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Pharmacological stress impairs working memory performance and attenuates dorsolateral prefrontal cortex glutamate modulation

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Abstract

Working memory processes are associated with the dorsolateral prefrontal cortex (dlPFC). Prior research using proton functional magnetic resonance spectroscopy (^{1}H fMRS) observed significant dlPFC glutamate modulation during letter 2-back performance, indicative of working memory-driven increase in excitatory neural activity. Acute stress has been shown to impair working memory performance. Herein, we quantified dlPFC glutamate modulation during working memory under placebo (oral lactose) and acute stress conditions (oral yohimbine 54mg + hydrocortisone 10mg). Using a double-blind, randomized crossover design, participants (N=19) completed a letter 2-back task during left dlPFC ^{1}H fMRS acquisition (Brodmann areas 45/46; 4.5cm^3). An automated fitting procedure integrated with LCModel was used to quantify glutamate levels. Working memory-induced glutamate modulation was calculated as percentage change in glutamate levels from passive visual fixation to 2-back levels. Results indicated acute stress significantly attenuated working memory-induced glutamate modulation and impaired 2-back response accuracy, relative to placebo levels. Follow-up analyses indicated 2-back performance significantly modulated glutamate levels relative to passive visual fixation during placebo but not acute stress. Biomarkers, including blood pressure and saliva cortisol, confirmed that yohimbine + hydrocortisone dosing elicited a significant physiological stress response. These findings support *a priori* hypotheses and demonstrate that acute stress impairs dlPFC function and excitatory activity. This study highlights a neurobiological mechanism through which acute stress may contribute to

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Contributors

EAW conducted the study, authored the manuscript, and developed the figures. EAW and JAS developed the ^{1}H fMRS experimental task, analyzed the data, and edited the manuscript. DK operated the MRI scanner and assisted with data collection. VAD assisted with experimental task development. MKG guided experimental design, pharmacological dosing, and safety protocol, and edited the manuscript. All authors have read and approved of this manuscript.

Conflict of Interest

All authors declare no conflict of interest with respect to the conduct or content of this work.

psychiatric dysfunction and derail treatment progress. Future research is needed to isolate noradrenaline vs. cortisol effects and evaluate anti-stress medications and/or behavioral interventions.

Keywords

Stress; Glutamate; ^{1}H fMRS; Working Memory; Noradrenaline; Dorsolateral; Prefrontal Cortex

INTRODUCTION

Working memory involves the maintenance of memoranda over a brief time period and is sub-served by phasic excitatory activity in the superior frontal sulcus (Goldman-Rakic, 1995). In non-human primate studies, spatial working memory is associated with persistent neuronal spiking in the dorsolateral prefrontal cortex (dlPFC) (Goldman-Rakic, 1995; Goldman-Rakic, 1999). Working memory traces are maintained by pyramidal cells in cortical layer III, which form feed-forward glutamatergic microcircuits (principally binding post-synaptic N-methyl-D-aspartate [NMDA] receptors) (Arnsten, 2009; Goldman-Rakic, 1995; Wang et al., 2013). However, these microcircuits, and associated working memory traces, can be disrupted by the local neurochemical environment. For example, noradrenaline levels alter spatial working memory proficiency (Arnsten, 2009). Emanating from the locus coeruleus, ascending noradrenergic projections terminate throughout the cortex, including the prefrontal cortex (Robbins, 2000). dlPFC neuronal spiking frequency and working memory response accuracy exhibit an inverted 'U'-shaped relationship with noradrenaline levels (reviewed (Arnsten, 2009)). Optimal dlPFC function is associated with moderate noradrenaline levels (i.e., alert) that primarily bind high-affinity post-synaptic α_2-adrenoceptors (Arnsten, 2009). However, insufficient (e.g., fatigue) or elevated (e.g., acute stress) noradrenaline levels suppress dlPFC neural spiking and impair working memory response accuracy (Arnsten, 2009; Birnbaum et al., 1999; Birnbaum et al., 2004; Ramos et al., 2005).

In humans, dlPFC function and working memory proficiency is often interrogated using the letter N-back task (Figure 1) (Owen et al., 2005). Human *in vivo* neuroimaging studies typically investigate the hemodynamic response (i.e., blood oxygen-level dependent [BOLD] response via functional magnetic resonance imaging [fMRI]) during letter N-back. Meta-analyses of BOLD fMRI data indicate N-back performance is reliably associated with robust bilateral dlPFC activation (Brodmann Areas 45/46) (Owen et al., 2005). However, the BOLD signal can be confounded by vascular and/or cardiac changes, such as elevated blood pressure and heart rate, which often accompany an acute stress response. Thus, to investigate the effects of acute stress on dlPFC function, we used a novel neuroimaging approach: functional proton magnetic resonance spectroscopy (^{1}H fMRS) (Stanley and Raz, 2018).

^{1}H fMRS facilitates *in vivo* measurement of neurochemistry at time scales less than one minute, and importantly, the ^{1}H fMRS signal is not confounded by neurovascular coupling. Seminal ^{1}H fMRS research in humans found *in vivo* glutamate levels were 2–4% higher in the occipital lobe during visual stimulation, relative to no stimulation (Mangia et al., 2007;

Schaller et al., 2013). Subsequent ^{1}H fMRS human studies found task-driven glutamate modulation throughout the human brain; occipital lobe (Apšvalka et al., 2015; Bednařík et al., 2015), motor cortex (Schaller et al., 2014b), anterior cingulate (Kühn et al., 2016; Taylor et al., 2015), and hippocampus (Stanley et al., 2017), using a variety of paradigms. Task- or stimulation-induced increase in glutamate levels reflect increased excitatory neurotransmission and metabolic activity (Sonnay et al., 2016). Thus, glutamate modulation measured via ^{1}H fMRS is a viable approach to investigate dynamic *in vivo* excitatory neural activity driven by task-related cognitive demand. Relevant to the present study, we recently found letter 2-back performance was associated with elevated *in vivo* glutamate levels (2.1–2.7%; 0.25–0.32 mmol/kg wet wt.) in the dlPFC of healthy subjects, relative to passive visual fixation, using ^{1}H fMRS (Woodcock et al., 2018b).

Study Aims and Hypotheses

In this study, we used ^{1}H fMRS to measure glutamate modulation in the left dlPFC during letter 2-back task performance during two experimental sessions on separate days. We used a double-blind, placebo-controlled and randomized crossover oral-dosing design: acute stress (Yohimbine [YOH; 54mg] + Hydrocortisone [HYD; 10mg]) vs. placebo (lactose 54mg + lactose 10mg). Oral pretreatment with YOH+HYD is a pharmacological stress-induction approach that mimics the neurochemical constituents (elevated levels of noradrenaline and cortisol) of a ‘natural’ physiological stress response (De Kloet et al., 2005). We hypothesized acute stress would impair 2-back response accuracy and attenuate 2-back-induced glutamate modulation relative to placebo levels, consistent with preclinical research (Arnsten, 2009). Specifically, we hypothesized 2-back performance would elevate glutamate levels during the placebo session (consistent with our previous findings (Woodcock et al., 2018b)) but not during the stress session.

MATERIALS AND METHODS

Participant Recruitment

The Wayne State University Institutional Review Board approved all study procedures (conducted in accordance with the Declaration of Helsinki [1964]). Male and female daily cigarette smokers were recruited via Craigslist advertisements and screened (for a parent study; full description in Supplemental Material). Briefly, volunteers without self-reported MRI contraindications, neurological or psychiatric conditions, high blood pressure, heart problems, history of seizure/stroke, and not currently taking psychoactive medications were invited to participate in a laboratory-based screening interview. Individuals without current psychiatric, cardiovascular, or MRI contraindications and not using illicit substances (urine screen; marijuana use allowed) were invited to participate after providing written informed consent.

Experimental Procedures

Participants (N=19) completed two experimental sessions (stress and placebo; randomized order) under double-blind, within-subject crossover conditions. Each experimental session was identical and was conducted between 11am (participant arrival) and 4pm (discharge). Participants could smoke cigarettes *ad libitum* prior to each session. Upon arrival, participant

sobriety was verified (expired breath alcohol <.02%). Saliva samples were collected, and vital signs (seated, resting blood pressure [BP] and heart rate [HR]) were measured, periodically throughout each session. At 11:30am, participants smoked 6 puffs over 5 minutes (experimental control for recent nicotine exposure; preferred cigarette brand). At 11:45am (75min prior to neuroimaging), participants swallowed the YOH oral dose (54mg or 0mg [placebo; 54mg lactose]). At 12:15pm (45min prior to neuroimaging), participants swallowed the HYD oral dose (either 10mg or 0mg [placebo; 10mg lactose]). From 1–2pm, participants completed the neuroimaging protocol (described below). Participants remained on site until 4pm for monitoring and additional testing.

Pharmacology

Yohimbine (YOH) is a presynaptic α_2-autoreceptor antagonist that disinhibits noradrenaline release (Doxey et al., 1984). Biomarkers of YOH dosing include BP and saliva α-amylase (indirect biomarker of β-adrenoceptor stimulation) (Ehlert et al., 2006; Murburg et al., 1991). Hydrocortisone (HYD) is a glucocorticoid/mineralocorticoid receptor agonist that dose-dependently increases plasma cortisol levels (Meikle and Tyler, 1977). Salivary cortisol is a well-validated correlate of plasma cortisol levels and hypothalamic-pituitary-adrenal (HPA)-axis activity (Kahn et al., 1988). In combination, YOH+HYD mimic the neurochemical response of the autonomic nervous system (ANS) and HPA-axis to a stressful event.

Neuroimaging Paradigm

Prior to the MRI scan, task instructions were provided verbally, and each subject practiced the task until deemed proficient by the experimenter (Figure 1). The task consisted of two phases: flashing grayscale checkerboard (3Hz for 208s) followed by five blocks of alternating periods of passive visual fixation (32s) and letter 2-back (64s). The flashing checkerboard was implemented to minimize dlPFC glutamate variability prior to investigation of task-related modulation (Lynn et al., 2018), consistent with our prior work (Woodcock et al., 2018b). Participants were instructed to relax and focus their gaze on screen during the flashing checkerboard. The passive visual fixation periods were prompted with on-screen instructions (“Rest”; 2s) prior to static, centered fixation cross (30s). Similarly, each block of letter 2-back was prompted with instructions (“2-back”; 4s) followed by serial presentation of 20 capitalized letters that were 3s apart and displayed on-screen for 500ms followed by blank screen for 2500ms. Each block had 6 target letters that were pseudo-randomly dispersed. Subjects responded via button press with the right-hand index finger if the current letter matched the letter presented two previously. For each block, response accuracy was quantified as the percentage of correct responses and feedback was not provided to participants.

Neuroimaging Protocol

Neuroimaging was conducted on a 3T Siemens Verio system (32-channel receive-only head coil). First, T_1-weighted structural scans were collected using the 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence with the following parameters: TR=2.2s, TE=3ms, TI=799ms, flip angle=13°, field-of-view (FOV)=256×256×160mm^3, matrix=176×256×160, and pixel resolution 1×1×1mm^3. Second, the left dlPFC B_0-field was

shimmed (25×25×25mm; FASTESTMAP (Tkáć and Gruetter, 2005)) to maximize homogeneity in a volume larger than the MRS voxel from which spectra were acquired. Third, the left dlPFC voxel was prescribed 1.5×2.0×1.5cm^3; 4.5 cm^3; Brodmann Areas 45/46; Figure 2A) based on our prior research (Woodcock et al., 2018a). Voxels were prescribed manually (guided by 2D anatomical images of optimal placement) for initial participant scans (n=6). Subsequent scans were guided (n=11), or fully-prescribed (n=21), using the automated voxel placement (AVP) method inside the shimmed volume (Woodcock et al., 2018a). Fourth, ^{1}H fMRS spectra were continuously acquired every 16s (PRESS with OVS and VAPOR, TE=23ms, TR=4.0s, 4 averages/spectrum, bandwidth = 2kHz, 2048 data points, no apodization) during letter 2-back totaling 43 spectra or 688s. The first spectra (16s) during the flashing checkerboard was excluded from analyses due to possible partial T_1 saturation effects resulting in 42 spectra. A relatively short TE reduced the influence of J-evolution and diffusion and the relatively long TR ensured minimal T_1-weighted effect on the acquired signal. Fifth, a ^{1}H fMRS spectrum without water suppression was acquired (TE=23ms, TR=10s, 2 averages) immediately after the 2-back task from the same voxel location. Finally, following ^{1}H fMRS spectra acquisition, participants completed 2 repetitions of the letter 2-back task during fMRI acquisition (gradient echo planar imaging sequence: TE=36ms, TR=2.83s, matrix=80×80, 40 interleaved slices, voxel size=2.9mm isotropic; identical task parameters as ^{1}H fMRS).

Analysis Strategy

^{1}H fMRS spectra post-processing and metabolite quantification steps were 100% automated (LCModel v6.3) (Provencher, 2008). Raw spectra were eddy current-corrected using the unsuppressed water signal (Klose, 1990), and phase- and shift-corrected prior to metabolite quantification. T_1-weighted images were B_1-field corrected and the brain image was segmented into partial volume maps of cerebrospinal fluid, gray matter, and white matter using FreeSurfer and FSL tools (e.g., FLIRT, BET, and FAST) (Dale et al., 1999; Smith et al., 2004). Finally, voxel tissue composition and appropriate correction factors from the published literature (e.g., T_1 and T_2 relaxation; (Posse et al., 2007)) were used to quantify absolute glutamate concentration (mmol/kg wet weight; (Gasparovic et al., 2006)). Consecutive spectra were phase- and shift-corrected and then averaged prior to LCModel fitting, which resulted in a 32s temporal resolution (8 averages/spectra). As such, each 64s 2-back task block was cleaved into ‘early’ (first 32s) and ‘late’ (final 32s) 2-back levels. Rationale for this approach was threefold: 1) We used a classical block design, typical in fMRI research. Thus, to match the temporal resolution of the passive visual fixation periods (32s) and avoid bias on the quantification due to poor signal-to-noise ratio (SNR), 2-back blocks were bisected into ‘early’ and ‘late’ 2-back. 2) This approach is consitent with our prior work (Woodcock et al., 2018b) and 3) facilitated examination of the temporal dynamics of glutamate across 2-back task blocks, consistent with prior research (Michels et al., 2012).

Prior to outcome analyses, skewness/kurtosis statistics and the Shapiro-Wilk test of normality were used to evaluate variable distributions. Extreme values were winsorized by replacing the extreme value with nearest value such that final distributions approximated normality prior to outcome analyses. *A priori* hypotheses for neurochemical, physiological,

and behavioral data were evaluated using within-subject two-way repeated measures analyses of variance (rmANOVAs) that contrasted experimental session (placebo vs. stress). Working memory-induced glutamate modulation was calculated as percentage change relative to the preceding passive visual fixation block: [(2-back – visual fixation) / (visual fixation)] * 100. One-way rmANOVAs, paired t-tests, and/or descriptive statistics were used to interpret significant main effects. Voxel overlap across participants was quantified using the 'avp_overlap' script (included in the AVP suite) (Woodcock et al., 2018a). 3D geometric voxel overlap percentage in template space was calculated between each subject's voxel and the template voxel (i.e., voxel placement accuracy).

In addition to the above *a priori* analyses, we conducted several exploratory analyses. First, two prominent metabolites hypothesized to be unaffected by working memory modulation, i.e. negative control metabolites, were evaluated. Working memory-induced modulation of N-acetyl-aspartate (NAA) and phosphocreatine plus creatine (PCr+Cr) was evaluated using two-way rmANOVAs separately for early and late 2-back modulation (Bonferroni-corrected threshold: $p<.025$). Second, the change in LCModel fit characteristic levels from the preceding block of visual fixation to early 2-back and from visual fixation to late 2-back were also evaluated using two-way rmANOVAs: full-width half-maximum (FWHM) and CRLB% of glutamate (Bonferroni-corrected threshold: $p<.025$). Third, Pearson correlations evaluated possible relationships between 2-back behavioral data, physiological indices, and glutamate modulation during the stress session (uncorrected threshold: $p<.05$). Descriptive statistics are presented as mean ± one standard deviation (M ± 1 SD) unless otherwise noted. In all figures, error bars depict ± one standard error of the mean (SEM).

RESULTS

Sample Characteristics

Subjects were 27.5 (±3.9) years old (range: 21–34), mostly male (84.2%), and African-American (78.9%).

Stress Response Biomarkers

Resting and seated BP, HR, and saliva cortisol and α-amylase were measured prior to YOH +HYD dosing, before the MRI scan, and after the scan. Two-way rmANOVAs indicated significant time by experimental session interactions confirming YOH+HYD significantly increased systolic and diastolic BP and saliva cortisol throughout the stress session, relative to placebo levels (ps<.05; see Supplemental Material; Table S1). Saliva α-amylase levels significantly increased during the stress session ($p<.05$; partial $\eta^2=0.17$ [moderate-to-large effect]), but not the placebo session ($p=.41$).

Experimental Session Order Effects

Glutamate modulation and 2-back proficiency were not affected by experimental session order (ps>.45).

Behavioral Data

As hypothesized, a two-way rmANOVA indicated acute stress significantly impaired response accuracy by ~10% relative to placebo levels ($F(1,40)=6.01$, $p=.034$; partial $\eta^2=0.38$ [large effect]; 87.1%±13.3% vs. 78.2%±15.1%; Figure 3A). Response accuracy significantly improved across task blocks during each experimental session ($F(4,40)=6.98$, $p<.001$; partial $\eta^2=0.41$ [large effect]; ~77% to ~86%), indicative of practice effects. Finally, a significant task block by experimental session interaction ($F(4,40)=2.60$, $p=.05$; partial $\eta^2=0.21$ [moderate-to-large effect]) indicated response accuracy increased more across task blocks during placebo than acute stress.

A two-way rmANOVA indicated response latency (ms) significantly decreased across task blocks ($F(4,40)=2.79$, $p=.039$; partial $\eta^2=0.22$ [moderate-to-large effect]; Figure 3B), indicative of practice effects. There were no effects of experimental session ($p=.97$) or task block by experimental session interaction ($p=.29$).

^{1}H fMRS Voxel Placement

Mean 3D geometric voxel overlap with the template voxel across subjects was 85.6±16.8%. Mean gray and white matter voxel composition was 34.5% and 62.7%, respectively, which did not differ between sessions ($ps\geq.10$). The voxel was co-located with BOLD fMRI activation during letter 2-back (>rest) collected immediately after ^{1}H fMRS acquisition (Figure 2B, 2C).

Glutamate Modulation

A two-way rmANOVA indicated that ‘basal’ glutamate levels during passive visual fixation blocks did not differ by experimental session ($ps>.50$).

Glutamate modulation was calculated separately for early and late 2-back as the percentage change in glutamate levels relative to preceding passive visual fixation levels for each task block. Two-way rmANOVAs were used to test the effects of acute stress on dlPFC glutamate modulation. Results indicated acute stress significantly attenuated early 2-back-induced glutamate modulation relative to placebo modulation ($F(1,72)=6.02$; $p=.025$; partial $\eta^2=0.25$ [large effect]; Figure 4A). As hypothesized, follow-up one-way rmANOVAs indicated early 2-back significantly modulated glutamate levels during the placebo session ($F(1,83)=8.12$; $p<.01$; partial $\eta^2=0.09$; [moderate effect]; early 2-back glutamate levels were 2.7% higher than visual fixation levels: 11.6±1.1 vs. 11.3±1.0 mmol/kg wet wt.; Figure 4C), but not different during the stress session ($p=.58$; 11.4±1.0 vs. 11.4±1.0 mmol/kg wet wt.; Figure 4D).

Results indicated no significant main effect of acute stress on late 2-back-induced glutamate modulation relative to placebo modulation ($p=.40$; Figure 4B). Interestingly, late 2-back glutamate modulation exhibited a ‘trend’-level task block by experimental session interaction ($F(4,72)=2.22$; $p=.075$; partial $\eta^2=0.11$ [moderate effect]; Figure 4B) suggesting glutamate modulation nominally increased across task blocks during placebo but not stress.

Exploratory Bivariate Correlations

Exploratory Pearson correlations examined relationships between glutamate modulation and physiological indices for the stress session. Systolic and diastolic BP levels were significantly negatively correlated with late 2-back glutamate modulation during task block 5 (Pearson r=−.54, p=.016 and r=−.51, p=.027, respectively). Saliva cortisol levels were 'trend'-level positively correlated with late 2-back glutamate modulation during task block 3 (Pearson r=.44, p=.07). No significant Pearson correlations were observed between glutamate modulation and 2-back response accuracy for either session.

^{1}H fMRS LCModel Fit Characteristics

Replicating the above analysis strategy, we calculated the percentage change in LCModel fit characteristics from visual fixation to early 2-back and from visual fixation to late 2-back separately for FWHM and glutamate CRLB%. Exploratory two-way rmANOVAs evaluated the percentage change in LCModel fit characteristics contrasted by experimental session. Two-way rmANOVAs indicate no significant differences between experimental sessions for early or late 2-back glutamate CRLB% or FWHM (ps>.10 and ps≥.044; respectively; Bonferroni-corrected threshold: p<.025). Task-induced linewidth (FWHM) narrowing, i.e. the 'BOLD' effect, was smaller in this study (0.1–0.2 Hz) than previous research (e.g., 0.5 Hz; (Mangia et al., 2007)). However, if the BOLD effect was present in this study, one would assume that both glutamate and NAA would be affected. And, since NAA was not modulated by 2-back performance (see below), the glutamate/NAA ratio provides a secondary test of nominal linewidth changes influencing glutamate findings. Two-way rmANOVA indicated acute stress significantly attenuated glutamate/NAA ratio modulation relative to placebo levels, F(1,72)=7.34, p=.014, consistent with above glutamate modulation findings. Thus, these findings reaffirm linewidth changes (BOLD effect) did not confound the working memory-induced glutamate modulation effects herein.

Neurochemical Specificity

Two-way rmANOVAs indicated acute stress did not alter early or late 2-back-induced modulation of PCr+Cr or NAA levels relative to placebo (ps>.15 and ps>.20, respectively).

DISCUSSION

In this study, we measured dlPFC glutamatergic response to working memory task performance during two oral-dosing conditions, acute stress (YOH 54mg + HYD 10mg) and placebo (lactose 54mg + lactose 10mg), using a within-subject, randomized crossover design. Principal findings were threefold. First, during placebo, working memory processes significantly increased left dlPFC glutamate levels by 2.7% relative to periods of passive visual fixation. Second, acute stress significantly attenuated working memory-induced glutamate modulation relative to placebo levels. Third, acute stress impaired 2-back response accuracy relative to placebo levels. These findings provide empirical support consistent with several putative neurobiological mechanisms: 1) working memory processes drove phasic increases in excitatory neurotransmission and metabolic activity in the left dlPFC as measured by ^{1}H fMRS glutamate modulation; and 2) acute stress impairs working memory function via disruption of phasic excitatory neural activity in the left dlPFC.

Throughout the remainder of this manuscript, we interpret our findings and discuss these putative mechanisms.

During placebo, our results indicate letter 2-back response accuracy was generally high across trials (87% correct). 2-back task performance significantly increased dlPFC glutamate levels by 2.7% above passive visual fixation levels, replicating our prior work (Woodcock et al., 2018b). We interpret elevated glutamate levels, as measured by ^{1}H fMRS, to reflect increased excitatory neurotransmission *and* increased metabolic activity driven by working memory-related cognitive demands. ^{13}C MRS research indicates a tight, nearly 1:1, coupling between glutamate-glutamine cycling rate (excitatory neurotransmission) and neuronal oxidative metabolism [CMR_{GLC}; reviewed (Rothman et al., 2011)]. Thus, phasic increases in excitatory neurotransmission likely correspond with increased metabolic activity. Indeed, a recent preclinical study reported direct evidence of this relationship. The authors in that study reported forepaw (front limb) stimulation drove a concurrent increase in somatosensory cortex glutamate levels, as measured via ^{1}H fMRS, and *in vivo* CMR_{GLC} (Sonnay et al., 2016). These findings are analogous to human findings indicating periodic finger-tapping modulated motor cortex glutamate levels measured via ^{1}H fMRS (Schaller et al., 2014a). In the present study, we speculate that working memory traces, i.e., the neural maintenance of 2-back letters prior to a match and participant response, drove a phasic increase in excitatory neural activity and oxidative metabolism in the dlPFC, consistent with primate research (Arnsten, 2009). Arnsten and colleagues (reviewed (Arnsten, 2009)) have repeatedly demonstrated that spatial working memory traces are associated with more frequent neural spiking activity in the dlPFC. Excitatory feed-forward microcircuits in the dlPFC are thought to maintain spatial information prior to primate response in spatial working memory tasks. We speculate our findings reflect a macroscopic index of similar neurobiological processes in humans.

During the acute stress session, oral pretreatment with YOH+HYD significantly increased resting systolic and diastolic BP, HR, saliva cortisol, and saliva α-amylase levels, indicative of a physiological stress response (see Supplemental Material; Table S1), consistent with prior research (Greenwald et al., 2013). In terms of magnitude, we estimate YOH+HYD dosing induced a physiological stress response comparable to robust psychosocial stress-induction approaches (public speaking + cognitive tasks) as indicated by qualitative comparison with the published literature. Mean effect sizes in the present study were higher for saliva cortisol (Cohen's d=1.61 vs. 1.09 (Dickerson and Kemeny, 2004)), nearly identical for systolic BP (Cohen's d=1.17 vs. 1.18 (Ginty et al., 2014)), and lower for HR (Cohen's d=0.26 vs. 1.53 (Ginty et al., 2014)), relative to the literature. Thus, our findings indicate YOH+HYD dosing elicited an acute stress response comparable to robust psychosocial approaches.

Consistent with *a priori* hypotheses, acute stress significantly impaired 2-back response accuracy and attenuated working memory-induced glutamate modulation, relative to placebo levels. In contrast to the placebo session, 2-back performance did not alter dlPFC glutamate levels relative to a benign condition, passive visual fixation, thought to reflect 'basal' glutamate levels (Lynn et al., 2018). It is well-established letter 2-back task performance is dlPFC-dependent (Owen et al., 2005). Thus, our results indicate acute stress impairs dlPFC

function as indicated by 2-back response accuracy and working memory-induced modulation of dlPFC excitatory neurotransmission activity, i.e., glutamate modulation. Our findings are strengthened by the within-subject design as each subject served as his/her own control. Moreover, glutamate levels during passive visual fixation were not altered by acute stress. Thus, acute stress effects were specific to working memory-induced glutamate modulation and did not alter 'initial' or 'basal' glutamate levels during passive visual fixation. Finally, other metabolites, NAA and PCr+Cr, were unaffected by acute stress – thus, highlighting the neurochemical specificity of this effect.

We speculate that YOH dosing and the subsequent increase in noradrenaline levels drove these acute stress effects (Figure 5A); not HYD dosing and elevated cortisol levels. Evidence in support of this hypothesis is fourfold. First, biomarkers measured in this study (BP and saliva α-amylase) indicated that YOH significantly increased noradrenaline levels. Second, during the stress session, bivariate correlations indicated negative relationships between physiological indices influenced by noradrenaline levels, e.g., systolic and diastolic BP, and working memory-induced glutamate modulation. Conversely, higher saliva cortisol levels during stress were 'trend'-level related to greater glutamate modulation during 2-back. Thus, higher BP (and presumably noradrenaline) levels were related to less dlPFC excitatory activity, while the converse was true of cortisol levels. Third, prior research in non-human primates indicated that spatial working memory response accuracy and dlPFC neuronal spiking frequency exhibited an inverted 'U'-shaped relationship with noradrenaline levels (Figure 5B) (Arnsten, 2009). Elevated noradrenaline levels (i.e. stress or YOH dosing) reliably suppress dlPFC spiking activity and impair working memory response accuracy (Arnsten, 2009). During acute stress, noradrenaline floods the synapse, saturates high-affinity α_2-adrenoceptors, and 'spills over' onto lower-affinity α_1- (Arnsten et al., 1999) and β_1-noradrenergic receptors (Ramos et al., 2005) which disrupt the excitatory microcircuits that maintain working memory traces, and thus, impair proficiency (Arnsten, 2009). Fourth, prior research indicates HYD dosing exhibits a range of working memory effects: impairment (Lupien et al., 1999), enhancement (Henckens et al., 2011), and no effect (Henckens et al., 2011; Wingenfeld et al., 2011)). We speculate the low HYD dose used herein (oral 10mg) was unlikely to significantly alter working memory proficiency. However, future studies are needed to confirm this hypothesis and rule out the possible contribution of HYD and cortisol effects.

Stress-induced disruption of dlPFC excitatory activity and cognitive function is important for numerous behavioral health conditions. The dlPFC is associated with cognitive processes central to sustained behavior change mandated by treatment interventions (i.e., self-control, delayed gratification, and goal-directed behavior (Figner et al., 2010; Hare et al., 2014; McClure et al., 2007; McClure et al., 2004)). Thus, stress-induced impairment of dlPFC function could derail behavioral interventions, which has sweeping healthcare implications. High-frequency repetitive transcranial magnetic stimulation and/or cognitive training may bolster one's resilience to acute stress effects. In addition, 'anti-stress' medications that block α_1- and β_1-noradrenergic receptor stimulation (prazosin + propranolol) may rescue dlPFC cognitive functions during acute stress (Greenwald, 2018). We believe this experimental approach – pharmacological stress-induction and task-induced ^{1}H fMRS glutamate modulation – has widespread applicability in the study of brain function in health

and disorders (Stanley and Raz, 2018). Unlike other neuroimaging approaches, this experimental approach facilitates direct investigation of neurochemical mechanisms that modulate cognitive processes, excitatory neural activity, and behavior.

This study had several noteworthy methodological strengths. First, participants were carefully screened for participation. Second, we used a rigorous experimental design: double-blind, placebo-controlled, and randomized crossover. Third, subjects typically (76%) completed both experimental sessions within seven days. Finally, study design and hypotheses were informed by strong preclinical evidence. Several limitations should be mentioned. First, as with all ^{1}H fMRS studies, partial volume effects were possible. However, these effects were minimized by reliable voxel placement. Second, due to SNR limitations, GABA and glutamine levels were not quantified reliably. Third, we did not measure neurochemistry from a “control” voxel location. Fourth, the mean spectral linewidth difference between the 2-back and fixation conditions (i.e. the BOLD effect) for the placebo and stress sessions were 0.1 and 0.2 Hz, respectively, which are smaller than previous studies conducted at higher field strength (e.g., 0.5 Hz; (Mangia et al., 2007)), and did not exceed the Bonferroni-corrected threshold (p<.025). Prior estimates (Mangia et al., 2006) indicate these subtle linewidth differences are unlikely to significantly alter glutamate levels, consistent with our glutamate/NAA ratio findings. Fifth, the sample size is small and has notable limitations. All subjects were cigarette smokers recruited for a parent study and thus, findings need to be replicated in healthy volunteers. Also, subjects were disproportionality African-American and male. Sixth, 2-back behavioral data were lost due to a data collection error (~23%).

In summary, our findings indicated that working memory processes modulated glutamate levels in the left dlPFC, replicating our prior research (Woodcock et al., 2018b). Task-induced glutamate modulation likely reflects increased excitatory neurotransmission and metabolic activity. This finding contributes to the growing literature indicating ^{1}H fMRS provides a framework, not confounded by neurovascular coupling, to investigate dynamic changes in task-driven excitatory neural activity (Stanley and Raz, 2018). Acute stress attenuated working memory-induced glutamate modulation and impaired 2-back response accuracy relative to placebo levels. We speculate that elevated noradrenaline levels disrupted dlPFC excitability and impaired working memory processes. Future studies are needed to confirm this hypothesis. Stress-induced impairment of dlPFC function is important for healthcare and behavior change interventions ranging from psychiatric disorders, e.g., substance use; stress-induced relapse, to medical conditions, e.g., obesity; stress-induced overeating. Supplementary information available at the *NeuroImage* website.

Supplementary Material

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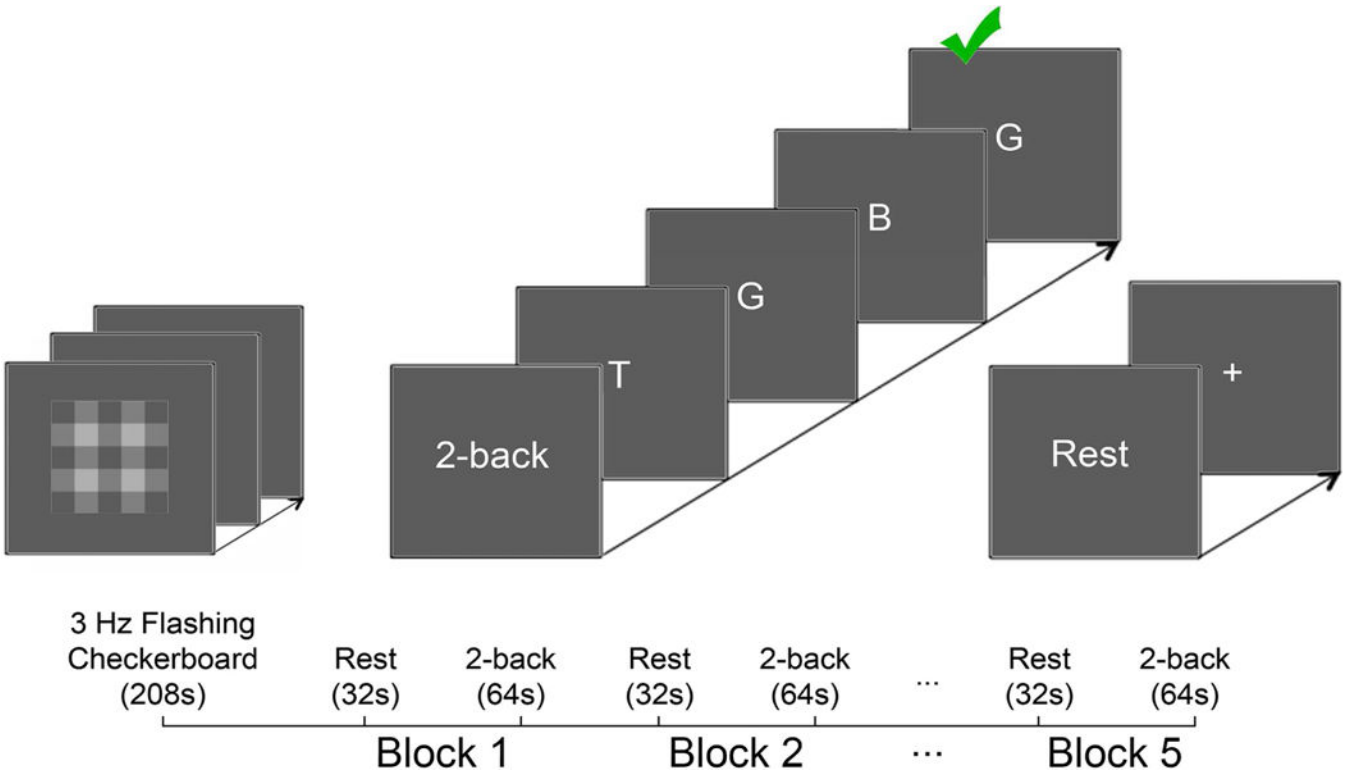

Figure 1–.

The experimental paradigm is depicted. Flashing checkerboard (3Hz; 208s) was followed by five repetitions (blocks) of alternating periods of passive visual fixation (32s; 2s instructions "Rest", 30s static fixation cross) and letter 2-back (64s; 4s instructions "2-back", 20 serial capitalized letters [3s/letter]).

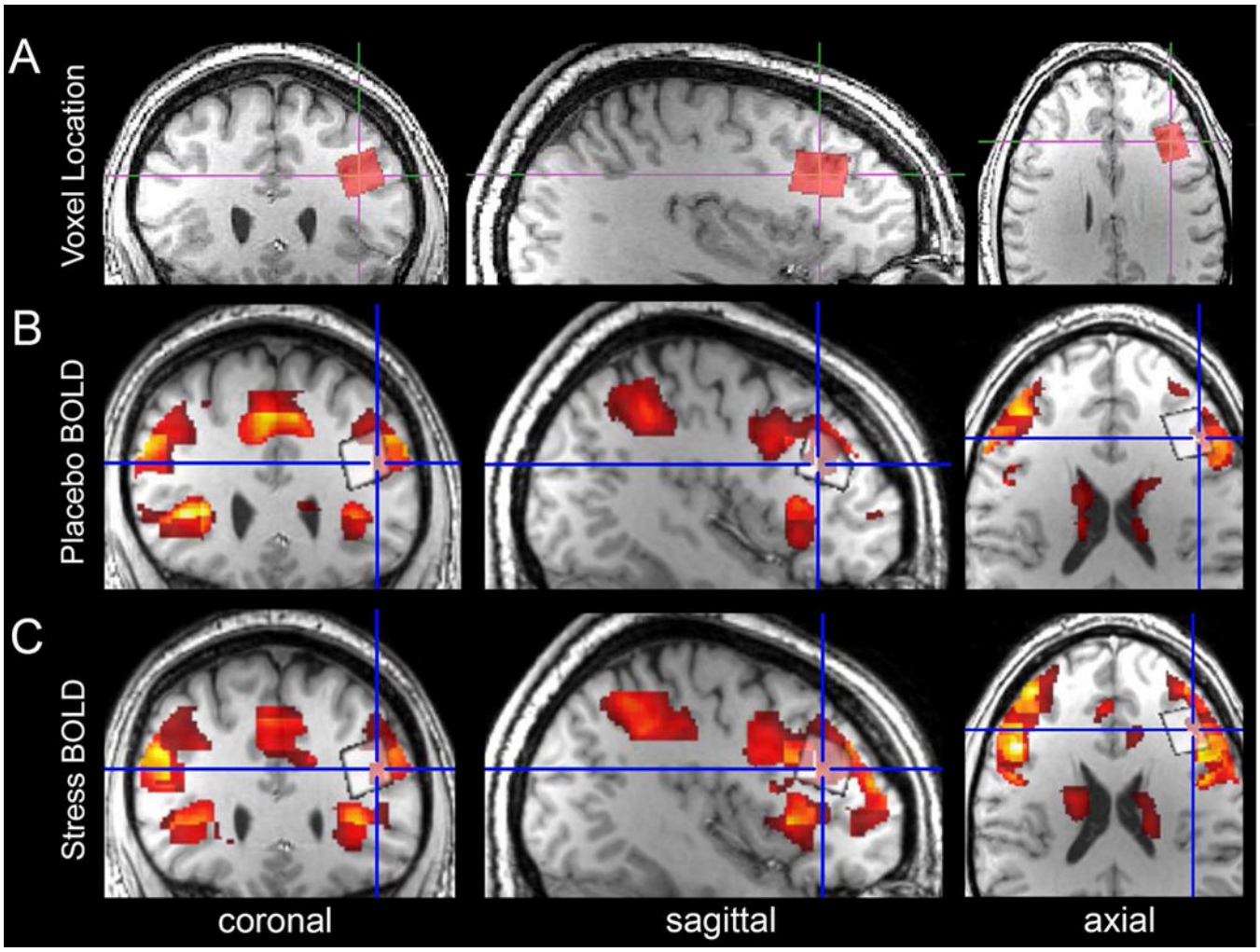

Figure 2–.

A) The voxel location is depicted in orthonormal slices. The voxel (15×20×15mm; 4.5cm^3) was placed in the left dlPFC (Brodmann Areas 45/46) based on our prior research (Woodcock et al., 2018b). B) Immediately following the ^{1}H fMRS letter 2-back task, BOLD fMRI data were collected during two repetitions of letter 2-back (identical task parameters) during both experimental sessions. BOLD fMRI activation (2-back > passive visual fixation) during the placebo session and the ^{1}H fMRS voxel are depicted on orthonormal slices. C) BOLD fMRI activation (2-back > passive visual fixation) during the stress session and the ^{1}H fMRS voxel are depicted on orthonormal slices.

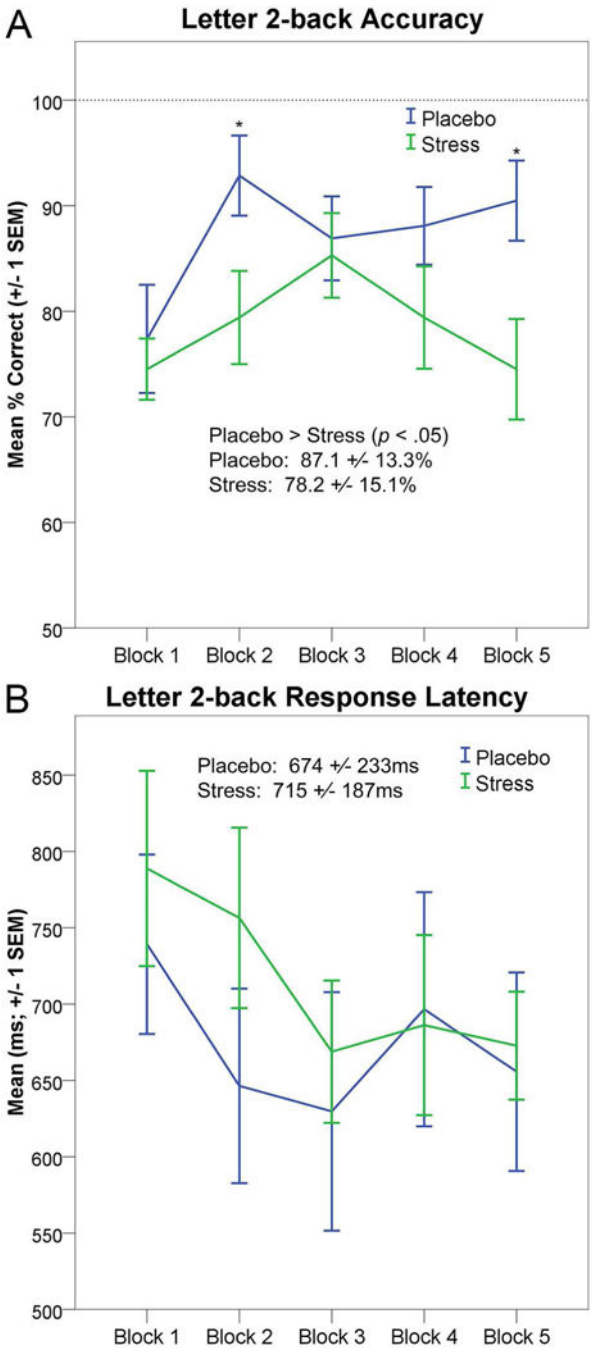

Figure 3–.

A) Letter 2-back response accuracy (% correct) across task blocks is depicted. Participants responded more accurately across task blocks on average during placebo (blue) than stress (green). Paired t-test: *$p < .05$, **$p < .01$. B) Letter 2-back response latency (ms) across task blocks is depicted. Response latency decreased across task blocks for both experimental sessions. Error bars: ± 1 SEM.

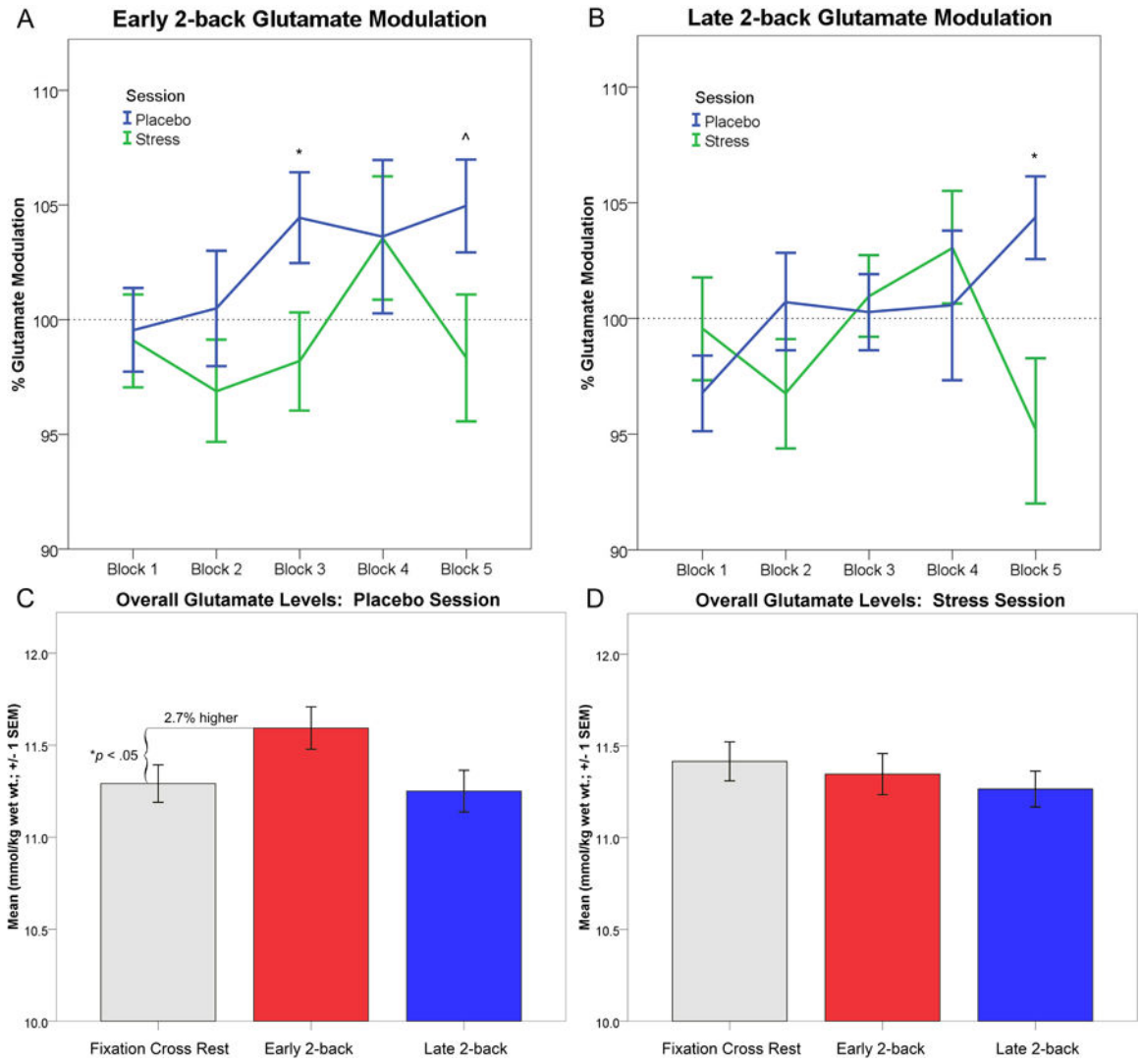

Figure 4–.

Glutamate modulation was calculated separately for early and late 2-back as the percentage change in glutamate levels during each task block relative to the preceding block of passive visual fixation. A) Mean early 2-back glutamate modulation is depicted across task blocks for the placebo (blue) and stress (green) sessions. B) Mean late 2-back glutamate modulation is depicted across task blocks for the placebo (blue) and stress (green) sessions. C) Mean glutamate levels for each task phase (passive visual fixation [light gray], early 2-back [red], and late 2-back [blue]) during the placebo session are depicted. D) Mean glutamate levels for each task phase during the stress session are depicted. Error bars: ± 1 SEM. ^Paired t-test; $p \leq .10$; *Paired t-test; $p < .05$.

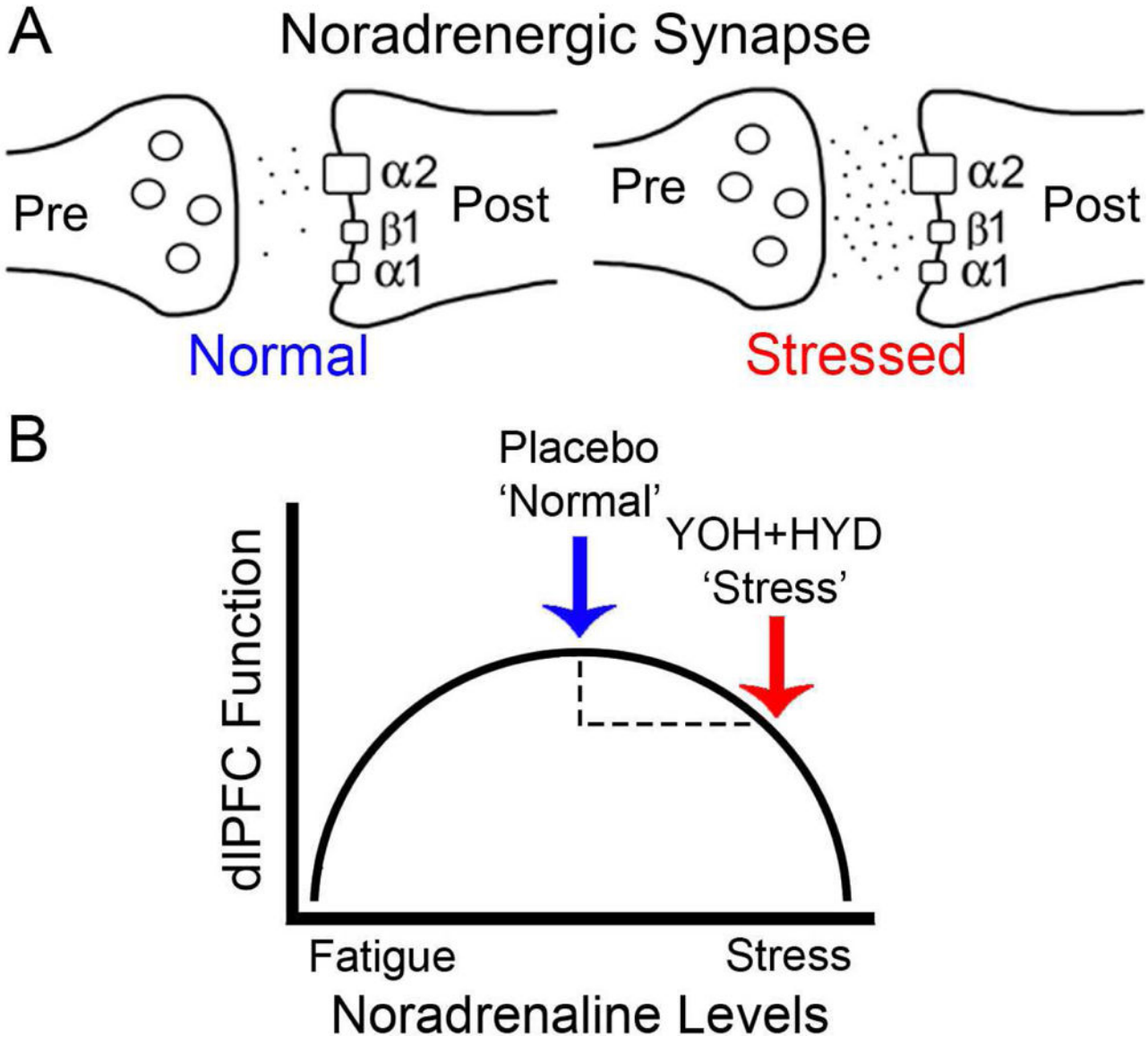

Figure 5–.

A) A cartoon illustration of a noradrenergic synapse during 'normal' (blue) and 'stressed' (red) conditions are depicted. Moderate levels of postsynaptic receptor stimulation are mediated primarily through the high-affinity α_2-adrenoceptor during 'normal' conditions. During acute stress (i.e., YOH dosing), the synapse is flooded with noradrenaline, which binds lower-affinity receptors, including α_1- and β_1-adrenoceptors. B) The theorized inverted 'U'-relationship between noradrenaline levels and dlPFC function is depicted. YOH +HYD dosing increased noradrenaline levels which impaired 2-back response accuracy and disrupted dlPFC engagement/excitatory activity (glutamate modulation).

Table 1:

LCModel fit characteristics

	Fit Characteristic	Early 2-back	Late 2-back	Visual Fixation
Placebo	Glutamate CRLB%	6.5 ± 0.9	6.6 ± 0.9	6.6 ± 0.9
	FWHM Hz	5.0 ± 0.7	5.0 ± 0.9	5.1 ± 0.8
	SNR	13 ± 2	13 ± 2	13 ± 2
Stress	Glutamate CRLB%	6.7 ± 0.8	6.8 ± 0.6	6.7 ± 0.9
	FWHM Hz	4.8 ± 0.5	4.8 ± 0.5	5.0 ± 0.5
	SNR	12 ± 2	12 ± 2	12 ± 2

Note: LCModel fit characteristics (32s temporal resolution; 8 averages/spectra) were contrasted by 2-back task phase (early and late 2-back vs. passive visual fixation). CRLB% = Cramer-Rao Lower Bound percentage, FWHM = full-width half-maximum, SNR = signal-to-noise ratio; Hz = Hertz.