrade LA, Duyckaerts C (2008) The black stuff and Konstantin Nikolaevich Tretiakoff. Mov Disord 23.

Lehrner A, Yehuda R (2014) Biomarkers of PTSD: Military applications and considerations. Eur J Psychotraumatol 5.

Li MH, Nishikawa T (2012) The relationship between active coping and trait resilience across U.S. and Taiwanese college student samples. J Coll Couns 15.

Liebe T, Kaufmann J, Li M, Skalej M, Wagner G, Walter M (2020) In vivo anatomical mapping of human locus coeruleus functional connectivity at 3 T MRI. Hum Brain Mapp 41.

Liu KY, Marijatta F, Hämmerer D, Acosta-Cabronero J, Düzel E, Howard RJ (2017) Magnetic resonance imaging of the human locus coeruleus: A systematic review. Neurosci Biobehav Rev 83.

Liu KY, Stringer AE, Reeves SJ, Howard RJ (2018) The neurochemistry of agitation in Alzheimer’s disease: a systematic review. Ageing Res Rev 43.

Maguen S, Cohen B, Ren L, Bosch J, Kimerling R, Seal K (2012) Gender differences in military sexual trauma and mental health diagnoses among iraq and afghanistan veterans with posttraumatic stress disorder. Women's Heal Issues 22:e61–e66.

Manaye KF, McIntire DD, Mann DMA, German DC (1995) Locus coeruleus cell loss in the aging human brain: A non-random process. J Comp Neurol 358.

Mann DMA (1983) The locus coeruleus and its possible role in ageing and degenerative disease of the human central nervous system. Mech Ageing Dev 23.

Mann DMA, Yates PO (1979) The effects of ageing on the pigmented nerve cells of the human locus caeruleus and substantia nigra. Acta Neuropathol 47.

McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP, Bruchas MR (2015) CRH Engagement of the Locus Coeruleus Noradrenergic System Mediates Stress-Induced Anxiety. Neuron 87:605–620 Available at: http://dx.doi.org/10.1016/j.neuron.2015.07.002.

McCall JG, Siuda ER, Bhatti DL, Lawson LA, McElligott ZA, Stuber GD, Bruchas MR (2017) Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior. Elife 6:1–23.

McLean CP, Asnaani A, Litz BT, Hofmann SG (2011) Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. J Psychiatr Res 45.

Melia KR, Rasmussen K, Terwilliger RZ, Haycock JW, Nestler EJ, Duman RS (1992) Coordinate Regulation of the Cyclic AMP System with Firing Rate and Expression of Tyrosine Hydroxylase in the Rat Locus Coeruleus: Effects of Chronic Stress and Drug Treatments. J Neurochem 58:494–502.

Metcalf O, Stone C, Hinton M, O'Donnell M, Hopwood M, McFarlane A, Forbes D, Kartal D, Watson L, Freijah I, Varker T (2020) Treatment augmentation for posttraumatic stress disorder: A systematic review. Clin Psychol Sci Pract 27:1–21.

Michopoulos V, Norrholm SD, Jovanovic T (2015) Diagnostic Biomarkers for Posttraumatic Stress Disorder: Promising Horizons from Translational Neuroscience Research. Biol Psychiatry.

Milad MR, Pitman RK, Ellis CB, Gold AL, Shin LM, Lasko NB, Zeidan MA, Handwerger K, Orr SP, Rauch SL (2009) Neurobiological Basis of Failure to Recall Extinction Memory in Posttraumatic Stress Disorder. Biol Psychiatry 66.

Minassian A, Geyer MA, Baker DG, Nievergelt CM, O'connor DT, Risbrough VB (2014) Heart rate variability characteristics in a large group of active-duty marines and relationship to posttraumatic stress. Psychosom Med 76:292–301.

Morey RA, Dunsmoor JE, Haswell CC, Brown VM, Vora A, Weiner J, Stjepanovic D, Wagner HR, Mid-Atlantic VA, LaBar KS, Brancu M, Marx CE, Naylor JC, Van Voorhees E, Taber KH, Beckham JC, Calhoun PS, Fairbank JA, Szabo ST (2015) Fear learning circuitry is biased toward generalization of fear associations in posttraumatic stress disorder. Transl Psychiatry 5.

Morris LS, McCall JG, Charney DS, Murrough JW (2020a) The role of the locus coeruleus in the generation of pathological anxiety. Brain Neurosci Adv 4.

Morris LS, Tan A, Smith DA, Grehl M, Han-Huang K, Naidich TP, Charney DS, Balchandani P, Murrough JW, Kundu P (2020b) Sub-millimeter variation in human locus coeruleus is associated with dimensional measures of psychopathology: An in vivo ultra-high field 7-Tesla MRI study. NeuroImage Clin.

Murphy PR, Robertson IH, Balsters JH, O'connell RG (2011) Pupillometry and P3 index the locus coeruleus-noradrenergic arousal function in humans. Psychophysiology 48:1532–

1543.

Naegeli C, Zeffiro T, Piccirelli M, Jaillard A, Weilenmann A, Hassanpour K, Schick M, Rufer M, Orr SP, Mueller-Pfeiffer C (2018) Locus Coeruleus Activity Mediates Hyperresponsiveness in Posttraumatic Stress Disorder. Biol Psychiatry 83:254–262.

Naidich TP, Duvernoy HM, Delman BN, Sorensen AG, Kollias SS, Haacke EM (2009) Duvernoy's Atlas of the Human Brain Stem and Cerebellum: High-Field MRI, Surface Anatomy, Internal Structure, Vascularization and 3 D Sectional Anatomy.

National Institute of Mental Health (2008) The National Institute of Mental Health Strategic Plan.

Nestler EJ, Mcmahon A, Sabban EL, Tallman JF, Duman S, Nestler EJ, Mcmahont A, Sabbant EL, Tallman JF, Duman RS (1990) Chronic Antidepressant Administration Decreases the Expression of Tyrosine Hydroxylase in the Rat Locus Coeruleus Source : Proceedings of the National Academy of Sciences of the United States of America , Published by : National Academy of Sciences Stable. Proc Natl Acad Sci U S A 87:7522–7526.

Nijdam MJ, Gersons BPR, Olff M (2013) The role of major depression in neurocognitive functioning in patients with posttraumatic stress disorder. Eur J Psychotraumatol 4.

Norrholm SD, Jovanovic T, Gerardi M, Breazeale KG, Price M, Davis M, Duncan E, Ressler KJ, Bradley B, Rizzo A, Tuerk PW, Rothbaum BO (2016) Baseline psychophysiological and cortisol reactivity as a predictor of PTSD treatment outcome in virtual reality exposure therapy. Behav Res Ther 82:28–37 Available at: http://dx.doi.org/10.1016/j.brat.2016.05.002.

O'Donnell ML, Bryant RA, Creamer M, Carty J (2008) Mental health following traumatic injury: Toward a health system model of early psychological intervention. Clin Psychol Rev

28.

Odh G, Carstam R, Paulson J, Wittbjer A, Rosengren E, Rorsman H (1994) Neuromelanin of the Human Substantia Nigra: A Mixed-Type Melanin. J Neurochem 62.

Orr SP, Roth WT (2000) Psychophysiological assessment: Clinical applications for PTSD. J Affect Disord 61:225–240.

Ozer EJ, Best SR, Lipsey TL, Weiss DS (2003) Predictors of posttraumatic stress disorder and symptoms in adults: A meta-analysis. Psychol Bull 129.

Papakostas GI, Nutt DJ, Hallett LA, Tucker VL, Krishen A, Fava M (2006) Resolution of Sleepiness and Fatigue in Major Depressive Disorder: A Comparison of Bupropion and the Selective Serotonin Reuptake Inhibitors. Biol Psychiatry 60.

Papakostas GI, Thase ME, Fava M, Nelson JC, Shelton RC (2007) Are Antidepressant Drugs That Combine Serotonergic and Noradrenergic Mechanisms of Action More Effective Than the Selective Serotonin Reuptake Inhibitors in Treating Major Depressive Disorder? A Meta-analysis of Studies of Newer Agents. Biol Psychiatry.

Paredes-Rodriguez E, Vegas-Suarez S, Morera-Herreras T, De Deurwaerdere P, Miguelez C (2020) The Noradrenergic System in Parkinson’s Disease. Front Pharmacol 11.

Pearson C, Zamorski M, Janz T (2014) Mental Health in the Canadian Armed Forces.

Pompili M, Sher L, Serafini G, Forte A, Innamorati M, Dominici G, Lester D, Amore M, Girardi P (2013) Posttraumatic stress disorder and suicide risk among veterans: A literature review. J Nerv Ment Dis.

Posner MI, Sheese BE, Odludaş Y, Tang YY (2006) Analyzing and shaping human attentional networks. Neural Networks 19:1422–1429.

Prince J (2008) Catecholamine dysfunction in attention-deficit/hyperactivity disorder an update.

J Clin Psychopharmacol 28.

Racagni G, Popoli M (2008) Cellular and molecular mechanisms in the long-term action of antidepressants. Dialogues Clin Neurosci 10.

Raskind MA et al. (2013) A trial of prazosin for combat trauma PTSD with nightmares in active-duty soldiers returned from Iraq and Afghanistan. Am J Psychiatry 170:1003–1010.

Raskind MA, Peskind ER, Chow B, Harris C, Davis-Karim A, Holmes HA, Hart KL, McFall M, Mellman TA, Reist C, Romesser J, Rosenheck R, Shih M-C, Stein MB, Swift R, Gleason T, Lu Y, Huang GD (2018) Trial of Prazosin for Post-Traumatic Stress Disorder in Military Veterans. N Engl J Med.

Ronzoni G, del Arco A, Mora F, Segovia G (2016) Enhanced noradrenergic activity in the amygdala contributes to hyperarousal in an animal model of PTSD. Psychoneuroendocrinology 70:1–9 Available at: http://dx.doi.org/10.1016/j.psyneuen.2016.04.018.

Rosenfield S (2000) Gender and dimensions of the self: Implications for internalizing and externalizing behavior. In: Gender and its effects on psychopathology., pp 23–36 American Psychopathological Association series. Arlington, VA, US: American Psychiatric Publishing, Inc.

Rothman RB, Baumann MH, Dersch CM, Romero D V., Rice KC, Carroll FI, Partilla JS (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 39.

Samuels E, Szabadi E (2008) Functional Neuroanatomy of the Noradrenergic Locus Coeruleus: Its Roles in the Regulation of Arousal and Autonomic Function Part I: Principles of Functional Organisation. Curr Neuropharmacol 6:235–253.

Sareen J (2014) Posttraumatic stress disorder in adults: Impact, comorbidity, risk factors, and treatment. Can J Psychiatry 59.

Sareen J, Cox BJ, Stein MB, Afifi TO, Fleet C, Asmundson GJG (2007) Physical and mental comorbidity, disability, and suicidal behavior associated with posttraumatic stress disorder in a large community sample. Psychosom Med 69.

Sasaki M, Shibata E, Tohyama K, Takahashi J, Otsuka K, Tsuchiya K, Takahashi S, Ehara S, Terayama Y, Sakai A (2006a) Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. Neuroreport.

Sasaki M, Shibata E, Tohyama K, Takahashi J, Otsuka K, Tsuchiya K, Takahashi S, Ehara S, Terayama Y, Sakai A (2006b) Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. Neuroreport 17:1215–1218.

Sayed S, Iacoviello BM, Charney DS (2015) Risk Factors for the Development of Psychopathology Following Trauma. Curr Psychiatry Rep 17.

Schmidt KT, Weinshenker D (2014) Adrenaline rush: The role of adrenergic receptors in stimulant-induced behaviors. Mol Pharmacol 85.

Schmidt U, Kaltwasser SF, Wotjak CT (2013) Biomarkers in posttraumatic stress disorder: Overview and implications for future research. Dis Markers 35.

Schneiderman AI, Braver ER, Kang HK (2008) Understanding sequelae of injury mechanisms and mild traumatic brain injury incurred during the conflicts in Iraq and Afghanistan: persistent postconcussive symptoms and posttraumatic stress disorder. Am J Epidemiol 167.

Schwarz LA, Luo L (2015) Organization of the locus coeruleus-norepinephrine system. Curr Biol 25:R1051–R1056 Available at: http://dx.doi.org/10.1016/j.cub.2015.09.039.

Shalev AY, Peri T, Canetti L, Schreiber S (1996) Predictors of PTSD in injured trauma survivors: A prospective study. Am J Psychiatry 153.

Sharma Y, Xu T, Graf WM, Fobbs A, Sherwood CC, Hof PR, Allman JM, Manaye KF (2010) Comparative anatomy of the locus coeruleus in humans and nonhuman primates. J Comp Neurol 518:963–971.

Sherin JE, Nemeroff CB (2011) Overview of psychological trauma, post-traumatic stress disorder, and biological markers.

Shibata E, Sasaki M, Tohyama K, Otsuka K, Endoh J, Terayama Y, Sakai A (2008) Use of Neuromelanin-Sensitive MRI to Distinguish Schizophrenic and Depressive Patients and Healthy Individuals Based on Signal Alterations in the Substantia Nigra and Locus Ceruleus. Biol Psychiatry 64.

Shibata E, Sasaki M, Tohyama K, Otsuka K, Sakai A (2007) Reduced signal of locus ceruleus in depression in quantitative neuromelanin magnetic resonance imaging. Neuroreport 18.

Shin LM, Wright CI, Cannistraro PA, Wedig MM, McMullin K, Martis B, Macklin ML, Lasko NB, Cavanagh SR, Krangel TS, Orr SP, Pitman RK, Whalen PJ, Rauch SL (2005) A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch Gen Psychiatry 62.

Simpson KL, Altman DW, Wang L, Kirifides ML, Lin RCS, Waterhouse BD (1997) Lateralization and functional organization of the locus coeruleus projection to the trigeminal somatosensory pathway in rat. J Comp Neurol 385:135–147.

Sofuoglu M, Sewell RA (2009) Norepinephrine and stimulant addiction. Addict Biol 14.

Southwick SM, Bremner JD, Rasmusson A, Morgan CA, Arnsten A, Charney DS (1999) Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. Biol

Psychiatry 46.

Southwick SM, Krystal JH, Bremner JD, Morgan CA, Nicolaou AL, Nagy LM, Johnson DR, Heninger GR, Charney DS (1997) Noradrenergic and serotonergic function in posttraumatic stress disorder. Arch Gen Psychiatry 54.

Stahl SM (1998) Basic psychopharmacology of antidepressants, Part 1: Antidepressants have seven distinct mechanisms of action. J Clin Psychiatry 59.

Stein DJ, Ipser JC, Seedat S, Sager C, Amos T (2006) Pharmacotherapy for post traumatic stress disorder (PTSD). Cochrane Database Syst Rev.

Stein MB, McAllister TW (2009) Exploring the convergence of posttraumatic stress disorder and mild traumatic brain injury. Am J Psychiatry 166.

Strawn JR, Geracioti TD (2008) Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. Depress Anxiety 25.

Sulzer D, Bogulavsky J, Larsen KE, Behr G, Karatekin E, Kleinman MH, Turro N, Krantz D, Edwards RH, Greene LA, Zecca L (2000) Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. Proc Natl Acad Sci U S A 97.

Sulzer D, Cassidy C, Horga G, Kang UJ, Fahn S, Casella L, Pezzoli G, Langley J, Hu XP, Zucca FA, Isaias IU, Zecca L (2018) Neuromelanin detection by magnetic resonance imaging (MRI) and its promise as a biomarker for Parkinson’s disease. npj Park Dis 4 Available at: http://dx.doi.org/10.1038/s41531-018-0047-3.

Swanson LW (1976) The locus coeruleus: A cytoarchitectonic, golgi and immunohistochemical study in the albino rat. Brain Res 110.

Szabadi E (2013) Functional neuroanatomy of the central noradrenergic system. J

Psychopharmacol 27.

Takahashi K, Kayama Y, Lin JS, Sakai K (2010) Locus coeruleus neuronal activity during the sleep-waking cycle in mice. Neuroscience 169.

Taylor B, Westlund K (2017) The Noradrenergic Locus Coeruleus as a Chronic Pain Generator. J Neurosci Res 95:1336–1346.

The Government of Canada (2020) Statistics of women in the Canadian Armed Forces.

Thompson NJ, Fiorillo D, Rothbaum BO, Ressler KJ, Michopoulos V (2018) Coping strategies as mediators in relation to resilience and posttraumatic stress disorder. J Affect Disord 225.

Trego L, Wilson C, Steele N (2010) A call to action for evidence-based military women's health care: Developing a women's health research agenda that addresses sex and gender in health and illness. Biol Res Nurs 12.

Trujillo P, Summers PE, Ferrari E, Zucca FA, Sturini M, Mainardi LT, Cerutti S, Smith AK, Smith SA, Zecca L, Costa A (2017) Contrast mechanisms associated with neuromelanin-MRI. Magn Reson Med 78.

Tsai J, Harpaz-Rotem I, Pietrzak RH, Southwick SM (2012) The role of coping, resilience, and social support in mediating the relation between PTSD and social functioning in veterans returning from Iraq and Afghanistan. Psychiatry(New York) 75.

Tully K, Bolshakov VY (2010) Emotional enhancement of memory: How norepinephrine enables synaptic plasticity. Mol Brain 3.

Valentino RJ, Van Bockstaele E (2008) Convergent regulation of locus coeruleus activity as an adaptive response to stress. Eur J Pharmacol 583.

Van Ameringen M, Mancini C, Patterson B, Boyle MH (2008) Post-traumatic stress disorder in Canada. CNS Neurosci Ther 14:171–181.

van Stegeren AH (2008) The role of the noradrenergic system in emotional memory. Acta Psychol (Amst).

Verma V, Rasmussen K, Dawe GS (2006) Effects of short-term and chronic olanzapine treatment on immediate early gene protein and tyrosine hydroxylase immunoreactivity in the rat locus coeruleus and medial prefrontal cortex. Neuroscience 143:573–585.

Vila M (2019) Neuromelanin, aging, and neuronal vulnerability in Parkinson's disease. Mov Disord 34.

Wakamatsu K, Murase T, Zucca FA, Zecca L, Ito S (2012) Biosynthetic pathway to neuromelanin and its aging process. Pigment Cell Melanoma Res 25.

Wallace DM, Magnuson DJ, Gray TS (1989) The amygdalo-brainstem pathway: Selective innervation of dopaminergic, noradrenergic and adrenergic cells in the rat. Neurosci Lett 97.

Wang W, Zhornitsky S, Zhang S, Li C-SR (2021) Noradrenergic correlates of chronic cocaine craving: neuromelanin and functional brain imaging. Neuropsychopharmacology 46:851–859 Available at: https://doi.org/10.1038/s41386-020-00937-9.

Waterhouse BD, Lin CS, Burne RA, Woodward DJ (1983) The distribution of neocortical projection neurons in the locus coeruleus. J Comp Neurol 217:418–431.

Weathers FW, Bovin MJ, Lee DJ, Sloan DM, Schnurr PP, Kaloupek DG, Keane TM, Marx BP (2018) The clinician-administered ptsd scale for DSM-5 (CAPS-5): Development and initial psychometric evaluation in military veterans. Psychol Assess 30:383–395.

Weathers FW, Keane TM, Davidson JRT (2001) Clinician-administered PTSD scale: A review of the first ten years of research. Depress Anxiety 13.

Weinshenker D (2008) Functional Consequences of Locus Coeruleus Degeneration in Alzheimers Disease. Curr Alzheimer Res 5.

Weinshenker D (2018) Long Road to Ruin: Noradrenergic Dysfunction in Neurodegenerative Disease. Trends Neurosci 41.

Weiss JM, Stout JC, Aaron MF, Quan N, Owens MJ, Butler PD, Nemeroff CB (1994) Depression and anxiety: Role of the locus coeruleus and corticotropin-releasing factor. Brain Res Bull 35:561–572.

Wengler K, Ashinoff BK, Pueraro E, Cassidy CM, Horga G, Rutherford BR (2021) Association between neuromelanin-sensitive MRI signal and psychomotor slowing in late-life depression. Neuropsychopharmacology 46.

Westlund KN, Dan Coulter J (1980) Descending projections of the locus coeruleus and subcoeruleus/medial parabrachial nuclei in monkey: Axonal transport studies and dopamine-β-hydroxylase immunocytochemistry. Brain Res Rev 2.

Wilens TE (2008) Effects of methylphenidate on the catecholaminergic system in attention-deficit/hyperactivity disorder. J Clin Psychopharmacol 28.

Yeo J (2021) Emotional Disturbances After Traumatic Brain Injury: Prevalence, Assessment, and Treatment. Curr Phys Med Rehabil Reports 9.

Zanoveli JM, Carvalho MC, Cunha JM, Brandão ML (2009) Extracellular serotonin level in the basolateral nucleus of the amygdala and dorsal periaqueductal gray under unconditioned and conditioned fear states: An in vivo microdialysis study. Brain Res 1294.

Zatzick D, Jurkovich GJ, Rivara FP, Wang J, Fan MY, Joesch J, MacKenzie E (2008a) A national US study of posttraumatic stress disorder, depression, and work and functional outcomes after hospitalization for traumatic injury. Ann Surg 248.

Zatzick DF, Jurkovich GJ, Fan MY, Grossman D, Russo J, Katon W, Rivara FP (2008b) Association between posttraumatic stress and depressive symptoms and functional outcomes in adolescents followed up longitudinally after injury hospitalization. Arch Pediatr Adolesc Med 162.

Zecca L, Bellei C, Costi P, Albertini A, Monzani E, Casella L, Gallorini M, Bergamaschi L, Moscatelli A, Turro NJ, Eisner M, Crippa PR, Ito S, Wakamatsu K, Bush WD, Ward WC, Simon JD, Zucca FA (2008) New melanic pigments in the human brain that accumulate in aging and block environmental toxic metals. Proc Natl Acad Sci U S A 105:17567–17572.

Zecca L, Fariello R, Riederer P, Sulzer D, Gatti A, Tampellini D (2002) The absolute concentration of nigral neuromelanin, assayed by a new sensitive method, increases throughout the life and is dramatically decreased in Parkinson's disease. FEBS Lett 510:216–220.

Zhang W, Phillips K, Wielgus AR, Liu J, Albertini A, Zucca FA, Faust R, Qian SY, Miller DS, Chignell CF, Wilson B, Jackson-Lewis V, Przedborski S, Joset D, Loike J, Hong JS, Sulzer D, Zecca L (2011) Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: Implications for progression of parkinson's disease. Neurotox Res 19.

Zohar J, Amital D, Miodownik C, Kotler M, Bleich A, Lane RM, Austin C (2002) Double-blind placebo-controlled pilot study of sertraline in military veterans with posttraumatic stress disorder. J Clin Psychopharmacol 22.

Zoladz PR, Diamond DM (2013) Current status on behavioral and biological markers of PTSD: A search for clarity in a conflicting literature. Neurosci Biobehav Rev 37:860–895 Available at: http://dx.doi.org/10.1016/j.neubiorev.2013.03.024.

Zucca FA, Bellei C, Giannelli S, Terreni MR, Gallorini M, Rizzio E, Pezzoli G, Albertini A, Zecca L (2006) Neuromelanin and iron in human locus coeruleus and substantia nigra during aging: Consequences for neuronal vulnerability. J Neural Transm 113.

Zucca FA, Segura-Aguilar J, Ferrari E, Muñoz P, Paris I, Sulzer D, Sarna T, Casella L, Zecca L (2017) Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease. Prog Neurobiol 155.

Zucca FA, Vanna R, Cupaioli FA, Bellei C, De Palma A, Di Silvestre D, Mauri P, Grassi S, Prinetti A, Casella L, Sulzer D, Zecca L (2018) Neuromelanin organelles are specialized autolysosomes that accumulate undegraded proteins and lipids in aging human brain and are likely involved in Parkinson's disease. npj Park Dis 4.