ABSTRACT

Stressed Out! A Review of the Neurobiological and Cognitive Effects of Stress

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While stress is a common experience, high levels of stress can be dangerous to both physical and mental health. Chronic stress is implicated in many psychological disorders such as generalized anxiety disorder, depression, and post-traumatic stress disorder, all of which contribute to lower quality of life and a substantial economic burden. The body's stress response is mediated by the hypothalamic-pituitary-adrenal (HPA) axis, which involves a cascade of signaling molecules such as corticotropin releasing hormone (CRH) and cortisol. Chronic dysfunction of the HPA axis alters brain structure and function, through effects of stress hormones. Exposure to chronic stress may result in cognitive deficits such as impaired decision making and memory, and emotional deficits such as heightened fear/anxiety and depressed mood. While specific treatments for stress-related disorders are in development, current treatments still lack efficacy. Therefore, further research is needed to understand better the root of stress-related disorders and improve treatment outcomes.

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STRESSED OUT! A REVIEW OF THE NEUROBIOLOGICAL AND COGNITIVE EFFECTS OF STRESS

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DEDICATION

I dedicate this thesis to my parents who have always been my greatest advocates and have supported me so lovingly throughout all of my academic endeavors.

CHAPTER ONE

The Importance of Studying Stress

Introduction

Stress is a nearly ubiquitous experience in our fast-paced society. At its best, small amounts of stress can lead to focus and efficiency for the task at hand. However, at its worst, stress can lead to debilitating psychological disorders such as anxiety, depression, and post-traumatic stress disorder, as well as taking a toll on one's physical health. While tests for measuring self-reported levels of stress, such as Depression Anxiety Stress Scale (DASS), the Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI), have aided in identifying individuals who may need treatments for stress-related disorders, many people with psychological disorders related to stress do not respond well to treatments (Crawford & Henry, 2003). Since stress can affect a wide range of people and can lead to serious consequences, it is important to investigate how stress impacts brain function, identify the neural mechanisms underlying related psychological disorders, and formulate more effective treatments to help people lead fuller, happier lives.

Stress and Health

While the most commonly thought of problems associated with stress are mental disorders, stress can also have a large impact on general physical health. High stress is associated with an increase in maladaptive health behaviors including drug use, risky sexual behaviors, and decreased exercise (Doom & Haeffel, 2013). For example, the

increased daily stress caused by entering the first year of college causes differential weight changes in males and females, with stressed males tending to lose weight and stressed females tending to gain weight (Economos, Hildebrandt, & Hyatt, 2008). Clinical studies indicate that stress is a contributing factor to risk of cardiovascular disease, atherosclerosis, and heart attack (Kivimaki & Steptoe, 2012). Stressful life events also correlate with increased risk of developing breast cancer (Kruk, 2012). These health risks can lead to decreased life span, greater health related costs, as well as general distress. Thus, it is important to study stress in order to mitigate its effects and promote better general health.

Stress can also have a large impact on mental health due to its effects on the secretion of stress hormones such as norepinephrine, corticotrophin releasing factor, and adrenocorticotropic hormone, which are modulated by the hypothalamic-pituitary-adrenal (HPA) axis (see Chapter 2). Changes in mechanisms involved with noradrenergic stress hormones have been found to contribute to anxiety disorders such as generalized anxiety disorder (GAD), post-traumatic stress disorder (PTSD), obsessive compulsive disorder (OCD), and phobias (Bremner, Krystal, Southwick, & Charney, 1996). High stress has also been found to contribute to the development of affective disorders such as depression. In a study of medical students, who tend to be highly stressed due to the demands of medical school and high work load, it was found that the rate of depression was 12.9% higher than the depression rate for the general population (Dahlin, Joneborg, & Runeson, 2005). The psychiatric consequences associated with stress will be considered further later in this chapter.

In addition to the typical psychological symptoms of stress-related psychiatric disorders, patients with anxiety disorders have an increased risk of somatic symptoms. For example, patients with GAD have a nearly fivefold increase in risk for developing peptic ulcers (Goodwin, 2002). An association between chronic pain and stress-related disorders such as anxiety disorders and depression has also been found. People with GAD are much more likely to experience chronic pain than the general population, and there is a relationship between the severity of psychiatric disorders (including, but not limited to GAD) and the likelihood of a person experiencing chronic pain-related disability (McWilliams, Cox, & Enns, 2003; Olfson & Gameroff, 2007). These factors can contribute greatly to the decreased quality of life experienced by those with stress-related disorders.

In addition to stress impacting physical health, a person's health can also affect their likelihood of developing stress related disorders. For example, people with diabetes (Type I or Type II) appear to be at an increased risk for anxiety disorders, with diabetic populations showing 20.5% lifetime prevalence of GAD, much higher than the 5.1% prevalence in the general population as noted above, and is increased anxiety symptoms in 40% of diabetic patients (Grigsby, Anderson, Freedland, Clouse, & Lustman, 2002).

Stress-Related Disorders

The stress response produced by the body is an attempt to regain equilibrium in response to environmental or internal challenges. While normal levels of stress are helpful for reacting to demanding or potentially dangerous situations, high and prolonged levels of stress can have a negative impact on one's health and happiness. In a recent

survey conducted by the American Psychological Association (APA), Americans reported average stress levels of about 1.2 points higher on a ten-point scale than what they considered to be healthy stress levels (around 4.9) (Anderson et al., 2015). Problems coping with acute stress, chronic stress, or intense stressors such as traumatic experiences can potentially lead to depression, anxiety disorders, panic disorder, obsessive compulsive disorder (OCD), post-traumatic stress disorder (PTSD), and substance abuse. Aside from the neuropsychiatric disorders associated with stress, stress can lead to other undesirable consequences. Work-related stress, for example, is thought to be a major cause of back pain, cardiovascular disease, and musculoskeletal disease, as well as decreased productivity due to burnout (Béjean & Sultan-Taïeb, 2005).

The lifetime prevalence of generalized anxiety disorder (GAD) is approximately 5.1% in the general population (Wittchen, 2002). Taken together with related anxiety disorders, GAD, panic disorder, and PTSD affect approximately 18.1% of the United States' population, equivalent to about 50 million people. Mood disorders, such as depression, affect approximately 9.5% of the population, and substance disorders including alcohol and drug abuse affect 3.8% (Kessler, Chiu, Demler, & Walters, 2005). These disorders have been found to be comorbid in many patients. In addition, many of those with GAD have been found to have an additional mental disorder diagnosis such as panic disorder, a particular phobia, major depressive disorder (MDD), or PTSD.

People diagnosed with anxiety disorders have a substantially lower overall quality of life than the general population, particularly those patients who have the additional diagnosis of MDD (Barrera & Norton, 2009). Recently the World Health Organization reported that depression is "the leading cause of disease burden for women" worldwide,

and is the third leading cause worldwide for all age groups and both genders (World Health Organization, 2008).

The lower quality of life due to stress-related disorders should not be surprising when considering the symptoms of these disorders. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM), the symptoms of Acute Stress Disorder include: intrusion symptoms in which the distress and memory of a traumatic event are recurrent and involuntary, avoidance of those memories, persistent negative mood, dissociative symptoms in which reality seems altered and memory of the stressful/traumatic event may be affected, irritability, and disturbances in ability to sleep or concentrate. PTSD has similar symptomology, with recurrent, involuntary and intrusive memories of the traumatic event, dissociative reactions including flashbacks, intense psychological stress due to either internal or external cues related to the initial event, avoidance of distressing memories, and alterations of mood, among other symptoms. For GAD, symptoms may include excessive, uncontrollable worry, restlessness, irritability, disturbed sleep, muscle tension, and difficulties concentrating. MDD symptoms may include persistent depressed mood, anhedonia, insomnia, fatigue, psychomotor problems, changes in weight, feelings of worthlessness, and suicidal thoughts (American Psychiatric Association, 2013). Symptoms for these disorders may vary significantly between patients, but regardless, the symptoms are highly intrusive into one's daily activities and contribute to decreased quality of life.

Economic Burden of Stress

In addition to the detrimental impact on the individual, these disorders also produce a substantial economic burden through lost productivity and cost of treatment, among other factors. Studies analyzing economic burden have found that anxiety disorders cost the United States approximately $42.3 to $46.6 billion annually, with the majority of costs due to lost productivity rather than treatment costs (DuPont et al., 1996; Greenberg et al., 1999). In another study, when compared to other conditions including hypertension, diabetes, and alcohol dependence, GAD was found to have the highest number of per capita work impairment days (Wittchen, 2002). When looking at a combination somatic and psychiatric disorders attributed by work-related stress (back pain, cardiovascular disease, and depression) in France, the estimated cost to the public health insurance system was between €1,167 to €1,975 million; it is more than likely that there is a similar financial burden due to work-related stress in the United States ((Béjean & Sultan-Taïeb, 2005). Personal health care costs are also a concern, with research showing that patients with both GAD and high chronic pain having significantly higher health care costs than the general population (Olfson & Gameroff, 2007).

CHAPTER TWO

Neurobiology of Stress

HPA Axis

The psychological response of an organism to stress is critically dependent on activity of the hypothalamic-pituitary-adrenal (HPA) axis (also known as the stress axis). HPA axis activity is activated by changes in environmental or psychological conditions (such as threats) that increase cytokine activity in the hypothalamus. Typically, the stress response is mediated by the activity of glucocorticoids, which inhibit the transcription of cytokines, which in turn inhibits inflammatory cells. However, the activity of glucocorticoids in the stress response is mediated by Type 2 glucocorticoid receptors (GR). These receptors are low affinity, so their activity is strongest when there are very high levels of glucocorticoids (high affinity, Type 1 mineralocorticoid receptors are important for circadian rhythms, and will not be discussed here) (O'connor, O'halloran, & Shanahan, 2000).

In response to acute physical or psychological stress, the mediating effects of GR allow for increased levels of secreted cytokines, such as interleukin-1 (IL-1) and interleukin-6 (IL-6). The cytokines act on receptors in the hypothalamus to cause the release of corticotropin-releasing hormone (CRH) from parvocellular neurons in the paraventricular nucleus (PVN) and arginine vasopressin (AVP) (Jessop, 1999; O'connor et al., 2000; Turnbull & Rivier, 1999). Once released into the median eminence, CRH and AVP travel to corticotroph cells in the anterior pituitary and bind to CRH receptors causing the production and release adrenocorticotropin (ACTH) as well as activating the

locus-coeruleus-norepinephrine (LC-NE) sympathetic nervous system, another branch of the stress response. LC-NE in turn activates more CRH release; the two systems thus act as a positive feedback mechanism amplifying the stress response. The entire process, from the initial activation of the HPA axis to the release ACTH into the bloodstream is quite rapid. After its release, ACTH travels from the pituitary into the blood stream to the adrenal glands, triggering the release of cortisol, adrenaline, and noradrenaline. These hormones then go on to activate the body's "flight or fight" response by producing physiological changes that help in adapting to potentially dangerous situations (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; O'connor et al., 2000). Adrenaline increases heart rate and helps to ensure that blood goes primarily to the brain and skeletal muscles, while noradrenaline causes vasoconstriction (Sargis, 2015). After initial activation of the stress response, the HPA axis is inactivated through a negative feedback loop. Glucocorticoids (like cortisol) enter back into the brain and act at the pituitary gland, hypothalamus, and hippocampus to decrease the synthesis of ACTH and CRH (Meyer & Quenzer, 2013).

Activation of the HPA axis and the flight or fight response of the sympathetic nervous system in dangerous situations can enhance chances of survival by allocating the body's resources to where they are most needed. However, prolonged activation and problems with regulation of this system in the absence of harm can have a myriad of negative effects. For example, one study showed that chronic mild stress in mice has been implicated in compromised feedback regulation of the HPA axis, indicated by increased levels of stress hormones following a stress procedure (Li et al., 2008).

Disruptions in the normal functioning of the HPA axis are thought to play a role in stress-related disorders. In many people with major depression, there is an increased amount of circulating cortisol, suggesting HPA axis hyperactivity. High circulating levels of cortisol may be due to problems in feedback inhibition. The loss of regulatory control of cortisol levels may involve either lower binding affinity of glucocorticoids for GRs, or decreased expression of GRs (Pariante & Lightman, 2008). The role of GR was further established in an experiment which showed that mice genetically modified to under-express GR showed an increased susceptibility to depression-like behaviors and increased levels of corticosterone (CORT; the rodent equivalent to human cortisol) following exposure to stress, while those that overexpressed GR were more resilient and had reduced peak levels of CORT (Ridder et al., 2005). This indicates that variability in GR expression may indicate degree of predisposition for developing depression.

Changes in the functioning of the HPA-axis are also implicated in the development of anxiety disorders. In children and adolescents diagnosed with an anxiety disorder, salivary concentration of cortisol was correlated with variations in self-reported daily levels of anxiety (Kallen et al., 2008). Physical symptoms of anxiety, however, were associated with decreased HPA-axis output, possibly due to increased activity of negative feedback of the HPA-axis in those individuals. Nonetheless, animal studies support a relationship between high anxiety and increased HPA output. For example, rats bred for high or low anxiety-like behavior behave differently on the elevated plus maze, a common measure of anxiety. Both ACTH and CORT were higher in rats bred for high anxiety-like behavior (Landgraf, Wigger, Holsboer, & Neumann, 1999).

Dendritic Remodeling

Stress has also been found to induce dendritic remodeling. Chronically stressing rats was found to cause a decrease in both the length of apical dendritic pyramidal neurons in the medial prefrontal cortex (mPFC) and the number apical branches, suggesting that stress can change the functional connectivity of the mPFC (Radley et al., 2004). In addition, treatment with CORT produced the same decrease in total length of apical dendrites in pyramidal neurons of the prelimbic and infralimbic cortices. However, there was no decrease in branch number (Cerqueira, Taipa, Uylings, Almeida, & Sousa, 2007). This indicates that CORT levels play a role in dendritic length, but branching is mediated through a different mechanism. Similarly, chronic intermittent stress caused dendritic atrophy in the hippocampus. Interestingly however, stress caused excitatory projection neurons of the basolateral amygdala (BLA) to hypertrophy (Vyas, Mitra, Rao, & Chattarji, 2002). The chronic intermittent stress-induced increase in spine density of BLA neurons is still present after one day, and spine density is gradually increased near the soma ten days later (Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005).

Not all of these dendritic changes are necessarily permanent. Indeed, one study showed that chronic intermittent stress produced the expected atrophy of hippocampus dendrites and hypertrophy of BLA dendrites in rats. However, after a twenty-one day recovery period in their home cage, the dendritic atrophy in the hippocampus was reversed, though both the behavioral effects of increased anxiety and dendritic hypertrophy of the BLA remained (Vyas, Pillai, & Chattarji, 2004). Similarly, stress-

related changes in PFC dendritic retraction were reversible after a several week rest period (Radley et al., 2005).

Amygdala

The amygdala is a small, almond-shaped structure of the brain that is particularly important for mediating neural processes related to emotion, especially the acquisition and expression of conditioned fear. Over-activity of the amygdala is a mechanism implicated in the exaggerated responses to emotion-inducing stimuli common to many stress-related disorders. For example, an fMRI study of people with PTSD found that there was a greater than normal response of the amygdala during presentation of emotionally relevant facial expressions and less habituation to fearful facial expressions. These changes in amygdala activity were accompanied by decreased activity in the prefrontal cortex, which will be discussed later in this chapter (Shin, Wright, Cannistraro et al, 2005).

Structural changes in the amygdala may underlie the increased fear behavior related to stress and anxiety. For example, prenatally stressed rats, which show increased fear and anxiety-like behavior, were found to have a 30% increase in the volume of the lateral nucleus of their amygdala compared to non-prenatally stressed controls. This greater volume was primarily due to an increase in both neurons and glial cells, and may allow for more inputs to the amygdala, thus leading to over-activation (Salm et al., 2004). Similar changes in amygdala volume have also been found in clinical studies. One study found that children brought up in orphanages, where care is typically of lower quality than normal and thus provide early life stress, had larger amygdala volumes and showed

a deficit on performance in a behavioral task that required emotional control. In addition, greater amygdala volume was associated with greater anxiety (Tottenham et al., 2010). A study of moderate to strong depression in women showed that amygdala volume was greater by approximately 13% compared to healthy controls (Lange & Irle, 2004). In contrast, amygdala volume reductions have been reported due to childhood anxiety disorders, especially the gray matter of the left amygdala. While this may seem contradictory considering that hyperactivity of the amygdala is associated with inappropriate stress response and emotional control, this finding may indicate excitotoxicity due to initial hyperactivity (Milham et al., 2005).

In addition to structural changes, circuit disruptions in the amygdala are also implicated in amygdala dysfunction related to stress. In an optogenetic study of amygdala circuitry, selectively activating inputs from the basal lateral amygdala (BLA) to the centrolateral nucleus of the amygdala (CeL) was found to decrease anxiety-like behavior in mice on both elevated-plus maze and open-field test, with selective inhibition of that same circuit demonstrating the opposite effect (Tye et al., 2011). These data highlight the importance of that circuit for control of anxiety, and how damage to this circuit may be implicated in stress-related disorders.

Circuits between the amygdala and other structures of the brain are also important in producing anxiety-like behavior. Data from an fMRI study of people with GAD has also shown circuitry disruption within the amygdala. Patterns of functional connectivity of the BLA and centromedial subregion of the amygdala (CMA) with cortical and subcortical targets, respectively, were decreased in patients with GAD compared to controls, though resting-state connectivity was not significantly affected (Etkin, Prater,

Schatzberg, Menon, & Greicius, 2009). In rodent it was also found that stress prevents stimulation of the BLA to produce long-term potentiation on the medial prefrontal cortex (Maroun & Richter-Levin, 2003).

Prefrontal Cortex

The prefrontal cortex (PFC) is an area in the brain associated with higher-order cognitive functions and top-down control over the amygdala, especially in responses to fearful or emotionally relevant stimuli. This was shown in the aforementioned study of people with PTSD showing greater activation of the amygdala accompanied by reduced activity of the PFC in response to happy or sad faces (Shin et al., 2005). Another study showed that conditioned fear responses in animals could be decreased by stimulation of the medial prefrontal cortex (mPFC) by inhibiting activity in the centromedial nucleus of the amygdala (CeM) (Quirk, Likhtik, Pelletier, & Paré, 2003). In humans, it has been demonstrated that weaker connections between the amygdala and the ventromedial PFC are associated with higher levels of anxiety, further highlighting the importance of the role of the PFC in modulating amygdala influence on responses to distressing stimuli in healthy subjects (Kim & Whalen, 2009). Problems in regulating negative emotion, a hallmark of depression, are also related to dysfunction of PFC regulatory control over the amygdala. During performance of a task that engages such top-down regulatory control, patients with depression were shown to have a more lateralized response of the ventrolateral PFC, demonstrated by increased activation of the right PFC than the left, along with inability to reappraise negative stimuli, even with increased effort. Non-depressed controls, on the other hand, showed more bilateral activation, indicating more

efficient regulatory activity and were able to reappraise negative stimuli (Johnstone, Reekum, Urry, Kalin, & Davidson, 2007).

In addition to top-down control over the amygdala, the prefrontal cortex also plays a role in regulating the activity of the HPA axis. In rodents, it was found that glucocorticoids bind to receptors in the PFC, promoting negative-feedback inhibition of HPA axis activity. In rats with bilateral lesions of the PFC, levels of circulating ACTH and corticosterone increased significantly both twenty and forty minutes after administration of acute stress compared to controls, indicating delayed clearance (Diorio, Viau, & Meaney, 1993).

The activity of PFC also plays a crucial role in the cognitive processes involved in decision making and attention (both of which will be looked at further in the next chapter). One study used threat-related distractors to measure the relationship between anxiety levels and attentional control mediated by activity of the lateral PFC. The lateral PFC was found to be important in controlling attention when those distractors were expected, while the anterior cingulate cortex (ACC) did so with unexpected distractors. As anxiety levels increased, the recruitment of the lateral PFC decreased, indicating poor ability to control attention when faced with a threat (Bishop, Duncan, Brett, & Lawrence, 2004). This finding may indicate a basis for hypervigilance and increased attention to threatening stimuli demonstrated in anxiety disorders. Decision making, on the other hand, relies on activity in the orbitofrontal cortex (OFC). This has been demonstrated in rats exposed to chronic pain, which reduces the amount of dopamine (DA) present in the OFC. These changes were associated with poorer performance on an experimental

gambling task compared to controls (Pais-Vieira, Mendes-Pinto, Lima, & Galhardo, 2009).

Hippocampus

The hippocampus is a structure located in the medial part of the temporal lobe that has central importance for the formation, consolidation, and retrieval of declarative memories (Purves et al., 2008). The hippocampus has also been found to be altered in stress-related disorders. Chronic stress in mice, which produces a cognitive deficit associated with hippocampal dysfunction, has been shown to cause decreased brain-derived neurotrophic factor (BDNF; important for neurogenesis), increased apoptosis, and neuronal damage in the hippocampus. These cellular changes in the hippocampus are thought to be due to neurotoxicity of increased circulating CORT, ACTH, and cytokines (Li et al., 2008).

In an assessment of hippocampal volumes in adults with PTSD due to childhood maltreatment, both hippocampal asymmetry and reduced hippocampal volumes were found compared to healthy controls. The hippocampal asymmetry was also found in children with PTSD from maltreatment, but the reduced volume was only in adults, suggesting that development is affected early on and continues to be affected after the traumatic experience causing PTSD (Woon & Hedges, 2008). An fMRI study further showed that in children with post-traumatic stress symptoms there is less activity in the right hippocampus during a verbal declarative memory task, accompanied by poorer performance on the task than controls. These results are specific to the retrieval component, since encoding showed similar activity in the hippocampus for both controls

and symptomatic patients (Carrión, Haas, Garrett, Song, & Reiss, 2009). The asymmetrical activity may indicate reduced efficiency in retrieval by those with post-traumatic stress symptoms compared to healthy individuals.

In postmortem analyses of depression, cellular changes in the hippocampus included increases in granule cell and pyramidal cell density in the dentate gyrus and hippocampal CA subfields, respectively, as well as decreased soma size in pyramidal cells. These changes all contribute to the decreased hippocampal volume found in depressed patients (Stockmeier et al., 2004). In addition, an fMRI study of comorbidity of depression and GAD showed that there appeared to be a shared change in microstructure of the CA1 and CA2-3 regions of the hippocampus. These abnormalities were associated with deficits in hippocampal functioning (Cha et al., 2016). Another study found that depression causes changes in hippocampal shape, particularly inward deformation of the subiculum which receives inputs from other parts of the hippocampus and projects to other regions of the limbic system and prefrontal cortex (Posener et al., 2003).

CHAPTER THREE

Cognitive and Behavioral Effects of Stress

While stress can be adaptive and actually improve cognitive functioning in some cases, one of the more troublesome effects of stress-related disorders and prolonged stress is their primarily negative impact on cognitive functioning, particularly related to memory, attention, and decision making. The extent and direction in which cognition is affected by stress is best understood by modeling it after the classic Yerkes-Dodson Law for motivation, learning, and difficulty of task. Just as the Yerkes-Dodson Law shows that motivation for learning increases as difficulty increases, then decreases when difficulty is too high, the effects of stress on cognition can be understood as moderate amounts of stress increasing learning in an adaptive manner, but high, prolonged levels of stress overwhelming the brain and causing deficits in cognition (Broadhurst, 1957; Lupien et al., 2007). In addition to changes due to stress-related disorders, stress experienced during the early development can also have a lasting impact on cognition and behaviors.

Effects of Stress on Attention

Selective attention toward a threat is a result of the normal response to stress. One study presented high threat, low threat, and neutral images to participants, finding that there was higher attentiveness toward the more threatening images (Mogg et al., 2000). In another study which looked at the cognitive affects following an acute stressor, participants were asked to play a psychologically stressful video game before undergoing

a selective attention task. Stress was found to slow reaction times and cause attentional inhibition (Skosnik, Chatterton Jr., Swisher, & Park, 2000). These finding imply that the attention is heightened for the tasks most relevant to survival at the expense of attention for other tasks. A comparisons of highly anxious and non-anxious participants found that those with higher anxiety displayed attention more biased toward threatening stimuli, accompanied by enhanced pre-attentive neural reactivity in the primary visual cortex (Eldar, Yankelevitch, Lamy, & Bar-Haim, 2010). This provides evidence supporting that the normal attentional response to threatening stimuli exaggerated in people with anxiety, leading to hypervigilance common to stress-related disorders.

The impact of stress on cognition is not only due to obvious, high intensity stressors, but has also been found to be brought about by daily fluctuations in stress. For example, a within-persons study found that on days in which participants encountered higher stress, their reaction times were slower than on days that were less stressful. In addition, it was found that cognitive performance on attention-requiring tasks decreased due to higher levels of stress (Sliwinski, Smyth, Hofer, & Stawski, 2006). When considering the evolutionary purpose of the stress response, that is, to be able to respond quickly to danger, this seems counterintuitive. However, these results looked at stress level for an entire day, not the effects of an acute stressor, implicating a difference in response to acute versus chronic stress. In addition, hypervigilance and attention biased toward threats decreases attentional resources toward other types of stimuli.

Stress and Decision Making

The ability to make decisions is also affected by stress. In a human study of decision making under psychological stress, subjects had to answer multiple choice questions. One group was under risk of unpredictable administration of harmless, yet painful electrical shock while another group was under risk of shock for poor performance. It was found that under either stressful condition, compared to unstressed controls, stressed subjects were less likely to consider all the available answer options, which further correlated with more errors (Keinan, 1987). While answering multiple choice questions impulsively in the lab may not be particularly consequential to a participant in the long run, poor decision making due to stress becomes more serious when it applies to things such as financial decisions. One study, in which participants were asked to perform a gambling task while undergoing acute stress (one hand placed in ice water for two minutes) found that, due to acute stress, participants tended to make more risky decisions in tasks where they were more likely to lose money, and less risky decisions in the tasks where they were more likely to gain money (Porcelli & Delgado, 2009). Taking these cognition studies into consideration, it appears that stress negatively influences the ability to perform on tasks requiring higher order cognition by lessening attention and causing more impulsive, rather than well-considered, decisions. This can be particularly consequential when it applies to important decisions such as finances, which can affect not only the stressed individual, but also their family and livelihood. Using MRI technology, it was found that while performing a risky decision making task in combination with a working memory task, stressed groups had higher activation of the anterior prefrontal cortex (Gathmann et al., 2014). In rats, decision making deficits

caused by chronic stress were studied and found to be due to changes in corticostriatal circuits. Specifically, associative circuits underwent atrophy, while the opposite occurred in sensorimotor circuits (Dias-Ferreira et al., 2009).

Stress and Memory

Stress can also have a negative impact on memory. In mice, chronic mild stress was found to cause a deficit in performance on an object recognition test, a behavioral assay used to model episodic memory. In addition, stressed mice showed a deficit in the ability to discriminate between new and familiar locations (Li et al., 2008). However, acute stress was found to actually enhance working memory in rats. This appears to be due to activation of glucocorticoid receptors, which enhances NMDA receptor and AMPA receptor function and trafficking, which go on to increase synaptic transmission in the prefrontal cortex (Feng et al., 2011). The effects of glucocorticoid activity on memory have also been investigated in humans. In a study of the interaction between circulating glucocorticoids and memory retrieval, researchers found that administration of a stress-level dose of cortisone caused decreased blood flow in the temporal lobe, especially in the parahippocampal gyrus, and impaired performance on a declarative memory recall task (De Quervain et al., 2003).

Stress also impairs long-term potentiation (LTP) in the hippocampus. Hippocampal cells of rats that have undergone restraint and tail shocks display impaired LTP compared to controls tested using *in vitro* electrophysiology (Foy, Stanton, Levine, & Thompson, 1987). In addition to the role of the hippocampus in stress-related memory changes, changes in other areas of the brain have also been implicated in memory

deficits. For example, acute inescapable stress inhibits LTP in both the amygdala and the prefrontal cortex (PFC), areas involved in memory formation and higher order cognitive functioning; this suggests the role of stress in producing amygdala-dependent automatic responses to stress (Maroun & Richter-Levin, 2003).

While chronic stress seems to induce memory deficits, the presence of an acute stressor may actually have a way of strengthening memory and learning, which in the most extreme cases, can be likened to the persistent, intrusive memories associated with disorders such as PTSD. This strengthening of memories for fear-inducing stimuli in non-pathological cases may be evolutionarily adaptive as a way of increasing chances of survival during future encounters with similar dangerous stimuli. One study found that, although a decrease in hippocampal neurogenesis was associated with chronic unpredictable stress, predictable shocks showed the opposite effect. Rats that experienced the predictable shocks treatment showed improved cognitive functioning, particularly in hippocampal-dependent memory, likely due to increased neurogenesis and dendritic growth in the hippocampus. In addition, those rats displayed less anxiety-like behavior compared to controls (Parihar, Hattiangady, Kuruba, Shuai, & Shetty, 2011). Post-learning stress has also been implicated in improving memory consolidation in humans. However, the relationship between increased stress hormone levels (particularly cortisol and norepinephrine) and improved memory was only found for emotionally arousing stimuli, showing an interaction between those hormones and the process of post-learning consolidation in emotionally salient contexts (Cahill, Gorski, & Le, 2003). Furthermore, there is evidence that increased noradrenergic activity in the lateral nucleus of the amygdala (LA), an important site of fear learning, contributes to the strengthening

of memory consolidation, which may be what is occurring with the intrusive traumatic memories associated with PTSD. Pharmacological evidence shows that blocking noradrenergic receptors in the LA impairs both consolidation and reconsolidation of fear memories (Dębiec & LeDoux, 2006).

Behavioral Changes Due to Early Life Stress

During periods of early development, stress can have lasting impact on behavior and cognition. Since circuits are forming and changing greatly during periods of development, sensitivity to the effects of stress may be greater during early life than at later points. These effects have been studied extensively in animals by introducing stressors prenatally and in the first few postnatal weeks (which when compared to humans, models development occurring during the last trimester). One of the long-term effects of prenatally stressed rats is an acceleration of HPA axis dysfunction that typically occurs as a result of aging, reflected by abnormal changes in glucocorticoid levels of middle-aged rats. In addition, 21 month old animals that had been prenatally stressed performed more poorly on spatial recognition memory tests, giving further evidence for interactions between glucocorticoids and mechanisms for memory (Vallée et al., 1999). Additional evidence for prenatal stress causing memory deficits has been found in a study showing deficits in spatial learning, which requires hippocampal activity, and less neurogenesis in the dentate gyrus both early in life and in adulthood of prenatally stressed rats (Lemaire, Koehl, Moal, & Abrous, 2000).

Prenatal stress also has been shown to cause weakened avoidance learning, which indicates increased emotionality, and in female rodents, less activity in an open field test

(Lehmann, Stöhr, & Feldon, 2000). In a guinea pig study of the effects of prenatal stress decreased open field activity was only found in males, suggesting sex-related differences that may vary between species. In addition, the study found that basal cortisol levels were increased in the prenatally stressed animals, but that they had a decreased adrenocortical response to being placed in the open-field than non-stressed animals and no significant difference in amount entries to the middle of the field compared to controls (a decrease in entries would indicate higher anxiety-like behavior) (Emack, Kostaki, Walker, & Matthews, 2008). Elevated plus-maze data has shown that prenatally stressed rats, when later exposed to acute stress, show more anxiety-like behavior, indicated by increased time in open arms of the maze, than rats that have only been acutely stressed, suggesting that stress early in development increases vulnerability to stress later in life (Estanislau & Morato, 2005). Another study done by the same lab showed that the anxiolytic effects of prenatal stress shown by behavior in the elevated plus-maze do not appear until adulthood. Interestingly, in late adolescence, prenatally stressed animals seem to exhibit less anxiety-like behavior and were more likely to investigate the open arms of the elevated-plus maze (Estanislau & Morato, 2006). This may be due to a resiliency effect from stress that is related to age at which a stressor is experienced.

Early life stress in humans, while much harder to study, has also been shown to have long term negative consequences. One study of childhood poverty showed that the chronic stressors children living in poverty experience may lead to poor functioning of emotion regulatory systems. MRI data of young adults showed that those who had lived in poverty during childhood had could not suppress amygdala activity in order to control negative affect, likely due to decreased activity in the prefrontal cortex (Kim et al., 2013).

These findings, along with the previously mentioned animal studies, indicate a strong case for the influence of early life stress on mental health outcomes in adulthood.

CHAPTER FOUR

Treatment of Stress-Related Disorders

While stress related psychiatric disorders such as anxiety and depression have a large impact on the well-being of individuals, their families, and even their workplace, there is much that is yet to be understood about these disorders, their mechanisms, and how to effectively treat them. Treatment for anxiety and mood disorders include both pharmacotherapy and cognitive behavioral therapy (CBT), though even with treatment, some patients suffering from these disorders show little or no improvement. In fact, for patients with mild to moderate depression, antidepressant drug treatment and placebo treatment had the same efficacy, and it was only in severely depressed patients that drug treatment proved significantly efficacious (Fournier JC, DeRubeis RJ, Hollon SD, & et al, 2010). For the treatment of anxiety, the use of the antidepressant drug paroxetine reduced anxiety for 68% of patients (compared to 46% for the placebo group), however remission was only achieved by 36% of patients, leaving a large number of patients with untreated symptoms and even more without remission (Rickels et al., 2003).

Cognitive Behavioral Therapy vs. Pharmacotherapy

The use of CBT for treatment of anxiety disorders is significantly more effective than placebo treatment, however there is still need for better, more effective treatments of anxiety disorders in order to help current-treatment resistant suffers (Hofmann & Smits, 2008). For depression, comparisons of treatment with pharmacotherapy versus psychotherapy show that they have about the same effectiveness, helping to alleviate only

24% and 28% of the "disease burden" respectively (Vos et al., 2004). One meta-analysis comparing pharmacotherapy and psychotherapy in the treatment of a range of anxiety and depressive disorders suggests that treatment efficacy varies largely depending on what specific disorder is being assessed. For example, it found that treatment of OCD with psychotherapy had a bigger effect than pharmacotherapy, but there was no significant difference in effect for treatment of panic disorder, MDD, and social anxiety disorder. In addition, the meta-analysis showed that the type of treatment was important; particularly that both tricyclic antidepressants (TCAs) and non-directive counseling were less effective than psychotherapy (Cuijpers et al., 2013).

While comparisons of the efficacy of pharmacotherapy versus psychotherapy are unclear in many respects, it is important to take into consideration the fact that psychotherapy avoids the unpleasant side effects often brought about by pharmacotherapy, which may affect patient adherence to treatment. For example, patients taking selective serotonin reuptake inhibitors and tricyclic antidepressants for treatment of MDD had a 14.4% and 18.8% drop-out rate due to side effects, respectively (Anderson & Tomenson, 1995). In addition, it is important to note that, like pharmacotherapy, CBT produces changes in the brain, such as modulation of the metabolic processes in cortical-limbic pathways (Goldapple, Segal & Garson et al., 2004). It has also been suggested that the combination of pharmacotherapy and psychotherapy increases the response rate to treatment and reduces the treatment drop-out rate (Pampallona, Bollini, Tibaldi, Kupelnick, & Munizza, 2004).

SSRIs

Selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed treatments for both depression and anxiety disorders. SSRIs exert their action by blocking serotonin transporters, thus preventing reuptake of serotonin (5-HT). When serotonin is released by a cell into the synapse, it is able to act on a serotonin receptor, of which there are seven different subtypes (all G-coupled protein except for 5-HT_3, which is ionotropic) (Squire et al., 2012). Areas with high serotonin receptor densities in the brain include layers III and V of the cerebral cortex and the raphe nucleus of the brainstem, though receptors are located in many other parts of the brain as well, including the amygdala and hippocampus (Pazos, Probst, & Palacios, 1987). The serotonin transporter, a protein with twelve transmembrane helices and an extracellular loop between the third and fourth helices, is also present in the synapse and acts to clear excess serotonin and transport it back into the cell, thus preventing extended action of serotonin at its receptors. The action of the serotonin transporter depends on the binding of both sodium and chloride ions, along with serotonin. When all three are bound, the protein undergoes a conformation change, allowing it to carry serotonin back into the cell. Blocking the transporter allows for serotonin to remain in the synaptic cleft and therefore continue to act at its receptors for a longer amount of time or to travel to more distant receptors (Sghendo & Mifsud, 2012).

A well know SSRI is fluoxetine, commonly known by its brand name, Prozac, which will be used here as a model for explaining how SSRIs work. Fluoxetine has a good oral bioavailability of approximately 72% and reaches its peak plasma concentration approximately six to eight hours after administration. A typical prescribed

dose of fluoxetine is from 5 to 20 mg/day (Sghendo & Mifsud, 2012). Fluoxetine is metabolized by the liver to an active compound called norfluoxetine. Both fluoxetine and norfluoxetine have fairly long half-lives which increase with continued administration, causing drug clearance to take up to several weeks (Leonard, 1995). Fluoxetine is able to effectively bind to serotonin transporters with higher than 80% rate of occupancy and it can remain bound for 50 hours, allowing for prolonged accumulation of 5-HT in the synapse (Sghendo & Mifsud, 2012). Although fluoxetine and other SSRIs are able to exert their drug action fairly quickly, the therapeutic effects may not be felt until after about two weeks of drug administration. This indicates that the therapeutic effects of SSRIs are likely related to other downstream changes that occur later, such as changes in gene expression or serotonin receptor adaptations which may vary at different brain regions (Stahl, 1998).

Benzodiazepines

Benzodiazepines (BDZs) are a commonly prescribed class of anxiolytic drugs, which also act as anticonvulsants, sedative-hypnotics, and cause relaxation of muscles (Tallman, Paul, Skolnick, & Gallager, 1980). These drugs act as an agonist for gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter of the brain by binding to a specific BDZ site on $GABA_A$ receptors. The $GABA_A$ receptor complex is ionotropic, allowing for influx of chloride ions. It consists of five subunits and, in addition to its binding site for GABA, has modulatory sites for other substances such as BDZs, barbiturates, and neurosteroids. While the BDZ binding site on the $GABA_A$

receptor complexes are widespread, there is a particularly high concentration in the amygdala, other limbic structures, and the cerebral cortex. (Meyer & Quenzer, 2013).

One type of BDZ is alprazolam (or Xanax), used here as a model for the action of this class of drugs. Alprazolam has good oral bioavailability of 80 to 100% (Greenblatt & Wright, 2012). It takes approximately one to two hours after administration for alprazolam to reach its peak plasma concentrations, and it has a half-life of nine to sixteen hours (Verster & Volkerts, 2004). A typical effective dose of alprazolam is 3 mg or less per day (Dawson, Jue, & Brogden, 2012). Alprazolam is metabolized in the liver and has at least twenty-nine different metabolites, though the majority of alprazolam is excreted by the kidney in its initial form. Alprazolam acts by binding to the BDZ site of $GABA_A$ receptors, where its modulatory effects allows for better binding of GABA to its receptor, in turn causing greater inhibitory activity in the brain. This increased inhibition is the basis for its sedative effects (Verster & Volkerts, 2004). In addition, the anxiolytic effects of benzodiazepines such as alprazolam may also be due to their interaction with serotonin and norepinephrine systems. In rats it was found that oxazepam (another BDZ) caused decreased turnover of both serotonin and norepinephrine. While the modulation of norepinephrine turnover underwent tolerance, that was not the case for serotonin, suggesting that this may be an important part of the efficacy of BDZs in treating anxiety disorders (Wise, Berger, & Stein, 1972).

While drugs such as SSRIs and benzodiazepines can be effective treatments for stress-related disorders, they are not completely effective in all populations and may also cause unpleasant side-effects such as nausea, drowsiness, headache, mood changes, and weight changes (among others) due to binding of drugs to receptors outside their target

regions. In order to mitigate this, newer, more highly selective drugs have been and continue to be developed.

CHAPTER FIVE

Research Models and Future Directions

Animal Models for Researching Stress

Stress research is a broad field, with labs around the world looking at various elements of stress and related disorders, from causes and risk factors such as early life stress, neurobiological changes, behavioral and cognitive effects, and mechanisms of treatment. Various manipulations for inducing stress responses in animals (mainly rodents) have been developed and found to be effective. These methods range from selective breeding for high or low anxiety-related behaviors, to exposure to various types of chronic or acute stressors. However, determining whether an animal is anxious or depressed poses a different problem when trying to produce valid models for studying these disorders. Psychological stress factors are extremely important for stress-related disorders, but unfortunately these cannot be evaluated in animals. Nonetheless, animal studies are still useful for researching the neurobiological factors of stress and methods for evaluating anxiety-like and depressive-like behaviors in animals have been developed using several different behavioral assays. The following behavioral assays are useful for measuring levels of anxiety from stressors or anxiolytic effects of drugs or other manipulations.

One common behavioral assay is the open-field test, which measures anxiety-like behavior by measuring the amounts of time spent near the walls of a large, usually square, arena, and the amounts of time spent in the center. The arena is brightly lit and unprotected, and the rat is placed in it alone, both of which are factors that create a

stressful environment for rodents. Since rodents tend to display thigmotaxis, exploration of the center, measured either by entries or time spent in that region, indicate less anxiolytic-like behavior. In addition, grooming behavior and the number of fecal boli may also be evaluated to determine anxiety-like behavior (Bailey & Crawley, 2009).

Elevated plus maze is another useful behavioral assay for evaluating levels of anxiety-like behavior in rodents. The maze consists of two arms that have high walls surrounding them, and two arms that are open. Greater number of entries or amount of time spent exploring the open arms indicates anxiolytic-like behavior, while entries or time in closed arms show the opposite. Another measure of anxiolytic-like behavior commonly used is head dips below the side of the open arms. One variation of this assay is the elevated zero maze, which eliminates ambiguity of how to score time spent in the center square of the plus maze (Hogg, 1996).

Rodents are naturally social creatures, and will therefore normally approach and actively interact with another rodent it has previously never encountered. However, these social interactions have been found to decrease as stress increases. It has been shown that when two unfamiliar rats are placed together, the amount of time actively interacting with each other is inversely related to the brightness or unfamiliarity of the testing arena (since rodents prefer dark places and tend to be xenophobic) (File & Hyde, 1978). This behavioral assay is particularly useful since its relationship to human behaviors related to stress is more obvious (for example, social withdrawal associated with depression or anxiety disorders).

One assay for testing the efficacy of antidepressants is the forced swim test. This test has been found to have high predictive validity of the antidepressant effect of

different drugs. In this test, rodents are placed in a cylindrical container filled with water so that they can neither touch the bottom and still keep their head above the surface, nor climb out of the container to escape the water, therefore forcing the animal to either float or swim (both of which they are naturally capable of doing). Behavior is evaluated based on the amount of time the animal is immobile (floating), which is typically thought to indicate "behavioral despair", or the amount of time swimming, climbing, or diving (active behaviors). Efficacy of antidepressant drugs is associated with greater amounts of time performing active behaviors and a longer latency to immobility (Castagné, Moser, Roux, & Porsolt, 2010).

Studies of Early Life Stress

There have been many advances in the specific effects of early life stress, as reflected by the current literature. It is important to study early life stress, because, while individuals may be able to make life changes to aid in coping with later-life stress, early life stressors cannot be controlled by the individual experiencing that stress, and can lead to problems in adulthood. It is possible that the development of ways to address early life stressors early on may help to mitigate the long-term effects of stress, leading to a better overall quality of life. However, in order to develop such treatments or interventions, more must be understood about the effects of early life stress and how they affect the brain.

The effects of prenatal stress are typically studied by stressing mother rats. The increased levels of cortisol induced in the pregnant dam cause decreased levels of glucocorticoid receptors in the hippocampus, hypothalamus, and the mPFC, which leads

to deficit in the negative feedback response of the HPA-axis and may underlie the previously discussed behavioral changes demonstrated by prenatally stressed rats (Bingham, Sheela Rani, Frazer, Strong, & Morilak, 2013). Early life stress can also be studied using maternal separation postnatally. Isolating a pup from its mother and siblings daily during the first few weeks of life has been shown to cause higher CORT levels in response to stressors and increased anxiety-like behavior later in life (Kalinichev, Easterling, Plotsky, & Holtzman, 2002).

Future Directions

One future direction for our lab is to investigate how early life stress affects decision making in adulthood, measured using a delayed discounting task. One hypothesis is that early life stress compromises decision making through bottom-up processing by increased amygdala activation leading to decreased PFC functioning. This study will have three aims: (1) show that early life stress can produce vulnerability to acute stress during adulthood, (2) elucidate the mechanism of stress-induced differences involving the PFC and its interactions with the amygdala, and (3) show that those stress-induced difference can be reversed by using optogenetic activation of the PFC. In addition, the effects of early life stress on synaptic reorganization in these regions could by investigated by measuring levels of PSD95, a marker for synaptic spine density.

In order to investigate the first aim, rats will be separated into four different groups: (1) chronic postnatal stress and acute restraint stress in adulthood, (2) postnatal stress only, (3), adult stress only, and (4) non-stressed controls. These four groups will then be compared using a delayed discounting test in order to evaluate prefrontal cortex-

dependent behaviors, with the hypothesis that the group receiving the combination of postnatal stress and adult stress will show the greatest deficit in performance on the delayed-discounting task compared to the other groups.

The second aim will be studied using both lesioning techniques and optogenetic techniques. Non-stressed rats will undergo one of four conditions: (1.) excitotoxic lesion of the PFC, (2) sham lesion, (3) optogenetic activation of amygdala inputs to the PFC, and (4) sham optogenetics. The hypothesis for this aim is that the treatment groups (1 and 3) will show deficits in delayed-discounting task performance compared to the sham controls, indicating that PFC activity is crucial for task performance, and that over-activity of the amygdala inhibits PFC functioning needed for the task.

Finally, the third aim will show that strengthening top-down control of the PFC over the amygdala will reverse the effects of stress on task performance. To do so, rats that have undergone both postnatal stress and adult stress will either undergo optogenetic activation of the PFC or sham optogenetics. It is hypothesized that by selectively activating the PFC, task performance in the stressed rats will be returned to baseline (non-stressed) levels. This experiment would be a novel way of determining the mechanisms through which interaction of the PFC and amygdala affect the stress response and it effect on higher-order cognition in animals vulnerable to stress due to early-life experience.

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