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Samuel Sangyoon Han April 13, 2020

Glutamatergic Stress Response Dysfunction in Major Depressive Disorder: Consideration of Stress Exposures

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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Bachelor of Arts with Honors

Department of Psychology

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Abstract

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Stress is a major factor in a variety of health outcomes, including Major Depressive Disorder. Although stress is seen to instigate the onset, severity, and duration of depression, its relationship with neurobiological abnormalities remains uncertain. Animal stress models show that response to chronic stress leads to structural damages in mPFC induced by glutamatergic neurotoxicity. This same stress-induced glutamatergic dysfunction may be responsible for the microdamage consistently seen in the mPFC of depressed patients. The goal of the present study is to determine the impact of previous stress exposure on mPFC glutamatergic stress response between a control and depressed group using MRS techniques and the comparison of two stress exposure categories: (1) perception of recent chronic stress and (2) cumulative life course stress. The findings indicate glutamatergic stress response to be associated with perceived stress only in the control group. This suggests a glutamatergic dysfunction in the depressed mPFC that fails to engage in adaptive stress response shown in the healthy mPFC. The demonstration of glutamate abnormality in the depressed mPFC provides insight to glutamate's role in the pathophysiology of depression and can allow advancements in non-monoamine based pharmaceutical treatment.

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Stress is a significant risk factor for physical and mental health (Thoits, 2010), particularly Major Depressive Disorder (MDD). Stressful life events have been found to precipitate the onset of depressive episodes as well as impact their severity, duration, and frequency of recurrence (Cohen et al., 2007; Kendler et al., 1999). While small doses of stress can promote adaptation within a changing environment, excessive stress can damage physiological functioning (McEwen, 2017a). This biphasic effect of stress is especially true when stress exposure becomes chronic (McEwen, 2017b, 2020). To date, many questions remain as to how stress may contribute to functional abnormalities and structural changes seen in the depressed brain.

Although the pathophysiology of MDD is highly heterogeneous, some replicable changes to brain structure and function have been observed; meta-analyses consistently demonstrate reduced medial prefrontal cortex (mPFC) volumes in patients with major depression (Arnone et al., 2012; Koolschijn et al., 2009; Zanatta et al., 2019), marking this brain area to be implicated in the pathophysiology of MDD. Findings indicate greater structural damages in the mPFC with higher number of prior depressive episodes, suggesting that these morphological deficits may be linked to stress exposure (Treadway et al., 2015). Yet, the underlying neurobiological effects of stress that are linked to these damages in the mPFC are still unclear.

In animal models, a wealth of data shows evidence for the mPFC as a site that is vulnerable to the deleterious effects of excessive stress exposure. Rodent studies involving repeated stressors reveal maladaptive neurostructural changes in the mPFC in response to stress that include damage to synaptic plasticity and dendritic morphology (Cerqueira et al., 2007;

Cook & Wellman, 2004; Radley et al., 2005; Radley et al., 2006). Importantly, these studies have implicated glutamate-mediated excitotoxicity as a potential mechanism for mPFC microdamage. Higher than normal concentrations of extracellular glutamate in response to environmental stress provoked neuronal degeneration, death, and structural remodeling in the mPFC, indicating regulatory processes of glutamate to be compromised by stress exposure. The evidence from rodent studies exhibit the effects of stress on morphological changes in the mPFC that are mediated by glutamate release and synaptic transmission, converging to illustrate a relationship between stress and glutamate abnormalities that precipitate microdamage in the mPFC. This may give insight to how the same brain area sustains deficits in MDD, pushing for the investigation of the glutamate neurotransmitter in relation to stress and mPFC of depressed individuals.

In general, changes in glutamate transmission is normative in responding to a stressor. A wide array of behavioral and physiological alterations occur in response to a stressor, collectively referred to as a stress response (Carrasco & Van de Kar, 2003; Chrousos & Gold, 1992). In this response to aversive stimuli, the neuroendocrine system maintains homeostasis by engaging with a collection of structures known as the hypothalamic-pituitary-adrenal (HPA) axis. Here, corticotropin-releasing factor (CRF), the principle regulator of the HPA axis, instigates successive events that release glucocorticoids, which are hormones responsible for the regulation of physiological changes in response to stress (Smith & Vale, 2006). What is important to note is of the effect of stress-induced glucocorticoids in glutamate transmission in the prefrontal cortex, including effects on glutamate release, glutamatergic receptors, and glutamate metabolism; the glucocorticoids released by the HPA-axis directly mediate glutamate neurotransmission in order to adaptively respond to stress (Popoli et al., 2012). However, the effects of stress seen in animal

stress models suggest an inappropriate stress response and subsequent structural damage to the mPFC that is mitigated by glutamate excitotoxicity.

In clinical studies, while past research on the neurochemistry of mood disorders has been dominated by monoamines (Bunney & Davis, 1965; Berton & Nestler, 2006; Schildkraut, 1965), more recent work has increasingly highlighted the role of the amino acid neurotransmitter glutamate, the primary excitatory transmission in the brain. With such a crucial role in excitatory processes, changes in glutamate influence a variety of brain functions (Sanacora et al., 2012). Indeed, research has provided evidence for glutamate dysfunction in MDD, with demonstrated effects of glutamate toxicity on neurogenesis, neuronal growth, and synaptic plasticity (Pittenger & Duman, 2008; Racagni & Popoli, 2008; Sanacora et al., 2008). Further, N-methyl-D-aspartate receptor (NMDA-R) antagonists—in which glutamatergic neurotransmission is stunted—have shown similar actions to antidepressants (Trullas & Skolnick, 1990), implicating the glutamatergic system to be implicated in depression's pathophysiology. Specifically, Ketamine hydrochloride, a potent NMDA-R antagonist, results in rapid and robust decreases in core depressive symptoms in depressed patients who are resistant to monoaminergic antidepressants, consonant with hypotheses of glutamatergic NMDA receptor dysfunction in depression (Abdallah et al., 2017; Berman et al., 2000; Murrough et al., 2013). Further, in relation to stress, the administration of ketamine reverted physiological alterations and anhedonia-like behavior induced by chronic stress in rats (Garcia et al., 2009). Coined as the 'neuroplasticity hypothesis', this shift towards a glutamatergic view of depression's pathophysiology and respective pharmaceutical treatment focuses on neuroplastic changes within and throughout brain areas in relation to glutamatergic synapses and circuitry.

As mentioned before, animal stress models have shown the mPFC to be an area susceptible to stress-induced neurostructural damage mediated by glutamate dysfunction. Accordingly, evidence from animal stress paradigms suggests that chronic stress alters glutamatergic synapses and dendrites in the mPFC, resulting in morphological changes such as atrophy, reduced density, and retraction in dendrites (for a review see: Sanacora et al., 2012). Here, stress induces alterations in glutamatergic mechanisms that subsequently harm the structure of animal mPFC, which raises the question of how humans—specifically, depressed patients—may undergo stress-induced glutamate changes.

A critical drive of the effects of stress on glutamatergic function is the duration of the stressor. Acute stress raises prefrontal glutamate tone (Lee et al., 2006; Lupinsky et al., 2010; Steciuk et al., 2000; Yuen et al., 2009), and such increase notably occurs in larger proportions in the mPFC than other regions related to stress response, indicating stress response as regionally selective to this brain area (Bagley & Moghaddam, 1997; Moghaddam, 1993). Chronic stress, however, results in the decrease of glutamate neurotransmission in the mPFC (Knox et al., 2010; Yuen et al., 2012). Following this pattern, continually applying a tail-pinch stressor in healthy rats displayed a habituation in glutamatergic release rather than the increase seen in acute stress, which indicate a sign of neurochemical adaptation of the mPFC in response to stress (Bagley & Moghaddam, 1997); this reduction in mPFC glutamate following chronic stress may be a protective mechanism in response to the repeated exposure (McEwen et al., 2015; McEwen, 2017). A clear dichotomy is seen in which glutamate signaling in mPFC normally elevates following acute stress, while the same neurotransmission decreases with exposure to chronic stress as an adaptive stress response against excitotoxicity.

In the context of psychopathology, some studies have indicated glutamate abnormalities in depression. An overall low concentration of glutamate has been shown in the mPFC depressed individuals (Luykx et al., 2012) and depressed patients with strong anhedonic symptoms retain lower glutamate concentration within the mPFC when compared to controls (Walter et al., 2009). However, the literature on glutamatergic abnormalities in depression is comprised of mixed findings, pushing for an investigation of the presence of glutamatergic dysfunction in MDD.

Although the separate alterations in glutamate in response to acute and chronic stress have been investigated by a number of animal investigations, how the magnitude of stress previously experienced influences the glutamatergic response to a current stressor has not been well-characterized, particularly in humans. Few studies have inquired the interaction between an ongoing stressor and the previous stress exposure in their effect towards glutamatergic stress response. Past literature has pointed to this fact, calling for future research to examine how the response of glutamate release to acute stressors may be modified by previous chronic stress with the use of distinct protocols (Armario et al., 2008; Sanacora et al., 2012). Only a handful of studies have explored such dynamic processes, with only research by Luczynski et al. (2015) involving the effects of chronic stress towards acute glutamate stress response in rodent mPFC exactly resembling this topic. Yet, literature still lacks research on the effect of previous stress exposure on the present glutamatergic stress response in humans, and even more so with the consideration of MDD in this paradigm.

An additional consideration in examining previous stress exposure's influence on biological stress response is the impact of subjective vs. objective stressors. Lazarus and Folkman (1984) posits that stress response is a two-way process in which how stress is perceived or appraised by the individual largely dictates the stress's effect. Indeed, studies reveal that

chronic stress that is deemed uncontrollable or inescapable confer risks for poor health outcomes and developing psychopathology (Dickerson and Kemeny, 2004; McEwen, 2017; Kendler et al., 1993, 2004; Kessler, 1997). Further, consistent exposure to this kind of chronic stress is seen to result in learned helplessness behavior that strongly reflects symptoms of anhedonia, a cardinal symptom of depression (Seligman et al., 1968; Willner et al., 1992a,b; Amat et al., 2008). With how stressors are perceived being integral to the subsequent effect of stress, investigating stress-induced glutamate response given a context of perceived chronic stress can indicate stress response as being mainly concerned with the subjectivity of stressors. Importantly, a recent study found a negative relationship in a healthy control group in which subjects with low levels of perceived chronic stress had increase in stress-induced mPFC glutamate levels, but a decline as recent stress exposure increased (Cooper et al., 2020). The same relationship was not present in the second group of depressed individuals, suggesting an adaptive glutamatergic stress response in healthy controls. Accordingly, one of the objectives of the current study was to replicate these findings.

However, the consideration of lifetime stress exposure in replicating these findings may display a stronger effect on how glutamate responds to a stressor. Stressors occurred across a life course exert a cumulative effect towards biological processes, increasing the risk for disease and health deficits (Lupien et al., 2009). Through repeated and prolonged engagement with biological responses to adversity and stress, allostatic load—or biological "wear and tear"—occurs, involving the overexertion of neural adaptive systems that maintain homeostasis (McEwen, 2006). These adaptive mechanisms, that involve neuromodulators like glucocorticoids to be released, can turn to long-term consequences of atrophy of neuronal processes when stressors become excessive or chronic, seen in mPFC microdamage from previously mentioned

animal stress models. This collective effect that endangers adaptive biological functioning is similarly seen in the stress sensitization model of depression, in which continual exposure to major life stressors gradually lead to the hypersensitivity to acute stress, resulting in a lower threshold to maladaptive stress responses (Monroe & Harkness, 2005). Subsequent depressive episodes become decoupled from stressors, possibly due to neurobiological changes caused by cumulative stress exposure. Thus, examining life course stress and its relationship to stress-induced glutamate may demonstrate the effect of allostatic load towards maladaptive functioning and glutamate dysfunction in MDD, and possibly display a stronger relationship to glutamate stress response than perceived chronic stress. The Stress and Adversity Inventory (STRAIN) is an instrument that directly measures the cumulative stress experienced throughout one's life and is found to predict reactivity to acute stress, cortisol levels, and mental health in the general population (Slavich & Shields, 2018), but the implementation of STRAIN or life course stress in general are nearly absent in empirical literature—even more so in literature on mood disorders and depression. Their implications for the aggregate effect of stress exposure on allostatic load make this a promising construct to investigate.

Taken all together, stress is consistently shown to be an underlying pathway to various neurobiological alterations, illustrated in microdamage in rodent mPFC and changes in glutamate transmission. Glutamate and mPFC retain a strong link, in which stress enacts changes in glutamate as part of an adaptive stress response that can become damaging to this brain structure with repeated, excessive stress exposure. What is still unclear is if the volumetric reductions in mPFC seen in depressed patients are brought on by glutamate-mediated microdamage in mPFC shown in animal models. Looking at stress response in relation to changes in mPFC glutamate becomes an investigation of possible glutamatergic dysfunction within the depressed mPFC that

may explain structural damage seen in that brain area. Further, the interaction between previously exposed chronic stress and a current acute stressor in relation to glutamate activity can investigate the adaptive mechanism of glutamatergic stress response. In addition, previous stress exposure and its subsequent stress response has been widely assessed by one's perception of chronic stress—and have been shown to have a relationship with stress-induced mPFC glutamate—but examining the cumulative stress exposed in a lifetime may illustrate the allostatic load placed upon the individual, providing a stronger relationship to stress response. Given the evidence reviewed above, the present study first attempted to replicate the findings of the study by Cooper et al. (2020). Then, the study explored life course stress in relation to stress-induced glutamate changes in the mPFC to examine whether it illustrates stronger effects in glutamatergic stress response than perceived chronic stress. Associations between life course stress and other available data were examined as well. STRAIN, a measure that assesses the cumulative stress exposure in a lifetime, was the main instrument used to explore relationships among variables.

The aims of the present study were five-fold. First, life course stress was predicted to be correlated with stress-induced mPFC glutamate change across all subjects. Second, it was hypothesized that the associations will be significantly different between two sample groups, Control and MDD, in which the only control group will have a negative association with glutamate change. In comparing with perceived stress, it was hypothesized that life course stress will display stronger association to stress-induced mPFC glutamate change than perceived stress. Should this hypothesis not be supported, it was predicted that an interaction effect will be present between perceived stress and life course stress. Finally, an exploratory hypothesis was conducted

to examine associations of life course stress with number of depressive episodes and affective and cortisol response to acute stress.

Methods

Participants

A total sample of 48 participants, between the ages of 18 and 65, were recruited for the study ($n_{control} = 26$, $n_{MDD} = 22$). The mean age of the control group was 27.96 (standard deviation [SD] = 7.85), while the average age for the depressed group was 29.14 (SD = 9.35). A full description of sample demographics is provided in **Table 1**. Healthy control subjects were recruited in response to community advertisements in Atlanta, GA. Those who were interested in the study were instructed to complete a prescreening survey on REDCap, a HIPAA-compliant web application. Exclusion criteria for these subjects included an assessment of current or past psychiatric disorders using the Structured Clinical Interview for DSM Disorders (SCID; First et al., 2005), with exceptions to specific phobias or past alcohol abuse.

The completion of an evaluation clinic protocol assessed the subject's eligibility to partake in the study. Before enrollment, screening test results and medical records of all referred subjects were reviewed by study staff to guarantee their health and eligibility. MDD patients were excluded for anything above the minimal risk for suicide indicated by SCID and the Columbia Suicide Severity Rating Scale (C-SSR; Posner et al., 2008). The rest of the depressed subjects were recruited in similar fashion to healthy subjects, in which a prescreen survey and SCID were administered.

Other exclusion criteria included substance use comprised of illegal drugs, psychotropic medications, or nicotine, confirmed with a urine drug screen prior to scanning. Subjects were also screened for any contraindications for participation in an MRI scanner.

Procedure

In the initial visit, researchers acquired informed consent from all participants before continuing with the study. Subjects underwent a Structured Clinical Interview (SCID) with a qualified research clinician to ensure all eligibility criteria were met. Then, they were asked to complete a number of questionnaires and tasks. Questionnaires included self-reports of demographic information, depressive symptoms, and stress history, including the STRAIN measure. Administered tasks involved behavioral tests to ensure the absence of any neurocognitive deficits in the subjects.

During the second visit, subjects completed a set of MRS scans at the Facility for Education and Research in Neuroscience (FERN) in Emory University's Department of Psychology. The scanning session consisted of a pre-stress MRS scan, a laboratory stressor (elaborated below), followed by a post-stress MRS scan. A mood assessment before, during, and after the stressor were completed. Saliva samples were collected at four separate time points throughout the session to assess the presence of a stress response.

Measures

Finger Tapping Task (FTT), Digit Symbol Task (DST; Wechsler, 1946), and Reaction Time Task (RTT). The FTT, DST, and RTT were administered to ensure an absence of any neurocognitive impairments or disorders in the subjects.

Columbia-Suicide Severity Rating Scale (C-SSRS). The questionnaire is used to assess the presence of suicidal ideation and respective behavior. The assessment is comprised of questions about suicide ideation, intensity, and behavior (Posner et al., 2008). MDD patients were screened

with the C-SSRS at every session to check for any indication of being above minimal risk of suicide during the study.

Beck Depressive Inventory-II (BDI-II). Beck Depressive Inventory-II is an effective measure of depressed mood in which it evaluates the presence and severity of symptoms of depression over the past two weeks (Beck et al., 1996).

Perceived Stress Scale (PSS). PSS is a psychological instrument involving the subject's perception of stress; the measure includes the extent to which events in the past month were considered stressful. Items examine the chronic stressors' predictability and controllability, as well as current levels of chronic stress (Cohen et al., 1994).

Stress and Adversity Inventory (STRAIN). The Stress and Adversity Inventory embodies the subject's full life-course experiences of stress, inquiring about a wide range of acute, chronic, and early-life stressors. The measure also includes ratings of severity, frequency, timing, and duration of these stressful events. STRAIN is a highly efficient online system that is able to appraise cumulative stress in a life-time, reflecting stress's exertion of allostatic load or biological "wear and tear" towards an individual's health. The instrument's assessment of a subject's life-time stressors has shown strong concurrent, discriminant, and predictive validity as well as test-retest reliability. The measure is significantly distinct from personality traits or social desirability characteristics and has illustrated strong associations with health and cognitive outcomes (Slavich & Shields, 2018). For the current study, the total count (StressCT) and severity of stressors (StressTH) were chosen from STRAIN for analysis. StressCT and StressTH are the main stress exposure indices that directly represent the aggregate amount and severity of stress experienced across all stressor categories throughout a lifetime.

Maastricht Acute Stress Task (MAST). Participants underwent the Maastricht Acute Stress Task that combines previous physical and social stressor paradigms together to induce optimal cortisol response and subjective stress reaction (Smeets et al., 2012). MAST is comprised of a cold pressor and social performance task involving moderately difficult arithmetic subtraction in front of an experimenter. The subjects were instructed to immerse their hand and wrist into ice water (1-5°C) in random time intervals that ranged from 30 to 90 seconds. When the hand was taken out between these intervals, subjects were required to perform serial subtraction beginning from the number 2043 and counting down by 17. If the subject's subtraction was incorrect, the experimenter demanded the subtraction to start over again, starting from the top. A total of five trials were administered. The experimenter deliberately maintained a neutral attitude and only provided negative feedback when errors were made by the subject. 4 serial subtraction blocks varying in duration were administered. To extend the effect of the stressor, it was implied to the subjects that the task will be done again in a later time due to lack in performance. Due to the nature of this study requiring the subject to be in the fMRI scanner, the MAST used for this study has been modified to accommodate the facility.

Visual Mood Scale (VAMS). In measuring affective responses to the MAST, all participants completed a mood rating through the visual analogue mood scale (VAMS; Stern et al., 1997). The scale contains five 1000mm horizontal lines with a bidimensional mood state: Happy-Sad, Relaxed-Tense, Friendly-Hostile, Sociable-Withdrawn, Quick Witted-Mentally Slow. Subjects used buttons attached to a computer cursor to designate a location within the line that best described their current mood state. VAMS was completed in the beginning before and after the MAST. After correcting for reverse scoring, a maximum of 100 indicated the highest positive mood possible.

Salivary Cortisol. Cortisol was measured through saliva collected at four separate time points. Using cortisol Luminescence Immunoassay (CLIA) from IBL-International, Germany, salivettes were centrifuged at 2,000g for 10 minutes in 20 Celsius.

Magnetic Resonance Spectroscopy (MRS). For all samples, MRS data were collected on the 3T Tim TRIO with a 32-channel phased-array design RF head coil operating at 123 MHz. Data was collected at FERN located in Emory University. A single 2x2x2 cm voxel in the mPFC was placed using High-resolution T1-weighted anatomical images so that the voxel's posterior edge was positioned directly in front of the anterior side of the corpus callosum. A modified J-resolved PRESS protocol (2D-JPRESS) employed by the Proton MRS collected PRESS MRS spectra at incremental echo-times (TE) to sample J-resolved periodicity of coupled metabolites like glutamate. Shimming was automatically done, with some manual shimming done to optimize voxel field homogeneity. The 2D-JPRESS sequence collected 22 TE-stepped spectra ranging from 30 to 350ms in fifteen ms increments. For acquisition, Repetition time was (TR)=2s, F1 acquisition bandwidth of 67hz, spectral bandwidth =2kHz, readout duration 512ms, NEXT = 16/TE-step, total scan duration of 12 minutes. The same sequence was done twice, before and after MAST.

Analyses

fMRS analysis. 22 TE-dropped free-induction decay (FIDs) were zero-filled out to 64 points, Gaussian-filtered, and Fourier transformed to quantify glutamate. Every J-resolved spectral extraction in a bandwidth of 67 Hz was fitted with LCModel (Provencher, 1993, 2001), consistent with validated methods (Jensen et al., 2009; Henry et al., 2010). The raw peak areas across the 64 J-resolved extractions for each metabolite was calculated to result in an integrated area under the 2D surface. Glutamate metabolites were shown as ratios to total creatine (Cr). %

ΔGlu was calculated using the formula [(Glu/CrDuringStress – Glu/CrPreStress) / Glu/CrPreStress].

Statistical Analysis. Data were analyzed using two main statistical approaches. The first approach used Pearson Correlation coefficients to examine relationships between the PSS, Strain and % ΔGlu. Fisher's and Steiger's Z tests were subsequently computed to compare correlations between groups and stress variables, respectively. The second approach used regression analysis and multiple interaction effects to examine a possible interaction between PSS and STRAIN in stress-induced glutamate change.

Results

First, a Pearson correlation coefficient was computed to assess the association between STRAIN and stress-induced change in glutamate across all subjects. Ten subjects were excluded in analyses due to unsuccessful scans at the facility. No significant correlation was found between StressCT and percent-change in glutamate, $r(36) = .09$, p = ns, two-tailed. Similarly, StressTH was uncorrelated to glutamate change, $r(36) = .05$, p = ns, two-tailed.

To examine possible differences in between groups, STRAIN's relationship to stress-induced change in glutamate was compared between control and MDD groups. Pearson correlation coefficients were computed. In controls, glutamate change was uncorrelated with StressCT, $r(19) = .11$, p = ns (**Figure 1A**), and StressTH, $r(19) = .01$, p = ns (**Figure 1C**). Similarly, there were no correlations in StressCT, $r(15) = .17$, p = ns (**Figure 1B**), or StressTH, $r(15) = .18$, p = ns (**Figure 1D**), in depressed patients.

Pearson correlation coefficients were calculated to also examine the potential relationship between PSS and glutamate change in the two groups. For healthy controls, PSS and glutamate change were correlated, $r(45) = -.43$, $p = .002$ (**Figure 2A**), while no correlation was present in

the MDD group, $r(21) = .13$, p = ns (**Figure 2B**). There were no group differences in baseline glutamate levels. To test whether the relationship between PSS and change in glutamate was dependent upon STRAIN, a possible interaction effect was examined through a multiple regression analysis in both groups. In controls, the interaction between StressCT and PSS was not a significant predictor of glutamate change, $\beta = .38$, p = ns, $R^2 = .072$. Similarly, the interaction between StressTH and PSS did not predict glutamate change, $\beta = .40$, p = ns, $R^2 = .09$. In the same manner, the MDD group did not show any significant interaction effects, with interaction between StressCT and PSS, $\beta = 1.23$, p = ns, $R^2 = -.32$, or StressTH and PSS, $\beta = 1.88$, p = ns, $R^2 = -.24$, both indicating not to be predictors of glutamate change.

STRAIN's potential relationships with other data from the same samples were examined. Specifically, STRAIN's associations with cortical thickness, cortisol levels, affective responses, and number of depressive episodes were tested. All of the following analyses were Pearson's correlation coefficient calculations with two-tailed significance and centered around the StressCT and StressTH dimensions of STRAIN.

For Cortisol, baseline levels and stress-induced changes were examined across all subjects. Two subjects were excluded due to insufficient cortisol samples. Results found STRAIN and baseline cortisol to be uncorrelated in both StressCT, $r(44) = -.20$, p = ns, and StressTH, $r(44) = -.17$, p = ns. Equally, STRAIN and change in cortisol after the acute stressor were uncorrelated in both StressCT, $r(44) = -.05$, p = ns, and StressTH, $r(44) = -.18$, p = ns.

Two variables of mood responses were examined across all subjects: baseline mood ratings and change in ratings with the introduction of the stressor. Three subjects were excluded due to missing data. Baseline mood response was uncorrelated to StressCT, $r(43) = -.04$, p = ns,

and StressTH, r(43) = -.14, p = ns. Change in mood ratings showed non-significant effects to StressCT, r(43) = -.13, p = ns, and StressTH r(43) = -.20, p = ns.

The potential association between STRAIN and number of depressive episodes was also tested among the MDD group. Three subjects were excluded due to their inability to provide an approximate number of episodes experienced. Results indicated depressive episodes to be correlated with StressCT, r(19) = .50, p = .028, and StressTH, r(19) = .49, p = .032.

Discussion

The present study investigated the relationship between life course stress exposure and mPFC glutamate transmission in response to an acute stressor. It was found that no association was present between life course stress and stress-induced glutamate change either across subjects or groups. Rather, the study found a relationship between recently perceived stress and change in glutamate concentration in the control group, in which perception of recent stress as more uncontrollable led to a decrease in stress-induced glutamate. Further, life course stress was not associated with any exploratory variables that were examined, with the exception of the relationship between the number of depressive episodes. The present findings illustrate the effect of acute stress on mPFC glutamate may be an adaptive response in healthy controls that is absent in patients with depression and replicates the findings of the study conducted by Cooper et al. (2020).

The relationship between perceived stress and glutamate change in healthy controls is important to note. First, the direction of this effect was negative, such that perception of chronic stress as being uncontrollable was associated with lower glutamate levels in response to the acute stressor. This evidence resembles the findings of the study conducted by Luczynski et al. (2015) that reported lower mPFC glutamate in acute stress response when rodents were previously

exposed to chronic stress. Typically, acute stress preferentially increases glutamate in the mPFC (Steciuk et al., 2000; Yuen et al., 2009), while chronic stress decreases its concentration (Knox et al., 2010; Yuen et al., 2012). Notably, excess glutamate release and transmission lead to neurotoxicity and maladaptive effects to brain morphology (Musazzi et al., 2011). The decrease shown in chronic stress is a display of tolerance in glutamatergic response, indicating an adaptive stress response of a healthy mPFC (Bagley & Moghaddam, 1997) that engage in downregulation to keep the glutamatergic system from excitotoxicity. The negative association found in the current study may reflect such adaptation, where recent exposure to chronic stress led to the subsequent adaptive response of the mPFC glutamate in the face of an acute stressor. This would follow evidence of the mPFC's role in mediating adaptive responses to stress and producing neurochemical regulation to continuing stressors (Amat et al., 2008; Bagley & Moghaddam, 1997).

The absence of any association with the STRAIN suggest that this mPFC glutamate adaptation may reflect perceptions of stress that are relatively recent. As such, it likely reflects how recent stress is being handled as opposed to the cumulative 'wear and tear' associated with lifetime stress exposure. The evidence is further supported by the absence of an interaction effect between perceived and life course stress, indicating that the effect seen between PSS and change in glutamate levels is not dependent upon STRAIN. In the context of healthy controls, this suggests the cumulative effect of stress exposure—allostatic load— may not be completely relevant to the present neurobiological stress response, but rather the individual's appraisal of recent chronic stress.

Interestingly, this adaptive pattern was not present in the MDD group. The effect of acute stress on glutamate change was not associated with perceived stress throughout MDD subjects.

Importantly, the present study did not observe any group differences in baseline glutamate levels, as also seen in the study by Cooper et al. (2020). Here, the data indicates that MDD stress response was not equally influenced by perception of recent stress as it did in the control group. This stark contrast suggests that glutamatergic stress responses in MDD subjects are insensitive to recent chronic stress and how they have been perceived. The difference may demonstrate glutamate dysfunction in the depressed mPFC, as the negative relationship seen in controls reveals that the adaptive mechanism in stress response is missing in depressed subjects. This may indicate a maladaptive response through learned helplessness, a behavior mediated by damages to the mPFC (Amat et al., 2008). The present data would corroborate extensive literature that posit the involvement of glutamatergic dysfunction in the pathophysiology of depression (Mitchell & Baker, 2010) and further implicate the mPFC's vulnerability to stress exposure (Treadway et al., 2015).

Under the aims of the current study, the absence of any associations between life course stress and stress-induced glutamate was unexpected. The data suggests that glutamatergic stress response involves a temporal dimension, with glutamate levels depending critically on recent stress context rather than collective stress exposure. In terms of exploratory variables, life course stress did illustrate a positive relationship with the number of depressive episodes. The present data suggests that stressful life events retain an association with increasing episodes. This association may be promising to investigate further as previous evidence suggests that although stressful life events are central to the precipitation of an initial depressive episode, their role gradually diminishes in subsequent episodes (Monroe & Harkness, 2005). It is interesting to note that under the stress sensitization model, individuals experiencing the first depressive episode reported higher levels of chronic stress than those who had recurrent episodes (Stroud et al.,

2010), which may reflect the disengagement of perceived stress in MDD seen in the present study.

In sum, the current study was able to demonstrate a specific relationship in which stress-induced mPFC glutamate's relationship with perceived stress is only shown in controls. Although baseline levels of glutamate showed no group differences, the same association is diminished in MDD, illustrating glutamatergic dysfunction and possibly maladaptive neurobiological stress response in the depressed mPFC. The study's investigation of glutamatergic stress response extends the current literature in two ways. First, stress-induced glutamate in relation to previous exposure to chronic stress or life course stress has not been well-characterized. Second, literature on glutamatergic stress response mainly examined animal models. The same stress response in mPFC has not been explored in humans, yet alone in depressed individuals.

The findings of the study support the notion of glutamatergic dysfunction in MDD, specifically under the context of neurobiological stress response within the mPFC. The findings of the study further supports the neuroplasticity hypothesis, reiterating the abnormalities in glutamatergic processes observed in depression. Identifying the absence of an adaptive glutamatergic stress response in MDD in the present study may continue to emphasize this neurotransmitter as integral to the pathophysiology of depression. In turn, mounting evidence of glutamatergic dysfunction can push for the implementation of NMDA-R antagonists, that directly interacts with glutamate systems, in pharmaceutical treatment for depression that shift away from conventional monoamine-based antidepressants (Berton & Nestler, 2006; Musazzi et al., 2011; Trullas & Skolnick, 1990).

A few limitations are present in the study. First, the findings are correlations and any causality cannot be inferred. The primary association in the study only confirms a relationship between stress-induced glutamate change and stress appraisal. Second, the sample sizes for both groups were moderate, running the risk of weaker power and generalizability of the study. Additionally, the sample size for PSS was larger than what was available in STRAIN, preventing strong comparison in associations. Further, the range of scores for STRAIN and PSS were restricted in the study. For STRAIN, the study's range of scores for StressCT and StressTH only encompassed a small portion of their possible ranges. Out of a possible range of 0 to 166 in StressCT scores, the groups resulted in scores as low as 2 and high as 45. The same limited range was seen in StressTH scores as well. For PSS, the two groups had range of scores that were distinctly different with no overlap, with the MDD group having higher scores. The restriction in the range of scores in both measures affect the correlational coefficients, which may have impacted the absence of associations between STRAIN and glutamate change or the comparison of associations between the control and MDD group in PSS. Third, the MRS measure extracts intracellular glutamate levels, and thus do not directly represent glutamate transmission. The hypotheses in this study were formed from animal studies that were able to directly measure glutamate transmission and synaptic release. The MRS utilized in this study measured glutamate metabolite concentration, which cannot make direct inferences about neurotransmission. Lastly, the correlation between life course stress and number of depressive episodes was extracted after excluding subjects who could not provide an approximate number of recurrent episodes. The omission of these outliers may have skewed the association.

The study shows a promising start in expanding the literature on glutamatergic dysfunction and mPFC abnormalities in MDD. The results of this study were correlational and

showcased distinct glutamatergic behavior in depressed subjects. Future research should continue to identify glutamatergic dysfunction in depression, specifically through methods that allow the inference of causality. Further, additional research should be implemented to investigate the specific damages of the depressed mPFC afflicted by glutamate abnormalities and whether such harm directly reflect those in animal models. Subsequent research should also examine the gamma-Aminobutyric acid (GABA) neurotransmitter and its relationship with glutamate under the context of abnormal stress response. Future studies should also continue to explore life course stress, particularly its relationship with depressive episodes. This association may offer important context to the decoupling of stressors throughout recurrence seen in the stress sensitization model. Ultimately, the continued research on MDD glutamatergic dysfunction will garner stronger understanding of depression's pathophysiology and illness progression, allowing subsequent advancements in the disease's pharmacological treatment.

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Table 1: Demographics of Sample Groups

Variables	Control		MDD	
	N	%	N	%
Gender				
Male	7	26.9	6	27.3
Female	19	73.1	16	72.7
Race				
Asian	8	30.8	1	4.5
Black/African American	3	11.5	4	18.2
White	15	57.7	17	77.3
Ethnicity				
Hispanic	2	7.7	3	13.6
Not Hispanic	22	84.6	16	72.7
No Response	2	7.7	3	13.6

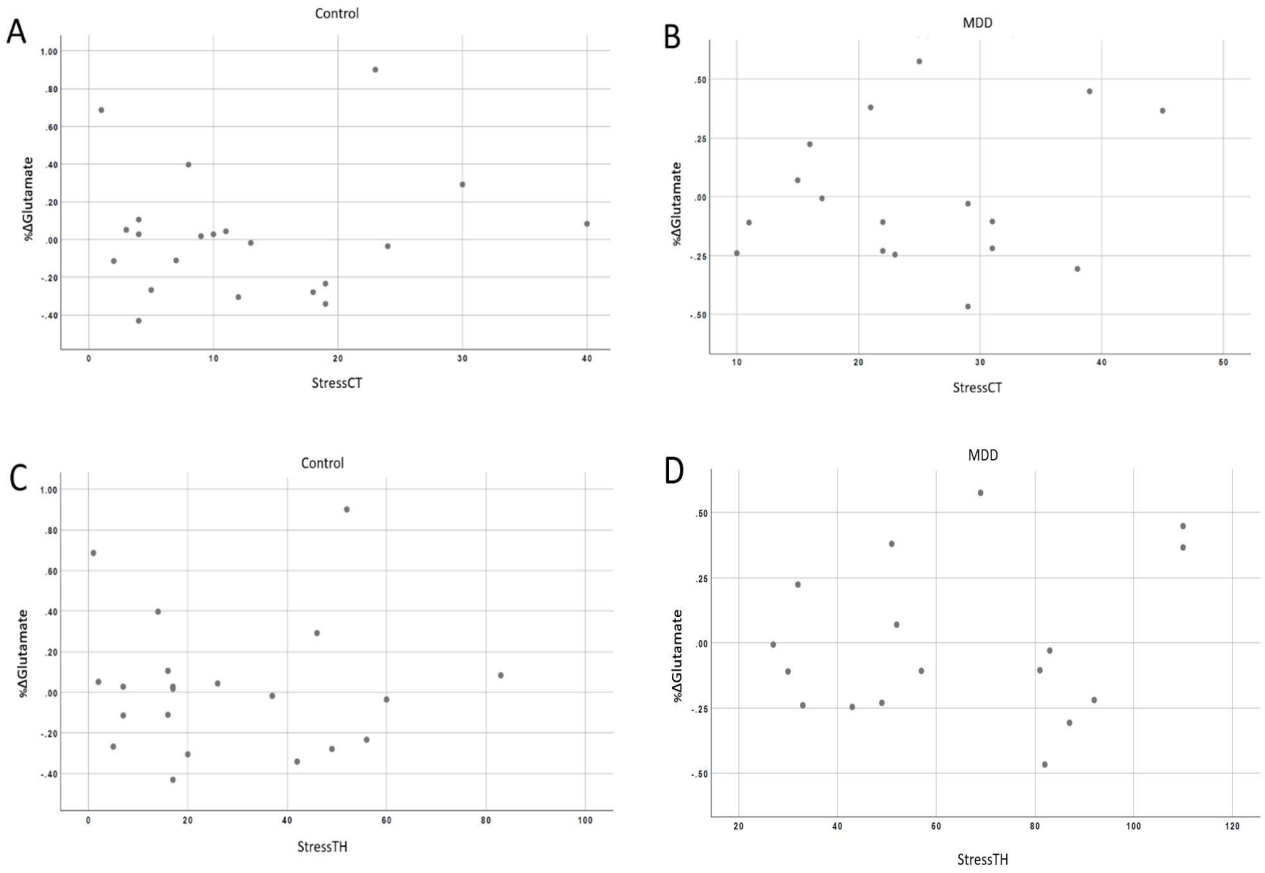

Figure 1: Changes in mPFC glutamate in relation to life course stress exposure (STRAIN). **A**. Association between total stress count (StressCT) and percent change in glutamate in control group ($r(19) = .11$, p = ns). **B**. Association between total stress count (StressCT) and percent change in glutamate in MDD group ($r(19) = .01$, p = ns). **C.** Association between total stress severity (StressTH) and percent change in glutamate in control group ($r(15) = .17$, p = ns). **D.** Association between total stress severity (StressTH) and percent change in glutamate in MDD group ($r(15) = .18$, p = ns).

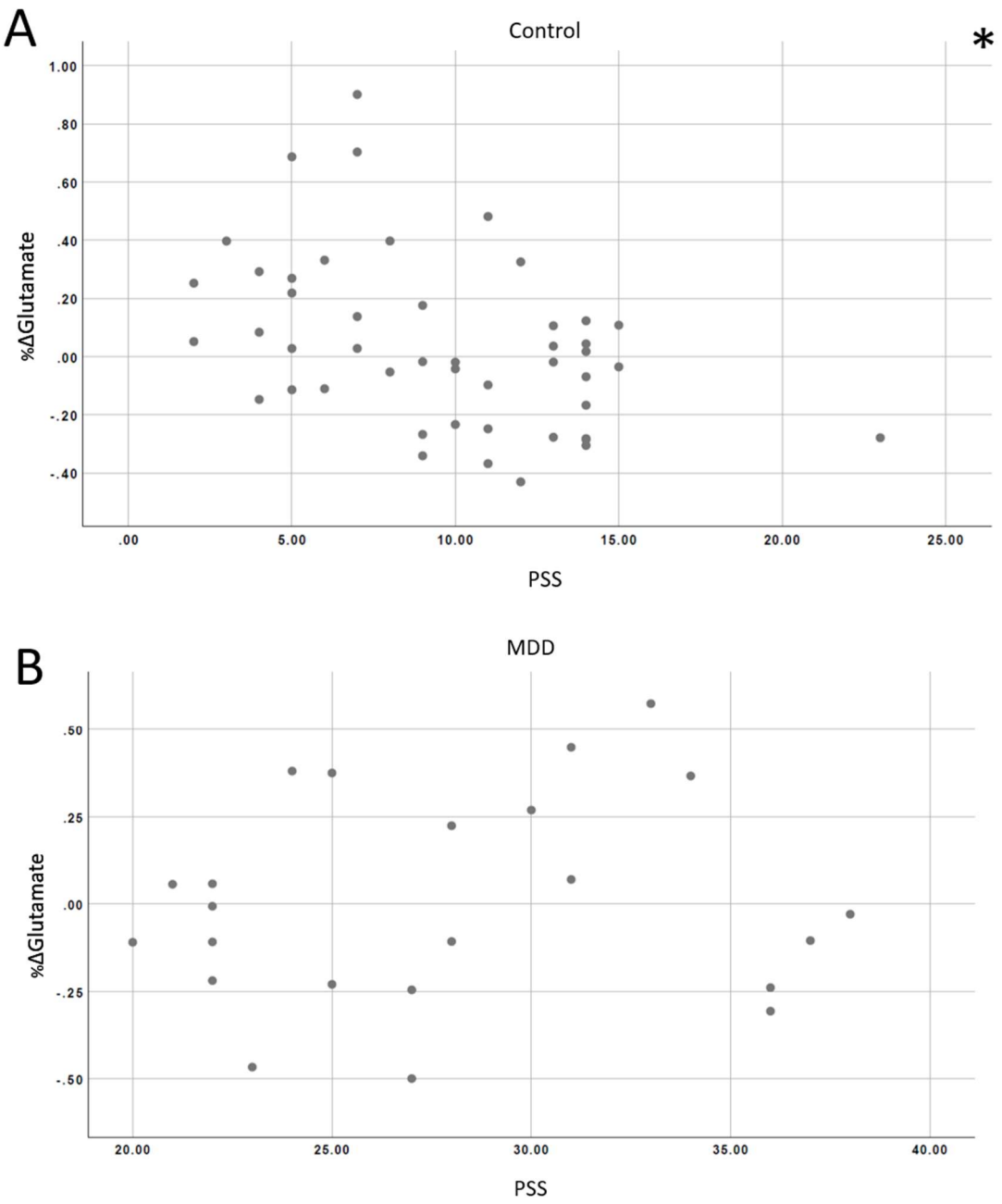

Figure 2: Changes in mPFC glutamate in relation to perceived chronic stress (PSS).

A. Association between PSS and percent change in glutamate in control group ($r(45) = -.43$, $p = .002$), *p < .05. *NB*: Effect remains significant when potential high-influence data point is removed. **B**. Association between perceived chronic stress (PSS) and percent change in glutamate in MDD group ($r(21) = .13$, p = ns).