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Biological Mechanisms Linking Stress and Anhedonia

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Abstract

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Colin Ho-Ming Stanton
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Evidence from research across species suggests that stress exposure is linked with anhedonia (loss of pleasure and/or decreased motivation). However, the mechanisms through which stress might impact anhedonia remain unclear. Chapters 1 and 2 of this dissertation review putative etiological pathways from stress to anhedonia and discuss stressor characteristics that could inform experimental models of stress-induced anhedonia. Chapter 3 describes an attempt to identify which types of stress are most associated with anhedonia using stress interview data from multiple datasets. Unexpectedly, we found no credible effects on anhedonic symptoms for stressor chronicity, severity, dependence on behavior, or interpersonal focus. Instead, number of stressors endorsed was the best predictor of anhedonic symptoms. Next, Chapters 4 and 5 report on two studies that tested possible biological mediators of the stress-anhedonia link. Chapter 4 describes an analysis of the UK Biobank dataset aimed at evaluating frontostriatal functional connectivity as a mechanism of stress-induced anhedonia. Although stress exposure predicted anhedonia, analyses uncovered no stable relation between frontostriatal connectivity and anhedonia, and no support for the proposed mediation model. Chapter 5 details a study that implemented a laboratory-based stressor to assess its potential impact on motivated behavior (thought to be a key component of anhedonia), and whether any such effects might be mediated by inflammatory

responding. Low concentrations of salivary cytokines suggested questionable validity of inflammatory assessment, and no effect of stress on inflammatory responding was observed. Additionally, stress produced no measurable changes in motivated behavior. Thus, analyses revealed no evidence consistent with inflammation as a mechanism of stress-induced anhedonia. Finally, Chapter 6 discusses conclusions and implications of the current findings, and provides ideas for future directions.

An Investigation of Biological Mechanisms Linking Stress and Anhedonia

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Table of Contents

Chapter 1: General Introduction

General Introduction

Anhedonia, defined as the loss of pleasure and/or motivation to engage in valued activities, is a debilitating condition observed in several psychiatric disorders, including major depressive disorder (MDD), post-traumatic stress disorder (PTSD), and schizophrenia (Barch & Dowd, 2010; Nawijn et al., 2015; Pizzagalli, 2014). Anhedonia has been linked to suicide completion among individuals with MDD (Fawcett et al., 1990) and is poorly treated by serotonergic antidepressants (Nutt et al., 2007). Additionally, disorders characterized by anhedonia exert a significant personal, societal, and economic toll worldwide (Whiteford et al., 2013). Reducing the burden of anhedonia could therefore lead to substantial benefit. However, the causal pathways to anhedonia remain poorly understood, limiting innovation in diagnosis, treatment, and prevention.

Focusing on the etiology of anhedonia, as opposed to a broader psychiatric phenotype such as MDD, may yield more reliable findings than applying widely-used diagnostic categories. Indeed, considerable heterogeneity in the presentation of these diagnostic categories likely hampers investigation into possible causal mechanisms, since current diagnoses accommodate a wide variety of symptom presentations with potentially disparate etiologies (Fried, 2017). For example, one study identified over 1,000 unique symptom profiles for individuals with MDD, some of which did not share a single common symptom (Fried & Nesse, 2015). Furthermore, these diagnostic categories are inherently descriptive in nature, and were derived from clinical observation rather than knowledge of causal mechanisms (Clark, Cuthbert, Lewis-Fernández, Narrow, & Reed, 2017). By contrast, delineating the mechanistic pathways that contribute to specific symptoms could produce more constrained, empirically-defined phenotypes.

One possible approach to identifying an etiological pathway to anhedonia is to begin with a precipitating factor, such as exposure to psychological stressors. Cross-species work suggests that stress exposure contributes to anhedonic-like (i.e., diminished reward-seeking) behavior in nonhuman animals (Anisman & Matheson, 2005; Hollon, Burgeno, & Phillips, 2015; Russo & Nestler, 2013) and decreased behavioral sensitivity to rewards as well as increased self-reported anhedonic symptoms in humans (Pizzagalli, 2014). (For more on the link between stress and anhedonia, see the literature review in Chapter 2.) Yet much remains unknown about the connection between stress and anhedonia.

For instance, little is known about the dimensions of stress that most strongly predict anhedonia. Nonhuman animal studies of stress-induced anhedonia vary in the chronicity, severity, and type of stress implemented (Anisman & Matheson, 2005). However, few studies in humans have addressed the effects of different types of stressors on anhedonia, and as a result the validity of these preclinical models remains unclear. Human subjects research by Keller, Neale, and Kendler (2007) using a large sample of twins suggests that anhedonia may be especially common following some types of stressors (e.g., death of a loved one or romantic loss) relative to others. Yet while this design tested the relative impact of different categories of stress on patterns of depressive symptoms, the roles of potentially important factors, such stress severity, were not assessed. Additionally, chronic stressors were grouped into a single category, while acute stressors were categorized according to the theme of the stressor (e.g., “personal failure or abandoned goals”), making it difficult to compare acute and chronic stressors directly. Thus, follow-up work is needed to investigate the impact of key dimensions of stress,

including severity and chronicity, on anhedonia. A more fine-grained understanding of how certain stressors are differentially associated with anhedonia could inform the implementation of ecologically-valid stress manipulations across species. This knowledge could also help identify which individuals are at risk for developing anhedonia, which could inform further research (e.g., using longitudinal high-risk designs) and preventative care.

Furthermore, the mechanisms through which stress may influence risk for anhedonia remain largely unknown. However, some evidence suggests that alterations in medial prefrontal cortex (mPFC) interactions with mesolimbic circuitry, including ventral tegmental area (VTA) and nucleus accumbens (NAc), could contribute to decreases in motivated behavior following stress (Russo & Nestler, 2013). As for how stress might perturb these frontostriatal dynamics, several hypotheses have been put forward. Some studies suggest that stress exposure could increase anhedonia risk by producing mPFC hypofunction (Covington et al., 2010; Ossewaarde et al., 2011; Treadway, Buckholtz, & Zald, 2013), whereas other findings suggest increased mPFC excitability could account for decreases in motivated behavior (Ferenczi et al., 2016; Moreines, Owrutsky, & Grace, 2017). These seemingly contradictory findings are further explored in Chapter 2. Nevertheless, evidence suggests that frontostriatal interactions are a worthy target for future study as a possible mechanism of stress-induced anhedonia. Many of the key studies described here were conducted in rodents (Chaudhury et al., 2012; Covington et al., 2010; Ferenczi et al., 2016; Moreines et al., 2017). Additionally, human studies have typically not tested whether frontostriatal connectivity statistically mediates the relation

between stress and anhedonia (Ossewaarde et al., 2011; Treadway et al., 2013). Thus, this model awaits further testing in a sufficiently large human-subjects sample.

In addition to frontostriatal functioning, inflammatory responses might play a role in stress-induced anhedonia, possibly by impacting dopaminergic reward responses (Felger & Treadway, 2017). This hypothesis and relevant findings are discussed in greater detail in Chapter 2. Briefly, in rhesus monkeys, chronic administration of interferon-α, a pro-inflammatory cytokine, decreased effort-based sucrose consumption and blunted dopamine release in the caudate following stimulation (Felger, Mun, et al., 2013). Follow-up investigation revealed that these deficits were abolished by administration of L-DOPA, the precursor to dopamine (Felger, Hernandez, & Miller, 2015). Together, these findings suggest that inflammation could produce anhedonia by disrupting dopamine synthesis. Additionally, following social defeat stress in rodents, the inflammatory cytokine interleukin-6 (IL-6) increases in blood circulation and appears to infiltrate nucleus accumbens. In conjunction with synaptic remodeling in NAc after stress (J. Wang et al., 2018), IL-6 infiltration across the blood-brain barrier may contribute to the development of anhedonic-like behavior in rodents (Hodes et al., 2014; Menard et al., 2017; J. Wang et al., 2018).

However, in human studies, evidence is considerably more mixed. In one fMRI study, stress-induced increases in IL-6 were associated with diminished reward prediction error-linked responses in ventral striatum following stress, but no behavioral effects emerged (Treadway et al., 2017). Additionally, a recent study found that stress-induced increases in IL-6 mediated the impact of stress on changes in reward responsiveness (Boyle, Stanton, Eisenberger, Seeman, & Bower, 2020). However, unexpectedly, stress-

induced IL-6 was associated with *increased* reward responsiveness (Boyle et al., 2020). Given promising findings in nonhuman animal models, more work is needed to clarify the connections between stress, inflammation, and subsequent reward processing.

In summary, research suggests that stress contributes to the onset of anhedonia. However, the impact of key dimensions of stress on anhedonia remains unclear, potentially limiting the ecological validity of preclinical models and hindering the search for plausible mediators. Additionally, few studies have assessed promising mechanisms of stress-induced anhedonia (e.g., frontostriatal connectivity, inflammatory responses) in humans. Addressing these empirical gaps could aid the elaboration of etiological pathways to anhedonia. The delineation of such pathways is essential to the creation of a more constrained, empirically-derived phenotype, which could facilitate diagnosis and provide targets for novel clinical interventions.

Overview of Dissertation Chapters

The broad goal of this dissertation is to better characterize the link between stress and anhedonia. Chapter 2 provides a literature review that covers putative biological mechanisms of stress-induced anhedonia and provides additional relevant background information. Chapter 3 presents an attempt to statistically model the impact of key dimensions of stress (including chronicity and severity) on anhedonia. Chapter 4 describes an investigation of mPFC functional connectivity with NAc, a key node of the mesolimbic reward circuit, to determine whether frontostriatal dynamics could plausibly mediate the effect of stress on anhedonia. Next, Chapter 5 details an examination of inflammatory responses to a laboratory stressor and their association with subsequent

motivated behavior. Finally, a synthesis of results and ideas for future directions are presented in Chapter 6.

Chapter 2: Literature Review

From Stress to Anhedonia: Molecular Processes through Functional Circuits

Adapted from:

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Abstract

Converging evidence across species highlights the contribution of environmental stress to anhedonia (loss of pleasure and/or motivation). However, despite a clear link between stress and the emergence of anhedonic-like behavior in both human and animal models, the underlying biological pathways remain elusive. Here, we synthesize recent findings across multiple levels, from molecular signaling pathways through whole-brain networks, to discuss mechanisms through which stress may influence anhedonia. Recent work suggests the involvement of diverse systems that converge on the mesolimbic reward pathway, including medial-prefrontal cortical circuitry, neuroendocrine stress responses, homeostatic energy regulation systems, and inflammation. We conclude by emphasizing the need to disentangle the influences of key dimensions of stress on specific aspects of reward processing, taking into account individual differences that could moderate this relationship.

Introduction

Our response to environmental stressors helps to guide decision-making in an evolutionary balancing act that pits the pursuit of rewards, crucial for survival and reproduction (e.g., food and mating opportunities), against potential threats (e.g., predators and pathogens) (A. J. Holmes & Patrick, 2018; Lima, 1998). Stressors can tip this balance by decreasing reward-seeking behavior (Anisman & Matheson, 2005). Seen through an evolutionary lens, decreased approach behavior in response to environmental threats may be highly adaptive in some contexts. For example, following physical harm or the threat of infectious disease, **anhedonia** (see **Glossary**) and social withdrawal may preserve resources for healing wounds and inhibit the spread of pathogens (A. H. Miller & Raison, 2016; Raison & Miller, 2016). However, the adaptive value of a given trait or behavior is sensitive to both environmental context and complex interactions across cognition, behavior, and genetics (Dingemanse, Kazem, Réale, & Wright, 2010; A. J. Holmes & Patrick, 2018). For some individuals with existing vulnerabilities, such as ruminative coping styles following a traumatic event (Michael, Halligan, Clark, & Ehlers, 2007), stress responses culminating in anhedonia can trigger the onset of psychiatric illness.

In this review we highlight some of the diverse circuit-level and molecular mechanisms through which stress could lead to anhedonia. In doing so, we adopt a multi-system, multi-level approach, in which we examine how the effects of stress may echo across levels of analysis (e.g., molecular processes and functional circuits) and involve interactions between diverse systems (e.g., immune responses that alter brain reward functioning). We first discuss likely contributors to stress-induced anhedonia at the level

of neurocircuitry, including systems that govern motivated behavior, neuroendocrine responses to stress, and energy **homeostasis**. Next, we review possible pathways to stress-induced anhedonia at the molecular level, with a particular focus on immune system signaling pathways. We conclude with a roadmap of promising future directions in the study of stress and anhedonia.

Impact of Stress on Anhedonia

Research across species, including rodents and humans, has demonstrated a link between stress and anhedonic-like behaviors (Anisman & Matheson, 2005; Hollon et al., 2015; Pizzagalli, 2014; Russo & Nestler, 2013). Here, we briefly review evidence of this relationship. Readers should note that more detailed reviews covering cross-species work on stress and anhedonia are available elsewhere (Anisman & Matheson, 2005; Hollon et al., 2015; Pizzagalli, 2014; Russo & Nestler, 2013).

Rodent studies have employed a variety of stress manipulations. These include social defeat stress, in which a rodent is placed in proximity to another, aggressive rodent and subjected to physical attack (Golden, Covington, Berton, & Russo, 2011); and chronic mild stress (CMS), in which rodents are exposed to an unpredictable series of stressors, including 24-hour light cycle, food deprivation, and damp bedding (Tye et al., 2013; Willner, 2005). Research groups employing these approaches have discovered associated decreases in reward-seeking behaviors, suggestive of decreased pleasure and/or motivation. Social defeat and CMS in rodents, for example, produce blunted sucrose preference and/or diminished social interaction (Hollon et al., 2015; Russo & Nestler, 2013; Willner, 2005).

Although considerably less work has focused on the study of stress and anhedonia in humans, results are broadly consistent with the nonhuman animal literature (Ironside, Kumar, Kang, & Pizzagalli, 2018; Pizzagalli, 2014). Following naturally-occurring stressors (e.g., medical residence examinations; Soares et al., 2012), individuals self-report decreased pleasure in daily activities (Berenbaum & Connelly, 1993) and exhibit lowered sensitivity to **reward devaluation** (Soares et al., 2012). Laboratory studies using threat of shock as a stressor (Berghorst, Bogdan, Frank, & Pizzagalli, 2013; Bogdan & Pizzagalli, 2006) have found decreased **response bias** toward rewarded outcomes (Bogdan & Pizzagalli, 2006) and diminished reward sensitivity (Berghorst et al., 2013). In support of this experimental evidence, large, observational studies have established a link between life stress and phenotypes that are often marked by anhedonia, such as major depressive disorder (MDD) (Brown & Harris, 1978; Hammen, 2005; Kendler, Karkowski, & Prescott, 1999). Notably, few large-scale studies have assessed the impact of stress on anhedonia *per se* (for an exception, see Keller et al., 2007), and therefore more work is needed to examine individual symptoms. In all, converging evidence across species suggests that stress can produce anhedonic behavior.

Putative Circuit-level Mediators of Stress-Induced Anhedonia

Effects of stress on motivated behavior depend on the interplay of systems spanning medial prefrontal cortex (mPFC), midbrain and striatum, amygdala, hypothalamus, brainstem, and other regions implicated in reward processing (Russo & Nestler, 2013) (Box 1). Due to space constraints, we focus on the contributions of three brain systems: mesocorticostriatal reward circuits (mPFC, midbrain, and striatum); subcortical stress response circuits (including hypothalamus and extended amygdala);

and brainstem-based energy homeostasis circuits (including GLP-1 neurons). Readers should note that although we discuss these separately for the purposes of organization, the distinctions are largely arbitrary. The amygdala, for instance, is involved in reward processing (Murray, 2007). Other reviews cover emerging research on the contributions of mu opioid systems (Ironside et al., 2018) and the lateral habenula (Yang, Wang, Hu, & Hu, 2018), and provide a more molecular-level focus on stress-induced changes in corticostriatal circuitry (Russo & Nestler, 2013).

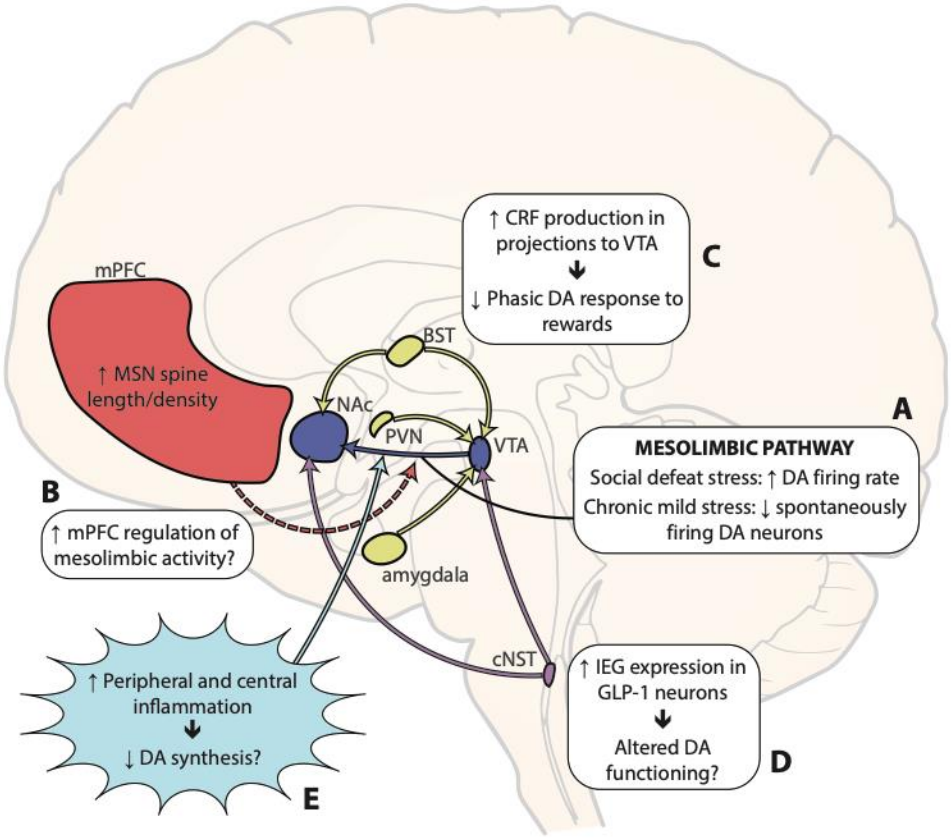


Figure 1. Putative circuit-level and molecular mechanisms of stress-induced anhedonia. (A) Social defeat stress (10 days) increased the firing rate of VTA dopamine neurons, but only in the "susceptible" group (those animals that developed anhedonic-like behaviors following stress) (Cao et al., 2010; Friedman et al., 2014; Krishnan et al., 2007). Chronic mild stress (4-7 weeks) decreased the number of spontaneously active VTA dopamine

neurons (Chang & Grace, 2014; Moreines et al., 2017; Tye et al., 2013). (B) Some work suggests that increased mPFC excitability could suppress activity in the mesolimbic pathway (Ferenczi et al., 2016; Moreines et al., 2017). (C) Endogenous CRF release in VTA seems to mediate the effect of restraint stress on motivation to work for food reward, likely by decreasing phasic dopamine responses to reward (Wanat, Bonci, & Phillips, 2013). (D) GLP-1 signaling appears to mediate the hypophagic effects of restraint stress (Maniscalco, Zheng, Gordon, & Rinaman, 2015), likely by decreasing the rewarding value of food (S. L. Dickson et al., 2012). GLP-1 neurons in cNST project directly to VTA and NAc (Alhadeff, Rupprecht, & Hayes, 2012), where they appear to influence dopaminergic functioning, although the direction of the effect is unclear (Fortin & Roitman, 2017; Mietlicki-Baase et al., 2014, 2013; X. F. Wang et al., 2015). More research is needed to assess whether GLP-1 neurons (and other homeostatic energy systems) contribute to stress-induced anhedonia. (E) Inflammation may inhibit dopamine availability (Felger, Hernandez, et al., 2015), either by inhibiting the function of enzymes in the dopamine biosynthetic pathway (see (Felger & Treadway, 2017)) or by creating oxidative stress through increased kynurenine (Dantzer, 2016). BST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; cNST, caudate nucleus of the solitary tract; CRF, corticotropin-releasing factor; DA, dopamine; GABA, γ-Aminobutyric acid; GLP-1, glucagon-like peptide-1; IEG, immediate-early gene; mPFC, medial prefrontal cortex; MSN, medium spiny neuron; NAc, nucleus accumbens; PVN, hypothalamic paraventricular nucleus; VTA, ventral tegmental area.

Stress and the mesolimbic reward circuit.

The mesolimbic reward circuit, which includes the ventral tegmental area (VTA) and nucleus accumbens (NAc), plays a key role in reward processing and motivated behavior (Box 1). A substantial literature has addressed whether the functioning of this circuit, in particular dopaminergic signaling, may mediate the effects of stress on subsequent anhedonic-like behavior (Cabib & Puglisi-Allegra, 2012; Hollon et al., 2015; Russo & Nestler, 2013).

Seemingly conflicting reports have emerged regarding VTA spontaneous dopaminergic firing and anhedonic-like responses to stress (Hollon et al., 2015). Several studies using social defeat stress have reported *increased* firing rates in “susceptible” mice—that is, those which exhibited decreased social interactions and decreased sucrose

preference (Cao et al., 2010; Friedman et al., 2014; Krishnan et al., 2007). Yet other studies in rats found that CMS *decreased* the number of spontaneously firing neurons, leading to decreased mobility in the forced swim test, which is thought to represent anhedonic-like behavior (Chang & Grace, 2014; Moreines et al., 2017). These studies generally found no significant change in firing rates (except Chang & Grace, 2014, Experiment 3). One possibility is that stress may induce increased firing rates in VTA dopamine neurons, but a decreased number of spontaneously active neurons.

Experimental manipulations of dopaminergic firing have also exerted apparently divergent effects on anhedonic behavior (Chaudhury et al., 2012; Tye et al., 2013). For example, induced phasic dopaminergic firing (via **optogenetics**) in VTA rescued decreases in sucrose preference caused by CMS (Tye et al., 2013). Yet a study of social defeat stress found that optogenetically-induced phasic dopaminergic firing rendered mice *more susceptible* to anhedonic effects of stress, as reflected in reduced sucrose preference and decreased social interaction (Chaudhury et al., 2012).

Numerous differences in study design could account for these apparently discrepant findings. As others have noted (Hollon et al., 2015), several parameters varied across studies, including stressor type (social defeat vs. chronic mild stress), chronicity (10 days of social defeat vs. 4-6 weeks of CMS), and measure of dopaminergic activity (number of spontaneously active neurons vs. neuronal firing rate). It is possible that stress could decrease the *number* of spontaneously active dopamine neurons (Chang & Grace, 2014; Moreines et al., 2017) while also increasing *firing rates* of the remaining, spontaneously active dopamine neurons (Cao et al., 2010; Friedman et al., 2014; Krishnan et al., 2007) in susceptible animals. However, few studies report both measures.

Also, studies of CMS often report main effects of stress, but rarely distinguish between susceptible and unsusceptible rodents.

Additionally, VTA contains phenotypically diverse dopamine neurons. Some VTA dopamine neurons co-release glutamate or GABA (Morales & Margolis, 2017), and VTA dopamine neurons follow diverse projection pathways (Ikemoto, 2007; Morales & Margolis, 2017). Thus, responses to stress may depend on the population of VTA neurons under study. For example, CMS produces a decrease in spontaneously active dopamine neurons in medial VTA (which primarily project to NAc) and central VTA, but not lateral VTA (Moreines et al., 2017); and susceptibility to social defeat increases after phasic stimulation of VTA projections to NAc, but not projections to mPFC (Chaudhury et al., 2012).

Taken together, existing literature suggests that stress impacts dopaminergic functioning, but the underlying mechanisms remain unclear. Further work may clarify which qualities of stressors affect dopaminergic firing rates vs. number of spontaneously active neurons, and whether such changes are necessary and sufficient to produce anhedonic behavior following stress.

Medial prefrontal cortex regulation of the mesolimbic circuit.

What is the mechanism through which stress impacts mesolimbic dopamine activity and perturbs reward functioning? Stress causes wide-ranging changes in brain structure and function, including in hippocampus, amygdala, and across PFC (Arnsten, 2009; McEwen et al., 2015). These interconnected regions may therefore mediate the effects of stress on anhedonia, especially given their roles in fear conditioning and responding (LeDoux, 2000; Milad & Quirk, 2002; Phelps, Delgado, Nearing, & LeDoux,

2004) and guiding behavior based on incentive value (Kable & Glimcher, 2007; Murray, 2007; Rangel & Hare, 2010). To highlight exciting recent work spanning rodents (Ferenczi et al., 2016; Moreines et al., 2017) and humans (Admon & Pizzagalli, 2015; Drysdale et al., 2017), this section focuses on the mPFC-mesolimbic circuit.

Stress may perturb mPFC-mesolimbic interactions via structural changes, such as dendritic remodeling, in mPFC (McEwen & Morrison, 2013). Specifically, in rats, chronic restraint stress or chronic immobilization cause dendritic shrinkage and spine loss in mPFC (prelimbic and infralimbic cortex) (McEwen & Morrison, 2013). In humans, stress increases risk for several psychiatric disorders that commonly involve anhedonic symptoms: MDD, schizophrenia, and post-traumatic stress disorder (PTSD) (Barch & Dowd, 2010; Nawijn et al., 2015; Pizzagalli, 2014). These disorders are also marked by decreased mPFC gray matter volume (MDD and PTSD) (Bremner, 2002; A. J. Holmes et al., 2012; Schmaal et al., 2017) or accelerated gray matter loss in mPFC (schizophrenia) (Cannon et al., 2015). These gray matter differences are relatively subtle in nature (Cannon et al., 2015; A. J. Holmes & Patrick, 2018; Schmaal et al., 2017). For instance, an estimated <1% annual change in cortical thickness characterizes converters to schizophrenia, although effect sizes for the comparison with high-risk non-converters and controls ranged from medium to large (Cannon et al., 2015). Additionally, mPFC volume may also relate to illness chronicity: As one example, left mPFC cortical thickness in patient populations is inversely related to number of depressive episodes (Treadway et al., 2015). Further work is needed to elucidate the mechanism through which these structural changes might give rise to alterations in mPFC function.

Given these stress-induced changes in mPFC structure, researchers have tested the hypothesis that stress produces anhedonia through mPFC hypofunction. In mice exhibiting anhedonic-like behavior (decreased sucrose preference and decreased social interaction) following social defeat stress, optogenetically-induced phasic firing in mPFC (prelimbic and infralimbic) attenuated these deficits (Covington et al., 2010). This result could suggest that induced phasic firing compensated for a deficit in mPFC activity. In one study, following social defeat stress, the firing rate of VTA dopamine neurons projecting to mPFC decreased by about 80% (Chaudhury et al., 2012), suggesting that decreased dopaminergic input to mPFC may directly contribute to mPFC hypoactivity. Work in humans also supports the mPFC hypofunction hypothesis: Perceived life stress (Treadway et al., 2013) and stress induced by aversive video clips (Ossewaarde et al., 2011) are associated with decreased blood oxygenation level dependent (BOLD) activity in mPFC during reward anticipation and receipt. By itself, this evidence is consistent with the notion that stress leads to mPFC hypofunction and related decreases in motivated behavior.

Yet other findings suggest a more complex relation between mPFC-striatal activity and motivated behavior. In a key study, researchers employed optogenetic techniques in rats to stably increase excitability of mPFC (primarily infralimbic) neurons (Ferenczi et al., 2016). Increased mPFC excitability led to reversible reductions in reward seeking, as evidenced by decreased sucrose preference and social interaction. Furthermore, increased mPFC excitability led to blunted BOLD responses in dorsal striatum following dopaminergic midbrain excitation. This result suggests that heightening mPFC excitability caused decreased reward-seeking behavior by altering

interactions between midbrain dopamine neurons and the striatum (Ferenczi et al., 2016) through an unknown mechanism. This interpretation was supported by a recent study examining CMS and dopaminergic functioning in rats (Moreines et al., 2017). In non-stressed rats, pharmacological activation of mPFC (infralimbic) selectively inhibited dopamine neurons in medial VTA. CMS decreased the number of spontaneously firing dopamine neurons in medial and central, but not lateral, VTA. This decrease was rescued by pharmacological inactivation of mPFC (infralimbic) (Moreines et al., 2017). However, following a social defeat paradigm, the synaptic strength of mPFC-to-ventral striatal connections did not significantly differ between stress-susceptible and resilient mice (Christoffel et al., 2015), suggesting no contribution of this pathway to subsequent anhedonic behavior. This seemingly discrepant finding could be explained by indirect, rather than direct, influences of mPFC function on mesolimbic activation (Moreines et al., 2017). Alternately, different types of stress—e.g., CMS vs. social defeat stress—may exert different impacts on corticostriatal pathways (see **Future directions in the study of stress and anhedonia**). Nevertheless, when taken together, these results provide evidence that mPFC hyperactivity may contribute to anhedonia following stress.

Recent work in humans also suggests that mPFC-striatal connectivity may contribute to anhedonia in individuals with MDD and remitted MDD (rMDD). One study examined individuals with rMDD using spectral dynamic causal modeling of BOLD functional connectivity (Admon & Pizzagalli, 2015), an analytic approach that uses Bayesian inference to estimate directional interactions between neural systems. In response to a naturalistic positive mood induction, individuals with rMDD exhibited less reciprocal mPFC-ventral striatal connectivity and were characterized instead by mPFC

modulation of ventral striatum (VS), relative to controls (see Box 1 for a discussion of corticostriatal structure and function). This pattern of functional connectivity in individuals with rMDD was accompanied by lower mood approximately 30 minutes following the induction (Admon & Pizzagalli, 2015).

A second study endeavored to characterize biological sub-phenotypes ("biotypes") in large samples of individuals with MDD using BOLD functional connectivity data and MDD symptoms (Drysdale et al., 2017). Hierarchical clustering analyses yielded four biotypes based on similar patterns of connectivity features. Hyperconnectivity in frontostriatal (and thalamic) networks characterized two of the biotypes, and this hyperconnectivity was associated with anhedonia and psychomotor retardation (Drysdale et al., 2017). These results are consistent with the hypothesis that mPFC interaction with mesolimbic circuitry regulates motivation.

In summary, evidence suggests that a) stress causes dendritic shrinkage and spine loss in mPFC; b) mPFC activity alters mesolimbic dynamics; and c) mPFC may mediate the impact of stress on mesolimbic function and anhedonia. Notably, to fully capture the role of mPFC in stress-induced anhedonia, it may be necessary to examine how various subregions of mPFC coordinate with other brain regions implicated in stress.

Neuroendocrine stress responses and mesolimbic reward processing.

Neuroendocrine stress responses induce a variety of physiological changes to cope with threat, such as mobilizing stored energy for use by muscle (Sapolsky, Romero, & Munck, 2000). In addition, neuroendocrine responses may inhibit or enhance reward seeking, depending on site of action and prior stress exposure (see below). Thus, neuroendocrine activity is also poised to mediate the link between stress and anhedonia.

Much of the research on this possibility has focused on corticotropin-releasing factor (CRF), a key component of HPA axis functioning (see Box 2 and Figure 2).

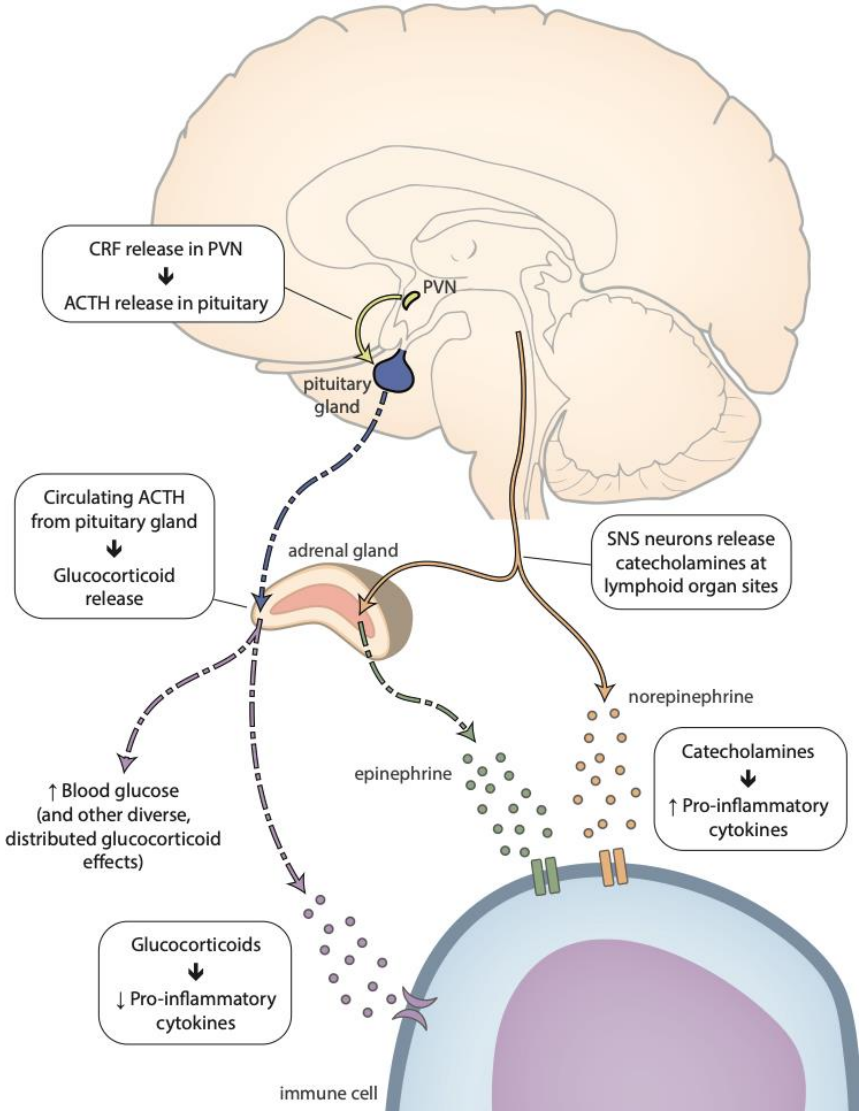


Figure 2. Neuroendocrine and inflammatory stress responses. The PVN releases CRF, which reaches the pituitary gland through the median eminence. CRF binding in the pituitary gland causes ACTH release into circulation (Smith & Vale, 2006). Circulating ACTH reaches binding sites in the adrenal cortex, releasing glucocorticoids (in humans, primarily cortisol) (Smith & Vale, 2006). Glucocorticoids act on immune cell receptors to downregulate pro-inflammatory cytokines, decreasing inflammation. At the same time, SNS terminals at release catecholamines at peripheral sites (Elenkov, Wilder, Chrousos, & Vizi, 2000). These neurotransmitters act on immune cell receptors, causing the release of cytokines that upregulate inflammation (Szelényi, 2001). ACTH, adrenocorticotropic

hormone; CRF, corticotropin-releasing factor; PVN, hypothalamic paraventricular nucleus; SNS, sympathetic nervous system.

CRF is released by neurons in regions such as the hypothalamic paraventricular nucleus (PVN), the bed nucleus of the stria terminalis (BST), and central nucleus of the amygdala (CeA) (Kono et al., 2017), which contribute to the expression of fear and anxiety (LeDoux, 2000; Shackman & Fox, 2016) and promote hormonal responses to threat (Herman & Cullinan, 1997). CRF-releasing neurons from these regions project to VTA and NAc (Dabrowska, Martinon, Moaddab, & Rainnie, 2016; Rodaros, Caruana, Amir, & Stewart, 2007) (Figure 1), where CRF influences dopamine release and motivated behavior, but with divergent effects in either region (Hollon et al., 2015), as discussed further below.

In the rat VTA, uncontrollable foot-shock stress causes CRF release (B. Wang et al., 2005). Injection of CRF into VTA dose-dependently increases the baseline firing rate of dopamine neurons (Wanat, Hopf, Stuber, Phillips, & Bonci, 2008), but decreases phasic dopamine response to food rewards (but not reward-predictive cues) (Wanat et al., 2013). Further, restraint stress decreases motivation to work for food reward in a progressive ratio task (Wanat et al., 2013) and biases decision making towards low effort/low reward choices (Bryce & Floresco, 2016), which can be blocked by a CRF **antagonist** injected into VTA (Bryce & Floresco, 2016; Wanat et al., 2013). Thus, CRF release in VTA appears to decrease reward motivation following stress, likely via dopaminergic changes.

CRF activity in NAc seems to exert rather different effects on motivated behavior (Lemos et al., 2012; Peciña, Schulkin, & Berridge, 2006). Injection of CRF into NAc

increased the ability of Pavlovian reward cues to enhance lever-pressing for sucrose rewards (Peciña et al., 2006) and caused conditioned place preference alongside increased dopamine release (Lemos et al., 2012). These results suggest that, unlike in VTA, CRF release in NAc enhances reward conditioning. However, severe forced-swim stress abolished the ability of CRF to increase dopamine release, and also switched the behavioral effect of CRF to conditioned place *aversion* (Lemos et al., 2012). Thus, impact of CRF release in NAc appears to be conditional on prior stress exposure.

CRF may exert its influence on mesolimbic functioning by gating the release of brain-derived neurotrophic factor (BDNF) following stress (Berton et al., 2006; Koo et al., 2016; Krishnan et al., 2007; Walsh et al., 2014). Numerous studies suggest that following 10-day social defeat stress, BDNF released from VTA acts on receptors in NAc to mediate the impact of stress on reward-seeking and social behavior (Berton et al., 2006; Koo et al., 2016; Krishnan et al., 2007). Even briefer, "subthreshold" social defeat stress, when combined with phasic, optogenetic stimulation of VTA-NAc neurons, produced social interaction deficits and increased BDNF levels in NAc (Walsh et al., 2014). Importantly, CRF antagonism in NAc prior to subthreshold stress and optogenetic stimulation prevented this increase in BDNF and rescued effects of stress on social interaction (Walsh et al., 2014). Thus, CRF release may be necessary to produce stress-induced alterations in NAc BDNF and decreased social interaction.

Preliminary human work supports an effect of CRF on reward processing and behavior. One study examined a **single-nucleotide polymorphism (SNP)** in the *CRHR1* gene that codes for the CRF receptor CRHR1 (Bogdan, Santesso, Fagerness, Perlis, & Pizzagalli, 2011). **Homozygosity** for the A allele of this SNP was associated with blunted

neural response to rewards (indexed by scalp-recorded electrophysiology) under acute stress (threat of shock). A/A individuals also exhibited a decreased behavioral response bias under stress. Decreased response bias in this task has been previously associated with MDD status, and with increased anhedonia symptoms in individuals with MDD (Pizzagalli, Iosifescu, Hallett, Ratner, & Fava, 2008). Though sample size in the genotyping study was relatively small (*n*=84) for detection of a gene by environment interaction, and the effects warrant replication, these results are concordant with animal literature suggesting an effect of stress-induced CRF on anhedonia.

In summary, neuroendocrine stress responses may recalibrate mesolimbic reward processing: CRF appears to decrease reward motivation via actions in the VTA, but enhances reward conditioning in NAc, except after prior severe stress exposure. CRF may exert these effects by moderating stress-induced BDNF release. More work in this domain will be crucial to understanding how HPA axis functioning and mesolimbic reward circuits coordinate during stress responses, and how dynamics in these systems contribute to stress-induced anhedonia.

Stress, energy homeostasis, and reward.

Maintaining energy homeostasis—e.g., by regulating feeding and satiety—requires flexible adjustments to reward seeking (Berthoud, 2012; Hayes & Schmidt, 2016). For example, rodents that have consumed food to satiety exhibit diminished motivation for food rewards (Colwill & Rescorla, 1985). To accomplish this, energy homeostasis systems coordinate with the mesolimbic reward system (Cassidy & Tong, 2017) (see below). Although energy homeostasis systems include diverse **central and peripheral** signaling pathways involving numerous peptides (Berthoud, 2012), for the

purposes of this review, we highlight the role of glucagon-like peptide-1 (GLP-1). GLP-1 signaling appears to decrease reward motivation (Hayes & Schmidt, 2016), and these pathways are activated by stress (Maniscalco et al., 2015)—see below. Thus, GLP-1 pathways and other energy homeostasis systems are well-positioned to mediate stress effects on anhedonia.

GLP-1-producing neurons originate almost exclusively in the caudal nucleus of the solitary tract (cNST) in the brainstem (Merchenthaler, Lane, & Shughrue, 1999). These projections play key roles in decreasing food intake both during satiety (Maniscalco, Kreisler, & Rinaman, 2012; van Bloemendaal, ten Kulve, la Fleur, IJzerman, & Diamant, 2014) and following stress (Maniscalco & Rinaman, 2017). GLP-1 signaling appears to decrease the rewarding value of food (S. L. Dickson et al., 2012) through direct projections to NAc and VTA (Alhadeff et al., 2012) (Figure 1). For instance, injection of a GLP-1 receptor **agonist** into rat VTA or NAc reduced lever pressing for food in a **progressive ratio task** (S. L. Dickson et al., 2012). Importantly, GLP-1-induced alterations in motivated behavior extend to alcohol and drug rewards (Hayes & Schmidt, 2016). For example, GLP-1 agonism in rats reduces the impact of alcohol reward on conditioned place preference (Shirazi, Dickson, & Skibicka, 2013). Similar reports have emerged for other drugs, including cocaine and nicotine (Hayes & Schmidt, 2016). Several studies in rodents suggest that GLP-1 alters reward functioning by influencing dopaminergic mesolimbic circuitry, although some studies report decreases and some increases in dopamine activity (Fortin & Roitman, 2017; Mietlicki-Baase et al., 2014, 2013; X. F. Wang et al., 2015). Taken together, these results suggest that GLP-1 signaling may decrease motivation for rewards in general.

GLP-1 signaling pathways are also activated by stress, including restraint and elevated platform stress (Maniscalco et al., 2015). Notably, peripheral inflammation in response to immune challenge also appears to activate GLP-1 neurons (Gaykema et al., 2009), highlighting the need for a multi-systems approach to bridge work on immune responses and homeostatic energy regulation pathways following stress (see also *Inflammation and reward processing*, below). GLP-1 neurons projecting to the PVN also mediate HPA axis responses to stress (Figure 1), including release of adrenocorticotropic hormone (ACTH) and glucocorticoids (Ghosal et al., 2017)—see Box 2 and Figure 2. These results suggest that GLP-1 pathways could interact with other putative mechanisms of stress-induced anhedonia. Importantly, recent work in rats suggests that GLP-1 signaling is crucial for restraint stress to induce hypophagia (decreased food intake), as an intraventricular GLP-1 receptor antagonist attenuated the hypophagic effects of restraint stress (Maniscalco et al., 2015). Given the involvement of mesolimbic reward circuits in producing GLP-1 mediated hypophagia (Alhadeff et al., 2012), this study suggests that GLP-1 could play a role in stress-induced alterations in reward behavior more generally. The possibility that GLP-1 signaling partially mediates the effect of stress on reward processing merits follow-up.

Not surprisingly, other energy homeostatic pathways may also contribute to stress-induced anhedonia. For instance, antagonism of a melanocortin receptor (MC4R) in NAc prevents anhedonic-like decreases in sucrose preference following chronic stress in mice (Lim, Huang, Grueter, Rothwell, & Malenka, 2012). Indeed, as research on the links between energy homeostasis systems and reward systems has expanded considerably in recent years (Cassidy & Tong, 2017), the list of possible stress-anhedonia

mediators has also expanded. Notably, anhedonia is frequently accompanied by appetite and weight changes in humans diagnosed with MDD (American Psychiatric Association, 2013). Thus, energy homeostasis systems in general are an exciting target for future research on stress-induced anhedonia.

Putative Molecular Signaling Pathways to Stress-Induced Anhedonia

In addition to circuit-level mechanisms that could connect stress and anhedonia, molecular signaling pathways also contribute to motivated behavior (Felger & Treadway, 2017; Russo & Nestler, 2013). Much recent work has focused on the cascade of inflammatory responses to stress (see Box 2) and possible effects on dopamine synthesis (Felger & Treadway, 2017). Other molecular signaling pathways are covered elsewhere (Russo & Nestler, 2013).

Inflammation and reward processing.

Numerous studies suggest that inflammation alters motivated behavior and mesolimbic function, possibly through dopaminergic changes. Although we highlight recent work in this domain, a more thorough treatment of this topic is available (see Felger & Treadway, 2017).

A series of studies in mice suggests that the pro-inflammatory cytokine interleukin-6 (IL-6), in particular, may be a key contributor to stress-induced anhedonia (Hodes et al., 2014; Menard et al., 2017; J. Wang et al., 2018). Social defeat stress produces a ~27-fold increase in peripheral IL-6 (Hodes et al., 2014). Additionally, social defeat appears to weaken the blood-brain barrier by reducing levels of Cldn5, a cell adhesion molecule, allowing IL-6 to infiltrate NAc **parenchyma** and producing

diminished social interaction (Menard et al., 2017). These studies suggest that stress-induced IL-6 infiltration, in conjunction with synaptic remodeling in NAc (see J. Wang et al., 2018), contributes to the development anhedonic-like behavior (Hodes et al., 2014; Menard et al., 2017; J. Wang et al., 2018). Increases in IL-6 and social interaction deficits were both rescued by bone marrow infusions from IL-6 knockout mice, suggesting that these immune and behavioral changes are mediated by bone marrow-derived, peripheral immune cells (Hodes et al., 2014). Interestingly, administration of the plant metabolites dihydrocaffeic acid (DHCA) and malvidin-3'-O-glucoside (Mal-gluc) blunted IL-6 responses to social defeat stress and promoted resilience to anhedonic-like behaviors (blunted sucrose preference and decreased social interaction) (J. Wang et al., 2018). DHCA inhibited IL-6 production via disrupted gene transcription, while Mal-gluc inhibited synaptic restructuring in NAc, and both changes were necessary to achieve therapeutic effects (J. Wang et al., 2018). Taken together, this work suggests that inflammatory responses, together with neurovascular and synaptic changes, promote susceptibility to anhedonic behavior. Moreover, these exciting studies illustrate how research spanning multiple systems (e.g., immune response and brain reward systems) may produce key insights about pathways to stress-induced anhedonia.

Despite this evidence linking inflammatory responses to brain reward systems, rodent studies on inflammation and dopaminergic function specifically have yielded mixed results. Rodent studies measuring the impact of interferon-α (IFN-α, a pro-inflammatory cytokine) on dopamine release have reported either increases or decreases in dopamine/dopamine metabolites (Felger & Treadway, 2017). These divergent findings may be partly due to differences in dosing, chronicity, and timeframe of exposure (Felger

& Treadway, 2017). However, some evidence suggests that recombinant human IFN-α does not bind to expected targets in rodents (Loftis, Hauser, Macey, & Lowe, 2006), and inconsistent use of species-specific IFN-α could therefore explain these mixed results in rodents (Felger & Treadway, 2017). By contrast, a recent study that administered IL-6 found decreased effortful responding for a preferred (vs. freely available) reward alongside decreased extracellular dopamine in NAc core, as assessed by microdialysis (Yohn et al., 2016), suggesting that behavioral changes were mediated by alterations in dopamine release.

Two studies in rhesus monkeys have assessed *in vivo* dopamine release in response to inflammation. Chronic administration of IFN-α decreased effort-based, but not freely available, sucrose consumption (Felger, Mun, et al., 2013) and *in vivo* microdialysis in these animals indicated decreased dopamine release in the caudate. Additionally, inflammation-induced deficits in dopamine release were abolished by administration of L-DOPA, the precursor to dopamine (Felger, Hernandez, et al., 2015). This finding suggests that IFN-α administration decreased dopamine release by reducing synthetic capacity.

In humans, a recent functional magnetic resonance imaging (fMRI) study of healthy female participants examined the effect of stress-induced inflammation on reward prediction errors (RPEs; see Box 1) in VS, which includes NAc (Treadway et al., 2017). First, participants completed a cold pressor task while performing serial subtraction in front of an experimenter. Blood levels of IL-6 were assessed before and after the stressor. During a second session, participants completed arithmetic problems of escalating difficulty while exposed to criticism from an unfriendly, impatient experimenter. They

then completed a probabilistic reward task during an fMRI scan. Analyses revealed that stress-induced increases in IL-6 during the first session were associated with diminished VS BOLD responses to RPEs during the second session, although there was no main effect of stress on BOLD RPE signals, and no behavioral effects were detected (Treadway et al., 2017).

An fMRI study of individuals with MDD investigated the association between resting-state functional connectivity, inflammatory markers, and anhedonic symptoms (Felger, Li, et al., 2015). Connectivity between VS and ventromedial PFC (vmPFC) negatively correlated with blood levels of C-reactive protein (CRP), a marker of inflammation. Furthermore, decreased VS-vmPFC connectivity was associated with higher anhedonia scores (Felger, Li, et al., 2015). These results suggest that resting-state fluctuations may capture alterations in the functional architecture of the corticomesolimbic circuit (see Box 1 and *Medial prefrontal cortex regulation of the mesolimbic circuit*) associated with inflammation and anhedonia.

Altogether, these findings suggest that inflammation impacts motivated behavior, possibly through changes in dopaminergic mesolimbic circuitry. Several plausible theories have been advanced to characterize these alterations at the molecular level.

Inflammation and dopamine synthesis.

Inflammation may decrease dopaminergic signaling by disrupting the biosynthetic pathway to dopamine (Felger & Treadway, 2017). Inflammation appears to decrease the availability of tetrahydrobiopterin (BH_4), an enzyme cofactor that is critical at two stages of dopamine synthesis: a) conversion of phenylalanine to tyrosine, and b) conversion of tyrosine to L-3,4-dihydroxphenylalanine (L-DOPA), the precursor to dopamine (Box 3).

To test this hypothesis, researchers have examined cerebrospinal fluid (CSF) and blood concentrations of phenylalanine, tyrosine, and the phenylalanine/tyrosine (Phe/Tyr) ratio as indirect measures of dopamine synthesis. The Phe/Tyr ratio was elevated in individuals receiving IFN-α as a treatment for hepatitis C (Felger, Li, et al., 2013), suggesting that inflammation impeded the conversion of phenylalanine to tyrosine (see Box 3). Although promising, interpretation of these findings is limited by small sample size, especially in the control group (9 individuals). Additionally, in a sample of elderly persons with chronic low-grade inflammation, elevated tyrosine, but not phenylalanine or the Phe/Tyr ratio, was associated with reduced motivation (Capuron et al., 2011). However, tyrosine levels were non-significantly associated with inflammatory markers (IL-6 and CRP). Thus, the role of inflammation in impeding the conversion of tyrosine to L-DOPA (see Box 3) remained unclear. Taken together, this important research has yielded hints that inflammation disrupts dopamine synthesis by decreasing BH_4 availability, consistent with some animal work (Kitagami et al., 2003). Given the potential significance of these findings, additional follow-up is merited.

Inflammation may also decrease dopamine synthesis through the kynurenine pathway by increasing oxidative stress (Dantzer, 2016)—see Figure 3 (for more detailed discussion, see Dantzer, 2016; Felger & Treadway, 2017). Importantly, dopamine neurons are especially vulnerable to inflammatory insult (Block, Zecca, & Hong, 2007). Moreover, xanthurenic acid, a kynurenine pathway metabolite, inhibits BH_4 synthesis (Haruki, Hovius, Pedersen, & Johnsson, 2016), suggesting that increased kynurenine production resulting from inflammation could interfere with dopamine synthesis through BH_4 depletion. Consistent with the kynurenine pathway theory, increased quinolinic acid

(QUIN) levels in CSF were associated with IFN-α treatment for hepatitis C (Raison et al., 2010), see Figure 3.

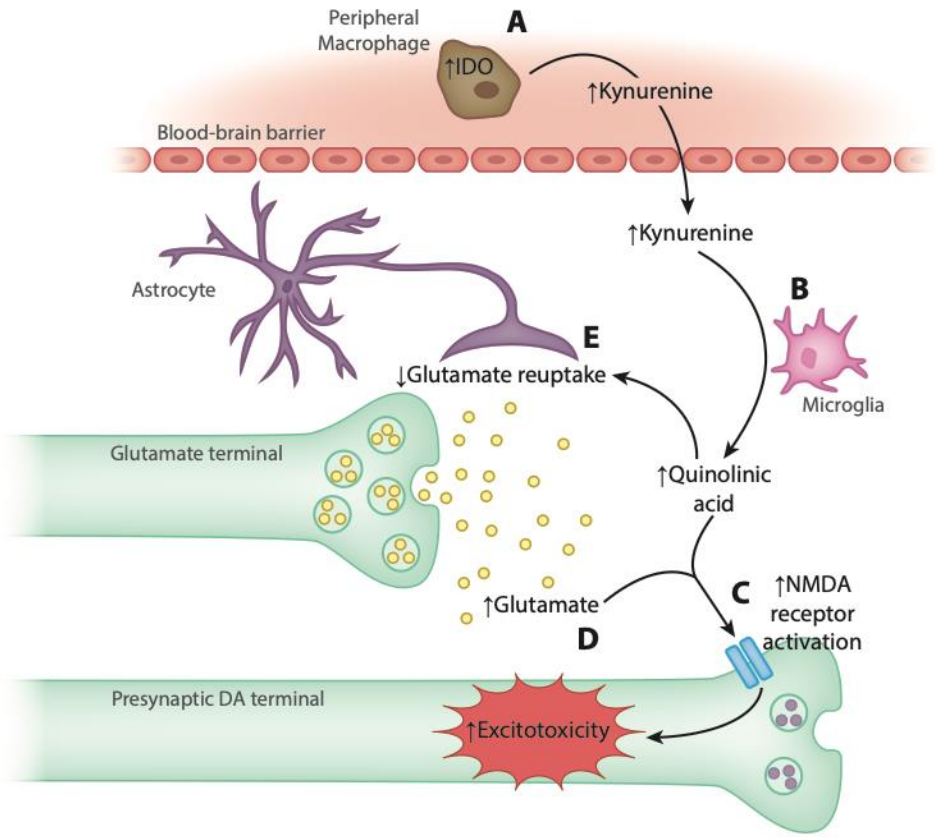


Figure 3. Inflammation-induced excitotoxicity through the kynurenine pathway. (A) Pro-inflammatory cytokines stimulate indoleamine 2,3-dioxygenase (IDO) in peripheral macrophages, resulting in kynurenine that crosses the blood-brain barrier. (B) Microglia convert kynurenine to quinolinic acid (QUIN). (C) In turn, QUIN exerts possibly neurotoxic effects, in part by (D) activating N-methyl-d-aspartate (NMDA) receptors, and (E) by decreasing glutamate reuptake, which increases glutamate release to potentially excitotoxic levels (Dantzer, 2016). DA, dopamine; IDO, indoleamine 2,3-dioxygenase; NMDA, N-methyl-d-aspartate.

Altogether, evidence suggests that inflammation could interfere with dopamine synthesis, possibly by preventing enzymatic activity in the biosynthetic pathway or by increasing neurotoxicity. However, whether *stress-induced* inflammation causes disrupted dopamine synthesis remains equivocal. Some work has examined the impact of

stress on dopamine depletion (Cabib & Puglisi-Allegra, 2012), although inflammation was not assessed. Moreover, it is not yet clear whether stress type (e.g., interpersonal stress; see (Slavich & Irwin, 2014)) may influence anhedonic responses via changes in inflammation.

Future Directions in the Study of Stress and Anhedonia

Consistent with our view, recent work suggests that stress contributes to anhedonic behavior through perturbations across diverse systems and multiple levels of analysis. For example, pro-inflammatory signaling molecules may cross the blood-brain barrier to infiltrate brain reward systems directly (Hodes et al., 2014; Menard et al., 2017; J. Wang et al., 2018), but could also affect motivated behavior through homeostatic energy regulation pathways (Gaykema et al., 2009). However, despite much promising work to date on the relations linking stress and anhedonia, important gaps in our collective knowledge persist.

Anhedonia and key dimensions of stress.

Examinations of stress-induced anhedonia have implemented paradigms that vary considerably across studies in terms of stress chronicity, severity, controllability, and type (Anisman & Matheson, 2005), making it difficult to synthesize results across labs. As a result, the implications of variability in these dimensions of stress are incompletely understood.

Experiments also vary in the severity of their stress manipulations as a function of the species under study. In rodents, chronic mild stress commonly entails stressors such as “strobe light illumination for 1 to 16 h” or “dark cycle (continuous darkness for 24 to

36 h)" (Tye et al., 2013, p. 542) twice per day for 8-12 weeks. Such stressors would be unethical to administer to human participants. As a result, human stress inductions generally take place on the order of minutes, not hours (e.g., Bogdan & Pizzagalli, 2006; Treadway et al., 2017) and accordingly are not matched for the chronicity (nor, likely, the severity) of many animal studies. Experimental work in humans assumes that relatively mild and acute stressors will produce alterations in reward function that are *reliably detectable* and *qualitatively similar* to alterations produced by severe and/or chronic stress. Yet the actual relation between stress and anhedonic processes could violate these core assumptions.

The impact of stressor chronicity also remains unclear. It is possible that stressors produce divergent effects on mesolimbic dopaminergic functioning depending on chronicity (see *Stress and the mesolimbic reward circuit*). Yet both acute stressors (e.g., death of a loved one) and chronic stress (e.g., ongoing financial difficulties) can lead to anhedonic symptoms (Keller et al., 2007). The impact of these events on subsequent symptoms may depend in part on regulatory responses. For certain individuals, such as those with a ruminative response style (Michael et al., 2007), even acute stressors may provoke chronic stress responses, leading to long-lasting and relatively inflexible anhedonic states.

Observational studies of responses to naturally-occurring severe/chronic stressors could supplement the important experimental work reviewed above. Both avenues of research are necessary, given the trade-off between experimental control and strength of causal inference vs. ecological validity. Large observational studies in humans could distinguish the differential impact of numerous types of stressors. Indeed, preliminary

evidence suggests that anhedonia may be especially prominent following interpersonal losses (e.g., death of a loved one or romantic loss) (Keller et al., 2007). Notably, the aforementioned study assessed the severity of anhedonia relative to other dysphoric symptoms, rather than an "absolute" measure of anhedonia (Keller et al., 2007). Thus, more work is needed to understand the impact of dimensions of stress, such as stressor chronicity, on anhedonia *per se*. A few important studies have compared the contributions of acute life events vs. chronic difficulties on broader phenotypes that may or may not include anhedonia, such as MDD (Muscatell, Slavich, Monroe, & Gotlib, 2009), or on depressive symptom aggregates (McGonagle & Kessler, 1990). However, such studies rarely examine the links between stress and anhedonia specifically, or indeed any individual symptoms (for an exception, see Keller et al., 2007). More work in this domain could help researchers continue to increase the ecological validity of experimental models of stress and anhedonia.

Finally, anhedonia is a multifaceted construct. Although anhedonia is often defined with reference to loss of pleasure or motivation (American Psychiatric Association, 2013), researchers have recently conceptualized anhedonic behavior in terms of a broader array of motivational and reward processes. Because decision-making requires the weighing of potential rewards against expected costs (Lima, 1998; Zald & Treadway, 2017), decreased reward seeking could result from changes to several facets of this process, e.g., reward devaluation and/or an increase in forecasted effort costs (Treadway & Zald, 2011). Future experiments can make use of paradigms that distinguish these facets of reward processing (Zald & Treadway, 2017).

Researchers studying stress and anhedonia must account for a counterintuitive relationship: Under certain circumstances, stress *increases* reward motivation and sensitivity to reward (Mather & Lighthall, 2012; Willner, 2005). Alcohol/substance use problems and obesity are linked with stress and (at least theoretically) involve increased reward seeking (Volkow & Wise, 2005), although increased sensation seeking may predispose individuals towards substance use (A. J. Holmes, Hollinshead, Roffman, Smoller, & Buckner, 2016), suggesting the importance of individual factors. How can we reconcile these apparent discrepancies?

One possibility is that certain individuals are more prone to seek out rewards rather than experience anhedonia in response to stress. Such between-individual variability is plausible from an evolutionary perspective, e.g. due to fluctuations in environmental demands that preclude the possibility of a single, "optimal" response profile (Dingemanse, Both, Drent, & Tinbergen, 2004; A. J. Holmes & Patrick, 2018). Indeed, sex may be a meaningful individual difference for stress responses: Following a cold pressor, men responded more quickly and "cashed in" more often in a decision-making task, whereas women responded more slowly and "cashed in" less (Lighthall et al., 2012). Still, between-individual factors are unlikely to fully account for variability in responses to stress. Some evidence in humans suggests that, within individuals, different events are likely to provoke more or less prominent anhedonia, and dissociable patterns of dysphoric symptoms in general (Keller et al., 2007). A comprehensive model of stress and anhedonia will incorporate both stable tendencies that vary across individuals (Sih, Bell, & Johnson, 2004) and within-individual variability (e.g., in response to different types of stress) (Dingemanse et al., 2010).

Concluding Remarks

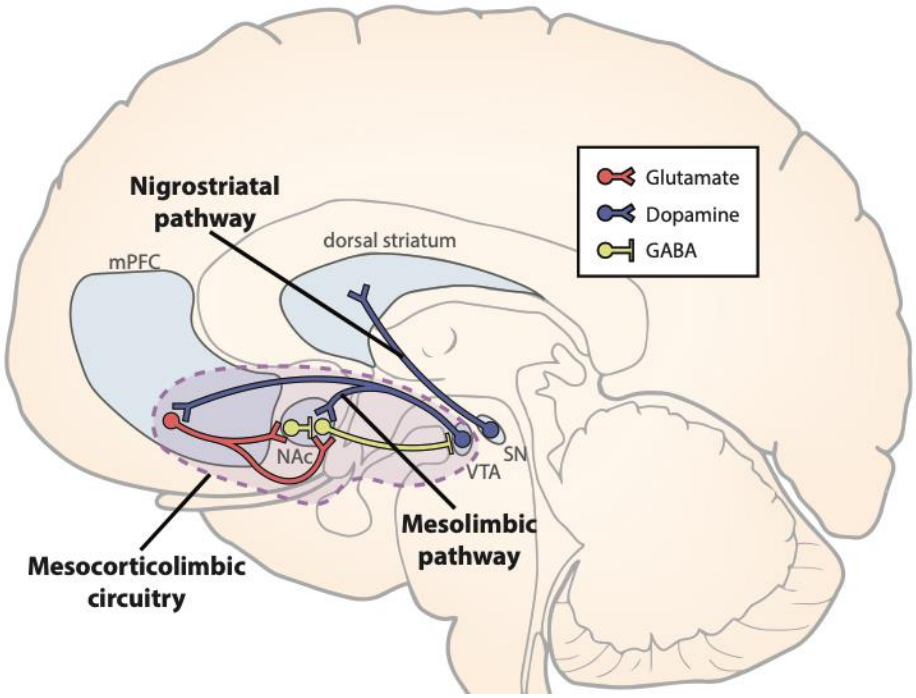
Delineating an anhedonic phenotype rooted in etiology represents an important goal for psychiatric research. Such a phenotype could help to increase homogeneity in clinical research samples, and the accompanying increase in statistical power could make it easier to identify critical vulnerability factors for chronic, severe anhedonia. In turn, identifying vulnerability factors and proximal causes of anhedonia could improve predictions of conversion to disorder and suggest novel targets for treatment.

Cross-species work has made considerable progress in illuminating plausible mechanisms that could bridge the occurrence of stress and onset of anhedonia. Yet seemingly contradictory patterns of results have emerged across lines of research. Better parsing heterogeneity in stressors and in reward processing could help to decipher puzzling results and yield crucial insights. Additionally, a unified model is needed to account for isolated findings across levels of analysis (e.g., molecular signaling, neural circuitry, behavior, subjective experience), including seemingly discrepant findings (e.g., stress decreases the number of spontaneously firing dopamine neurons in VTA, but increases firing rates).

Furthermore, the full realization of a multi-system, multi-level approach to psychopathology will require more inter-disciplinary collaboration, drawing on psychology, neuroscience, immunology, endocrinology, and economics, among other fields. Unraveling pathways to complex psychiatric phenotypes requires research that cuts across levels—from genetics to molecular signaling pathways, functional circuits, cognition, behavior, and culture. We believe that this approach holds the potential to yield much-needed answers for individuals experiencing debilitating psychiatric illness.

Box 1. Reward Processing, Dopamine, and the Mesolimbic System

Reward processing recruits diverse circuits spanning numerous regions, including the basal ganglia, medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), and amygdala (Haber & Knutson, 2009). Here, we provide a brief review focused on the dopaminergic mesolimbic pathway, given its well-established relation to anhedonia (Russo & Nestler, 2013), although other circuits likely contribute. The stress-initiated mechanisms described in this review converge on the mesolimbic pathway (see main text, Figure 1).



Box 1, Figure I. Dopaminergic and mesocorticolimbic circuitry. GABA, γ-Aminobutyric acid; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; SN, substantia nigra; VTA, ventral tegmental area.

The mesolimbic pathway consists of dopaminergic neuronal projections from the ventral tegmental area (VTA) to nucleus accumbens (NAc) (Figure I). Dopamine

neurons, including those of the mesolimbic pathway, play a key role in reward processing (Berridge, Robinson, & Aldridge, 2009; Mirenowicz & Schultz, 1994; Schultz, 2016). Their specific role may vary based on time (Schultz, 2016) and on the particular population of dopamine neurons (e.g. Howe & Dombeck, 2016). For instance, researchers have argued that an initial component of the dopamine response is sensitive to stimulus salience, whereas a subsequent component encodes a reward prediction error (RPE) (Schultz, 2016). That is, the dopaminergic RPE signal spikes following unpredicted rewards, and is sensitive to reward value. As rewards become more predictable (e.g., due to association with predictive cues), dopaminergic firing spikes in response to reward-predicting cues and is suppressed when expected rewards are omitted (Schultz, Dayan, & Montague, 1997). Recent evidence suggests that mesolimbic dopaminergic firing encodes the value of working for a reward, and thus may signal reward value and influence motivation (Hamid et al., 2016).

Distinct populations of dopamine neurons appear to differentially impact reward processing vs. locomotion, suggesting heterogeneity of dopamine subpopulations (Howe & Dombeck, 2016). Dopamine also acts on functionally heterogeneous receptors: D1-type receptors, which are excitatory, and D2-type receptors, which inhibit neuronal firing. Spikes in mesolimbic dopamine activity (e.g., in response to unexpected rewards) excite the D1 receptor-expressing “direct” pathway, leading to behavioral reinforcement (Hikida, Kimura, Wada, Funabiki, & Nakanishi, 2010; Kravitz, Tye, & Kreitzer, 2012). By contrast, suppressed dopaminergic firing disinhibits the D2 receptor-expressing “indirect” pathway to facilitate learning from non-reward/punishment (Hikida et al., 2010; Kravitz et al., 2012).

Activity in the mesolimbic pathway appears key for reward learning and for anhedonic responses following stress (see *Stress and the mesolimbic reward circuit*). However, other dopaminergic projections also likely contribute to reward processing. For example, the nigrostriatal pathway consists of dopaminergic projections from substantia nigra to dorsal striatum (Lynd-Balta & Haber, 1994). Dorsal striatal activity appears to play a relatively larger role in goal-directed and habit-based reward learning, whereas ventral striatum (including NAc) predominantly contributes to associative learning (Liljeholm & O'Doherty, 2012).

mPFC is closely interconnected with mesolimbic regions (Haber & Knutson, 2009) (Figure I). mPFC receives dopaminergic projections from VTA (Lidow, Goldman-Rakic, Gallager, & Rakic, 1991) and sends glutamatergic projections to NAc (Britt et al., 2012). The vast majority of NAc neurons are GABAergic (Meredith, 1999), including fast-spiking interneurons (Pennartz, Groenewegen, & Lopes da Silva, 1994) and projections to VTA (both direct and indirect through the ventral pallidum) (Haber, 2016). Connections among these regions form a mesocorticolimbic circuit, which is implicated the computation of reward value and motivation (Kable & Glimcher, 2007; Liljeholm & O'Doherty, 2012; Rangel & Hare, 2010). Thus, mPFC is ideally situated to influence mesolimbic dynamics, either directly (via glutamatergic synapses on NAc interneurons, or on NAc projections to VTA) or indirectly (via wide-ranging projections of mPFC). Notably, mesolimbic regions are connected with a host of other regions implicated in reward processing, including orbitofrontal cortex (OFC), amygdala, and hippocampus (Haber & Knutson, 2009).

Box 2. Stress, Inflammation, and the HPA Axis

Although mammalian stress responses are complex, we provide a brief and schematized review of neuroendocrine and inflammatory responses here (for more detailed reviews, see Sapolsky et al., 2000; Slavich & Irwin, 2014; Smith & Vale, 2006). Stress upregulates activity in two key systems: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) (Elenkov et al., 2000). HPA axis responses originate in the hypothalamic paraventricular nucleus (PVN), which releases corticotropin-releasing factor (CRF) into the median eminence. CRF binding in the pituitary gland causes the release of adrenocorticotropic hormone (ACTH) into the blood (Smith & Vale, 2006). Circulating ACTH reaches binding sites in the adrenal cortex, causing the release of glucocorticoids (in humans, primarily cortisol) (Smith & Vale, 2006). Glucocorticoids promote a variety of effects at numerous sites of action, including boosted glucose concentration in the bloodstream, which provides fast energy resources to cope with potential threat (Sapolsky et al., 2000).

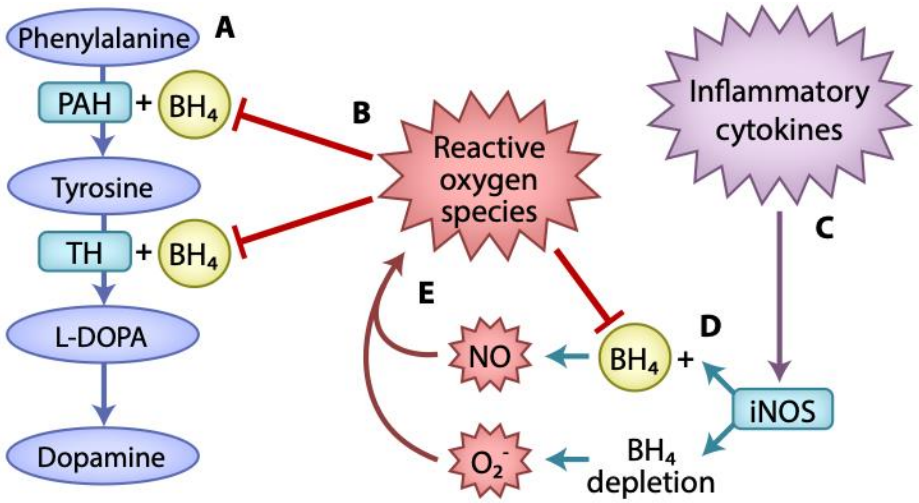
At the same time, sympathetic nervous system projections from the brainstem release catecholamines (including norepinephrine, epinephrine, and dopamine) at peripheral sites (including lymphoid organs, such as the thymus, spleen, and lymph nodes) (Elenkov et al., 2000). Cathecholamines then act on immune cell receptors at these sites, causing the release of cytokines that upregulate inflammation (Szelényi, 2001).

Broadly speaking, glucocorticoids suppress inflammatory cytokine activity (Sapolsky et al., 2000) that might interfere with “fight or flight” behavioral coping (Slavich & Irwin, 2014). However, as glucocorticoid responses wane, inflammation may

produce “sickness behaviors” (e.g., decreased feeding and socializing) which are thought to facilitate recovery processes (e.g., wound healing) (A. H. Miller & Raison, 2016; Raison & Miller, 2016).

Box 3. Inflammation May Inhibit Dopamine Synthesis via BH_4 Oxidation

Inflammation may impede the biosynthetic pathway to dopamine. For a detailed review, see Felger and Treadway (2017). Briefly, dopamine synthesis requires the conversion of phenylalanine to tyrosine by phenylalanine hydroxylase (PAH). A second enzyme, tyrosine hydroxylase (TH), converts tyrosine to L-DOPA, the human precursor to dopamine. For these conversions, both enzymes require the cofactor BH_4 (Figure I).



Box 3, Figure I. Inflammatory disruption of dopamine synthesis through BH4 oxidation. (A) Dopamine synthesis requires the conversion of phenylalanine to tyrosine by the enzyme PAH. A second enzyme, TH, converts tyrosine to L-DOPA, the human precursor to dopamine. For these conversions, both enzymes require the cofactor BH_4. (B) BH_4 facilitates production of NO by the enzyme iNOS. In the absence of BH_4, iNOS increases production of a reactive oxygen species, superoxide (O_2^-). (C) NO and O_2^- react to produce peroxynitrite ($ONOO^-$), a powerful oxidant. BH_4 is susceptible to oxidation by peroxynitrite and O_2^-, which further decreases BH_4 availability. (D) Inflammation may drive BH_4 oxidation by increasing the activity of iNOS. BH_4, tetrahydrobiopterin; iNOS, inducible nitric oxide synthase; L-DOPA, L-3,4-dihydroxyphenylalanine; PAH, phenylalanine hydroxylase; TH, tyrosine hydroxylase.

BH_4 also facilitates production of nitric oxide (NO) by nitric oxide synthases (NOSs). In the absence of BH_4, NOSs increase production of a reactive oxygen species, superoxide (O_2^-). BH_4 is susceptible to oxidation by O_2^-. Furthermore, NO and O_2^- react to produce peroxynitrite ($ONOO^-$), a powerful oxidant, which may cause neuronal death in addition to BH_4 oxidation (Sharma & Nehru, 2015). Thus, initial BH_4 depletion could cause still greater BH_4 deficits through oxidative loss (Figure I).

Inflammation may drive BH_4 oxidation by increasing the activity of inducible NOS (iNOS), an NOS type produced in peripheral macrophages and brain glial cells (Galea, Feinstein, & Reis, 1992) (Figure I). Accordingly, one study of rats suggests that peripherally-administered IFN-α decreases brain levels of BH_4 and dopamine through NO that is thought to cross the blood-brain barrier (Kitagami et al., 2003).

Chapter 3: Key Dimensions of Stress and Contributions to Anhedonia

Abstract

Although past work suggests a link between stress exposure and anhedonia (Keller et al., 2007; Pizzagalli, 2014), the types of stressors that most strongly promote anhedonia remain unknown. A clearer understanding of which types of stress are most associated with anhedonia in humans could inform preclinical models and further elucidate the etiology of anhedonia. Thus, the present study applied Bayesian modeling techniques to investigate which dimensions of stress are mostly closely associated with anhedonic symptoms. To do so, we compiled life stress interview data from multiple studies that assessed stressor severity, chronicity, dependence on participants' behavior, and interpersonal focus. Unexpectedly, analyses uncovered no evidence that these stressor characteristics were substantially associated with anhedonic symptoms. Instead, the best-fitting model treated all stressors equally, and number of stressors endorsed within a 6-month time window predicted anhedonic symptoms. Based on the posterior estimates from this relatively simple model, the impact of each individual stressor on anhedonic symptoms was modest: Each stressor endorsed was associated with an increase of approximately 0.48 on the MASQ Anhedonic Depression subscale (overall *SD* = 19.18). More work is needed to assess whether this result holds in larger samples, while also examining other potentially important dimensions of stress and other (e.g., narrower) time windows.

Introduction

Substantial cross-species work suggests a link between stress and anhedonia (Pizzagalli, 2014; Russo & Nestler, 2013; Stanton, Holmes, Chang, & Joormann, 2019). However, the types of stress that most strongly promote anhedonia *per se*, rather than a broader phenotype that includes anhedonia, such as major depressive disorder (MDD), are unknown. Investigating which types of stress are most associated with anhedonia would inform both etiological understanding of anhedonia and methodological practice. For example, if chronic stress is most strongly associated with anhedonia, this outcome would suggest that repeated activation of biological stress responses contributes to the onset of symptoms more strongly than acute stress responses. Additionally, a detailed understanding of how stressor types differentially predict anhedonia could inform the design of ecologically-valid stress manipulations in both humans and nonhuman animals.

Examinations of stress-induced anhedonia have varied considerably in the chronicity, severity, controllability, and type of stress implemented (Anisman & Matheson, 2005), but the implications of variability in these dimensions of stress are incompletely understood. Findings from one study in humans (Keller et al., 2007) suggested that anhedonia may be especially common following some types of stressors (e.g., death of a loved one or romantic loss) relative to others. This study examined the contribution of stressors to individual depressive symptoms in a large sample of twins who reported experiencing a dysphoric episode in the past year. However, this design tested the relative impact of different *categories* of stress, while the impact of stress severity remained unclear. Furthermore, the analysis examined the impact of stress on patterns of symptoms, rather than focusing on individual symptoms, such as anhedonia

(Keller et al., 2007). Follow-up work is needed to a) investigate the replicability of these results, and b) extend them by investigating the (possibly interactive) impact of severity and chronicity as well as other important dimensions of stress.

Although few studies have focused on anhedonia *per se*, researchers have devoted considerable effort to examining how dimensions of stress contribute to MDD (see Hammen, 2005), MDD subtypes (e.g., Harkness & Monroe, 2006), or categories of MDD symptoms (e.g., somatic vs. cognitive symptoms; Monroe, Harkness, Simons, & Thase, 2001). As a result, prior work has identified several dimensions of stress that appear to contribute to the onset of depressive symptoms. In general, stressors of greater severity are associated with higher risk for MDD onset (Kendler, Karkowski, & Prescott, 1998). Additionally, higher numbers of stressors endorsed within the same month increase risk for MDD onset (Sullivan, Kessler, & Kendler, 1998), suggesting a possible effect of chronicity. However, the literature on stressor chronicity and MDD is mixed, with some studies finding that chronic stressors are more predictive of depressive symptoms (McGonagle & Kessler, 1990), some finding that acute events are more predictive (Muscatell et al., 2009), and some finding no significant differences (Rojo-Moreno, Livianos-Aldana, Cervera-Martínez, Dominguez-Carabantes, & Reig-Cebrian, 2002). This inconsistent literature may at least partly reflect differences in methodology, since time thresholds for determining chronic stress have varied from 4 weeks (Muscatell et al., 2009; Rojo-Moreno et al., 2002) to 1 year (McGonagle & Kessler, 1990). Additionally, stressors that are judged to be dependent—that is, plausibly influenced by the participant's own behavior—are more strongly associated with MDD onset than likely independent events, after controlling for stressor severity (Kendler et al., 1999). Some

evidence also suggests that stressors whose primary focus is interpersonal may contribute more strongly to depressive symptoms, at least for a subset of individuals who are especially sensitive to interpersonal stress (Hammen, Marks, Mayol, & DeMayo, 1985).

Despite knowledge about the types of stress that increase risk for the onset of MDD as a syndrome, little work has focused on the types of stress that predict anhedonia specifically. As noted above, one study examined the impact of different categories of stressors on patterns of MDD symptoms (Keller et al., 2007). However, this study did not address the *severity* of stress exposure or of anhedonia (only presence or absence) and did not examine past predictors of MDD status, such as stressor independence or interpersonal focus. Additionally, although nonhuman animal models of anhedonia tend to use chronic stressors (Anisman & Matheson, 2005; Russo & Nestler, 2013) the role of stress chronicity in human anhedonia remains unclear. Thus, additional work is needed to determine whether the stressor characteristics that most strongly predict MDD—which may or may not include anhedonia as a symptom (American Psychiatric Association, 2013)—are also predictive of anhedonia *per se*.

To adequately assess stressor characteristics, detailed life stress interviews may provide the most detailed and comprehensive data. While stress checklists (e.g., Brugha & Cragg, 1990; T. H. Holmes & Rahe, 1967) are readily scalable to large sample sizes due to brevity and low implementation cost, they may omit potentially relevant information. As others have noted (Harkness & Monroe, 2016), checklists: a) often miss crucial life events, possibly because they include a restricted set of life events and often do not allow for the reporting of more than one event in the same category; b) rely on participants' subjective interpretation of event severity (if they assess severity at all),

thereby reducing measurement standardization and conflating stress exposure with stress responses; c) generally do not contain detailed information about event timing, rendering the selection of time windows and clarification of temporal precedence more difficult; and e) tend not to include ratings of chronicity, dependence, or interpersonal focus, which may be relevant to the impact of stress on anhedonia (see above). By contrast, the Life Events and Difficulties Schedule (LEDS-2; Bifulco et al., 1989) is a life stress interview that inquires about a wide range of acute and chronic stressors; uses rating panels that are blind to diagnostic status to assess stressor severity, independence, and interpersonal focus, rather than relying participants' judgments; and guides severity ratings using standardized anchors for each stressor. The LEDS also yields information on stressor timing. Thus, there are *a priori* reasons to expect that the detailed information provided by an intensive stress interview may be needed to assess which stressors are most associated with anhedonia. However, collecting a sufficient sample of interview data presents a particular challenge, since administering the LEDS requires considerable expertise, resources, and time.

Additionally, to model the effects of several potentially relevant stressor characteristics requires an analytic strategy capable of handling complex models with numerous parameters. Bayesian approaches are especially suited to estimating models with many parameters (Gelman et al., 2013). Furthermore, Bayesian approaches intuitively account for uncertainty when estimating effects, since they describe the credibility of parameter values using a posterior probability distribution, after taking both prior beliefs and the observed data into account (e.g., as opposed to a point estimate that maximizes the likelihood of the data). A hierarchical model may be especially suited to

characterize stress data, since stressor characteristics are thought to be interrelated—e.g., interpersonal stressors are assumed to be usually dependent on participants' behavior (Kendler et al., 1999). In addition, some stressor subtypes may be relatively rare, and hierarchical modeling would allow for information about one category of stressors (e.g., chronic stressors) to inform estimates for a particular subcategory for which there may exist few examples (e.g., chronic, dependent, noninterpersonal stressors). However, to our knowledge, Bayesian hierarchical modeling has not been applied to the question of which stressor characteristics are most linked to anhedonia.

To address the empirical gaps described above, we examined the contributions of key stress dimensions to anhedonia using intensive interview data compiled from multiple prior studies. Using Bayesian hierarchical modeling, we constructed a model to evaluate the contributions of stressor severity, chronicity, independence, and interpersonality (interpersonal vs. noninterpersonal focus) to anhedonic symptoms. Additionally, we compared the outcome of the resulting "full" model to a "simple sum" model that treats all stressors equally and uses number of stressors endorsed to determine the impact on anhedonic symptoms. We hypothesized that, if the contribution of stress to anhedonic symptoms mirrors the work that has been conducted on stress and MDD, then dependent, interpersonal stressors (Hammen et al., 1985; Kendler et al., 1999) would have the greatest impact on anhedonic symptoms. As a competing hypothesis, we considered whether a model that treats all stressors as equal might better account for anhedonic symptoms (given the stress dimensions tested and the available data).

Methods

Sample Composition

The present sample comprises four datasets from studies conducted by Kate Harkness and colleagues (see Harkness et al., 2010; Harkness & Monroe, 2006). Table 1 characterizes each of these four samples in terms of age, sex, and diagnostic status. Because differences in diagnostic category, age, sex, and study protocol could influence our results, we controlled for these factors in each model (see *Model structure*). After excluding individuals with missing stress interview or anhedonia self-report data, and individuals with diagnoses besides MDD or remitted MDD, the final sample for this analysis consisted of 635 individuals. Of this sample, 82.4% of individuals identified as white or Caucasian, 1.6% as Black or Afro-Canadian, 8.7% as Asian or Asian-Canadian, 0.8% as First Nations (indigenous peoples of Canada), and 5.5% race unknown or not reported. Additionally, 1.3% of individuals identified as Hispanic or Latinx.

Table 1. Sample characteristics by study

Study	Age		Sex (*n)*		Diagnostic Category		
	Range	*M* (*SD*)	Female	Male	HC	MDD	rMDD
ARP	13-21	15.55 (1.46)	83	44	50	74	3
BSP	15-33	20.97 (3.13)	206	59	104	143	18
LSD	18-57	36.77 (10.56)	74	None	None	74	None
PAD	12-21	16.31 (2.23)	127	42	71	76	22
Total:	12-57	20.49 (7.70)	490	145	225	367	43

Note. ARP = Adolescent Risk Project; BSP = Blue Sky Project; LSD = Life Stress and Depression; PAD = Predictors of Adolescent Depression. HC = healthy control; MDD = Major Depressive Disorder; rMDD = remitted Major Depressive Disorder.

Materials and Measures

Life stress interview. The Life Events and Difficulties Schedule (LEDS-2; Bifulco et al., 1989) is a semi-structured interview and rating system that assesses recent stressful life events (over the 5-year period prior to the interview, for the present data). The LEDS inquires about events from 10 domains: education, occupation, housing, finances, role changes, legal issues, health, romantic relationships, other relationships, and deaths. All interviews were audiotaped, and these tapes were used by a research assistant to prepare vignettes of each event, omitting information about diagnostic status and participants' emotional reactions to events. Using the vignettes, a panel of 2-4 blind raters coded the severity of each event according to a set of standardized criteria. Severity was rated on a 5-point scale in terms of long-term threat to the individual (1 = marked, 2a = high moderate, 2b = low moderate, 3 = some, 4 = little/none), anchored by examples for each severity level of each stressor. This procedure allows for measurement of stress exposure *per se*, avoiding potential bias due to knowledge of diagnosis or participant reactions to stress. Discrepancies among raters were discussed to arrive at a consensus rating, which was used for all analyses.

The same panels also rated event *independence* (whether a participant's own behavior likely influenced an event). Events judged to be entirely or almost entirely independent of a participant's behavior were coded as likely independent (e.g., lost job due to factory closing); otherwise, the event was coded as plausibly dependent (e.g., quit job).

Additionally, panels rated *interpersonality*, where an event was coded as interpersonal only if the stressor centered primarily on an interpersonal relationship (e.g.,

divorce). Events that did not primarily focus on a relationship were coded as noninterpersonal, even if they involved another person (e.g., receiving a failing grade, although that grade was assigned by a teacher). Notably, many potentially noninterpersonal events could be coded as interpersonal if a relationship was the key element (e.g., being publicly berated by a teacher over a failing grade).

Anhedonia measure. Anhedonia was assessed using the Anhedonic Depression subscale of the Mood and Anxiety Symptom Questionnaire (MASQ; Watson, Weber, et al., 1995). The MASQ has previously exhibited good internal consistency in clinical and non-clinical samples ($\alpha > .80$) and both convergent and discriminant validity (Watson, Clark, et al., 1995).

Analytic Approach

We adopted a Bayesian hierarchical linear modeling approach to characterize the association between stress dimensions and anhedonia. The Bayesian framework is preferred for the present application because: a) the focus is on estimating parameter values themselves, rather than estimating the likelihood of the data given a null hypothesis; b) Bayesian inference provides intuitive quantification of uncertainty for parameter estimates; and c) Bayesian approaches are relatively well-suited to fitting complex models with many parameters (Gelman et al., 2013).

Bayesian models start with a *prior* distribution that represents our confidence in the possible values of the parameters of interest, before taking the data into account. An example of a parameter might be a regression weight for the effect of interpersonal stress severity on anhedonia. Some function is selected (e.g., a linear function) to estimate the *likelihood* of the data for all plausible parameter values. Finally, using Bayes' Rule,

analyses take into account prior expectations and also the likelihood of the data, producing *posterior* distributions for each parameter. These distributions describe our confidence in parameter values, given the priors used and the observed data (Gelman et al., 2013; Kruschke, 2014).

We employed Hamiltonian Markov Chain Monte Carlo (Hamiltonian MCMC) to sample from the posterior distribution. Hamiltonian MCMC is a widely used method that a) does not assume a particular shape for the posterior, and b) can generate representative samples from the posterior of even highly complex models (Gelman et al., 2013; Kruschke, 2014). To implement Bayesian modeling via Hamiltonian MCMC, models were defined using the Stan programming language (Stan Development Team, 2021) via interface with R version 3.5.3 (R Core Team, 2020). Model fitting was conducted using four chains of 10,000 steps each, with a warm-up of 500 steps.

When evaluating the estimated posteriors for a given model, we here consider a parameter to have contributed substantially to the outcome if the 95% high density interval (HDI) for the posterior of that parameter does not contain zero.

Data Preparation

For LEDS data, acute life events rated as having "little to no long-term threat" to the individual can include events that are often viewed as beneficial, such as a marriage (Bifulco et al., 1989). Thus, these events were excluded from analysis. The resulting severity ratings were re-coded such that higher values indicated increasing severity: some long-term threat = 0, low moderate = 1, high moderate = 2, and marked = 3. Similarly, chronic difficulties rated as "very mild" were dropped, and the resulting ratings were re-coded to fit the same scale as acute life events: mild = 0, low moderate = 1, high

moderate = 2, and marked = 3. Independence ratings were dichotomized in accordance with prior work (Harkness et al., 2010).

Because prior work suggests that events within a 6-month time window prior to MDD onset are of the greatest etiological relevance (Harkness et al., 2010; Kendler et al., 1998), we examined stressors within a 6-month time window prior to each participant's interview date, when the MASQ was administered. Chronic difficulties were included if any part of the difficulty's time span included the 6-month window of interest.

We sorted participants into age groups rather than treat age as a continuous variable, since we do not necessarily expect a linear effect of age. To facilitate comparisons with prior work (Harkness et al., 2010), we used the following age groups: a) adolescence (ages 12-17); b) young adulthood (ages 18-29); c) middle adulthood (ages 30-49); and d) upper middle adulthood (ages 50-65).

Model Structure

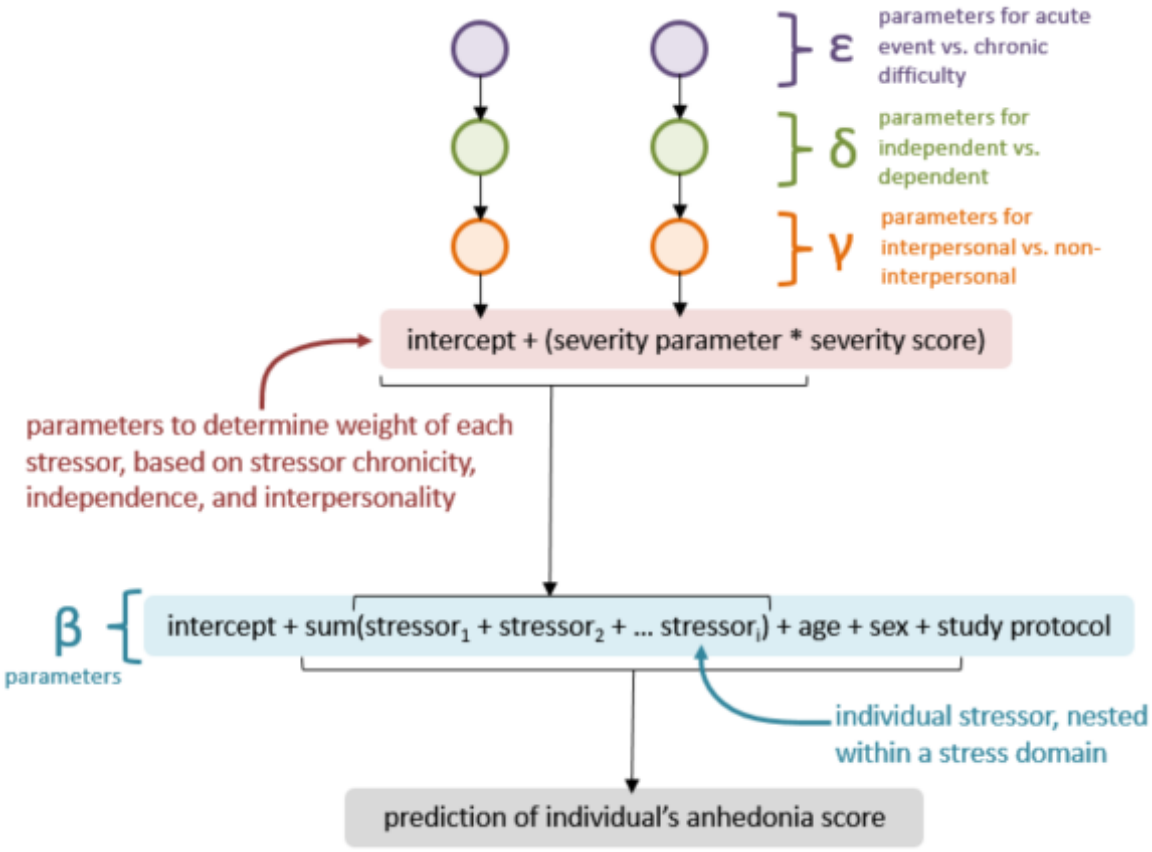


Figure 1. "Full" model schematic diagram.

"**Full" model.** This study primarily aimed to disentangle the possible effects of stressor chronicity, severity, independence, and interpersonality. Thus, we constructed a model—referred to here as the "full" model—that attempts to account for these dimensions of stress when predicting anhedonic symptoms. See Figure 1 for a schematic summary of the "full model" structure, and Appendix A for a more detailed diagram. To predict anhedonia score for each participant, the "full model" included a) an overall intercept, added to b) summed values, one for each stressor endorsed by that participant, which were weighted based on the characteristics of each particular stressor, and c) parameters for age group, sex, and study protocol. Overall intercepts were fit for each

diagnostic category; thus, the model estimated the impact of other effects over and above diagnostic status. The weight for each stressor was defined as the sum of two parameters: a) a term that was added when the stressor was present, regardless of stressor severity; and b) a term that was multiplied by the stressor severity value (0-3). Each of these two parameters was influenced hierarchically by: 1) whether the stressor was rated as interpersonal vs. non-interpersonal; b) whether the stressor was plausibly dependent vs. likely independent; and c) whether the stressor was an acute life event or chronic difficulty. Because some stressors were missing independence or interpersonality ratings (see *Descriptive Statistics*, below), missingness was treated as an additional data category for these variables.

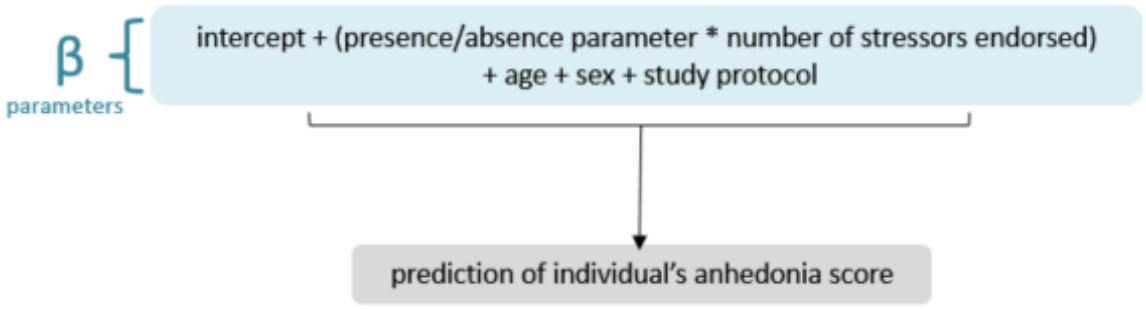


Figure 2. "Simple sum" model schematic diagram.

"**Simple sum" model.** For comparison purposes, we also fit a model that accounts only for stressor presence/absence. See Figure 2 for a minimal schematic of this "simple sum" model, and Appendix A for a more detailed diagram. To predict anhedonia score for each participant, the "simple sum model" included a) an overall intercept, added to b) a single parameter multiplied by the sum number of stressors endorsed by the participant within the time window, regardless of stressor characteristics, and c) parameters for age

group, sex, and study protocol. As in the "full model," overall intercepts were fit for each diagnostic category.

Priors. For all models, $y_{i,g} \sim \text{skew\_normal}(x_i, \omega_g, \alpha_g)$, where x_i represents the location value for subject i, ω_g represents the scale parameter for diagnostic group g, α_g represents the skew parameter for diagnostic group g, and $y_{i,g}$ represents the anhedonia score for subject i from diagnostic group g. A skew-normal prior was applied here to account for skew in the data, since individuals in the healthy control group were skewed towards lower anhedonia scores, and individuals in the MDD group were skewed towards higher scores. Priors for ω_g were set for each diagnostic group g, where $\omega_g \sim \text{normal}(\text{SD}_g, 1)$ and SD_g indicates the empirical standard deviation of anhedonia scores for diagnostic group g. For the skew parameter, $\alpha_g \sim \text{normal}(0,1)$ for each diagnostic group. The location value x_i was defined as the sum of β values for each subject i (see Figure 1 below for a schematic representation and Appendix A for more detail).

At the β level of each model, an overall intercept β_0 for each diagnostic group was included, and $\beta_{0,g} \sim \text{normal}(\text{M}_g, \text{SD}_g * 5)$ where M_g is the empirical mean of anhedonia scores for group g. For β parameters for age, sex, and study protocol, normal priors were specified with mean of 0 and variance of 1. In the "simple sum" model, an additional β parameter was added to capture the impact of number of stressors endorsed, for which a normal prior was specified with mean of 0 and variance of 1.

In the "full" model, priors for parameters at the ϵ level were specified as follows: $\epsilon_c \sim \text{normal}(0,1)$ for each type of chronicity c (acute, chronic). At the δ level, priors

were specified as follows: $\delta_{d,c} \sim \text{normal}(\epsilon_c, 1)$ for each type of independence d (possibly dependent, likely independent, missing independence rating) and chronicity c. Finally, at the γ level, priors were specified as follows: $\gamma_{p,d,c} \sim \text{normal}(\delta_{d,c}, 1)$ for each type of interpersonality p (noninterpersonal, interpersonal, missing interpersonality rating), independence d, and chronicity c.

Results

Descriptive Statistics

On the LEDS, participants endorsed a total of 2,314 stressors (*M* = 3.64 stressors per participant, *SD* = 3.11) that met criteria for inclusion (see *Data Preparation*). Of these, 243 were missing interpersonality ratings (10.5% of all stressors) and 89 were missing independence ratings (3.6% of all stressors). Prevalence of particular types of stressors are listed in Table 2.

Table 2. Stressor prevalence by stressor type

Stressor Type	Total Count	Per Participant Count	
		M	*SD*
Chronicity			
Chronic difficulty	983	1.55	1.71
Acute life event	1,331	2.10	2.09
Independence			
Possibly dependent	1,276	2.01	2.16
Likely independent	954	1.50	1.56
Interpersonality			
Noninterpersonal	1,222	1.92	1.97
Interpersonal	849	1.34	1.59
Severity (long-term threat)			
Some	1,203	1.89	1.83
Low moderate	601	0.95	1.21
High moderate	378	0.60	0.96
Marked	132	0.21	0.61

For the anhedonia subscale of the MASQ, the mean score for the total sample was 69.23 (*SD* = 19.18). Details by diagnostic category are listed in Table 3.

Table 3. MASQ Anhedonic Depression subscale score by diagnostic category

Diagnostic Category	*M*	*SD*
HC	49.95	11.44
MDD	81.26	12.78
rMDD	67.40	13.12

Note. HC = healthy control; MDD = Major Depressive Disorder; rMDD = remitted Major Depressive Disorder.

Model Fitting

Before fitting the models to the data, we confirmed that each model worked as expected by fitting them to simulated data. The simulated dataset was constructed by using the independent variables from the actual dataset, where the dependent variable (anhedonia score) was replaced with fictitious data that were computed to include arbitrary effects of certain stressor types (e.g., an effect of chronic, dependent, interpersonal stress) or control variables (e.g., age group). Thus, the simulated data included the real-world stressors endorsed by participants, so that the relative prevalence of stressor types would remain realistic. Models were able to extract effects from simulated data as expected. When fit to the actual, observed data, Rhat < 1.01 for each parameter across both models, suggesting convergence. Additional diagnostics (e.g., autocorrelation and density plots for each parameter) are available in Appendix B.

To evaluate relative model fits, we applied leave-one-out cross-validation with Pareto-smoothed importance sampling (PSIS-LOO; Vehtari, Gelman, & Gabry, 2017) to calculate the expected log pointwise predictive density (ELPD). The difference in ELPD between the "simple sum" and "full" models was -324.8, approximately 34.4 standard errors from zero, suggesting that the "simple sum" model would be expected to better predict novel data.

Parameter Estimates

"**Full" model.** See Figures 1 and 2 for visualizations of the estimated posterior distributions for parameters that index the impact of stressors on anhedonic symptoms in the "full" model. Figure 1 contains estimates for parameters that index the impact of

stressor presence *per se*—that is, whether the participant endorsed that stressor, regardless of how severe the stressor was rated. Figure 2 contains estimates for parameters that represent the linear impact of stressor severity. Notably, for each of these parameters, the 95% high density interval (HDI) contains zero. Thus, based on model estimates, these parameters are unlikely to exert substantial predictive power with respect to anhedonic symptoms. These results provide no evidence that the stressor types assessed (severity, chronicity, independence, and interpersonality) contribute to anhedonia, after accounting for diagnostic status, age, sex, and study protocol. For additional model diagnostics, including posterior estimates for other parameters, see Appendix B.

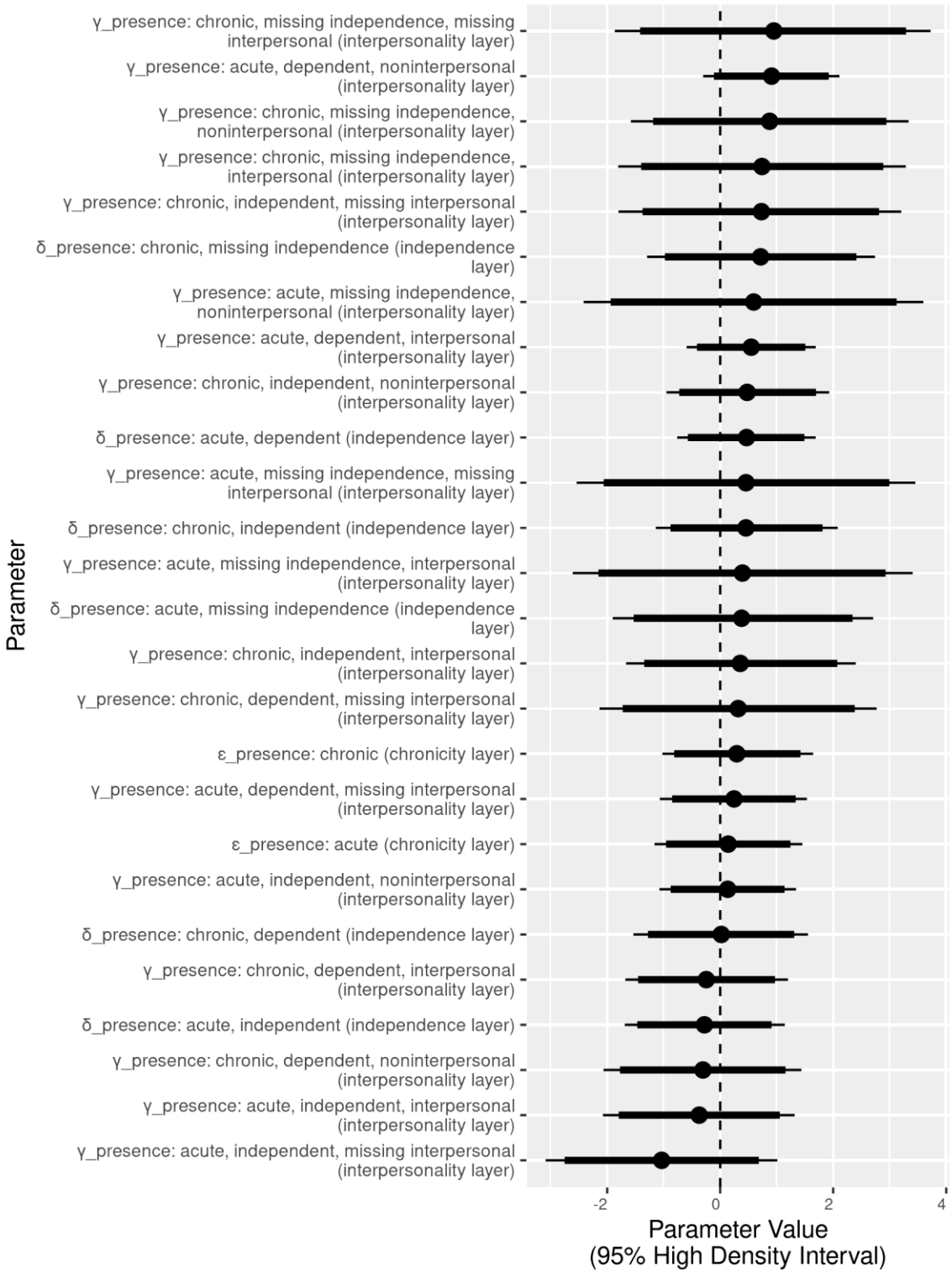


Figure 1. Posterior estimates for "full" model parameters that index stressor presence/absence. Thin lines denote 95% high density interval (HDI); thick lines denote 90% HDI; points denote the median of posterior samples.

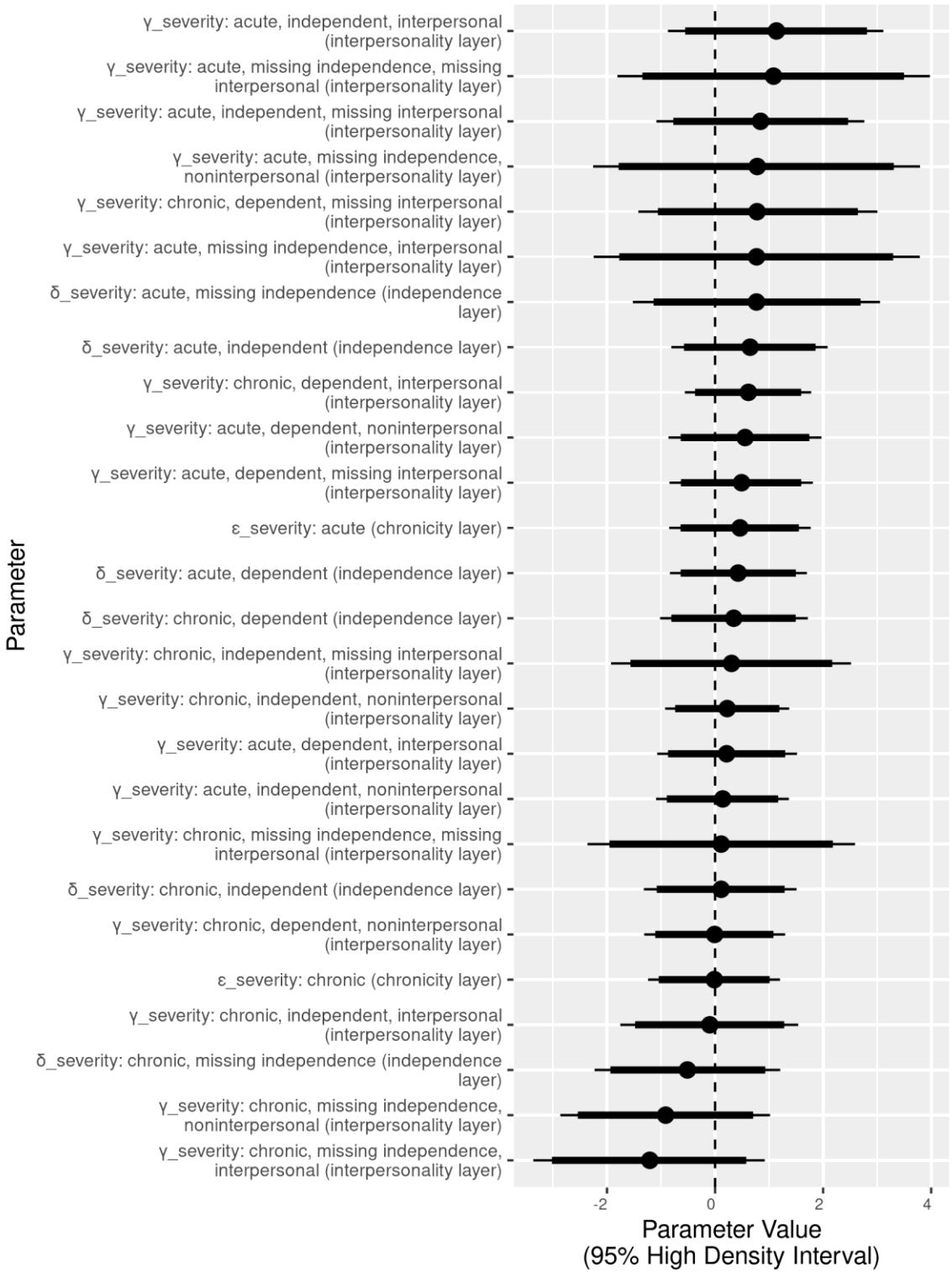


Figure 2. Posterior estimates for "full" model parameters that index the linear impact of stressor severity. Thin bands denote 95% high density interval (HDI); thick bands denote 90% HDI; points denote the median of posterior samples.

"Simple sum" model. The "simple sum" model specified only one parameter to account for the effect of stress on anhedonic symptoms, treating all stressors as equal. Figure 3 visualizes the posterior estimate for this parameter. Notably, the 95% HDI did not contain zero, suggesting that number of stressors endorsed within the time window (regardless of stressor attributes) significantly predicted anhedonic symptoms (median = 0.48, 95% HDI [0.14, 0.81]). The median of the posterior samples was 0.48, suggesting that each stressor endorsed was associated with an increase of approximately 0.48 on the MASQ Anhedonic Depression subscale. For additional model diagnostics, including posterior estimates for other parameters, see Appendix B.

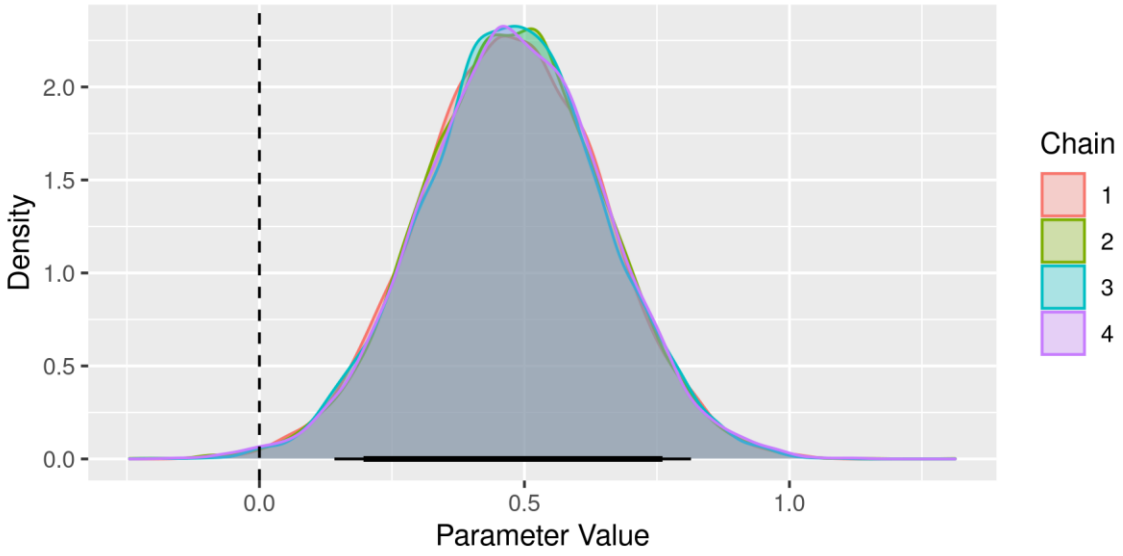


Figure 3. Posterior estimate for the stress parameter of the "simple sum" model. Thin bands denote 95% high density interval (HDI); thick bands denote 90% HDI.

Discussion

This study applied Bayesian modeling techniques to investigate which dimensions of stress are most closely associated with anhedonic symptoms. We compiled samples from multiple studies that used the LEDS life stress interview, which assessed stressor

severity, chronicity, independence, and interpersonal focus. However, we uncovered no evidence that any of these characteristics significantly predicted anhedonic symptoms. Analyses suggested that the best-fitting model was the simplest model, which treated all stressors equally. Based on posterior estimates from this model, each stressor endorsed within the time window (6 months prior to interview) produced a modest increase in anhedonic symptoms.

Several plausible explanations could account for why the "full" model failed to identify any stressor dimensions that are especially linked with anhedonia. One possibility is that this investigation was underpowered to characterize the specific subtypes of stress examined here. For instance, there may not be enough examples of chronic, severe, dependent, interpersonal stress to estimate the impact of this particular type of stress. Indeed, based on estimates from the "simple sum" model, the impact of stress on anhedonic symptoms is expected to be fairly modest: Each stressor endorsed was associated with an increase of approximately 0.48 on the MASQ Anhedonic Depression subscale, where the overall standard deviation in anhedonia scores was 19.18. Although the hierarchical design of the "full" model was designed to mitigate the problem of rare stress subtypes, this strategy may not have been enough to overcome sample size limitations. Thus, a larger sample size may be needed to detect potentially subtle effects of stressor characteristics.

However, a failure to detect significant effects could also be due to suboptimal model specification. A differently specified model might be capable of extracting effects of stressor characteristics on anhedonia, if such effects exist. For example, in the "full" model, we specified a linear effect of severity. Yet perhaps a nonlinear effect of stressor

severity would better fit the data, e.g., in which only the most severe stressors contribute to anhedonia risk. Testing other model specifications could therefore be a useful future direction.

Additionally, our investigation of the impact of stress severity may have been limited by the stressors that are assessed on the LEDS. For reasons of feasibility, no self-report assessment of stress can inquire about every possible stressful occurrence, no matter how minor. Thus, to be assessed via the LEDS, stressors must necessarily pass some threshold of severity. It is possible that once life experiences cross a given threshold, they exert similar effects on anhedonia, and the LEDS does not inquire about enough experiences on the less-severe side of this threshold to distinguish between impactful and non-impactful stressors.

Our measurement of stressor chronicity may also be overly narrow. Here, stressor chronicity referred to the time span over which a particular stressor unfolded (e.g., financial concerns that lasted for months). However, numerous acute stressors of different types, experienced over a relatively brief time window, could also be considered more chronic stress exposure than a single event. Indeed, results from the "simple sum" model indicate a non-zero linear slope for number of stressors endorsed within a 6-month time window, such that a greater number of stressors was associated with more severe anhedonia. Thus, in the sense that more stressors are linked to more severe illness, stress chronicity (when considering all stressors together, rather than the chronicity of a single stressor) could be said to contribute to anhedonia.

Notably, we examined the impact of key stress dimensions on severity of anhedonic symptoms. Many of the stress dimensions tested here were identified based on

research that focused on MDD onset. Thus, differences between our findings and those of the literature on stress and MDD could be accounted for in several ways. First, an individual may meet for MDD without endorsing anhedonia (American Psychiatric Association, 2013), so that MDD and anhedonia are not synonymous. Additionally, prior work has generally examined MDD onset rather than MDD severity. In the present study, we hypothesized a dose-response relation, such that more stress of a given type would be expected to predict higher anhedonia scores, even after accounting for diagnosis. In addition to temporal precedence, such a dose-response relation would be consistent with a causal effect. Here, we found a dose-response relation such that a greater number of stressors endorsed within the 6-month time window was linked with more severe anhedonic symptoms, when all stressors were treated as equal. Finally, earlier work examined stressors that occurred in a time window prior to MDD onset. In the present analysis, we considered stressors in the 6 months prior to assessment of anhedonia severity using the MASQ, which took place following MDD onset for individuals with MDD. In doing so, we examined stressors that were temporally proximal to the assessment of anhedonic symptoms, whereas using a pre-onset window would involve investigating more distant events for individuals with a longer course of illness. However, with this caveat in mind, future work could investigate whether the current pattern of results holds if only individuals with MDD are considered, and the time window is limited to events prior to MDD onset.

The present analysis raises questions that could be addressed in future research. First, additional studies could test whether the effects of certain stress dimensions are moderated by factors such as age, ~~and~~ sex, and early-life stress exposure. For example,

prior work suggests that sex differences in rates of stressful events prior to MDD onset are strongest in young adulthood (Harkness et al., 2010). Furthermore, key studies in individuals with MDD have been conducted using entirely female-assigned-at-birth samples (e.g., Kendler et al., 1999). Additionally, evidence from multiple lines of research suggests that early-life stress may increase interactions between peripheral inflammation and key neural circuitry, thereby elevating risk for a host of mental and physical disorders (Nusslock & Miller, 2016). Thus, early-life stress could also plausibly moderate the impact of stress on anhedonia. In the present study, we avoided including interaction terms for age, sex, early-life stress, and diagnostic status to maximize the comparability of the two models, since the "simple sum" model would require only an interaction with number of stressors endorsed, whereas examining interactions in the "full" model would necessitate dozens of additional parameters. However, investigating potential moderators represents an important area of future study.

The results of the present analysis suggest that, when examining the link between stress exposure and anhedonia, accounting for the number of stressors endorsed provides a more parsimonious and predictive model than one which accounts for stressor severity, independence, interpersonal focus, and chronicity. However, we take care to note that these results do not, by themselves, support the use of checklist measures over stress interviews. Of note, stress checklists often provide a very limited accounting of life stressors owing to their relative brevity, and tend to lack fine-grained information about stressor timing, rendering it difficult to establish temporal precedence. However, our results call into question the utility of assessing certain dimensions of stress when considering the impact on anhedonia severity (but not MDD onset, which was not

investigated here). Further investigation is needed in a larger sample to verify the results obtained here.

Despite these results, additional work is needed that uses detailed life stress information to predict anhedonic severity. Follow-up studies in larger samples could help to verify the present finding that number of stressors, rather than particular types of stress, may best account for variance in anhedonic symptoms. Alternatively, if different results emerge for larger samples, different time windows, or particular subgroups (e.g., certain age or diagnostic groups), that information would provide important guidance for preclinical models of stress-induced anhedonia, and might inform the development of new predictive tools and clinical interventions.

Chapter 4: Frontostriatal Connectivity as a Possible Mediator of Stress-Induced Anhedonia

Abstract

Anhedonia (loss of pleasure/lack of motivation) is a debilitating condition that cuts across diagnostic categories, yet little is known about the biological etiology of stress-induced anhedonia. We hypothesized that frontostriatal functional connectivity would statistically mediate the stress-anhedonia link in a sample of older adults (ages 40-69) from the UK Biobank database, split into discovery (n = 6,144) and replication (n = 4,101) samples. Bootstrapped regression analyses indicated that self-reported financial stress over the past 2 years was associated with self-reported anhedonia across both the discovery (B = .22; SE = .04; 95% CI: [.14, .31]) and replication (B = .25; SE = .05; 95% CI: [.16, .35]) samples. Additionally, number of stressors endorsed significantly predicted anhedonia score across the discovery (B = .06; SE = .01; 95% CI: [.06, .08]) and replication (B = .06; SE = .01; 95% CI: [.04, .09]) samples. Next, functional parcellation of cortex facilitated the identification of medial prefrontal cortex (mPFC) parcels with the highest functional connectivity to anatomically-defined nucleus accumbens (NAc). However, bootstrapped mediation models found no evidence that frontostriatal functional connectivity mediated the stress-anhedonia association, either for financial stress or number of stressors endorsed. Additionally, associations between frontostriatal connectivity and anhedonia scores were unstable across the discovery and replication samples, suggesting no reliable relation between frontostriatal connectivity and anhedonia. These null findings may have resulted from incorrect hypotheses, unreliable measurements, or insufficiently detailed measurement of psychological stress exposure. Large samples with more detailed psychiatric phenotyping alongside key biological

measures are sorely needed to advance understanding of the etiology of anhedonia, as well as the effects of stress on human psychiatric functioning more broadly.

Introduction

Anhedonia, the loss of pleasure and/or motivation observed in several types of psychiatric problems (Husain & Roiser, 2018), presents a debilitating emotional burden and is associated with increased suicide risk in individuals with mental illness (Fawcett et al., 1990). Cross-species work suggests that psychological stress leads to decreased motivated behavior in animals (Hollon et al., 2015; Russo & Nestler, 2013) and is associated with anhedonic symptoms in humans (Keller et al., 2007). Although the biological mechanism through which stress may influence anhedonia remains unclear, substantial work suggests that stress-induced alterations in frontostriatal functioning—particularly interactions between medial prefrontal cortex (mPFC) and mesolimbic circuitry—could impact motivated behavior, possibly contributing to anhedonia (Russo & Nestler, 2013). However, few studies have assessed whether frontostriatal interactions statistically mediate the effects of stress on anhedonia in humans.

Notably, stress causes wide-ranging changes in brain structure and function, including in hippocampus, amygdala, and across prefrontal cortex (Arnsten, 2009; McEwen et al., 2015). These interconnected regions, which are not limited to frontostriatal circuitry, may all contribute to stress-induced anhedonia, given their roles in fear conditioning and responding (LeDoux, 2000; Milad & Quirk, 2002; Phelps et al., 2004) and guiding behavior based on incentive value (Kable & Glimcher, 2007; Murray, 2007; Rangel & Hare, 2010). However, given promising work on mPFC-mesolimbic dynamics and motivated behavior that spans rodents (Ferenczi et al., 2016; Moreines et al., 2017) and humans (Admon & Pizzagalli, 2015; Drysdale et al., 2017), the present study focuses on the role of frontostriatal functioning.

Nonhuman animal models suggest that stress could alter motivated behavior, which is thought to be an important contributor to anhedonia (Pizzagalli, 2014; Zald & Treadway, 2017), through structural and functional changes in mPFC-mesolimbic circuitry. Stress exposure in rodents leads to well-documented structural alterations in mPFC, including dendritic shrinking and spine loss (McEwen & Morrison, 2013). Additionally, rodent work suggests that optogenetic manipulations to mPFC excitability can decrease motivated behavior and diminish blood oxygenation level-dependent (BOLD) striatal responses to midbrain dopaminergic activity (Ferenczi et al., 2016). Furthermore, in a separate study, chronic mild stress (CMS) in rats decreased the number of spontaneously firing dopamine neurons in medial and central ventral tegmental area (VTA; Moreines et al., 2017). Pharmacological inactivation of mPFC using tetrodotoxin prevented these changes (Moreines et al., 2017), suggesting that mPFC activity may mediate the impact of stress on dopaminergic midbrain functioning, which plays a key role in reward responding and motivated behavior (Berridge et al., 2009; Mirenowicz & Schultz, 1994; Schultz, 2016).

In humans, fMRI studies have found that mPFC-striatal connectivity may contribute to anhedonia among individuals with MDD and remitted MDD (rMDD). One study attempted to delineate biological sub-phenotypes ("biotypes") using BOLD functional connectivity data and MDD symptoms (Drysdale et al., 2017). Hierarchical clustering analyses suggested four biotypes determined by similar patterns of connectivity features. Hyperconnectivity in frontostriatal and thalamic networks characterized two of the biotypes. Additionally, frontostriatal hyperconnectivity was linked to anhedonia and psychomotor retardation (Drysdale et al., 2017). These results

suggest that frontostriatal connectivity, as assessed in humans via fMRI, may be associated with anhedonia status. This hypothesis is also consistent with findings from another study of rMDD individuals, in which patterns of frontostriatal functional connectivity predicted positive emotional responses to a naturalistic mood induction (Admon & Pizzagalli, 2015).

Despite cross-species work suggesting that frontostriatal interactions are perturbed by stress and associated with motivated behavior, positive affect, and anhedonic symptoms, we are aware of no studies that have assessed whether frontostriatal connectivity mediates the relation between stress and anhedonia in humans. We propose to address this hypothesis by examining resting-state functional connectivity (RSFC) between mPFC and ventral striatum (part of the mesolimbic pathway) in a subsample of the UK Biobank study (Sudlow et al., 2015; see additional details below).

RSFC assesses temporal correlations in BOLD signal oscillations across brain regions while participants are asked to rest quietly in the scanner. Several studies have found correspondence between resting-state and task-based activity (Cole, Ito, Bassett, & Schultz, 2016). As a result, researchers have argued that resting-state and task-based activity draw on a common functional network architecture (e.g., Cole, Bassett, Power, Braver, & Petersen, 2014). Thus, examining differences in patterns of resting-state activity following stress may reveal changes to the brain's functional architecture that increase anhedonia risk.

However, some evidence suggests that the direction of the resting-state correlation between two regions (i.e., positive or negative) may not match the direction of the task-evoked relation between those same regions. For example, during emotion-

related tasks, stronger inverse ventromedial PFC-amygdala functional connectivity is associated with lower anxiety (Hare et al., 2008; H. Kim et al., 2004; Urry et al., 2006). Yet at rest, *positive* functional connectivity between these regions is linked to lower anxiety (Burghy et al., 2012; Hahn et al., 2011; M. J. Kim, Gee, Loucks, Davis, & Whalen, 2011). In this case, the same phenotype is associated with inverse functional connectivity between two regions during a relevant task, but positive RSFC between the same two regions at rest. Increased, positive mPFC-striatal functional connectivity has been observed in rodents that exhibited heightened mPFC excitability and decreased motivated behavior (Ferenczi et al., 2016) as well as in humans with anhedonia (Drysdale et al., 2017). Thus, we predicted that the same pattern would hold in the present study, such that anhedonia would be associated with positive frontostriatal RSFC. We also hypothesized that changes in frontostriatal connectivity would mediate the association between stress and anhedonia.

As an exploratory follow-up, we investigated whether frontostriatal RSFC might mediate the stress-anhedonia relation for some subgroups of individuals. Some work suggests that frontostriatal reward processing changes with age, such that midbrain dopaminergic processing is less associated with prefrontal reward-related activity in older adults (Dreher, Meyer-Lindenberg, Kohn, & Berman, 2008) and older adults exhibit a decreased tendency to shift striatal BOLD activity from reward receipt to reward anticipation (Vink, Kleerekooper, van den Wildenberg, & Kahn, 2015). Additionally, sex differences in rates of MDD are well-documented (Kessler et al., 2003), and anhedonia is a primary symptom of MDD (American Psychiatric Association, 2013), leaving open the possibility that the mechanism of stress-induced anhedonia could vary based on sex

assigned at birth. Thus, we will examine whether frontostriatal connectivity might mediate the link between stress and anhedonia differently for younger vs. older individuals, and for individuals assigned female at birth vs. assigned male at birth.

Methods

Participants

UK Biobank is a cohort study of more than 500,000 individuals from the United Kingdom, recruited to participate at one of 22 sites across the UK (Sudlow et al., 2015). Participants were aged 40-69 when recruited between 2006-2010, and completed a wide variety of measures, including blood, urine, and saliva samples, physical measurements, and self-report questionnaires. In 2014, UK Biobank invited 100,000 of the original participants to participate in brain magnetic resonance imaging (MRI), including resting-state functional MRI, which can be used to investigate RSFC. At the time this analysis was conducted, scans from 10,472 individuals had been preprocessed using a pipeline developed by collaborators (see below).

MRI Data Acquisition and Preprocessing

Functional MRI acquisition. As part of the UK Biobank study, participants completed T1-weighted, T2-FLAIR, susceptibility-weighted (swMRI), and diffusion-weighted MRI (dMRI). A detailed summary of the UK Biobank brain imaging protocols are available elsewhere (K. L. Miller et al., 2016). Briefly, resting-state functional MRI data were collected at three dedicated imaging centers, each using a 3T Siemens Skyra (software platform VD13) with the standard Siemens 32-channel head coil. Acquisition parameters were as follows: 2.4-mm spatial resolution, 88 x 88 x 64 field-of-view matrix,

TR = 0.735 s, TE = 39 ms, flip angle = 52°, GE-EPI with 8x multiband acceleration, fat saturation. To facilitate motion correction and alignment, a single-band reference image with higher tissue-type image contrast was acquired. During the 6 min 10 s resting-state scan, participants were instructed to keep their eyes fixated on a crosshair, relax, and "think of nothing in particular" (K. L. Miller et al., 2016).

Preprocessing of structural and functional MRI data. Structural and functional MRI data were preprocessed through a collaboration with the Holmes Laboratory at Yale University. Structural MRI data from 10,472 UK Biobank participants were processed via a structural MRI pipeline using FreeSurfer version 6.0 (Fischl, 2012). Because evidence suggests that the use of face-stripped anatomicals may lead to shifts in FreeSurfer-based estimates of cortical anatomy (A. J. Holmes et al., 2015), the present pipeline applied a modified version of UK Biobank structural preprocessing (https://git.fmrib.ox.ac.uk/falmagro/UK_biobank_pipeline_v_1) to provide anatomical images without face blurring for segmentation in FreeSurfer. Anatomical data underwent gradient distortion correction, reduction of the anatomical image field of view, and joint linear and non-linear registration to a UK Biobank custom 1mm MNI152 "nonlinear 6th generation" group atlas. To reduce interpolation error and image distortions, linear and non-linear transforms to standard space were performed in a single combined transformation.

To extract RSFC data from cortical regions, surface-based preprocessing was conducted according to a previously published pipeline (https://github.com/ThomasYeoLab/CBIG) (Kong et al., 2019; Li et al., 2019). Echo-planar imaging (EPI) frames with frame-wise head-motion greater than 0.3mm or

DVARS greater than 75 were identified as outliers. These frames, along with one frame before and two frames after a detected movement, were considered censored data and later interpolated (see below). FreeSurfer's boundary-based registration software was used to align processed structural data with functional images, and a high-contrast EPI volume provided a functional reference. Additionally, BOLD run segments consisting of fewer than 5 contiguous frames were flagged for removal. Runs where more than 50% of frames were outliers were excluded from further analysis. Variance related to global signal, average white matter signal, average CSF signal, average ventricular signal, six head motion estimates (3 rotational, 3 translational), and all corresponding temporal derivatives were removed via linear regression. Censored frames were excluded from this nuisance regression step, and then interpolated using least-squares spectral estimation. The resulting data were bandpass filtered ($0.009\ Hz \leq f \geq 0.08\ Hz$) and linear trends were removed. Next, the preprocessed volumetric data were projected onto fsaverage6 surface space, smoothed with a 2mm full-width half-maximum kernel, and downsampled into fsaverage5 vertex space.

To generate estimates of resting-state functional activity from cortical data, the same procedure was conducted on non-bandpassed volumetric data. HCP Workbench (Marcus et al., 2011) was applied to generate parcellated surface-based estimates of resting-state functional activity (standard deviation of BOLD time course) and functional connectivity. Resting-state functional activity values were Z-transformed within individuals, and each participant's 200x200 matrix of RSFC estimates was Fisher's *Z* transformed.

To examine resting-state activity in nucleus accumbens, the preprocessing procedures described above were applied to resting-state functional activity estimates from nucleus accumbens. Volumetric delineations for bilateral nucleus accumbens were derived from FreeSurfer's subcortical segmentation procedure (https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/AnatomicalROI).

Additional fMRI data reduction. Individuals with missing thickness or resting-state estimates across all 200 cortical parcels, or with average cortical thickness or RSFA more than ±3 SD from the mean, were excluded from further analysis. Individuals with outlier (±3 SD) white matter lesion volume were censored from further analyses.

Additionally, participants were excluded for inverted T1-weighted signal-to-noise ratio > 3 SD above the mean (n = 131) or white matter lesion volumes (residualized for total gray and white matter volume) > 3 SD above the mean (*n* = 218). Of the remaining participants, individuals were excluded for mean run-wise frame-to-frame head motion during resting-state functional MRI > 3 SD above the mean (*n* = 51) and inverted resting-state signal-to-noise ratio > 3 SD above the mean. Participants were also excluded if their data could not be processed through FreeSurfer (*n* = 30).

Brain Parcellation and Regions of Interest

We adopted a cortical parcellation method that relies on a gradient-weighted Markov Random Field (gwMRF) model (Schaefer et al., 2017). This particular model integrates a global similarity approach (which groups brain locations according to similarity in functional MRI time course) with a local gradient approach (which detects RSFC patterns that change abruptly from one location to a nearby location). The model

also includes a spatial distribution term that encourages locations within a parcel to remain close to the parcel center (Schaefer et al., 2017).

In the present study, to minimize signal dropout and increase signal-to-noise ratio, we examined functional activity in NAc (part of VS) using an anatomical mask rather than using a functional parcellation of striatum. We identified mPFC regions of interest based on a) guidance from prior work and b) statistical strength of correlations with NAc within the present dataset. Specifically, prior studies have indicated mPFC regions with particularly strong correlations to VS in terms of both functional connectivity (Choi, Yeo, & Buckner, 2012) and gene co-expression (Anderson et al., 2018).

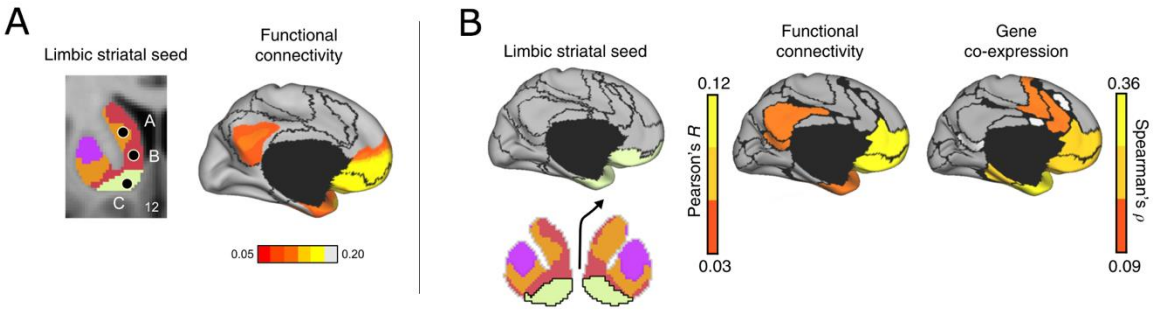


Figure 1. VS functional connectivity and gene co-expression in mPFC. (A) Analyses from Choi et al. (2012) revealed functional connectivity with VS in a region of mPFC that extends to perigenual cortex (indicated by an arrow). Figure part A adapted from Choi et al. (2012). (B) Anderson et al. (2018) found overlap between VS functional connectivity and gene co-expression in perigenual mPFC (indicated by arrows), among other areas. Figure part B adapted from Anderson et al. (2018).

Additionally, in the present dataset, we identified 13 mPFC parcels whose functional connectivity estimates with NAc were greater than 2 SD above the mean for all cortical parcels (Table 1). Estimates of functional connectivity with mPFC were similar between left and right NAc (Figure 2). Thus, we used the bilateral average of NAc functional activity to derive mPFC-NAc RSFC estimates, with the goal of increasing NAc signal reliability.

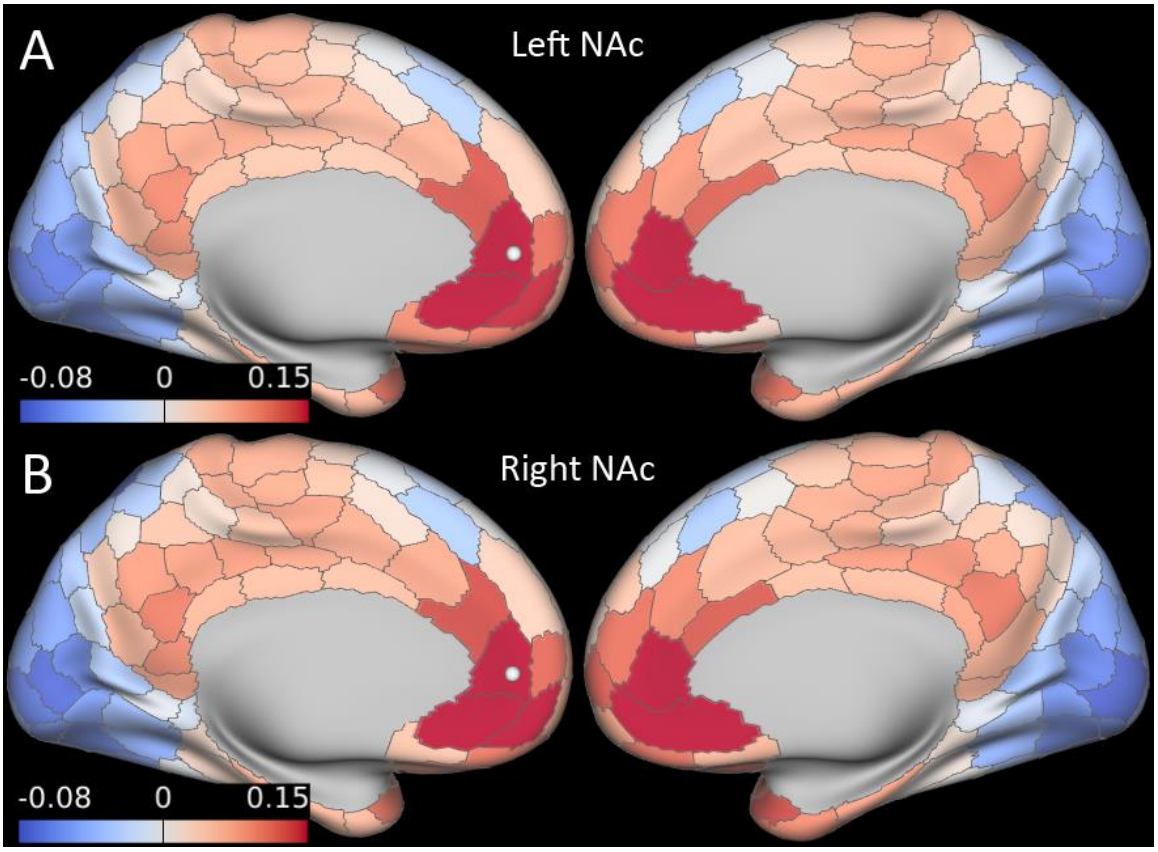


Figure 2. Functional correlations with medial cortical parcels are similar between left (A) and right (B) nucleus accumbens.

Self-Report Questionnaire

During the imaging visit, participants completed a battery of self-report assessments touching on a wide range of topics, including diet, physical activity, sociodemographics, and mental health. The full questionnaire is accessible on the UK Biobank website (https://www.ukbiobank.ac.uk/). To assess life stress, we examined participants' self-report regarding whether they had experienced any of the following in the past 2 years: a) "Serious illness, injury or assault to yourself"; b) "Serious illness, injury or assault of a close relative"; c) "Death of a close relative"; d) "Death of a spouse or partner"; e) "Marital separation/divorce"; f) "Financial difficulties"; g) "None of the above". Participants were allowed to choose more than one answer for this item.

Participants who responded “Prefer not to answer” (*n* = 16) were excluded from analyses. As a measure of anhedonia, we investigated participant responses to the following question: “Over the past two weeks, how often have you had little interest or pleasure in doing things?” Responses were scored as follows: “Not at all” = 0; “Several days” = 1; “More than half the days” = 2; “Nearly every day” = 3. Participants were allowed to select only one option for the anhedonia item (UK Biobank, 2018). Individuals who selected “Do not know” (*n* = 169) or “Prefer not to answer” (*n* = 16) for the anhedonia item were excluded from analyses. Additionally, participants with missing data on household income (*n* = 12), which was used as a control variable (see *Statistical Procedures*), were excluded from analysis.

Discovery and Replication Samples

To safeguard against the over-interpretation of spurious effects, we split the UK Biobank imaging dataset into separate discovery and replication samples. To avoid demographically-skewed samples that might result from simple random sampling, we applied spatially balanced sampling techniques (M. M. Dickson, Benedetti, Giuliani, & Espa, 2014) using the *BalancedSampling* package for R (Grafström & Lisic, 2020). Subsamples were balanced according to the following dimensions: sex; handedness; age; household income; head motion; number of self-reported episodes of depressed mood and number of self-reported episodes of disinterest/lack of pleasure (to control for chronicity of mood and anhedonic symptoms); and fluid intelligence (to control for cognitive function; see Bakrania et al., 2018 for more details on cognitive assessment). The discovery sample consisted of 60% of the overall sample after data cleaning (*n* = 6,124), and the replication sample comprised the remaining data (*n* = 4,097).

Statistical Procedures

All analyses controlled for the following variables, included as covariates: sex; age; household income; number of self-reported episodes of depressed mood; number of self-reported episodes of disinterest/lack of pleasure; and fluid intelligence (as a measure of cognitive function). Additionally, all analyses involving imaging data controlled for handedness and head motion.

We first investigated whether frontostriatal RSFC (i.e., between mPFC and VS) was statistically related to anhedonia score in any of the mPFC parcels of interest. Because anhedonia scores were strongly skewed in favor of lower anhedonia severity (see *Descriptive Statistics*), and thus violated assumptions of normality, we adopted a bootstrapped linear regression approach using the *boot* package for R (Canty & Ripley, 2020). A series of bootstrapped regressions was conducted, each one using frontostriatal RSFC *Z*-score from a different mPFC parcel of interest as the predictor and anhedonia score as the criterion, yielding a total of 13 bootstrapped regressions (one for each mPFC parcel of interest). For each regression, coefficients were obtained within each of 10,000 bootstrapped samples, and BC_a bootstrap confidence intervals (DiCiccio & Efron, 1996) were constructed for each coefficient. RSFC was considered to have a significant effect on anhedonia score for a parcel in mPFC if the bootstrap confidence interval for its regression coefficient did not contain zero.

Next, we characterized the relation between stress and anhedonia. We conducted a bootstrapped linear regression using dummy coded variables for each type of stressor (0 = absent, 1 = present) as the predictors and anhedonia score as the criterion. Additionally, in separate analyses, we examined whether the number of stressors endorsed by a

participant predicted anhedonia score. The procedure described above was used to generate bootstrapped samples and confidence intervals, as well as to evaluate statistical significance.

In addition, we applied bootstrapped mediation techniques (Preacher & Hayes, 2004) using the *mediation* package for R (Tingley, Yamamoto, Hirose, Keele, & Imai, 2014) to examine whether frontostriatal connectivity could plausibly mediate the relation between stress and anhedonia score. We constructed mediation models as follows: a) the predictor in each model was a stressor that significantly predicted anhedonia score; b) the putative mediator in each model was the frontostriatal connectivity *Z* score from an mPFC parcel whose connectivity score significantly predicted anhedonia score; c) the outcome variable was anhedonia score. Models were estimated for all combinations of significantly-predictive stressors and significantly-predictive frontostriatal connectivity estimates. In separate models, we also evaluated whether frontostriatal connectivity mediated the relation between number of stressors endorsed and anhedonia score. Mediation by frontostriatal connectivity was considered statistically significant if the 95% bootstrap confidence interval for the proportion mediated, or $c - c'$ (Preacher & Hayes, 2004), did not contain zero.

Finally, on an exploratory basis, we constructed moderated mediation models. We investigated whether frontostriatal connectivity might mediate the stress-anhedonia relation for subgroups of participants based on age or sex assigned at birth. To do so, we split the sample and applied mediation analyses to each subsample. For sex assigned at birth, we split the sample into female vs. male assigned at birth, and fit mediation models to each of those subsamples. For age, we used a median split to create two subsamples

and fit mediation models to each. Again, we assessed whether the 95% bootstrap confidence interval of $c - c'$ contained zero for each subsample.

Once all analyses were finalized in the discovery sample, we conducted the same statistical procedure in the replication sample to assess reliability of results.

Results

Descriptive Statistics

Tables 1, 2, and 3 present descriptive statistics for the overall sample (discovery and replication samples together). Table 1 includes the number of individuals who endorsed each response on the anhedonia item, as well as the number of individuals who endorsed each type of stressor over the 2 years prior to the imaging visit. Table 2 provides descriptive statistics for continuous demographic variables. Descriptive statistics for categorical demographic variables are listed in Table 3.

Participants endorsed 0.48 stressors per person on average (SD = 0.69).

Table 1. Descriptive statistics for self-reported stressors and anhedonia

Self-Report Item	n Participants Endorsing Item	Percentage of Overall Sample
Stressors endorsed (past 2 years)		
Serious illness, injury or assault to yourself	603	5.9%
Serious illness, injury or assault of a close relative	1,291	12.6%
Death of a close relative	2,132	20.9%
Death of a spouse or partner	134	1.3%
Marital separation/divorce	171	1.7%
Financial difficulties	579	5.6%
None of the above	6,302	61.7%
Anhedonia (past 2 weeks)		
Not at all	8,602	84.2%
Several days	1,355	13.3%
More than half the days	163	1.6%
Nearly every day	97	0.9%

Note. Participants were allowed to endorse as many stressors as applied to them.

Table 2. Descriptive statistics for continuous demographic variables

Self-Report Item	*M*	*SD*
Age at imaging visit	62.66	7.39
Lifetime number of depressive episodes	1.60	13.74
Lifetime number of anhedonic episodes	1.06	6.37
Fluid intelligence score	6.08	2.95

Table 3. Descriptive statistics for categorical demographic variables

Self-Report Item	*n* Participants Endorsing Item	Percentage of Overall Sample
Sex assigned at birth		
Female	5,384	52.7%
Male	4,833	47.3%
Handedness		
Right-handed	9,099	89.1%
Left-handed	968	9.5%
Use both right and left hands equally	148	1.5%
Annual household income		
Less than £18,000	1,177	11.5%
£18,000 to £30,999	2,634	25.8%
£31,000 to £51,999	2,861	28.0%
£52,000 to £100,000	2,068	20.2%
Greater than £100,000	540	5.3%
Prefer not to answer	637	6.2%
Do not know	300	2.9%

Relation between Frontostriatal Connectivity and Anhedonia

Discovery sample. The mPFC-NAc connectivity *Z*-scores of two mPFC parcels significantly predicted anhedonia score: LH_Limbic_OFC_3 (*B* = -.05; SE = .02; 95% CI: [-.10, -.01]) and LH_Default_PFC_4 (*B* = -.06; SE = .03; 95% CI: [-.11, -.01]). Contrary to our hypotheses, the estimated slopes for the impact of frontostriatal connectivity on anhedonia score were both negative, such that more negative frontostriatal connectivity scores were weakly predictive of higher anhedonia scores.

Replication sample. Frontostriatal connectivity did not significantly predict anhedonia score for any of the identified mPFC parcels. This result suggests that, given the present data and analytic strategy, frontostriatal functional connectivity did not reliably predict anhedonic symptoms.

Associations between Self-Reported Stress and Anhedonia

Discovery sample. Four stressors emerged as significant predictors of anhedonia score: a) "Serious illness, injury or assault to yourself" (*B* = .07; SE = .04; 95% CI: [.01, .15]); "Serious illness, injury or assault of a close relative" (*B* = .06; SE = .02; 95% CI: [.02, .11]); and "Financial difficulties" (*B* = .22; SE = .04; 95% CI: [.14, .31]). Additionally, number of stressors endorsed significantly predicted anhedonia score (*B* = .06; SE = .01; 95% CI: [.06, .08]).

Replication sample. Only one individual stressor emerged in the replication sample as a significant predictor of anhedonia score: "Financial difficulties" (*B* = .25; SE = .05; 95% CI: [.16, .35]). This outcome suggests that, of stressors endorsed over a 2-year period prior to assessment, only financial difficulties reliably predicted anhedonia scores. However, as in the discovery sample, number of stressors significantly predicted anhedonia score (*B* = .06; SE = .01; 95% CI: [.04, .09]).

Frontostriatal Connectivity as a Putative Mediator of the Stress-Anhedonia Association

Discovery sample. We examined frontostriatal connectivity in the two mPFC parcels that exhibited a significant association with anhedonia score in the discovery sample. In the model testing functional connectivity between the parcel LH_Limbic_OFC_3 and NAc as a mediator of the relation between financial difficulties

and anhedonia score, frontostriatal connectivity emerged as a significant mediator ($c - c'$ = -.01, 95% CI: [-.03, -9.63]). Contrary to our predictions, the estimated value of $c - c'$ was negative, such that the relation between stress and anhedonia was stronger when accounting for frontostriatal connectivity. This result is inconsistent with the mediation hypothesis, which would predict that the relation between stress and anhedonia weakens when frontostriatal connectivity is taken into account. Results for the other medial prefrontal parcel of interest (LH_Default_PFC_4) revealed no significant mediation (95% confidence interval for $c - c'$ contained zero). When examining frontostriatal connectivity as a mediator of the relation between number of stressors endorsed and anhedonia, no evidence for mediation emerged (95% confidence interval for $c - c'$ contained zero).

Replication sample. Because frontostriatal connectivity did not significantly predict anhedonia score for any mPFC parcels in the replication sample, no mediation models were tested in the replication sample. Thus, the results of the significant mediation model from the discovery sample cannot be confirmed, and cannot be considered reliable. Additionally, because frontostriatal connectivity did not significantly mediate the relation between number of stressors endorsed and anhedonia in the discovery sample, we did not evaluate this model in the replication sample.

Moderated Mediation

Discovery sample. We tested moderated mediation models for each parcel that was significantly associated with anhedonia scores (see *Frontostriatal connectivity Predicting Anhedonia Score*, above). For models testing whether frontostriatal connectivity mediated the stress-anhedonia relation in participants in the younger half of the sample (ages 45-63) and the older half of the sample (ages 64-80) separately, all 95%

confidence intervals for *c – c'* contained zero, providing no evidence of mediation. The same pattern emerged for testing mediation in assigned female at birth and assigned male at birth participants separately, such that 95% confidence intervals for *c – c'* all contained zero. Thus, we uncovered no evidence that frontostriatal connectivity mediates the stress-anhedonia relation for subgroups of individuals.

Discussion

This study provided no evidence to support the hypothesis that stress impacts anhedonic symptoms via changes in frontostriatal functioning. In our sample, one stressor type ("financial difficulties") was related to anhedonic symptoms, consistent with the literature on stress-induced anhedonia. However, frontostriatal RSFC was not consistently associated with anhedonic symptoms in any of the medial prefrontal parcels assessed. Furthermore, our analysis does not support frontostriatal RSFC as a statistical mediator of the stress-anhedonia association, either for the overall sample or for subgroups of individuals (i.e., younger vs. older individuals, assigned female vs. male at birth).

Several explanations could plausibly account for these null results. First, frontostriatal function may not act as a mechanism of stress-induced anhedonia. Although stress leads to structural changes in mPFC (McEwen & Morrison, 2013), these alterations may not contribute to anhedonia, or may influence anhedonic symptoms through other mechanisms besides altered frontostriatal functioning.

Additionally, data for the present study were collected as part of a larger investigation (the UK Biobank study) that was not specifically designed to assess the impact of stress and brain function on anhedonic symptoms. As a result, stress exposure

and anhedonic symptoms were minimally assessed, and may not provide the reliability and/or validity of standardized stress measures. For instance, some researchers have argued that the Life Events and Difficulties Schedule (LEDS; Bifulco et al., 1989) should be the gold standard for measuring the impact of stress exposure on psychopathology (Harkness & Monroe, 2016), due to its assessment of a large number of stressors and examination of key stress characteristics, such as timing, chronicity, and severity. This study also did not include an anhedonia measure with established reliability and/or validity, such as the Mood and Anxiety Symptoms Questionnaire (MASQ) Anhedonic Depression subscale (Watson, Weber, et al., 1995). Thus, the measures of stress and anhedonia included in this study may be insufficient to address the study's key questions. Notably, the UK Biobank dataset provides a freely-available, large sample with diverse measures, which rendered the present study feasible. At the same time, the brevity of the symptom inventories included (which facilitated their administration to thousands of participants) represented a significant methodological barrier to the present investigation of stress and anhedonia.

Moreover, the stress questionnaire used in the present study inquired about 6 stressful events over a 2-year period prior to assessment. Inquiring about a 2-year period may provide insufficient temporal resolution to identify stressors of maximal etiological importance to anhedonia. Prior work in individuals with MDD suggests that events within 6 months prior to MDD onset are of greatest etiological relevance to MDD (Harkness et al., 2010; Kendler et al., 1998). Furthermore, a more comprehensive inquiry about stress exposure might take into account stressors that are neglected in the present assessment. For example, researchers have suggested that targeted rejection events

particularly contribute to depressive symptoms (Slavich, Thornton, Torres, Monroe, & Gotlib, 2009), and these may be incompletely assessed using the 6 questionnaire items from the UK Biobank dataset.

Analyses suggested that self-reported financial stress over the past 2 years reliably predicted self-reported anhedonia severity, even after controlling for a number of demographic variables, depression/anhedonia chronicity, and several other stressors. Additionally, number of stressors endorsed also predicted anhedonia. These findings are consistent with other work that indicates a link between stress and anhedonia in humans (e.g., Keller et al., 2007). Although financial stress emerged as a predictor of anhedonia above and beyond the other stressors assessed, we caution against placing particular emphasis on financial stress as a contributor to anhedonia, for several reasons. First, only a small number of stressors were assessed, leaving open the possibility that other stressors could have stronger links with anhedonia. Additionally, as noted above, the 2-year time window for stressors assessed was quite broad, and as a result our analysis may not have detected the effects of stressors that exert greater influence over a more constrained time window. Finally, the present data did not allow for us to examine the impact of key dimensions of stress (e.g., chronicity, severity) which may better account for any pattern in stressors that contribute strongly to anhedonic symptoms.

Overall, this study did not uncover evidence that frontostriatal function mediates the effect of stress on anhedonia. At the same time, the null results of the present study cannot not rule out this hypothesis. Future work could better address this problem through the use of large datasets that include more detailed stress and symptom inventories, while also incorporating measures of putative biological mediators, such as

functional connectivity. Despite the practical challenges involved in collecting such a dataset, these efforts could be essential to understanding the impact of stress on psychiatric symptoms and accompanying biological mechanisms.

Chapter 5: Inflammation as a Potential Mediator of Stress-Induced Changes in Motivated Behavior

Abstract

Prior work suggests that stress exposure is linked with anhedonia, the loss of pleasure and/or motivation, a debilitating psychiatric condition (Pizzagalli, 2014; Russo & Nestler, 2013). Emerging research suggests that immune responses to stress may mediate this stress-anhedonia link through the regulation of inflammatory cytokine activity (Felger & Treadway, 2017). However, human subjects work has produced mixed results (Boyle et al., 2020; Treadway et al., 2017) that require clarification. The present study applied a well-validated psychosocial stressor (Kirschbaum, Pirke, & Hellhammer, 1993a) or corresponding control task, and investigated potential changes in inflammatory cytokine concentration and reward- and loss-based learning. Relative to the control group, the stressor produced a significant increase in negative affect (ATS = 15.18, df = 2.61, $p < .001$), suggesting a successful stress manipulation. Nevertheless, unexpectedly, no differences emerged in salivary concentration of pro-inflammatory cytokines. These results are inconsistent with the findings of meta-analyses of studies using human blood serum (Marsland, Walsh, Lockwood, & John-Henderson, 2017) and saliva (Szabo, Slavish, & Graham-Engeland, 2020). Low concentrations of some pro-inflammatory cytokines (< 1 pg/mL for IL-6, e.g.) suggest problems with sample storage, which may account for these null results. Additionally, no significant effects of stress on reward- or loss-based learning emerged, consistent with some prior work that found no effect of stress on behavior using similar tasks (Treadway et al., 2017). Altogether, this study provided no evidence for mediation of the stress-anhedonia link by inflammatory processes in humans, possibly due to methodological constraints or the absence of a true effect.

Introduction

Several debilitating psychiatric disorders, including major depressive disorder (MDD), schizophrenia, and post-traumatic stress disorder (PTSD), are linked to stress and characterized by anhedonia, defined as the loss of pleasure and/or motivation to engage in valued activities (Barch & Dowd, 2010; Nawijn et al., 2015; Pizzagalli, 2014). These disorders cause a significant personal, societal, and economic burden worldwide (Whiteford et al., 2013). However, the biological pathways linking stress and anhedonia remain poorly understood. The present study aims to address this critical issue by examining inflammatory processes as a potential biological mediator of the stress-anhedonia link.

Considerable work has focused on inflammatory responses to stress and subsequent effects on motivated behavior—an important component of anhedonia (Treadway & Zald, 2011)—and the impact of stress on relevant neural circuitry (Felger & Treadway, 2017). Preclinical models suggest that pro-inflammatory cytokines, such as interleukin-6 (IL-6), may contribute to stress-induced anhedonia (Hodes et al., 2014; Menard et al., 2017; J. Wang et al., 2018). For instance, social defeat stress in rodents appears to weaken the blood-brain barrier by reducing levels of Cldn5, a cell adhesion molecule, allowing IL-6 to infiltrate nucleus accumbens (NAc) and producing diminished social interaction (Menard et al., 2017). Stress-induced IL-6 infiltration, in conjunction with synaptic remodeling in NAc (J. Wang et al., 2018), appears to contribute to the development of anhedonic-like behavior (Hodes et al., 2014; Menard et al., 2017; J. Wang et al., 2018).

In humans, a study of healthy female participants examined the effect of stress-induced inflammation on reward prediction errors (RPEs) in ventral striatum (VS; includes NAc) using fMRI (Treadway et al., 2017). Stress-induced increases in IL-6 were associated with diminished RPE-linked responses in VS following stress (Treadway et al., 2017). Additionally, a recent study of healthy young women found that stress-induced increases in IL-6 mediated the impact of stress on changes in reward responsiveness (Boyle et al., 2020). However, contrary to the researchers' expectations, results indicated that stress-induced IL-6 was associated with *increased* responsiveness (Boyle et al., 2020). Given the importance of evaluating stress-induced inflammation as a biological mechanism of stress-induced anhedonia, these surprising results call for verification in an independent sample.

In summary, while work in rodents has established a plausible biological pathway through which stress could produce anhedonic behavior through inflammatory activity (Hodes et al., 2014; Menard et al., 2017; J. Wang et al., 2018), human work has been mixed, with at least one study finding an increase in behavioral reward sensitivity (Boyle et al., 2020), whereas another study found changes in NAc activity but no behavioral differences (Treadway et al., 2017). These conflicting behavioral results following stress-induced inflammation are consistent with mixed results using other inflammatory inductions, such as vaccination, which has produced reports of blunted (Harrison et al., 2016) or enhanced (Boyle et al., 2019) reward learning.

The present study aims to clarify these inconsistent results and evaluate the impact of stress-induced inflammation on motivated behavior. This study is similar to prior work that adopted a group-based design (Boyle et al., 2020) with both a stress and

control group, to mitigate the possible impact of task learning effects when examining behavior pre- and post-stressor (Treadway et al., 2017). However, we extend prior work by evaluating both reward-based and loss-based learning (Pessiglione, Seymour, Flandin, Dolan, & Frith, 2006) within a group design, following evidence that inflammation may differentially impact learning from rewards versus losses (Harrison et al., 2016). Notably, although reward learning is thought to be especially relevant to anhedonia, motivated decision-making is a complex and multifaceted process, and behavioral changes that culminate in anhedonia could result from disruptions in several different subprocesses (Zald & Treadway, 2017), including loss-based learning.

We predict that the stress manipulation will increase the salivary concentration of pro-inflammatory cytokines. Additionally, we expect that because the stressor increases inflammation, it will decrease reward sensitivity across behavioral tasks, but enhance sensitivity to losses, consistent with prior work (see Harrison et al., 2016). Finally, we hypothesize that stress-induced changes in pro-inflammatory activity will mediate the relation between stress and reward/loss learning. Because of evidence that an individual's history of life stress may influence their inflammatory responding to novel stressors (Fagundes & Way, 2014; Pace et al., 2006), we will also investigate whether these effects are moderated by life stressor history.

In addition to our main aims, this study provides an opportunity to explore the impact of stress-induced anti-inflammatory processes on motivated behavior. Some pre-clinical evidence suggests that anti-inflammatory activity is decreased relative to chronic stress in rodents (Rossetti et al., 2016), and clinical trials have targeted anti-inflammatory pathways in treatments for depression (Köhler et al., 2014; Lee et al., 2020). However,

we are aware of no studies that examine anti-inflammatory responses following stress and their association with motivated behavior. Therefore, we will perform exploratory analyses to a) characterize the anti-inflammatory response to an acute laboratory stressor; b) examine associations between anti-inflammatory responses and motivated behavior; and c) investigate anti-inflammatory responses as a possible mediator of the impact of stress on motivated behavior.

Methods

Participants

The Yale University Institutional Review Board approved all procedures, and participants received monetary compensation for their time. A total of 88 adult participants were enrolled in the study after screening procedures, and 86 completed the experiment. Two participants declined to continue their participation in the study after learning about the stressor task. Participants were recruited via flyering and Craigslist advertisements. Participant ages ranged from 18 to 56 (M = 27.37, SD = 9.30 years), and 49.2% of participants identified as white, 23.8% as Black or African American, 17.5% as Asian or Asian American, 3.2% as Middle Eastern or Arab, and 6.3% as mixed race or other. Additionally, 12.3% of participants identified as Hispanic or Latinx.

Given sex differences in inflammatory and hormonal responses to stress (Kirschbaum, Wüst, & Hellhammer, 1992; O'Connor et al., 2009; Welsh, Woodward, Rumley, & Lowe, 2008), and consistent with prior work (Treadway et al., 2017), the participant sample was restricted to individuals assigned female at birth. Menstrual cycle stage impacts inflammatory cytokine concentrations (O'Connor et al., 2009), and

restricting the sample by birth sex enables statistical control for this important contributor to inflammatory processes.

Additionally, due to potential influence on inflammatory markers (O'Connor et al., 2009), participants were excluded for nicotine use in the past month; use of selective serotonin reuptake inhibitors (SSRIs), statins, or antihypertensive medications in the past 6 months; or self-reported inflammatory disease (e.g., rheumatoid arthritis, diabetes mellitus). Because inflammatory markers were assessed in saliva, individuals with self-reported periodontal health problems, such as gingivitis (see Slavish, Graham-Engeland, Smyth, & Engeland, 2015), were also excluded from the study.

Stress and Control Tasks

Modified Trier Social Stress Test (TSST). Participants who were randomized to the stress condition underwent a modified version of the Trier Social Stress Test (TSST), a well-validated laboratory stress induction designed to induce social-evaluative threat (Kirschbaum, Pirke, & Hellhammer, 1993b). Prior work suggests that the TSST reliably elicits subjective and physiological stress responses (Dickerson & Kemeny, 2004; Kirschbaum et al., 1993b), including inflammatory responses (Izawa et al., 2013; Slavich, Way, Eisenberger, & Taylor, 2010). Like in other variations of the TSST (Yoon & Joormann, 2012), the stress induction involved one speech and one arithmetic task. The stressor tasks were performed in front of an experimenter wearing a lab coat and carrying a clipboard, who maintained a cold, flat demeanor throughout the stressor. For consistency, the same male experimenter conducted the TSST or control tasks for all participants. In the stressor tasks, participants were told that both tasks assessed aspects of intelligence, and that a panel of their peers would rate their videotaped performance.

Participants were asked to spend 3 min preparing a speech on why they were "an ideal job candidate," and then give a 5-min speech while standing in front of the experimenter and a video camera. Unbeknownst to participants, they were not actually recorded. Next, participants were asked to count backwards from 2083 to zero in increments of 17, out loud. Each time a mistake was made, the experimenter responded "incorrect," and instructed the participant to restart from the beginning. Participants engaged in this arithmetic task for 5 min.

Non-evaluative control tasks. By contrast, participants in the control condition engaged in tasks designed to approximate the physical demands of the TSST, but without the social evaluative threat (Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009). Participants spent 3 min thinking about a movie, novel, or recent trip that was relatively neutral in terms of affective content, and then stood up and talk about the chosen topic for 5 min while alone in the room. Next, participants spent 5 min counting up from zero in increments of 15. Participants were explicitly told they were not being recorded and that no one would be able to hear them complete the tasks.

Self-Report Measures

Positive and Negative Affect Scales – Short Form (PANAS-SF). The PANAS-SF (Watson, Clark, & Tellegen, 1988) comprises two widely-used 10-item mood scales which are internally consistent and stable over a 2-month period. The two subscales measure positive affect (e.g., "enthusiastic," "proud," "determined") and negative affect (e.g., "distressed," "upset," "afraid," "angry"), respectively (Watson et al., 1988).

Stress and Adversity Inventory (STRAIN). The STRAIN (Slavich & Epel, 2010; Slavich & Shields, 2018) assesses lifetime exposure to acute and chronic stress.

The adult version of the STRAIN inquires about 55 different types of stressors that cover all major life domains (e.g., health, intimate relationships, friendships, education, etc.) and several social-psychological characteristics (e.g., interpersonal loss, physical danger, role change, etc.). Users are presented with one question at a time. For each stressor endorsed, users are asked a series of follow-up questions to assess the severity, frequency, timing, and duration of the stressor. In prior work, the STRAIN has demonstrated convergent validity with other inventories of stress exposure and excellent reliability (Slavich & Shields, 2018).

Biological Measures

Cytokine assays. Inflammatory markers were measured using saliva samples. Following collection, samples were stored in a -80ºC freezer until they could be sent for multiplex assaying (Milliplex high-sensitivity immuno-assays; MilliporeSigma). The immune multiplex assessed 11 cytokines: interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Cytokines with > 20% of data outside the lower and upper thresholds of detection were excluded from further analysis, leaving six cytokines: IL-1β, IL-4, IL-6, IL-8, IL-10, and TNF-α. Among these cytokines, IL-1β, IL-6, IL-8, and TNF-α are considered broadly pro-inflammatory, while IL-4 and IL-10 are categorized as anti-inflammatory (Curfs, Meis, & Hoogkamp-Korstanje, 1997), although the role played by any given cytokine may vary depending on context (Cavaillon, 2001). Intraclass correlations for these candidate cytokines ranged from .81 for IL-1β to .95 for IL-10, suggestive of “good” (.75 to .90) to “excellent” (> .90) reliability. Once the

candidate cytokines were selected, data were cleaned as follows. Subjects with no sample values within the limits of detection for a particular cytokine were excluded from analysis for that cytokine. As a result, cytokine data was excluded for certain participants: For analyses of IL-1β, two participants' data was excluded; IL-6, nine participants; TNF-α, seven participants; IL-10, one participant; and IL-4, three participants. For the remaining data, samples above the upper threshold of detection were treated as missing data, and samples below the threshold of detection were set to half the lower threshold, consistent with other longitudinal analyses of inflammatory cytokines (Allswede, Yolken, Buka, & Cannon, 2020).

Reward Tasks

Pessiglione reward task. A reinforcement learning task designed by Pessiglione and colleagues (the "Pessiglione task," for ease of reference; Pessiglione et al., 2006) has previously been used to examine responses to vaccination-induced inflammation (Harrison et al., 2016) and psychological stress (Treadway et al., 2017). The task consists of three blocks of 72 trials each, with each block using three new pairs of abstract symbols (Figure 1).

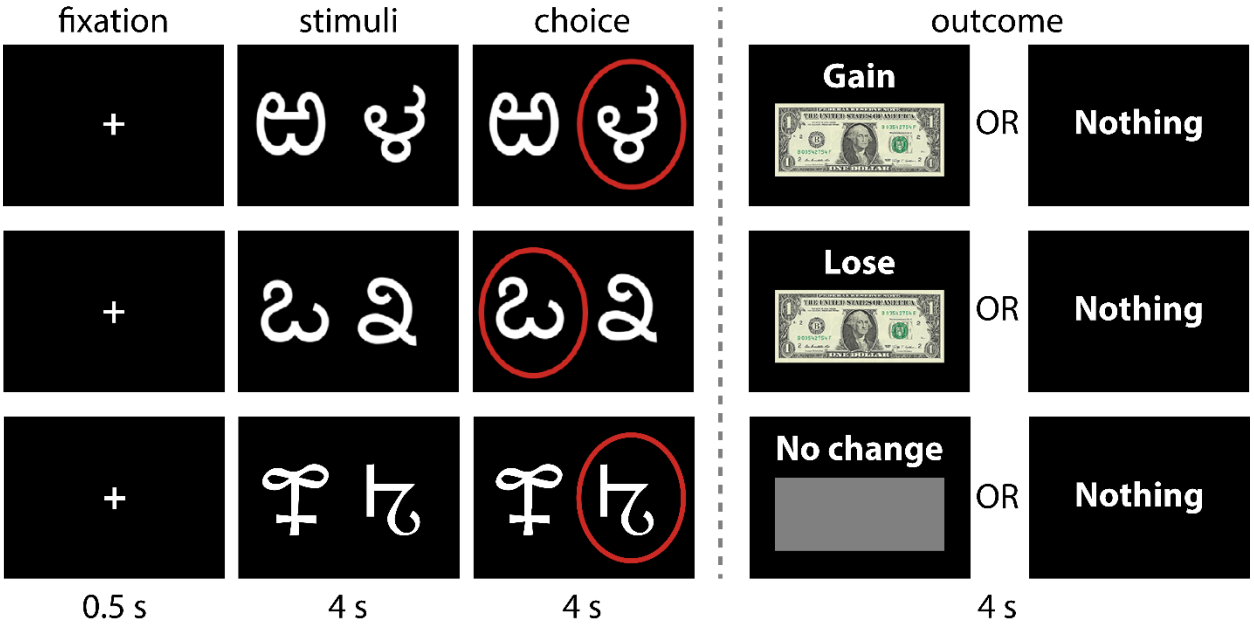


Figure 1. Pessiglione task. Figure adapted from Harrison et al. (2016).

Within each block, each pair of symbols is associated with a different set of outcomes: in the "gain" condition, either $1 or $0; in the "loss" condition, either -$1 or 0; in the "no change" condition, either look at gray square or nothing. In the gain condition, one stimulus is associated with a .8 probability of reward and a .2 probability of nothing (.8/.2), and the other stimulus has a reversed distribution (.2/.8). The same pattern holds for the "loss" and "no change" conditions (i.e., one .8/.2 stimulus and one .2/.8 stimulus). On each trial, one pair of stimuli is presented, with one stimulus on the left and the other on the right. Left/right position is randomized for each trial. Participants have 4 s to respond, after which the choice is circled in red for 4 s and the outcome is presented for 4 s ("gain," "loss," "nothing," or "gray square"). In keeping with prior work (Harrison et al., 2016), we examined participant choices by condition (potential reward vs. potential loss) in the last 50% of trials in each block. Percentage of high-reward-probability choices (the "correct" choice in the reward condition) provides a measure of reward

sensitivity. Conversely, percentage of low-loss-probability choices (the "correct" choice in the loss condition) provides a measure of punishment sensitivity.

Probabilistic Reward Task (PRT). In the PRT (Pizzagalli et al., 2008; Pizzagalli, Jahn, & O'Shea, 2005), participants view a mouthless cartoon face on the screen (Figure 2). After a delay of 500 ms, the "mouth" (a straight line) appears for 100 ms. Participants are required to guess, via key press, whether they have seen the "short" (11.5 mm) or "long" (13 mm) version of the mouth. The mouths are presented equally often, in pseudorandom order. No more than three instances of each mouth are presented consecutively. The present version of this task (Whitton et al., 2015) consists of two blocks of 100 trials each. On 40 of 100 trials per block, participants receive reward feedback for 1750 ms following correct responses. Participants earn 20 cents for each reward.

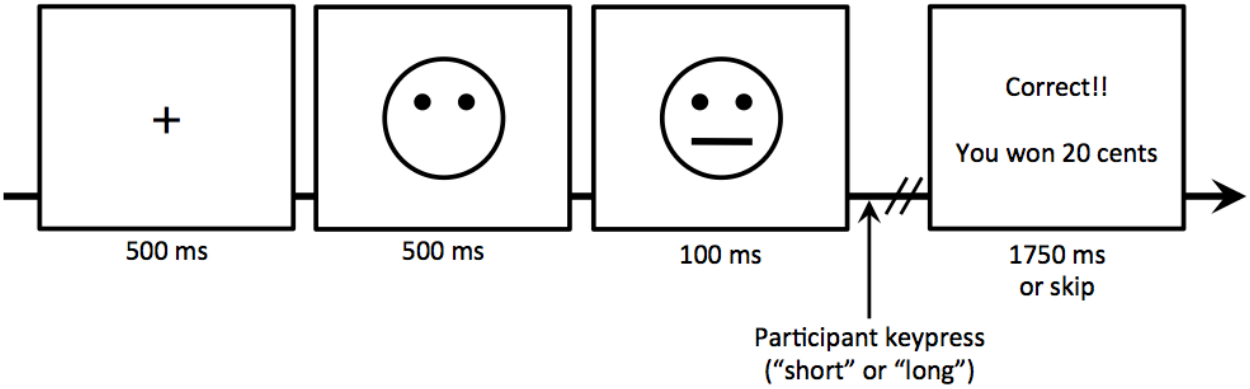


Figure 2. Signal detection task. The two keys that indicate a guess of "short" or "long" are counterbalanced, as is which mouth constitutes the "rich" vs. "lean" stimulus. Figure adapted from Pizzagalli et al. (2005).

Rewards are delivered in an asymmetrical ratio: One mouth type (the "rich" stimulus) is associated with three times more reward than the other (the "lean" stimulus). This task is designed to elicit a response bias that increases from the first to the second block. Here,

response bias (log *d*) is intended to measure the tendency to choose the disproportionately rewarded stimulus, quantified by the equation:

$$logd = \frac{1}{2} log \left(\frac{Rich_{correct} \times Lean_{incorrect}}{Rich_{incorrect} \times Lean_{correct}} \right)$$

where $Rich_{correct}$ represents the number of correct responses to the rich stimulus, and $Lean_{incorrect}$ represents the number of incorrect responses to the lean stimulus, etc. Thus, response bias provides a measure of the tendency to shift responses to favor frequently rewarded outcomes.

Procedure

Participants were asked to refrain from eating or drinking besides water for an hour before the study. Consistent with prior work (Treadway et al., 2017), participant sessions were conducted between 11am-4pm, to control for diurnal variation in inflammatory markers and cortisol. Following informed consent procedures, participants completed baseline questionnaires, including affect ratings using the PANAS-SF. Next, participants provided the first set of saliva samples. Participants were asked to passively drool through a straw into a vial. At each saliva assessment, participants filled a 1-mL vial, which were respectively used to assess cortisol (not examined here) and cytokine concentration.

Participants were then randomly assigned to undergo stress (TSST) or control tasks. To reduce the possibility that stress/control condition could influence baseline measures, research staff were blinded to stress/control condition until directly before the stress/control tasks were conducted. Additionally, participants completed the stress/control tasks in a different room that was only used for those tasks.

Following the stress/control tasks, participants completed additional questionnaires, including the STRAIN. Once 40 minutes had elapsed following the end of the stress/control tasks, participants completed their second saliva sample and set of affect ratings. Participants then completed both reward tasks (the Pessiglione task and the PRT). Finally, participants completed their third saliva sample and set of affect ratings, after which they were fully debriefed and paid.

Statistical Analysis

Statistical analysis and data visualization were performed using R 3.6.3 (R Core Team, 2020) supplemented by the *tidyverse* package ecosystem (Wickham et al., 2019). In cases where data violated the assumptions of normality and homoscedasticity, we adopted a nonparametric approach. For categorical variables in a repeated-measures analysis, we applied the ANOVA-type statistic (ATS; Brunner, Dette, & Munk, 1997), a rank-based approach that is robust to violations of normality and homoscedasticity. Unlike other widely used rank-based statistics (e.g., Kruskal, 1952), the ATS accommodates interaction terms for repeated-measures designs. The ATS is implemented in the R package *GFD* (Friedrich, Konietschke, & Pauly, 2017).

However, in some cases, practical limitations prevented the application of the ATS for non-normally distributed data. In particular, investigations of inflammatory data typically account for covariates such as analysis plate, menstrual stage, and contraceptive type. However, using the ATS, it is not presently possible to specify covariates without adding interaction terms into the model (S. Friedrich, personal communication, March 1, 2021), which in this case would involve estimating dozens of superfluous parameters. Thus, to examine inflammatory data, we fit linear mixed models (LMMs) using the *lme4*

package (Bates, Mächler, Bolker, & Walker, 2015) with nonparametric case bootstrapping (van der Leeden, Meijer, & Busing, 2008) to estimate 95% confidence intervals for effects of interest. Bootstrapped LMMs do not require the assumption of normality or homoscedasticity (van der Leeden et al., 2008), are well-suited to repeated-measures designs since they are capable of fitting both fixed and random effects, and can accommodate covariates. In bootstrapped LMMs, effects were considered statistically significant if the bootstrap 95% confidence interval for that effect did not contain zero.

Results

Manipulation Check

Self-reported affect. We first examined self-reported affect on the PANAS-SF negative and positive affect subscales by stress/control condition and time point. Because subgroups of this data violated assumptions of normality (Shapiro-Wilk $p < .05$) and sphericity (Mauchly's test of sphericity $p < .05$), the ATS was implemented. As expected, analyses indicated a significant 3-way interaction of Valence (Negative, Positive) × Condition (Stress, Control) × Time (Baseline, Post-stress, Pre-task, Post-task) (ATS = 13.85, df = 2.42, $p < .001$), and subsequent analysis was conducted to better characterize this interaction.

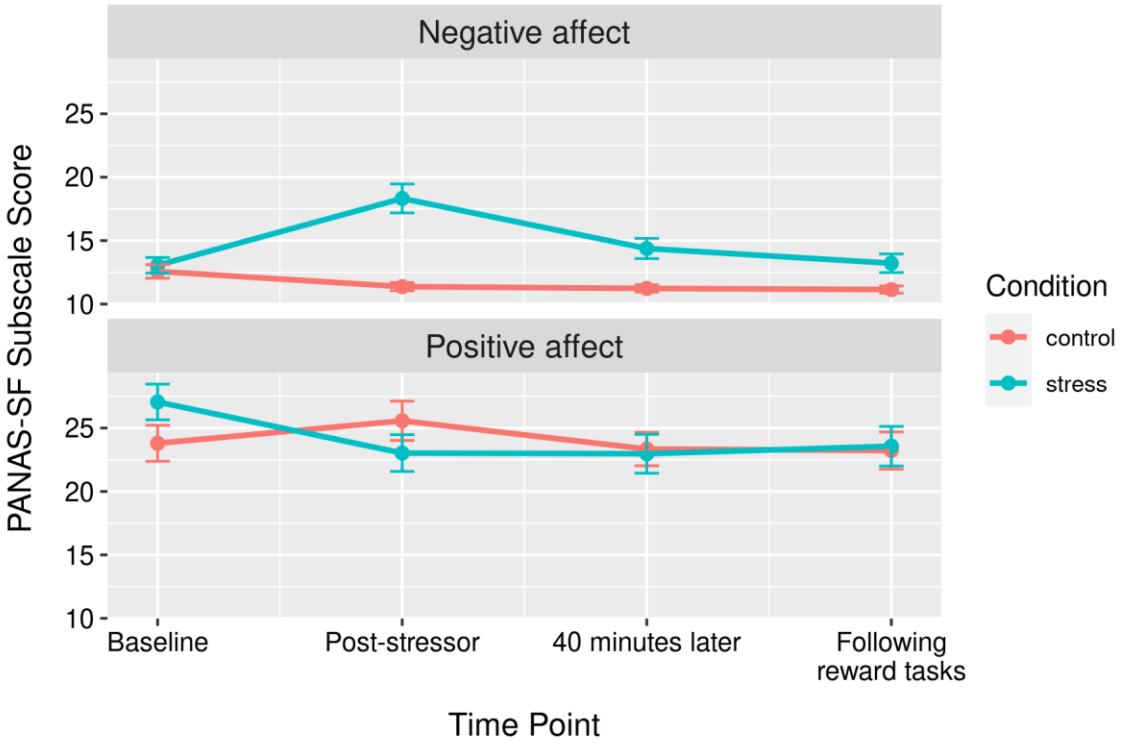


Figure 3. Affect ratings by valence and time.

For negative affect, follow-up analysis revealed a 2-way interaction of Condition × Time (ATS = 15.18, df = 2.61, $p < .001$), indicating that the time course of negative affect significantly differed between the stress and control groups. Figure 3 visualizes the time course of negative affect in the stress and control conditions. Kruskal-Wallis tests conducted for each time point found no significant differences between the stress and control groups at baseline ($H = .27$, $p = .604$). However, following the stressor/control tasks, the stress group reported significantly greater negative affect ($H = 22.60$, $p < .001$), and this difference persisted 40 min later ($H = 6.92$, $p = .009$). By the end of the reward tasks, group differences in negative affect no longer reached the threshold of statistical significance ($H = 3.62$, $p = .057$). As expected, these results suggest that the TSST

provoked a negative affective reaction relative to the control tasks, which decreased over the course of the experiment.

For positive affect, the 2-way interaction of Condition × Time was also significant (ATS = 5.10, df = 2.24, p = .004). The interaction significance appeared to be driven by a crossover effect between positive affect measured at baseline and following stress/control tasks (see Figure 3). However, the stress and control groups did not significantly differ on positive affect at any single time point (p > .05).

Time Course of Inflammatory Markers following Stress

We next examined the impact of the stress manipulation on the time course of inflammatory markers. Because subgroups of the data violated the assumption of normality (Shapiro-Wilk p < .05), we applied bootstrapped LMMs to these data.

Pro-inflammatory cytokines. Significant effects of time emerged for two pro-inflammatory cytokines: IL-1β (β = 0.21, SE = 0.04, 95% CI: [0.12, 0.29]) and IL-8 (β = 0.23, SE = 0.06, 95% CI: [0.12, 0.34]). These results suggest that concentrations of IL-1β and IL-8 increased with time across the stress/control groups. However, unexpectedly, no stress/control condition by time effects reached the level of statistical significance for any pro-inflammatory cytokine (95% CIs contained zero). Thus, we uncovered no evidence that the stress manipulation modulated pro-inflammatory responding.

Anti-inflammatory cytokines. Analyses indicated a significant effect of time for IL-10 (β = 0.24, SE = 0.04, 95% CI: [0.15, 0.32]), suggesting that IL-10 increased over time across all groups. A stress/control condition by time interaction emerged for IL-4 (β = -0.18, SE = 0.07, 95% CI: [-0.32, -0.03]) and IL-10 (β = -0.16, SE = 0.06, 95% CI: [-

0.29, -0.03]), suggesting that these anti-inflammatory cytokines decreased following stress, relative to the control condition. These results are visualized in Figure 4.

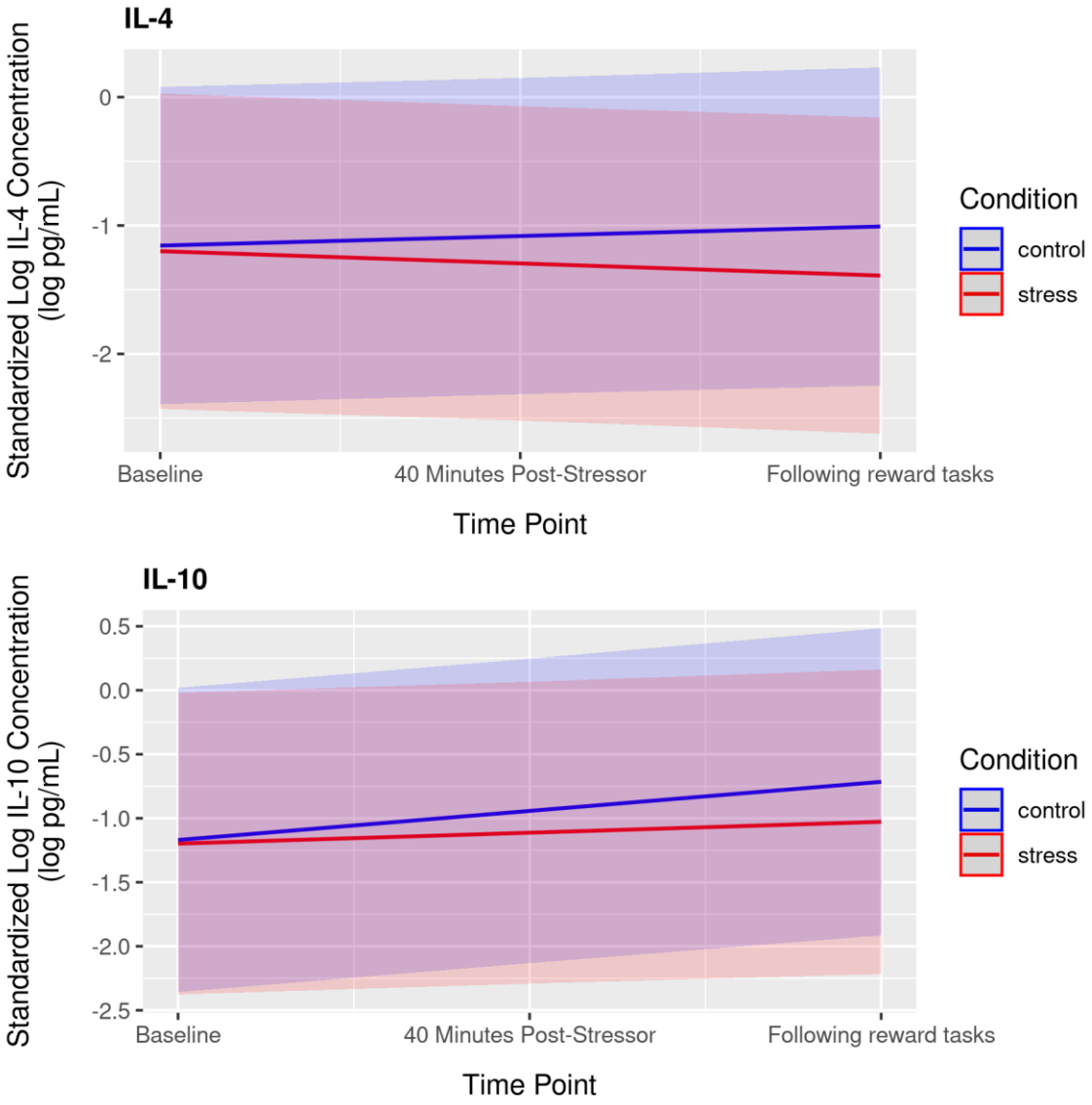


Figure 4. Model estimates of anti-inflammatory cytokine concentration, by time and stress/control condition. Graphs depict model estimates of marginal effects based on observed parameter values.

Reward- and Loss-Based Learning following Stress

Pessiglione reward task. When examining percentage optimal choice in the second half of the task (when participants are likely to have learned choice-outcome

contingencies), Shapiro-Wilk tests indicated that subgroups of the data were non-normally distributed ($p < .05$), and subsequent analyses were conducted using the ATS. The ATS indicated an unanticipated main effect of trial type (reward vs. loss trials; ATS = 10.63, df = 1, $p = .001$), such that participants were more likely to respond optimally on reward trials than on loss trials. Prior reports of results from this task have focused on group by trial type interaction effects, and to our knowledge, this main effect has not been publicly reported (Harrison et al., 2016; Pessiglione et al., 2006). Unexpectedly, no stress/control condition by trial type interaction emerged in our study ($p > .05$), yielding no evidence that the stress manipulation differentially influenced reward versus loss learning in the Pessiglione task.

PRT. Although data cleaning strategies for the PRT are relatively similar across studies, specific parameters for data removal vary (Boyle et al., 2020; Pizzagalli et al., 2008, 2005; Whitton et al., 2015). To facilitate comparison, we adopted data cleaning parameters from a study that also examined the impact of inflammation (Boyle et al., 2020). Subjects were excluded if they met the following criteria: a) > 80% of trials with response time < 150 ms or > 2500 ms; b) >= 16 trials more than ±3 SD from the mean; c) rich/lean reward ratio <= 2.4; d) < 50% accuracy overall. This process removed 10 subjects from the stress group and 10 from the control group, yielding a valid sample of $N = 64$. Next, individual trials were rejected if response time was < 150 ms or > 2500 ms, resulting in the exclusion of 273 out of 12,800 total trials across participants.

Following data reduction, Shapiro-Wilk tests provided no evidence that response bias was non-normally distributed within stress/control condition and task block ($p > .05$), and Levene's test did not indicate heteroscedasticity ($p > .05$). Thus, a repeated-

measures ANOVA would be an acceptable choice for this analysis, although the ATS produces nearly identical results under these conditions. Unexpectedly, neither the ANOVA nor the ATS revealed any significant effects ($p > .05$). In the absence of a Condition (Stress, Control) × Block (1, 2) interaction effect, we accept the null hypothesis that the stress manipulation did not influence response bias in the PRT as a measure of reward learning.

Associations with change in negative affect following stress. Because the stressor may not affect all individuals identically, we conducted bootstrapping regressions to examine whether change in negative affect following stress predicted reward/loss learning. Within the stress group, change in negative affect from pre- to post-stressor did not significantly predict reward or loss accuracy in the Pessiglione task or change in response bias from block 1 to block 2 in the PRT (95% confidence intervals contained zero). These results provide no evidence for altered reward or loss learning, even in individuals with robust affective responses to the stressor.

Cytokine Activity and Reward/Loss Learning

Analyses indicated that levels of IL-1β, IL-8, and IL-10 increased over time, but we observed no significant differences in the time course of salivary concentration for these cytokines based on stress/control condition. On an exploratory basis, we examined whether changes in concentration of these three cytokines over time (regardless of stress exposure) were associated with reward/loss learning. We applied bootstrapping regression models. In each model, change in IL-1β, IL-8, or IL-10 (from baseline to post-reward task) served as the predictor variable, and the outcome was either a) reward accuracy in the second half of the Pessiglione task; b) loss accuracy in the second half of

the Pessiglione task; or c) response bias in the PRT. Analyses revealed no significant associations between change in cytokine concentration and reward/loss learning on any task (95% confidence intervals contained zero).

Additionally, because analyses suggested that the stress manipulation may have decreased IL-4 concentrations relative to the control condition, we investigated whether change in IL-4 (from baseline to post-reward task) was associated with reward/loss learning on the Pessiglione task or PRT. Models were constructed using the same outcome variables as for IL-1β, IL-8, and IL-10, above. Again, analyses revealed no significant associations between change in IL-4 concentration and reward/loss learning (95% confidence intervals contained zero).

Mediation Analyses

Because no evidence emerged for an association between the stress manipulation and reward/loss learning, or between inflammatory responding and reward/loss learning, no analyses were conducted to explore inflammatory responding as a mediator of stress-induced changes in reward/loss learning.

Exploratory Analyses: Lifetime Stress Exposure as a Moderator of Stress-Induced Cytokine Activity

We applied bootstrapped LMMs to test the 3-way interaction of stress/control condition by time by life stressor count for all cytokines. A significant 3-way interaction in this case would suggest that the stress manipulation differentially impacted the time course of cytokine responding depending on prior life stress exposure.

Significant interaction terms emerged for the pro-inflammatory cytokines IL-1β (β = -0.15, SE = 0.07, 95% CI: [-0.31, -0.03]), IL-6 (β = -0.21, SE = 0.10, 95% CI: [-0.41, -0.01]), and IL-8 (β = -0.18, SE = 0.08, 95% CI: [-0.34, -0.02]). Results for pro-inflammatory cytokines are visualized in Figure 5. Notably, estimates of interaction terms for pro-inflammatory cytokines were all negative, such that models predicted a steeper increase in pro-inflammatory cytokines in the stress condition (relative to the control condition) when individuals had experienced fewer life stressors. By contrast, the models predicted more negative slopes for changes in pro-inflammatory cytokines in the stress condition (relative to the control condition) when individuals endorsed more life stressors.

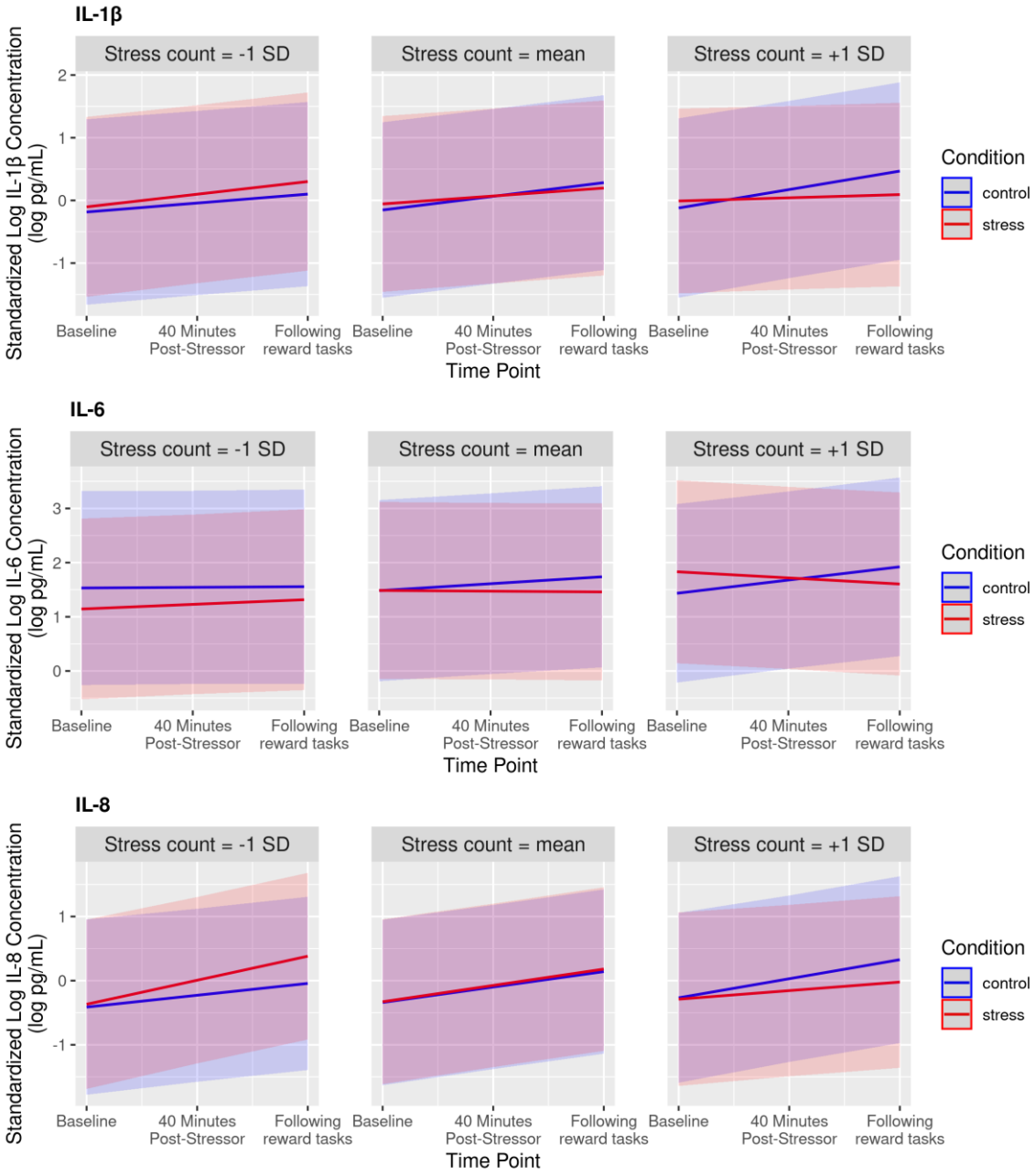


Figure 5. Moderation of stress effects on the time course of pro-inflammatory cytokine concentration. Graphs depict model estimates of marginal effects based on observed parameter values.

Additionally, analyses revealed a significant interaction term for the anti-inflammatory cytokine IL-4 (β = 0.22, SE = 0.08, 95% CI: [0.07, 0.41]), and these results are visualized in Figure 6. The interaction term for this anti-inflammatory cytokine was

negative. Thus, the model predicted more negative slopes for the change in anti-inflammatory cytokines in the stress condition (versus more positive slopes in the control condition) for individuals who endorsed fewer life stressors. For individuals who endorsed more life stress, the model predicted flatter slopes in both conditions.

Figure 6. Moderation of stress effects on the time course of anti-inflammatory cytokine concentration. Graph depicts model estimates of marginal effects based on observed parameter values.

Exploratory Analyses: Lifetime Stress Exposure as a Possible Moderator of Stress-Induced Changes in Reward and Loss Learning

To test whether prior stress exposure might moderate a relation between the stress manipulation and reward/loss learning, we applied bootstrapped LMMs.

For the Pessiglione task, we tested the 3-way interaction between stress/control condition, trial type (reward vs. loss), and life stressor count, with percent optimal choice as the outcome variable. The 95% bootstrap confidence interval contained zero. Therefore, we accept the null hypothesis that the stress manipulation did not differentially impact reward vs. loss learning in a different manner for those with high vs. low prior stress exposure. In summary, analyses revealed no evidence that prior stress exposure

moderated the impact of the stress manipulation on instrumental learning in the Pessiglione task.

For the PRT, we examined the 3-way interaction between stress/control condition, task block (1 vs. 2), and life stressor count. Response bias served as the outcome variable. Again, the 95% bootstrap confidence interval contained zero, yielding no evidence that the stress manipulation affected reward learning in the PRT differentially based on prior stress exposure.

Follow-up Analyses: Associations between Self-reported Affect and Inflammatory Changes

Because earlier analyses produced results that were unexpected given the existing literature (e.g., failing to indicate an effect of stress on pro-inflammatory responding), we conducted follow-up tests. Bootstrapping regressions revealed no association between change in self-reported affect pre- to post-stressor with change in cytokine concentrations from baseline to 40 min post-stressor or from baseline to following reward tasks, for any cytokine (all 95% confidence intervals contained zero). These analyses provided no evidence to suggest that the individuals who were most impacted by the stressor in terms of self-reported affect also experienced greater changes in inflammatory responding.

Discussion

This study examined inflammatory responses to stress as a possible mediator of the impact of stress on motivated behavior, thought to be an important component of anhedonia (Pizzagalli, 2014; Treadway & Zald, 2011). However, our investigation

uncovered no association between the experimental stressor and pro-inflammatory responses, and furthermore, did not find an effect of stress on reward/loss learning.

Several explanations may account for these null findings. One possibility is that the stressor applied here was not strong enough to induce changes in inflammatory responding. However, the TSST has produced pro-inflammatory responding in numerous other studies (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014). Additionally, individuals in the stress condition reported increased negative affect following the stressor, relative to individuals who underwent control tasks, suggesting that the stressor was potent enough to produce affective responding. Another possibility is that saliva provided an invalid measure of inflammatory responding. Notably, saliva represents a noninvasive method of assaying inflammatory markers that can be collected without training in venipuncture, and a number of studies have reported an impact of stress on inflammatory markers in saliva (see Szabo et al., 2020 for a review). However, one report suggested that the correlation between markers in saliva and blood plasma is low for most cytokines (Williamson, Munro, Pickler, Grap, & Elswick, 2012). These low correlations may raise questions about the validity of markers in saliva. Finally, the concentrations of salivary cytokines reported here are considerably lower than in some studies that have reported significant effects of stress on salivary cytokines, even at baseline measurement. For instance, Slavich and colleagues (2010) reported baseline log-transformed IL-6 concentrations between 1.2 and 1.4 log pg/mL on average, whereas the baseline mean in the present study was -1.4 log pg/mL. Maintaining cytokine samples requires storage at low temperatures (-80ºC), and problems with storage may have led to low cytokine

concentrations. Any or all of these factors may have contributed to null findings in the present study.

In addition, the present study found no evidence to support an effect of stress or stress-induced inflammation on reward/loss learning. Notably, some prior studies that reported an association between stress and reward sensitivity have used different stressors than were applied here, such as threat of shock (Berghorst et al., 2013; Bogdan & Pizzagalli, 2006). As a result, we cannot rule out the possibility that a different stressor may have led to the expected differences in motivated behavior.. However, some other prior studies have also failed to show an effect of stress on motivated behavior (Treadway et al., 2017), and one study that applied the TSST found only an indirect effect through inflammation, but no direct effect on behavior (Boyle et al., 2020). Thus, null findings in the present study may be best understood in the context of mixed prior literature in humans. Such inconsistent past findings may result from the absence of a true effect. On the other hand, it is important to note that the stressors that participants undergo in human studies tend to be mild and time-limited relative to those employed in nonhuman animal studies, due to practical and ethical constraints (Stanton et al., 2019). Thus, mixed findings could also reflect a weak or unstable effect, possibly due to necessary ethical limitations of human-subjects research. Additional studies with greater statistical power (e.g., larger sample sizes) could help to provide clarity, since such studies would have greater statistical power to characterize a potentially small effect.

In light of concerns regarding the validity of inflammatory assessment in the present study, our exploratory findings should be interpreted with caution. Our model estimates suggested a decreased change slope for anti-inflammatory cytokines (IL-4 and

IL-10) in the stress group (see Figure 4). However, these results are unexpected based on the past literature, since meta-analyses of studies using both blood serum (Marsland et al., 2017) and saliva (Szabo et al., 2020) suggest that an *increase* in IL-10 following stress relative to controls would be expected. Furthermore, we found that for individuals who endorsed more prior life stressors, the laboratory stressor was associated with a decreased change slope for broadly pro-inflammatory cytokines, such as IL-6 (see Figure 5). However, by contrast, past work suggests that more early stress exposure is associated with an *increased* inflammatory response following subsequent stress exposure (Fagundes & Way, 2014). Again, problems with measurement of cytokine concentration in the present study may have contributed to these unexpected results.

Overall, results from this study do not support the hypothesis that inflammatory responding mediates the relation between stress and anhedonia, but also cannot rule out this possibility. This study was limited by several issues, some which are inherent to experimentation with human subjects (i.e., ethical concerns that limit the severity and chronicity of stressors that can be applied), and some of which may have resulted from implementation difficulties (e.g., possible degradation of samples in storage leading to low cytokine concentrations; small sample size). Future studies could mitigate these limitations, e.g., by collecting larger sample sizes.

Laboratory studies in humans provide a relatively well-controlled method for examining the effects of stress on biology and behavior. At the same time, such work is necessarily limited in the types of stressors that can be studied. Supplementing experimental work with longitudinal, observational studies in large samples could shed additional light on how severe, real-world stressors relate to inflammatory signaling and

anhedonic behavior/self-reported symptoms. These studies are difficult to undertake, since they require repeated follow-ups and intensive life stress assessment, and involve the deployment of considerable resources. Still, such work may provide key etiological information that could inform therapeutic intervention and preventative care. Thus, continued research on the pathways to anhedonia represents a crucial step in furthering understanding of and treatment for this debilitating condition.

Chapter 6: General Discussion and Future Directions

Synthesis and Conclusions

The present set of studies aimed to contribute to a clearer understanding of the etiological pathway to stress-induced anhedonia. The first study (described in Chapter 3) sought to determine whether certain types of stress are especially strongly associated with anhedonia. The next two studies tested potential biological mediators of the link between stress and anhedonia: frontostriatal connectivity (Chapter 4) and inflammatory activity (Chapter 5).

Anhedonia was associated with number of stressors endorsed

Overall, our results are consistent with prior work (e.g., Keller et al., 2007) suggesting a link between stress and anhedonia. Across two studies (see Chapters 3 and 4), a greater number of stressors endorsed was linked to more severe anhedonia. This effect was detectable over a 6-month time window, using a detailed stress assessment (Chapter 3) as well as over a 2-year time window, with a much larger sample size and much more limited inquiry into potential life stressors (Chapter 4). Indeed, compared with a more complex model designed to account for key dimensions of stress, such as chronicity, severity, independence, and interpersonal focus, only number of stressors endorsed appeared to provide a non-zero contribution to prediction of anhedonic symptoms (Chapter 3). Taken at face value, these results might appear consistent with the notion that "more stress leads to more severe anhedonia."

However, several caveats apply. First, in both studies, assessment of both stress exposure and anhedonic symptoms relied, at least to some extent, on self-report. Thus, this association remains vulnerable to (some) biases that can occur in self-report data. For instance, based on the present data, we cannot rule out the possibility that individuals

who endorse more stressful life events also endorse more anhedonic symptoms due to a tendency to report negative life circumstances in general, absent any causal link. Notably, individuals with MDD exhibit a bias towards recall of negatively-valenced material (Matt, Vázquez, & Campbell, 1992), although this bias may be specific to sad material rather than threat stimuli (Gotlib et al., 2004). At the same, both studies (Chapters 3 and 4) inquired about specific events rather than relying on free recall, which may mitigate risk of recall bias, although it cannot be entirely ruled out. Additionally and crucially, the LEDS (see Chapter 3) uses diagnosis-blind rating panels to assign stressor severity ratings instead of relying on participants' judgment of stressor severity. Thus, participants' stress responses (including emotional reactions) do not factor into the assessment of stress exposure. Nevertheless, overall, our results are consistent with findings that negative life events are associated with MDD onset (Kendler et al., 1999) and linked to anhedonia (Keller et al., 2007).

Of note, the estimated effects of number of stressors endorsed were relatively small across studies. In our study of stressor characteristics predicting anhedonic symptoms (Chapter 3), the median posterior estimate of the effect of number of stressors endorsed was 0.48 (*SD* of anhedonia scores = 19.18), suggesting a very small predicted increase in anhedonic symptoms for each stressor endorsed. In our study using UK Biobank data (Chapter 4), the effect of number of stressors endorsed was similarly small (B = 0.06 across the discovery and replication samples, where anhedonia was measured on a 0-3 scale).

No evidence of a link between acute, mild stress and reward sensitivity

Although we identified an association between stress and self-reported anhedonia, our work did not detect an effect of stress on reward sensitivity, either on its own or in relation to loss sensitivity (Chapter 5). Links between MDD and diminished reward sensitivity (Pizzagalli et al., 2008) and decreased allocation of effort towards accruing rewards (Treadway, Bossaller, Shelton, & Zald, 2012) have been previously documented and are thought to contribute to anhedonia (Pizzagalli, 2014; Treadway & Zald, 2011). Additionally, prior laboratory studies using threat of shock as a stressor have reported an effect of decreased reward sensitivity (Berghorst et al., 2013; Bogdan & Pizzagalli, 2006). By contrast, in our own work, several possibilities could explain the failure to find a relation between stress and sensitivity to rewards. For example, we applied a social-evaluative stressor rather than using threat of shock. Thus, differences from the stressors used in prior work may have contributed to null findings, possibly due to insufficient stressor severity or duration.

On the other hand, our findings may be limited by the behavioral tasks chosen to assess anhedonic-like changes in behavior following stress. That is, assuming that stress alters neurobiological/behavioral processes that contribute to anhedonia, the tasks we chose may be insensitive to these alterations, at least under circumstances of mild stress. To facilitate comparison with existing work, our experimental study (Chapter 5) made use of established tasks with associations with MDD (Pizzagalli et al., 2008) and vaccine-induced inflammation (Harrison et al., 2016). However, as others have noted, anhedonia may represent a multifaceted and potentially heterogeneous construct (Treadway & Zald, 2011). Future research could incorporate measures that take a different approach to characterizing reward processing deficits that may be associated with stress and

anhedonia (see further discussion in **Unresolved Questions and Future Directions**, below).

No support for frontostriatal connectivity or inflammatory responding as a mechanism for stress-induced anhedonia

We also found no support for two potential biological mechanisms of stress-induced anhedonia: altered frontostriatal functioning (Chapter 4) and inflammatory responding (Chapter 5). However, we are unable to rule out these mechanisms due to limitations of the studies included here. In our study of frontostriatal connectivity as a potential mediator of the stress-anhedonia link, we were limited by minimal assessment of both anhedonia, which was measured using a single self-report item, and life stress, which was based on yes/no responses to only six potential stressors over a broad 2-year time window. Additionally, our study of the impact of stress-induced inflammation on anhedonic behavior was limited by questionable validity of inflammatory assessment. Concentrations of inflammatory cytokines were considerably lower than in past work, raising the possibility that samples degraded during the storage and analysis process. Additional work could clarify whether frontostriatal function and inflammatory responding are indeed plausible mediators (see below).

Unresolved Questions and Future Directions

Perhaps regrettably, the studies included here raise more questions than they answer. More research is needed, both to address the methodological limitations of this dissertation and also to expand the study of stress-induced psychopathology in general. The sections below discuss unanswered questions and suggest ideas for future work.

Is "number of stressors endorsed" a proxy for chronicity or severity of stress exposure?

As noted above, we found that number of stressors endorsed was associated with self-reported anhedonia (Chapters 3 and 4). However, it remains unclear whether this finding reflects the impact of multiple kinds of stressors, or may instead be a proxy for effects of stressor chronicity or severity. Indeed, one explanation that could account for this finding is that more chronic (i.e., more frequent) exposure to stress leads to greater anhedonic symptoms. However, using stress interview data (Chapter 3), analyses did not yield a credible non-zero effect of single stressors that are chronic in nature (e.g., financial stress lasting at least 4 weeks). Still, evidence from other work suggests that the definition of chronic stressors could plausibly influence the strength of the effect. Indeed, a study that used a higher length cutoff (1 year) to define chronic stressors found that they were more strongly associated with depressive symptoms than acute events (McGonagle & Kessler, 1990), whereas studies that have used a lower cutoff (4 weeks) have found that acute events are more robustly associated with depression severity (Muscatell et al., 2009) or no difference (Rojo-Moreno et al., 2002). One way to address this seeming discrepancy using the LEDS data from Chapter 3 would be to limit the data to only individuals who endorsed chronic stressors, and test whether stressor length is related to anhedonia scores. Such an analysis is feasible with the datasets used in this dissertation, and we plan to pursue this possibility as a future direction.

Another possibility is that multiple concurrent stressors create a more severe experience of stress. Yet analyses revealed no evidence of an effect of stress severity (Chapter 3). Notably, we included only events above a certain severity threshold in our analyses, and stressors must necessarily meet a certain threshold of severity to be

included in a life stress assessment. Accordingly, perhaps stressors above a certain level of severity contribute in a comparable fashion to anhedonic symptoms. However, an alternate explanation is that once an individual has developed substantial anhedonic symptoms, additional stressors exert a diminished impact on anhedonia. Thus, severity may have an impact, but only for individuals with milder symptoms. One way to test this possibility would be to examine diagnostic status as a moderator of the impact of stress severity on anhedonia. If a "ceiling effect" is present, we would expect stress severity to be associated with anhedonic symptoms in individuals without MDD, but not individuals who are already experiencing MDD. Additionally, for individuals with MDD, we might expect that stress exposure in the 6 months prior to MDD onset (as opposed to 6 months prior to assessment) exerts the strongest effect on anhedonic symptoms. However, this hypothesis rests on the assumption that that anhedonic symptoms remain constant throughout a depressive episode, which is the reason such analyses were omitted from the present dissertation. Even so, this hypothesis merits follow-up work.

Are there other, important dimensions of stress that were omitted here?

In our study using the UK Biobank sample (Chapter 4), financial difficulties (discovery $B = 0.22$ and replication $B = 0.25$) appeared to predict anhedonia scores more strongly than number of stressors endorsed ($B = 0.06$ across the discovery and replication samples). This result raises the question of whether some stressors do have a larger impact than others, even though our work may have failed to identify the key factors that categorize less impactful vs. more impactful stressors. For instance, using the dimensions of stress that have historically predicted MDD onset (such as independence and interpersonal focus), we found no effects on anhedonia (Chapter 3). Thus, we cannot rule

out the hypothesis that some stressors may be more influential than others, and we may have simply failed to test the dimensions of stress that most efficiently parse stressors with respect to their impact on anhedonia. Notably, the LEDS stress interview categorizes stressors according to 10 categories that were not included in the model, such as education, occupation, housing, and finances. We plan to test whether these domains contribute to anhedonic symptoms in future research efforts.

Why did we fail to find support for frontostriatal connectivity and inflammation as mediators of stress-induced anhedonia?

While our work failed to find support for altered frontostriatal functioning or inflammatory responding as mediators of the relation between stress and anhedonia, we may have failed to detect an effect due to study limitations. With respect to our investigation of frontostriatal connectivity (Chapter 4), the UK Biobank sample included only minimal assessment of life stress and anhedonia. In this sense, our study suffered from a common trade-off in large-scale research: Because the time and resources necessary for a particular assessment scale with sample size, and because such efforts often have broad aims, these projects frequently opt for many, briefer assessments rather than few but thorough measures. However, progress in etiological research will require large samples that provide both adequate statistical power and also thorough, detailed stress measurements. Since stress exposure is relevant to virtually all psychopathology, large-scale projects that include relatively detailed stress measurements could be vital to elucidating the etiology of multiple forms of mental illness. Such an undertaking could make use of judicious methodological compromises. For instance, the STRAIN (Slavich & Epel, 2010; Slavich & Shields, 2018) is a self-report instrument that inquires about a

large number of stressors and also assesses the frequency, timing, and duration of each stressor. This measure omits some of the features of gold-standard stress assessment. For example, it relies on participants' personal judgment to capture the severity of stressors, in contrast to the LEDS, which uses standardized anchors and blind rating panels (Harkness & Monroe, 2016). However, the STRAIN is considerably briefer than the LEDS (~30 minutes to complete as opposed to potentially several hours), and still assesses more stressors and provides more temporal detail than several widely-used stress checklists (e.g., Brugha & Cragg, 1990; T. H. Holmes & Rahe, 1967). A large study that included a measure like the STRAIN, alongside well-validated clinical inventories and biological measures of broad interest (such as resting-state functional connectivity or inflammatory markers), could provide a useful resource for advancing the study of psychopathology in general. However, such large-scale efforts would require a substantial mobilization of resources, likely coordinated across multiple sites and research teams.

In the meantime, smaller studies of stress-induced inflammation and motivated behavior could focus on different behavioral assessments. In our work, we relied on tasks that have been previously associated with MDD and vaccine-induced inflammation. However, future work could make use of tasks that probe other facets of motivated decision-making, such as habitual vs. "cognitive" or model-based reinforcement learning. Indeed, basic research on stress and decision-making suggests that stress may shift learning towards more habitual strategies (Otto, Raio, Chiang, Phelps, & Daw, 2013; Schwabe et al., 2007; Wirz, Bogdanov, & Schwabe, 2018). Notably, it remains unclear whether such conceptualizations of reinforcement learning offer a generative framework

for elucidating the etiology of anhedonia. However, basic research on the effects of stress on decision-making may offer compelling candidate mechanisms that could then be tested in clinical populations.

Finally, we may have failed to uncover evidence for mediation of stress-induced anhedonia by frontostriatal connectivity or inflammatory responding because no true effects exist. Stress-induced anhedonia may instead be mediated through a different pathway, such as through altered activity in energy homeostasis systems (see Chapter 2). For instance, GLP-1 neurons are thought to at least partially mediate hypophagic responses to stress (Maniscalco et al., 2015). Yet GLP-1 activity also appears to diminish motivation for other rewards, such as alcohol and drug rewards (Hayes & Schmidt, 2016), possibly via projections to NAc and VTA (Alhadeff et al., 2012). Thus, GLP-1 activity (and/or other energy homeostasis mechanisms) could mediate the link between stress-anhedonia. However, these pathways have received little attention in the anhedonia literature. In part, the lack of research may be due to difficulties with studying energy homeostatic pathways in humans. As an example, GLP-1 neurons originate largely in the brainstem (Merchenthaler et al., 1999), and brainstem imaging requires special considerations, such as the use of ultrahigh-field MRI scanners (Sclocco, Beissner, Bianciardi, Polimeni, & Napadow, 2018). Nevertheless, exploring additional candidate mechanisms for stress-induced anhedonia may yield important insights for the diagnosis and treatment of this condition.

Concluding Remarks

At present, widely-used diagnostic systems for psychiatric disorders rely on descriptive classifications rooted in clinical consensus (Clark et al., 2017). To make

much-needed advances in diagnosis, treatment, and prevention, it will be crucial to develop empirically-supported etiological theories for psychiatric problems such as anhedonia. This dissertation attempted to elucidate a causal pathway to anhedonia by examining the impact of particular types of stress and putative mechanisms through which stress might influence anhedonia. We were unable to find support for the mechanisms in question, or to effectively classify stressors that are especially associated with anhedonia. However, we propose that additional work is merited to examine how complex adaptations that unfold in response to stress could give rise to hedonic and motivational changes. We acknowledge that the pathway toward greater etiological knowledge will be lined with discarded hypotheses and null results, but hope that future work will lead to a revolution in care for those individuals whose lives are impacted by debilitating anhedonia.

Appendices A and B

Appendix A (Detailed Model Diagrams for “Key Dimensions of Stress and Contributions to Anhedonia”) and Appendix B (Model Diagnostics for “Key Dimensions of Stress and Contributions to Anhedonia”) are available online: https://yale.box.com/s/01x7dbeuk2a0f2wrby57zpeugzp5dmjo

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