The Relationship Between Neocortical ^{1}H MRS Metabolites and Subjective Experience in Healthy Adults

By

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This thesis by Meghan Anne Gonsalves is accepted in its present form by the Department of Behavioral and Social Sciences as satisfying the thesis requirements for the degree of Master of Science

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Abstract

Introduction: Glutamate (Glu) is the major excitatory neurotransmitter in the brain, yet its direct contributions to subjective experience in healthy humans have yet to be explored. Research involving glutamatergic antagonists' effects on subjective experiences in clinical populations and animal models has been well established, demonstrating a relationship between Glu and positive emotion. Thus, we hypothesized that Glu agonist, i.e. psychostimulant, induced changes in glutamatergic compounds would be positively associated with positive affective states. *Methods:* Using ^{1}H MRS, we recorded drug-induced changes in glutamatergic compounds, such as Glu, glutamate and glutamine (pooled as Glx), glutamine (Gln), creatine (tCr), myo-inositol (Ins), and choline (Cho), in the dorsal anterior cingulate cortex (dACC) of healthy male and female adults (M = 22.3 years). In a placebo-controlled, double-blinded, within-subjects crossover design, healthy participants (N = 26) were administered single clinically relevant doses of d-amphetamine (AMP; 20 mg), methamphetamine (MA; Desoxyn®, 20 mg), and placebo (PBO) on three separate test days, each serving as their own control. At 140-150 m post ingestion, ^{1}H MRS was conducted in the dACC. Self report measures of subjective experience were administered outside the scanner 15 mins before taking the capsule and at half hour intervals after ingestion, for a total of eight completion points during each test session. *Results:* Using principal components analysis, four AMP induced subjective experiences in comparison to PBO: Activated Positive Affect, Somatic Sensations, Activated Negative Affect, and Anxious Distress. We also found three MA induced effects in comparison to PBO: Activated Positive Affect, Activated Negative Affect, and Somatic + Low Negative Affect. In females, AMP-induced changes in Glu were significantly correlated with activated positive affect, r = .609, p < .05, and aversive Somatic Sensations were negatively correlated with AMP-induced changes in tCr, r = -.721, p < .01, and duration of drug liking, r = -.806, p < .01. In males, a negative association was found between AMP induced changes in Gln and Activated Negative Affect, r = -.624, p < .05. Similarly in females, MA induced changes in Gln were negatively related to Activated Negative Affect, r = -.705, p < .05. AMP induced changes in Gln were positively related to Anxious Distress in females, r = .613, p < .05. *Conclusions:* These findings corroborate the literature on heightened responsivity to the subjective emotional impact of changes in glutamatergic compounds after psychostimulant exposure in females. Future studies should investigate the relationship between ovarian hormones and psychostimulant-induced changes in glutamatergic compounds.

Introduction

Despite glutamate's (Glu) importance as the major excitatory neurotransmitter in the brain (Meldrum, 2000), direct contributions of Glu to subjective, conscious experience in humans is not well understood. Pharmacological challenge is a research strategy that can provide traction on this issue. Such an approach can include the use of Glu agonists to understand the cognitive and emotional ramifications of potentiation of glutamatergic function in humans and the utilization of Glu antagonists to understand the effect of glutamatergic down-regulation. Glutamatergic agonists, such as psychostimulants (Kalivas, 2007), and glutamatergic antagonists, such as ketamine (MacDonald, Miljkovic, & Pennefather, 1987), can be safely administered and manipulated in healthy adult volunteers.

Glu antagonists have been long implicated in the influence of subjective experiences. Specifically, research has revealed that glutamatergic *N*-methyl-D-aspartate (NMDA) receptor antagonists have profound effects on perception of time, mood, and dissociation. For example, the NMDA antagonist memantine has been shown to significantly increase feelings of highness, forgetfulness, detachment, lightheadedness, impaired motor coordination, and feelings of being "buzzed" (Jackson et al., 2009; Bisiaga & Evans, 2004; Krishnan-Sarin et al., 2015). Ketamine, another NMDA antagonist, significantly distorts the subjective experience of time, as measured by the Clinician-Administered Dissociative States Scales (Coull et al., 2011) and simulates the negative symptoms of psychosis, i.e. the blunting of emotion and catatonia, in healthy volunteers (Krystal et al., 2005). Neuroimaging studies using magnetic resonance spectroscopy (^{1}H MRS) indicate that ketamine positively modulates glutamine levels in the posterior cingulate cortex of depressed individuals (Lener et al., 2017). When

administered to healthy volunteers, low-doses of ketamine have been shown to induce phasic increases in Gln and Glu in the anterior cingulate cortex (ACC; Rowland et al., 2005). These effects appear to be functional, as 55 minute resting-state functional magnetic resonance imaging (fMRI) revealed ketamine induced decreased neuronal activation in comparison to placebo the dorsal anterior cingulate cortex (dACC) in healthy volunteers (Hoflich et al., 2017). The decrease in blood oxygenation level dependent (BOLD) response indicates that drug-induced reduction in Glu may relate to functional changes in brain activity. This literature collectively illustrates a negative association between glutamatergic antagonist-induced changes in Glu and mood.

Despite a plethora of research on glutamatergic antagonists in humans, glutamatergic agonists have not been as well studied in the context of either (a) brain levels of Glu or (b) subjective emotional experience. What has been reported primarily focuses on animal models and clinical populations. Using ^{1}H MRS, Yang et al. (2015) found that rhesus monkeys undergoing withdrawal after chronic methamphetamine self-administration had decreased glutamine (Glx) and myo-inositol (Ins) levels in the ACC. Similarly, chronic methamphetamine users in their first week of abstinence displayed decreased Glu and Glx levels in posterior cingulate compared to non-drug users. Decreased levels of glutamatergic compounds were also associated with heightened scores on the Beck Depression Inventory (O'Neill et al., 2015), indicating that decreased levels of Glu were associated with depressed mood in individuals during acute withdrawal from the long-term use of psychostimulants.

Human neocortex and conscious, subjective experience can also be informed by the examination of resting levels of Glu in clinical populations. Research using ^{1}H MRS

suggests that individuals with bipolar disorder have elevated glutamatergic neurotransmission in the ACC compared to healthy controls (Eastwood & Harrison, 2010). Conversely, other studies have demonstrated that individuals with diagnosed affective disorders, such as major depression and bipolar I disorder (BD), have decreased glutamatergic levels in various neocortical structures compared to controls (Poletti et al., 2016). These findings suggest that hypofunctionality of the glutamatergic system is associated with dysregulation of mood, as in major depression. Additionally, Gerhardt Soeiro-de-Souza et al. (2015) found heightened glutamine (Gln) levels in the ACC in individuals with euthymic BD, a state of BD characterized by stable mood states, compared to healthy controls. These findings collectively indicate that decreased levels of glutamatergic compounds are associated with negative affect in individuals with mood disorders.

Although the relationship between glutamatergic compounds and subjective mood has primarily been studied in clinical populations, recent literature has investigated this phenomenon in the neocortex of healthy individuals. Specifically, White et al. (2018) acutely administered *d*-amphetamine (AMP), methamphetamine (MA), and a placebo (PBO) to healthy volunteers, revealing that psychostimulants increased Glu and Glx levels in the dACC. The drug-induced increases in Glu predicted subjective drug effects such as drug liking and feelings of being high. Little is known about the direct effects of Glu levels on other subjective emotional responses, such as elation, anger, negative affect, and incentive motivation. To explore these phenomena, the present analysis builds on the prior project and examines relationships between drug effects on Glu levels in the dACC of healthy adults and relationships with drug effects on subjective emotion.

Subjective responses were assessed using a battery of validated self-report questionnaires administered at multiple time points before and after drug exposure.

The goal of the present analysis is to increase knowledge of the potential relationship between acute changes in neurometabolites in the human dACC and their relationship with acute changes in subjective emotion. It was hypothesized that an acute increase in glutamatergic compounds in the dACC, induced by MA and AMP, would predict the extent of the subjective experience of positive emotion after drug exposure. Specifically, we expected a positive relationship between a drug-induced rise in Glu, the major excitatory amino acid transmitter in the brain, and the magnitude of the drug-induced rise in elevated positive emotion. This relationship was expected to be strongest in females, given the heightened glutamatergic response in females compared to males (see White et al., 2018).

Methods

Study Design

The methods and materials used in this study have been previously published in White et al. (2018). In brief, the study used a double-blinded, placebo-controlled, within-subjects crossover design, and administered 20mg of methamphetamine hydrochloride (MA; Desoxyn®), 20mg of *d*-amphetamine sulfate (AMP), and a placebo (PBO; dextrose) to participants. Order of administration was fully counterbalanced across participants.

Recruitment

Inclusion and exclusion criteria were established in White et al. (2006) and White et al. (2007). Exclusion criteria included (1) ages < 18 and > 35, (2) BMI < 18 and > 30, (3) left-handedness, (4) lack of English fluency, (5) no high school diploma or GED, (6) current chronic medical conditions, (7) past or current diagnosis of an Axis I psychiatric disorder as defined by the DSM 5, (8) abnormal EKG, (9) smoking > 5 cigarettes per week, and in women, (9) lactation and current or intended pregnancy. Participants completed an electrocardiogram and physical exam; see White et al. (2018).

IRB Compliance

All participants provided written informed consent before participating. The Institutional Review Board for research with human subjects at Brown University and Memorial Hospital of Rhode Island approved of this study. This study was in accordance with the Code of Federal Regulations (Title 45, Part 46) “Protection of Human Subjects” used by the National Institutes of Health and the Office of Protection from Research Risks, and also in agreement with the National Advisory Council on Drug Abuse

Recommended Guidelines for the Administration of Drugs to Human Subjects and the Helsinki Declaration of 1964 (revised in 2013).

Participants

Twenty-six participants were eligible and completed the study. The sample consisted of 13 males and 13 females with a mean age of 22.3 years (*SD* = 3.27, range = 18-28) and normal body weight (mean weight = 145.1, *SD* = 17.4; mean BMI = 22.3, *SD* = 1.86). Males and females did not significantly differ in weight (Male: *M* = 150.9, *SD* = 16.6; Female: *M* = 139.4, *SD* = 16.9; $t(24) = 1.74$, $p = .10$) or age (Male: *M* = 22.37, *SD* = 3.67; Female: *M* = 22.04, *SD* = 2.86; $t(24) = .25$, $p = .11$) . Overall, participants were well educated, with 8% reporting having a high school diploma or GED, 61% reporting having some college, and 31% reporting having earned a bachelor's degree or more. The sample consisted of 73% White, 19% Asian, 4% African American, and 4% Multiracial/Other. Of the sample, 23% identified as Latino/Hispanic. Participants who completed the study received $220 in monetary compensation. Those who did not complete all three MRI sessions (n = 2) received prorated payment, i.e. $70 per session; see White et al. (2018).

Procedure

Participants were asked to complete seven separate sessions over multiple days. Sessions were in the following order: psychological screening via the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998), a medical screening involving an EKG, an orientation session, three MRI test sessions (AMP, MA, and PBO, fully counterbalanced across participants), and an exit session during which participants were debriefed. There was an average of nine days (± eight days) between the MRI test

sessions, with at least three days between AMP and MA administration to avoid sensitization. To ensure that participants were blind to the test condition, researchers told them the capsules could contain one of several kinds of drugs, such as placebo, anti-depressants, stimulants, or sedatives. Each testing day was approximately 5.5 hours long, and to control for circadian effects, was conducted at a fixed time of day in each subject. Two hours prior to each test session participants consumed a standardized meal. To ensure that participants had no drugs or alcohol in their system, they completed and passed a urine toxicology screening and breath alcohol level test; see White et al. (2018).

Drugs and Dosing

Study drugs were orally ingested and consisted of a placebo (PBO, dextrose), *d*-amphetamine sulfate (AMP), and methamphetamine hydrochloride (MA; Desoxyn®). AMP and MA were individually mixed with dextrose filler and provided to participants in opaque gelatin capsules. The PBO capsules only contained dextrose. AMP and MA were administered in 20 mg doses. The dosage was chosen because it is well studied in healthy participants (Wachtel & de Wit, 1999; Wachtel, Ortengren, & de Wit, 2002), within the recommended range of doses for ADHD, is equipotent across MA and AMP (Lamb & Henningfield, 1994), and increases subjective measures of stimulant effects (Martin et al., 1971; Mayo & de Wit, 2015). Both AMP and MA were included due to their differential effects on monoamine transporters. Research has demonstrated that MA has a greater impact on serotonin transporters than AMP, while norepinephrine and dopamine transporters are sensitive to AMP (Rothman & Baumann, 2003; Kahlig et al., 2005; Pifl et al., 1999). The order of drug administration was counterbalanced and randomized across participants, and resulted in six drug orders: AMP-PBO-MA, AMP-

MA-PBO, MA-PBO-AMP, MA-AMP-PBO, PBO-MA-AMP, and PBO-AMP-MA. During each test session, MRS was administered 140-150 min after drug ingestion; see White et al. (2018).

Structural Imaging

MR data was collected via the Siemens 3T TIM Trio system (Siemens Medical Solutions, New York, NY). At the beginning of each MRI session, using an MPRAGE sequence, whole-brain T1-weighted images were taken in the sagittal plane (resolution = 1 mm × 1 mm × 1 mm; TR = 1900 ms; TI = 900 ms, TE = 2.98 ms; flip angle = 9°; FOV = 256 × 256). SPM12 was used to process the structural T1 images (Edden et al., 2013) and voxel construction was completed via Gannet Coregister (Ashburner & Friston, 2005; Harris, Puts, & Edden, 2015); see White et al. (2018).

Spectroscopy

In the dACC, metabolites were investigated using single-voxel, point resolved spectroscopy (PRESS). Voxel size was 15x15x10mm (10 mm in SI direction). Using three plane reconstructions, voxel size was placed on the T1-weighted anatomical image. The voxel was placed on the dACC immediately anterior and parallel to the corpus callosum (see Figure 1). For the sagittal view, the midline slice where the corpus callosum was most distinct was selected. The voxel was then placed anterior and superior to the corpus callosum and rotated to be tangential to the corpus callosum. For the axial, coronal, and sagittal views, the voxel was aligned anterior and parallel to the corpus callosum making it so that the posterior edge of the voxel was in line with the horns of the lateral ventricles. For the coronal view, the voxel was right lateralized to capture connectivity with the salience network (Grayson et al., 2014) and minimize CSF

contamination from the longitudinal fissure. Using posthoc voxel reconstruction in subject specific anatomical space, voxel placement was confirmed. Using subject specific anatomical landmarks, reconstructed MRS voxels were checked to confirm agreement with voxel placement protocol for individual anatomy. During the MRS sessions standard first-order auto shimming was implemented, line width checked and shimming manually adjusted to minimize the free water line width. Prior to acquisition of spectral data, non-water suppressed datasets were obtained to provide for eddy current compensation and to provide a water reference. Repetition time (TR) was 3000 ms, echo time (TE) was 30 ms, with 128 averages taken for a total acquisition time of 6 m 24 s for water-suppressed data. The TR of 3000 ms reduced differential T1 effects to less than 12% and the T2 differentiation was minimized by the echo time of 30 ms (Traber et al., 2004). Using LCModel, spectra were processed and metabolites quantified (Provencher, 1993). All of the data received zero- and first-order phase correction as part of the regular LCModel process, done automatically by LCModel with no user intervention. Data were compensated for differential T1 effects according to individual metabolite T1 values at 3 Tesla (Ethofer et al., 2003; Mlynarik et al, 2001; Traber et al, 2004). Only metabolites with Cramer-Rao lower bound (CRLB) less than or equal to 20% were evaluated. All metabolites met CRLB criteria, except for three instances for Gln, which were excluded; see White et al. (2018) and Methods (below). Example spectra are shown in Figure 2.

^{1}H MRS Measures

Primary Measures. Metabolites reliably assessed by ^{1}H MRS include Glx, a composite measure of the amino acids Glu and Gln primarily comprised of Glu (Goryawala et al., 2016). Glu is an excitatory amino acid neurotransmitter involved in

learning and memory (Riedel, Platt, and Micheau, 2003). Gln, a measure of glutamine, is an amino acid that is a precursor to Glu and GABA, and is thought to play a role in learning (Jongkees, Colzato, and Immink, 2017). tCr is a combined measure of creatine and phosphocreatine, and is thought to improve brain function and enhance energy metabolism in neural tissue (Allen, 2012; Andres et al., 2008). Cho, a measure of choline-containing compounds glycerophosphocholine and phosphocholine (GPC+PCh), is a precursor of acetylcholine and increases attention and reward in ^{1}H MRS studies (Nordahl et al., 2002). Ins, a measure of myo-inositol, has a role in the second messenger cascade for catecholamines (Gamper & Shapiro, 2007). Means and standard deviations for each ^{1}H MRS outcome are presented in Table 1. Reliability for these metabolites is well established (Baker et al., 2008; Geurts et al., 2004; Provencher, 1993). All metabolites were quantified relative to water in institutional units (i.u.) per standard practice (Zhu & Barker, 2011). Ratio-based measures were considered but decided against, to avoid interpretive confounds should metabolites change simultaneously (Haga et al., 2009; Li et al., 2003; Rae, 2014). Metabolites were corrected for partial volume effects using the formula [*1/(1- fCSF)], where fCSF is the voxel volume fraction of cerebrospinal fluid therefore providing a measure of metabolite concentration in tissue within the voxel (per methods of Brandt et al., 2016; Larsen et al., 2016; Maltezos et al., 2014). Gray matter and white matter in the voxel did not differ across the three sessions, indicating high fidelity of voxel composition across the experiment (see White et al., 2018). Voxel placement reliability was assessed using dice coefficients (Dice, 1945; Sørensen, 1948; see White et al. (2018).

Subcomponent Measures of Glx. Glu and Gln were evaluated separately to

provide information on the specificity of relationships with Glx. LCModel analyzes Glu and Gln using the entire in vivo spectrum in relation to the complete model spectra produced by metabolites at known concentrations in vitro, and provides good discrimination between Glu and Gln when FWHM are on the order of 0.05 ppm (Provencher, 1993). The acquired spectra were uniformly well resolved with an average FWHM of 0.06 ppm and a modal FWHM of 0.05 ppm. Mean (±SD) CRLB uncertainty was 5(±1)% for Glx, 5(±1)% for Glu and 13(±2)% for Gln; see White et al. (2018).

Measures of Subjective Response

To characterize participants' conscious emotional states after drug ingestion, participants completed multiple self-report psychological questionnaires outside of the MRI scanner during each test session. Assessments were administered at half hour intervals throughout each session (i.e. 15 mins before taking the capsule and at half hour intervals after ingestion), for a total of eight completion points during each test session. Measures were administered in the following order: Positive and Negative Activation Rating Scales (Morrone et al., 2000), Positive and Negative Affect Schedule (Watson, Clark, & Tellegen, 1988), Visual Analogue Scale (Wewers & Lowe, 1990), Addiction Research Center Inventory (Haertzen, 1966), Profile of Mood States (McNair, Lorr, & Droppleman, 1971), and Drug Effects Questionnaire (Morean et al., 2013). Because the Drug Effects Questionnaire (DEQ) and its relationship with Glu have already been evaluated in the parent study (see White et al., 2018), this measure was not included in the present analyses. The remaining questionnaires and potential relationships with drug-induced changes in neurometabolites have not yet been evaluated, and were targeted for assessment in the present analysis.

Positive and Negative Activation Rating Scales. Positive and Negative Activation (Morrone) is a self-report questionnaire that measures the presence and intensity of positive and negative activated emotion. Participants were presented with two separate scales. The "positive activation" scale asked participants to rate their current emotional state on a scale of 0 to 9, with 0 = "depressed/sluggish," 1 = "dull/tired," 2 = "pleasant/fresh," 3 = "cheerful/lively," 4 = "delighted/energetic," 5 = "enthused/peppy," 6 = "thrilled/strong," 7 = "exuberant/vigorous," 8 = "elated/exhilarated," to 9 = "ecstatic/invincible." The "negative activation" scale asked participants to rate their current emotional state on a scale of 0 to 9, with 0 = "placid," 1 = "calm," 2 = "relaxed," 3 = "annoyed," 4 = "tense," 5 = "nervous," 6 = "distressed," 7 = "jittery," 8 = "hostile," to 9 = "contemptful." The Positive Activation measure has been validated in the context of the experimental manipulation of incentive motivation, specifically demonstrating increased levels of positive activation in response to dynamic film material; see Morrone et al. (2000).

Positive and Negative Affect Schedule. The Positive and Negative Affect Schedule (PANAS) is an internally consistent, reliable self-report questionnaire that measures the dimensions of positive and negative affect. The PANAS contains 20 items: a 10 item positive affect (PA) scale (i.e. attentive, interested, alert, enthusiastic, excited, determined, proud, inspired, active, and strong) and a 10 item negative affect (NA) scale (i.e. upset, distressed, irritable, afraid, angry, hostile, guilty, ashamed, jittery, and nervous). Participants are asked rate the extent to which they feel each statement at the present moment on a scale of 1 to 5, with 1 indicating "very slightly or not at all," and 5 indicating "extremely" (Watson, Clark, & Tellegen, 1988). The PANAS can be reliably

used as a measure of both positive and negative responses to psychostimulant administration (Fernandez-Serrano et al., 2011).

Visual Analogue Scale. The Visual Analogue Scale (VAS) is a sensitive, valid, and reliable self-report questionnaire that measures 11 bodily sensations/symptoms. These include separate items that query the subjective states of stimulation, interest, queasiness, contentment, drowsiness, anxiety, elation, nausea, sedation, hunger, and the desire to drink alcohol. For each item, participants are presented with 100 mm horizontal line and mark that line to indicate how they feel on a scale of 0, "not at all," to 100, "extremely" (Wewers & Lowe, 1990). The VAS has established validity in identifying psychostimulant-induced subjective effects, demonstrating increased positive scores with AMP administration in comparison to placebo (Jayaram-Lindstrom et al., 2008).

Addiction Research Center Inventory. The Addiction Research Center Inventory (ARCI) is a 53 item self-report questionnaire that evaluates subjective drug effects characteristic of multiple classes of drugs, such pentobarbital, chlorpromazine and alcohol, benzedrine, amphetamine, morphine and benzedrine, LSD, and marijuana. Each class of drug has its own set of questions, creating six factors of subjective drug response. The pentobarbital, chlorpromazine, and alcohol group (PCAG) scale contains 15 items that assess states of low motivation, fatigue, and sluggishness. The benzedrine group (BG) scale contains 13 items that assess states of energy and intellectual efficiency. The amphetamine scale contains 11 items that assess states of increased energy and sense of wellbeing. The morphine and benzedrine group (MBG) scale contains 16 items that assess states of euphoria, happiness, pleasantness, and popularity. The LSD scale is comprised of 14 items that assess states of depersonalization, tension, anxiety, and

difficulty with concentration. The marijuana scale contains 12 items that measure bodily sensations such as blurred vision and dry mouth. On each ARCI item, participants indicate whether each statement is "true" or "false" based on how they feel at the time of assessment (Haertzen, 1966). The ARCI has established validity in measuring psychostimulant-induced subjective responses, such as significant increases in MBG, LSD, and BG effects after AMP administration (White, Lott, & de Wit, 2006).

Profile of Mood States. The Profile of Mood States (POMS) is an internally valid, reliable 72-item self-report Likert-type questionnaire. Individual items on the POMS provide a specific mood descriptor (i.e. "friendly," "sad," "spiteful," etc.). Participants are then asked to rate each item in terms of how they are currently feeling on a scale of 0 to 4, with 0 indicating "not at all," and 4 indicating "extremely." The 72 items of the POMS are summarized into 10 factors, which provide an index of the subjective states of anxiety, depression, anger, vigor, fatigue, confusion, friendliness, elation, arousal, and positive mood, respectively; see (McNair, Lorr, & Droppleman, 1971). In healthy volunteers, the POMS has established validity in measuring increased psychostimulant-induced subjective responses, such as anger (Yarosh et al., 2015).

Quality Control Procedures.

Quality control (QC) was implemented in three steps for metabolites and an additional step for self-report measures. QC for Metabolites. *Step 1*: All MRS spectra were visually inspected for quality by an MRS data acquisition and analysis expert (co-author Woods). *Step 2:* Using coregistration tools from the Gannet analysis package (Harris et al, 2015), voxels were reconstructed in each anatomical scan and segmented using SPM12 (Ashburner et al, 2005) to allow for quantification of the proportion of

white matter (WM), gray matter (GM) and CSF in the voxel. Segmentation indicated a mean ± *SD* tissue fraction of .70 ± .06 GM, .13 ± .09 WM, and .18 ± .05 CSF within the dACC voxel (White et al., 2018). Tissue fractions of GM (Wald $\chi^2(2) = 1.752$, $p = .42$), WM (Wald $\chi^2(2) = 3.254$, $p = .20$), and CSF (Wald $\chi^2(2) = 4.157$, $p = .13$) did not differ between sessions (White et al., 2018). Data were corrected for partial volume effects using the formula [*1/(1- fCSF)], per established methods (Brandt et al, 2016; Larsen et al, 2016; Maltezos et al, 2014). *Step 3:* Data for individual metabolites were excluded where the CRLB exceeded 20% SD. These procedures indicated that the majority of the data were of high quality: from a total of 73 acquired scans in 26 participants, 7 scans were excluded for lack of water reference files, and CRLB (Gln) cutoffs were exceeded in 3 instances. Thus 66 scans in 24 participants were available for analysis, with additional exclusions for Gln (63 scans); see White et al., 2018. QC for Subjective Responses. Self-reports on each questionnaire (Morrone, PANAS, VAS, ARCI, and POMS) were evaluated for missing data prior to data reduction. This quality control process indicated that there were no missing data at any time point for any participant. In total, all 26 participants' subjective responses were included in the analyses.

Statistical Analysis

The relationship between subjective responses and metabolites was analyzed using SPSS Version 24 (IBM Corp, 2016).

Data Reduction. Self-report questionnaires were scored using standard methods (see references all above with scoring), producing a total of 31 subjective outcome measures per time point per participant. There were 3 sessions x 8 time points x 31 measures, creating a total of 744 data points per subject. To reduce the number of

statistical tests, the time-series data were reduced to two sets of summary measures in each participant. This data reduction involved two steps. First, time series data for each of the 31 original subjective measures were reduced to single scores for each test session using the trapezoidal method (Leventhal et al., 2017). This method provides an area under the curve (AUC) measure that summarizes the response over the entire period of testing on each study day. Delta (∆) scores were then calculated for each AUC measure, to provide a summary of the drug effect on each measure. This calculation involved subtracting the AUC on the PBO session from the AUC on the AMP and MA session, respectively, to produce two sets of ∆ scores for each subjective self-report measure: ∆AMP-PBO and ∆MA-PBO. Third, we conducted a principal components analyses (PCA) using a promax rotation on the delta scores, in order to identify the underlying structure of subjective responses to AMP and MA compared to PBO. Two principal components analyses were conducted: (1) promax PCA on the ∆AMP-PBO measures, and (2) promax PCA on the ∆MA-PBO measures. Scree plots were evaluated for deflection point for each analysis, and factors from each PCA were limited to those with an eigenvalue ≥ 2 (see Figure 3). Interpretation of each PCA factor was determined by items loading on the factor greater than or equal to |0.4|. Factors having three or items with factor loadings greater than |0.4| were retained in the analysis, see Table 2 and 3.

Gender Checks. To evaluate gender differences in subjective responses, independent sample t-tests were conducted to compare males and females on the PCA factors for ∆MA-PBO and ∆AMP-PBO.

Relationship between Neurometabolites and Subjective Emotion. The relationship between the principal components factors and neurometabolites were

evaluated using Pearson correlations. Analyses were restricted to metabolites that had significant drug effects at the sample level (i.e., Glx, Glu, tCr; see White et al, 2018). Several metabolites had significant results that did not survive Bonferroni correction (i.e., Cho, Ins; White et al., 2018). These metabolites were retained as exploratory outcomes in the present analysis. Gln, a component of Glx, was also retained for purposes of specificity. tNAA was excluded from analysis as there were no drug effects identified for this metabolite (White et al., 2018). Correlations were evaluated one-tailed given a-priori directional hypotheses for each metabolite (described above).

Gender Differences. Due to drug by gender interactions in the glutamatergic response in the dACC (see White et al., 2018), the relationship between drug effects on metabolites and drug effects of subjective response was evaluated separately by gender. Correlations in males and females were also assessed for differences in magnitude and direction using the Fisher r-to-z transformation (one-tailed), given our expectation that females would more easily display a positive relationship between glutamatergic and subjective drug responses, due to their heightened glutamatergic response compared to males (White et al., 2018).

Relationship with Drug Liking and Drug High. The relationship between the principal components subjective response factors, duration of drug liking, and magnitude of drug high were evaluated using Pearson correlations. Rationale. Duration of drug liking to AMP (ΔAMP-PBO Time-to-Peak DEQ "Drug Liking") and magnitude of drug high to MA (ΔMA-PBO Peak DEQ "Feel High") had previously been found to be moderately to strongly related to drug-induced changes in Glx, Glu and tCr (White et al., 2018). Hypothesis. We predicted a positive relationship between PCA factors of

subjective AUC response to AMP and MA, and these summary measures of drug liking and drug high, particularly in females for whom glutamatergic responses were heightened compared to males (see White et al., 2018). Tests were conducted at the group level and for males and females separately. Tests were evaluated one-tailed due to a-priori directional hypotheses of a positive relationship between glutamatergic and emotional response (above). Scatterplots of significant findings were inspected for outliers; findings free of high influence points are reported below.

Results

Principal Components Analysis

∆AMP-PBO. PCA for ∆AMP-PBO AUC measures identified four factors of subjective response to amphetamine. The first factor was labeled as "Activated Positive Affect," for it included positive loadings from items indicative of positive affect/mood, arousal, vigor, interest, and elation, and negative loadings from items indicative of fatigue, confusion, anxiety, and depression. The second factor was labeled as "Somatic Sensations," because it contained positive loadings from items indicative of queasiness, drowsiness, nausea, ARCI Marijuana, and ARCI MBG scales, and negative loadings from items indicative of wanting to drink alcohol. The third factor was labeled as "Activated Negative Affect," for it contained positive loadings from PANAS Negative Affect, items indicative of interest, and a want to drink alcohol, and negative loadings from items indicative of nausea and negative activation. The fourth factor was labeled as "Anxious Distress," for it included positive loadings from items indicative of anxiety and anger. These factors are summarized in Table 2.

∆MA-PBO. PCA for ∆MA-PBO AUC measures identified three factors of subjective response to methamphetamine. The first factor was labeled as "Activated Positive Affect," for it contained positive loadings from items indicative of positive affect/mood, vigor, friendliness, elation, arousal, interest, stimulation, and a want to drink alcohol and negative loadings from items indicative of depression, confusion, and drowsiness. The second factor was labeled as "Activated Negative Affect," because it included positive loadings from items indicative of negative affect, anxiety, arousal, and queasiness, and negative loadings from items indicative of contentment and sedation. The

third factor was labeled as "Somatic + Low Negative Affect," for it included positive loadings from items indicative of ARCI LSD and marijuana, and negative loadings from items indicative of negative affect, anger, and anxiety. See Table 3 for details.

Gender Checks

∆AMP-PBO. There were no significant differences between males and females in the PCA response to AMP (i.e., Activated Positive Affect (factor one): $t(22) = -.090$, $p = .929$; Somatic Sensations (factor two): $t(22) = -1.069$, $p = .297$; Activated Negative Affect (factor three): $t(22) = -1.332$, $p = .197$; Anxious Distress (factor four): $t(22) = -.560$, $p = .581$). This pattern of results indicates that male and female participants did not differ significantly in the subjective response to AMP.

∆MA-PBO. There were no significant differences between males and females in the PCA response to MA (i.e. Activated Positive Affect (factors one): $t(22) = .168$, $p = .868$; Activated Negative affect (factor two): $t(22) = -1.963$, $p = .062$; Somatic + Low Negative Affect (factor three): $t(22) = .140$, $p = .890$). This pattern of results indicates that males and females did not differ significantly in the subjective response to MA.

Relationship Between Subjective and Neurometabolic Responses

∆AMP-PBO. Gender sub-analyses indicated differential relationships between metabolites and emotion in males and females. Females. There was a significant positive correlation between drug effects on Glu and drug effects on Activated Positive Affect (factor one) in females, $r = .609$, $p < .05$ (see Figure 4), and a significant negative correlation between drug effects on tCr and drug effects on Somatic Sensations (factor two), $r = -.721$, $p < .01$ (see Figure 5). Furthermore, a significant correlation was found between drug effects on Gln and Anxious Distress (factor four) in females, $r = .613$, $p <$

.05 (see Figure 6). Males. In males, significant negative relationships were identified between drug effects on Glx, Gln, and drug effects on Activated Negative Affect (factor three; *Glx*: r = -.664, $p < .05$; *Gln*: r = -.624, $p < .05$,) (see Figure 7). The correlation analysis of drug effects on neurometabolites and subjective emotion indicated a lack of significant effects when males and females were combined (Full Sample; see Table 4). These findings are summarized in Table 4.

∆MA-PBO. Significant positive correlations were found between drug effects on Glx (see Figure 8), Glu (see Figure 9), and Activated Positive Affect (factor one; *Glx*: r = .382, $p < .05$, *Glu:* r = .438, $p < .05$) when males and females were combined at the sample level. These effects were primarily due to effects in females. Females. Significant positive correlations were found between drug effects on Glx and Activated Positive Affect, r = .526, $p < .05$ (see Figure 8), and drug effects on Glu and Activated Positive Affect (factor one), r = .520, $p < .05$ (see Figure 9). There was a significant negative correlation between drug effects on Gln and Activated Negative Affect (factor two), r = -.705, $p < .05$ (see Figure 10). A significant positive relationship was found between drug effects on Cho and Activated Negative Affect (factor two), r = .662, $p < .01$ (see Figure 10). Males. There were no significant relationships between drug effects on neurometabolites and PCA ∆MA-PBO factors in males. These results are summarized in Table 5.

Magnitude of Gender Differences

∆AMP-PBO. Fisher r-z transformations revealed significant gender difference in the magnitude of the relationship between subjective responses and the metabolic response to AMP. Specifically, significant differences were found between drug effects

on Glx, Gln, tCr, and Somatic Sensations (factor two; *Glx, Gln, & tCr*: $p < .05$), and between drug effects on Gln and Activated Negative Affect (factor three), $p < .05$. The magnitude of the correlation between AMP-induced effects on Glx, Glu, and Positive Affect (factor one) did not differ between males and females even though the directions of the correlations were of opposite sign. See Table 4 for details.

Δ MA-PBO. Fisher r-z transformations also revealed a significant gender difference in the magnitude of the relationship between subjective responses and the metabolic response to MA. Significant differences were found between drug effects on Gln, Cho, and Activated Negative Affect (factor two; *Gln:* $p < .05$, *Cho:* $p < .01$), and drug effects on Cho and Ins, and Sedation + Low Negative Affect (factor three; *Cho*: $p < .05$, *Ins:* $p < .05$). The magnitude of the correlations did not significantly differ between MA-induced effects on Glx, Glu, and Positive Affect (factor 1) in males and females, as both sets of correlations were positive in direction; see Table 5 for details.

Phenomenological Overlap with Drug Liking and Drug High

ΔAMP-PBO. In females, there was a significant negative correlation between the summary score of duration of drug liking for AMP (ΔAMP-PBO Time-to-Peak DEQ "Drug Liking") and ratings of aversive bodily sensations after drug consumption (factor two), $r = -.806$, $p < .01$ (see Figure 11). This effect was specific to females, and was not significant in males, $p > .43$, or at the level of the total sample, $p > .14$.

ΔMA-PBO. There was no relationship between the summary score of magnitude of drug high for MA (ΔMA-PBO Peak DEQ "Feel High") and the PCA factors of other subjective responses to MA (*full sample*: $p > .06$, n.s.; *males:* $p > .24$, n.s.; *females:* $p > .30$, n.s.).

Discussion

This study yielded four main findings. First, a robust relationship was observed between drug effects on glutamatergic compounds in dACC and drug effects on activated positive affect. This relationship was observed for both study drugs and appeared to be due to an elevated glutamatergic response to psychostimulants in females. Second, aversive somatic sensations were negatively related to duration of drug liking in amphetamine in women, and were negatively associated with drug effects on tCr. These findings indicate an elevated responsivity to the positive emotional impact of glutamatergic changes after drug exposure in females. Third, there was a consistent relationship between drug effects on Gln and activated negative affect in both males and females, and anxious distress in females. These four findings are discussed in turn below.

The primary finding was a strong, positive relationship between the drug-induced rise in glutamatergic neurometabolites and the drug-induced rise in activated positive affect. This was observed in response to both study drugs. In response to amphetamine, AMP-induced rise in Glu was positively correlated with AMP-induced rise in activated positive affect (Figure 4), and in response to methamphetamine, MA-induced rise in Glu and Glx was positively correlated with MA-induced rise in activated positive affect (Figures 6 and 7). These findings indicate that drug-induced increases in levels of Glu and Glx in the dACC are associated with increased ratings of a wide variety of self-reports of activated, positive emotion in healthy volunteers, including subjective assessments of vigor, stimulation, interest, elation, and euphoria. These findings are consistent with both the clinical and animal literature. In animal work, Richard & Berridge (2011) have found that blockade of specific glutamatergic receptors, using

microinjections of mGlu2/3 antagonist LY341495, in the nucleus accumbens of rats engenders specific aversive affective reactions, reduced affective "liking" of rewards, and decreased motivation. A similar relationship between drug effects on Glu and activated positive affect has also been demonstrated in clinical samples, such as chronic methamphetamine drug users. During the first week of abstinence, MA users have decreased Glx and Glu levels in the posterior cingulate cortex, increased levels of depression, and a negative relationship between Glx, Glu and depressive symptomology (O'Neill et al., 2015). In accordance with the literature, we found a significant relationship between psychostimulant-induced changes in Glu and positive emotions.

The above positive relationship between AMP- and MA-effects on Glu and activated positive affect was modulated by gender, as findings were only significant in females. This finding indicates that females have an increased sensitivity to the positive affective impact of drug changes in glutamatergic compounds compared to males. This finding is corroborates similar findings in the animal and in clinical literature. For instance, in rodents female individuals show heightened behavioral sensitivity to psychostimulants compared to male individuals (McCormick, 2005; Van Swearingen et al., 2013). In humans, female drug users are more vulnerable to psychostimulant abuse and addiction than males (Anker & Caroll, 2010). According to national epidemiological reports, women initiate cocaine and amphetamine use at earlier ages, and progress to drug dependence at a faster rate than men (Becker & Hu, 2008). Enhanced sensitivity and behavioral responsiveness to psychostimulants has been hypothesized to be due to heightened levels of circulating estrogen in females compared to males (Lynch, Roth, & Carroll, 2002). In comparison to the luteal phase, during the follicular phase of the

menstrual cycle, females experience increased feelings of positive subjective effects of amphetamine such as euphoria, increased energy, and desire. Within the follicular phase, women's AMP-induced positive emotions were positively related to pre-drug levels of salivary estradiol and negatively related to salivary progesterone (White, Justice & de Wit, 2002). Future studies should measure female hormone levels to further explain the robust relationships between Glu and Activated Positive Affect.

In women, there was a significant negative correlation between amphetamine-induced changes in tCr and AMP-induced effects on aversive bodily sensations (Factor 2; Figure 5). This finding indicates that individuals who display the greatest sensitivity to tCr, i.e., the greatest increase in tCr compared to PBO, are those for whom the sedative and aversive bodily sensations of the drug are relatively low in frequency and intensity. The AMP-induced change in tCr, which reflects an increase in energy metabolism in neural tissue (Andres et al., 2008) under drug compared to PBO, is associated with reduced levels of queasiness, drowsiness, nausea, and dry mouth, as indicated by positive scores on the Somatic Sensations factor (Figure 5). This finding is consistent with the negative relationship between AMP-induced somatic sensations and AMP-induced drug liking in females. Essentially, the greater the feelings of aversive sensations, the less likely females were to like AMP.

There were several similarities between males and females, including a robust negative relationship between drug-induced changes in Gln and activated negative affect. This finding emerged in different contexts in males and females, and as it concerned changes in Gln, must be considered exploratory due to the relatively high CRLB uncertainty for Gln in the present study (White et al., 2018). In males, there was a

significant negative relationship between Gln and activated negative affect after AMP (Figure 7). The relationship was not outlier driven and explained 38.9% of the variance in activated negative affect. As seen in Figure 10, females also displayed a robust negative relationship between Gln and activated negative affect after MA. This relationship was also not outlier driven and explained 49.7% of the variance in activated negative affect. The similarity in the magnitude and direction of the findings suggest that Gln may play an important role in the modulation of activated negative affect in both males and females. This relationship could further be explained through the Gln to GABA pathway, which could increase inhibition under psychostimulants (Struzynska & Sulkowski, 2004). Additionally in women, the activated negative affective response to MA was related to drug-induced changes in Cho. One prior study has found a relationship between Cho and self-reports of positive affect, with a negative association between resting Cho and positive affect as measured by the PANAS (Jung et al., 2002).

In women, AMP induced changes in Gln were also separately related to drug-induced changes in anxious distress (see Figure 6). This finding suggests that acute changes in Gln may be related to multiple, disparate subjective states, with acute increases in Gln associated with rises in anxiety, and acute decreases in Gln associated with acute reductions in activated states of negative emotion, such as anger, in women (see MA Factor 2). Modi et al. (2014) have also identified a positive relationship between Gln and anxious distress, finding an association between increased Gln levels in the ACC of healthy volunteers and trait measures of anxiety. Malgorzata et al. (2017) have suggested that in rodents, chronic restraint stress alters the cortical glutamatergic system, negatively affecting feedback mechanisms that regulate the hypothalamic-pituitary-

adrenal axis and behavioral processes involving depressive and anxious symptomatology. Further research is required to disentangle Gln's relationship with specific negative emotions such as anxiety and anger.

The strengths of this study include its diverse battery of psychological measures of subjective experiences; the within-subjects, placebo controlled crossover design; the sample was gender balanced; the testing of subjective effects over an extended period of time and on multiple sessions; and the robust methods for data reduction. The battery of subjective measures included numerous separate assessments of subjective experience, relevant to activated positive affect, activated negative affect, and bodily sensations. Additionally, our measurements of positive and negative affect explored multiple psychological dimensions of these phenomena, such as activation, arousal, and intensity. The within-subject, placebo controlled crossover design allowed for robust interpretations of psychostimulant effects on subjective experience. A researcher who was blinded to drug condition performed the MRS Voxel placement, and drug condition was randomized to eliminate bias in voxel placement. Also, voxel overlap (64%) did not differ between conditions and is on the order of that achieved by automated voxel placement (Lee et al, 2013), indicating excellent within-subject reliability of voxel placement (see White et al., 2018). By utilizing a fully gender-balanced sample, our results have substantial generalizability and enable us to draw firm inferences about gender-specific relationships between neurometabolites and emotion. Our data collection technique provided numerous measures of drug induced subjective experience over time on each test session, providing a rich, dimensional dataset on subjective experience after drug exposure. Finally, our approach to data reduction allowed us to summarize a large amount of information into

three to four factors of drug-induced change in subjective experience.

The limitations of the study included the relatively high CRLB uncertainty for Gln due to PRESS acquisition and the modest sample size. Since Gln is difficult to distinguish from Glu at the chosen TE and 3T, future studies should utilize acquisition parameters that differentiate Gln from Glu (see White et al., 2018). By using a standard MRS PRESS acquisition, we were unable to measure neurometabolites such as GABA and glutathione, which require spectral editing. Also, the measures of Glx include both metabolic pools and neurotransmitter levels of Glu and Gln, for MRS supplies data on the total tissue metabolite in the voxel. Robust differences were found in the relationship between drug induced subjective experience and neurometabolites in females. Such findings are consistent with the literature on heightened female sensitivity to psychostimulant induced positive emotion. To further unpack this phenomenon, future research should explore the effects of estrogen, progesterone and testosterone on psychostimulant-induced glutamatergic responses via a salivary assessment (White, Justice, & de Witt, 2002). Finally, studies involving larger sample sizes are suggested to better increase statistical power. Despite these limitations, this study provides substantial insight to the relationship between acute changes in neocortical glutamatergic compounds and subjective emotion in healthy adults.

In summary, a robust positive relationship was observed between the psychostimulant-induced rise in glutamatergic compounds in the dACC and changes in activated positive affect. This relationship appeared to be primarily due to a heightened glutamatergic response to psychostimulants in females. This finding is consistent with the clinical and animal literature indicating females have increased sensitivity to the

psychostimulant-induced positive affective states (McCormick, 2005; Van Swearingen et al., 2013; Anker & Caroll, 2010; Becker & Hu, 2008). Second, aversive somatic responses were negatively related to the duration of drug liking after amphetamine, and were negatively associated with the drug-induced increase in tCr in women. Third, there was a significant positive relationship between psychostimulant effects on Gln and activated negative affect in both genders. Drug-induced changes in Gln were positively related to anxious distress in women. To our knowledge, this is the first study to provide in-depth information on the relationship between psychostimulant-induced changes in subjective experiences and acute changes in the concentration of glutamatergic compounds in the human neocortex. Future studies can use larger sample sizes, salivary assessment of ovarian hormones in female participants, and additional neuroimaging methods, such as 3D MRSI, to provide information on additional brain regions involved in these effects. Future research that incorporates these recommendations will extend our understanding of the glutamatergic mechanisms of subjective experience in healthy adults.

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Table 1
CSF—Corrected Metabolite Concentrations

	PBO		AMP		MA	
	Mean	SD	Mean	SD	Mean	SD
Primary Measures						
Glx	12.92	1.44	13.91***[a]	1.51	13.16	1.49
Glu	8.62	0.84	9.32****[a]	1.01	8.80	1.08
Gln	4.41	0.87	4.59	0.85	4.42	0.95
tCr	6.28	0.54	6.56**[a]	0.64	6.34	0.53
Exploratory Measures						
Cho	1.80	0.19	1.91*	0.19	1.82	0.20
Ins	4.80	0.50	5.12*	0.55	4.86	0.58

Legend: The CSF—corrected metabolic concentrations are in institutional units (i.u.). "*" represents different from placebo at $p < .05$, "**" at $p < .01$, "***" at $p <. 0005$, and "****" at $p < .0001$. "[a]" indicates survival of a Bonferroni correction for multiple comparisons at alpha = .007. There were 63-66 MR spectra per outcome; n = 24

Table 2
∆AMP-PBO PCA Factor Loading Matrix

	Component			
	Activated Positive Affect	Somatic Sensations	Activated Negative Affect	Anxious Distress
PANAS: Positive Affect	**.911**	-.028	.218	.042
PANAS: Negative Affect	.077	-.393	**.805**	.071
Morrone: Positive Affect	**.458**	.134	-.382	.345
Morrone: Negative Affect	-.014	-.381	**-.581**	.170
ARCI: PCAG	**-.876**	.223	.319	-.028
ARCI: Benezedrine	**.852**	.079	-.119	.192
ARCI: Amphetamine	**.566**	.369	-.234	.052
ARCI: MBG	**.688**	**.582**	.083	-.024
ARCI: LSD	**-.632**	.055	-.297	.131
ARCI: Marijuana	-.135	**.734**	.116	-.089
POMS: Anxiety	**-.566**	.287	.159	**.578**
POMS: Depression	**-.614**	.356	.307	.180
POMS: Anger	**-.512**	.399	**.435**	**.482**
POMS: Vigor	**.873**	.241	.029	.109
POMS: Fatigue	**-.778**	.364	.272	-.117
POMS: Confusion	**-.804**	.109	.321	-.284
POMS: Friendliness	**.864**	.167	.186	-.122
POMS: Elation	**.868**	.361	.055	-.096
POMS: Arousal	**.912**	-.023	-.165	.300
POMS: Positive Mood	**.931**	.225	-.033	-.133
VAS: Stimulated	**.696**	.014	.254	.065
VAS: Interested	**.811**	.059	**.427**	-.127
VAS: Queasy	-.117	**.720**	-.371	-.120
VAS: Content	**.748**	.083	.361	-.164
VAS: Drowsy	-.332	**.566**	.254	-.029
VAS: Anxious	.033	.031	.299	**.849**
VAS: Elated	**.563**	.356	.357	-.202
VAS: Nauseated	-.252	**.769**	**-.450**	-.032
VAS: Sedated	-.293	.231	.103	-.180
VAS: Hungry	.190	.106	-.087	**.763**
VAS: Want Alcohol	.166	**-.408**	**.482**	.090

Legend: Bolded values indicate a factor loading of |0.4| or greater.

Table 3
ΔMA-PBO PCA Factor Loading Matrix

	Component		
	Activated Positive Affect	Activated Negative Affect	Somatic + Low Negative Affect
PANAS: Positive Affect	**.935**	.065	-.007
PANAS: Negative Affect	-.087	**.591**	**-.411**
Morrone: Positive Affect	**.514**	**.472**	-.062
Morrone: Negative Affect	.023	.394	.204
ARCI: PCAG	**-.899**	.109	.107
ARCI: Benezedrine	**.923**	.152	-.075
ARCI: Amphetamine	**.790**	.055	.384
ARCI: MBG	**.872**	.056	.162
ARCI: LSD	-.117	**.636**	**.471**
ARCI: Marijuana	**.410**	**.517**	**.509**
POMS: Anxiety	-.269	**.835**	-.153
POMS: Depression	**-.434**	.026	-.368
POMS: Anger	-.036	.358	**-.783**
POMS: Vigor	**.888**	.295	-.047
POMS: Fatigue	**-.722**	-.026	.025
POMS: Confusion	**-.648**	-.111	.377
POMS: Friendliness	**.897**	-.004	-.199
POMS: Elation	**.940**	.007	.101
POMS: Arousal	**.849**	**.401**	-.162
POMS: Positive Mood	**.942**	.000	.180
VAS: Stimulated	**.693**	.034	.053
VAS: Interested	**.751**	-.170	.043
VAS: Queasy	-.112	**.787**	.241
VAS: Content	**.611**	**-.436**	-.094
VAS: Drowsy	**-.512**	.349	.233
VAS: Anxious	-.332	**.632**	**-.443**
VAS: Elated	**.646**	-.039	-.178
VAS: Nauseated	-.162	**.825**	.279
VAS: Sedated	-.017	**-.451**	.296
VAS: Hungry	.035	**.425**	.022
VAS: Want Alcohol	**.624**	.006	-.251

Legend: Bolded values indicate a factor loading of |0.4| or greater.

Table 4
ΔAMP-PBO PCA Factor Neurometabolite Correlations

Full Sample

	Activated Positive Affect	Somatic Sensations	Activated Negative Affect	Anxious Distress
Primary Measures				
ΔAMP-PBO Glx	-.023	.319	-.217	.099
ΔAMP-PBO Glu	.273	.059	-.342	-.105
ΔAMP-PBO Gln	-.120	.378	-.090	.274
ΔAMP-PBO tCr	-.141	-.150	-.334	.019
Exploratory Measures				
ΔAMP-PBO Cho	-.097	-.173	-.044	-.155
ΔAMP-PBO Ins	-.173	-.189	-.276	-.107

Females

Primary Measures				
ΔAMP-PBO Glx	.328	-.336 Φ	-.091	.124
ΔAMP-PBO Glu	.609*	-.378	-.366	.092
ΔAMP-PBO Gln	.201	-.353 Φ	.273 Φ	.613*
ΔAMP-PBO tCr	-.168	-.721** Φ	-.456	-.161
Exploratory Measures				
ΔAMP-PBO Cho	-.052	-.476	-.084	.092
ΔAMP-PBO Ins	-.283	-.260	-.396	-.202

Males

Primary Measures				
ΔAMP-PBO Glx	-.265	.481 Φ	-.664*	.049
ΔAMP-PBO Glu	-.028	.216	-.534	-.197
ΔAMP-PBO Gln	-.369	.562 Φ	-.624* Φ	.198
ΔAMP-PBO tCr	-.077	.164 Φ	-.236	.046
Exploratory Measures				
ΔAMP-PBO Cho	-.156	-.026	-.028	-.326
ΔAMP-PBO Ins	.052	-.188	.020	-.115

Legend: "*" represents $p < .05$, "**" represents $p < .01$, "Φ" indicates significant difference ($p < .05$) between males and females

Table 5
ΔMA-PBO PCA Factor Neurometabolite Correlations

Full Sample

	Activated Positive Affect	Activated Negative Affect	Somatic + Low Negative Affect
Primary Measures			
ΔMA-PBO Glx	.382*	.056	.161
ΔMA-PBO Glu	.438*	.095	.253
ΔMA-PBO Gln	.159	-.175	.017
ΔMA-PBO tCr	-.035	-.041	-.194
Exploratory Measures			
ΔMA-PBO Cho	.053	-.029	.258
ΔMA-PBO Ins	.196	.032	.183

Females

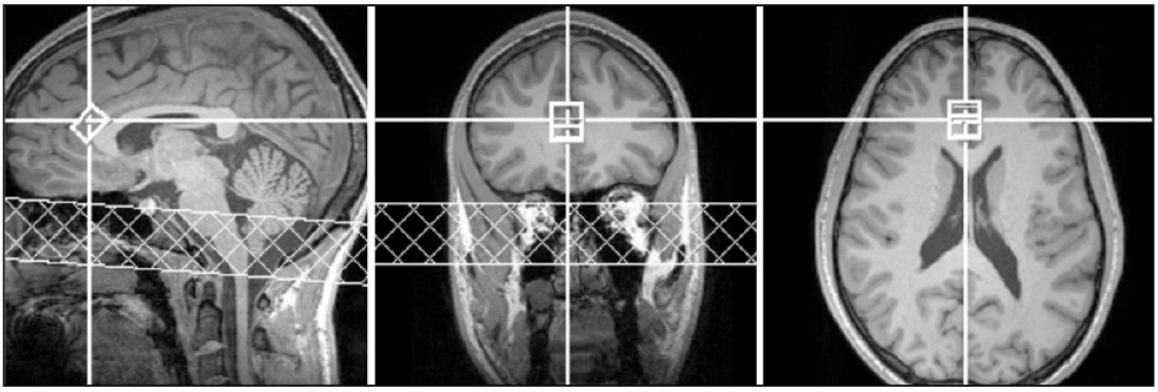
Primary Measures			
ΔMA-PBO Glx	.526*	-.135	.262
ΔMA-PBO Glu	.520*	-.018	.498
ΔMA-PBO Gln	.331	-.705* Φ	.005
ΔMA-PBO tCr	-.009	.112	-.245
Exploratory Measures			
ΔMA-PBO Cho	.006	.662** Φ	.470 Φ
ΔMA-PBO Ins	.281	.381	.461 Φ

Males

Primary Measures			
ΔMA-PBO Glx	.098	.028	-.075
ΔMA-PBO Glu	.307	-.101	-.124
ΔMA-PBO Gln	-.169	.166 Φ	.004
ΔMA-PBO tCr	-.048	-.104	-.183
Exploratory Measures			
ΔMA-PBO Cho	.162	-.430 Φ	-.345 Φ
ΔMA-PBO Ins	.079	-.224	-.397 Φ

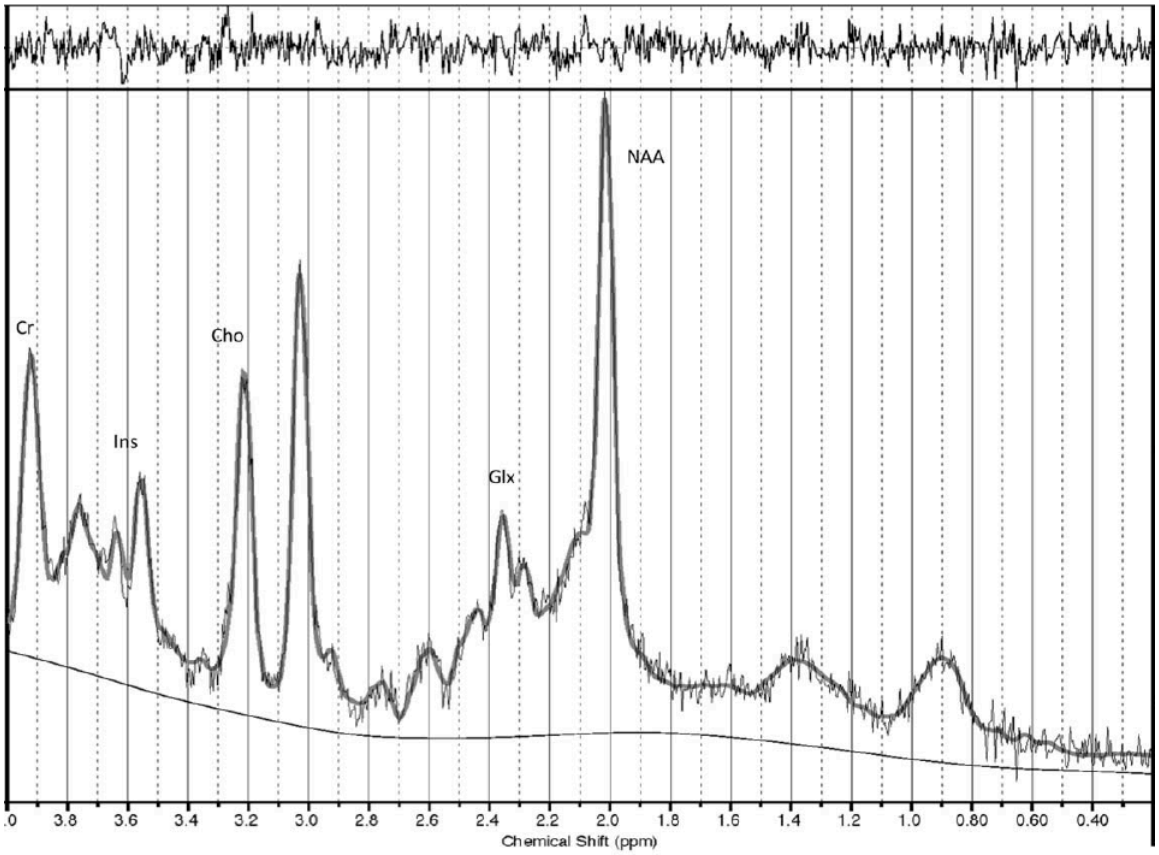
Legend: "*" represents $p < .05$, "**" represents $p < .01$; "Φ" indicates significant difference ($p < .05$) between males and females

Figure 1
Example of dACC MRS Voxel Placement



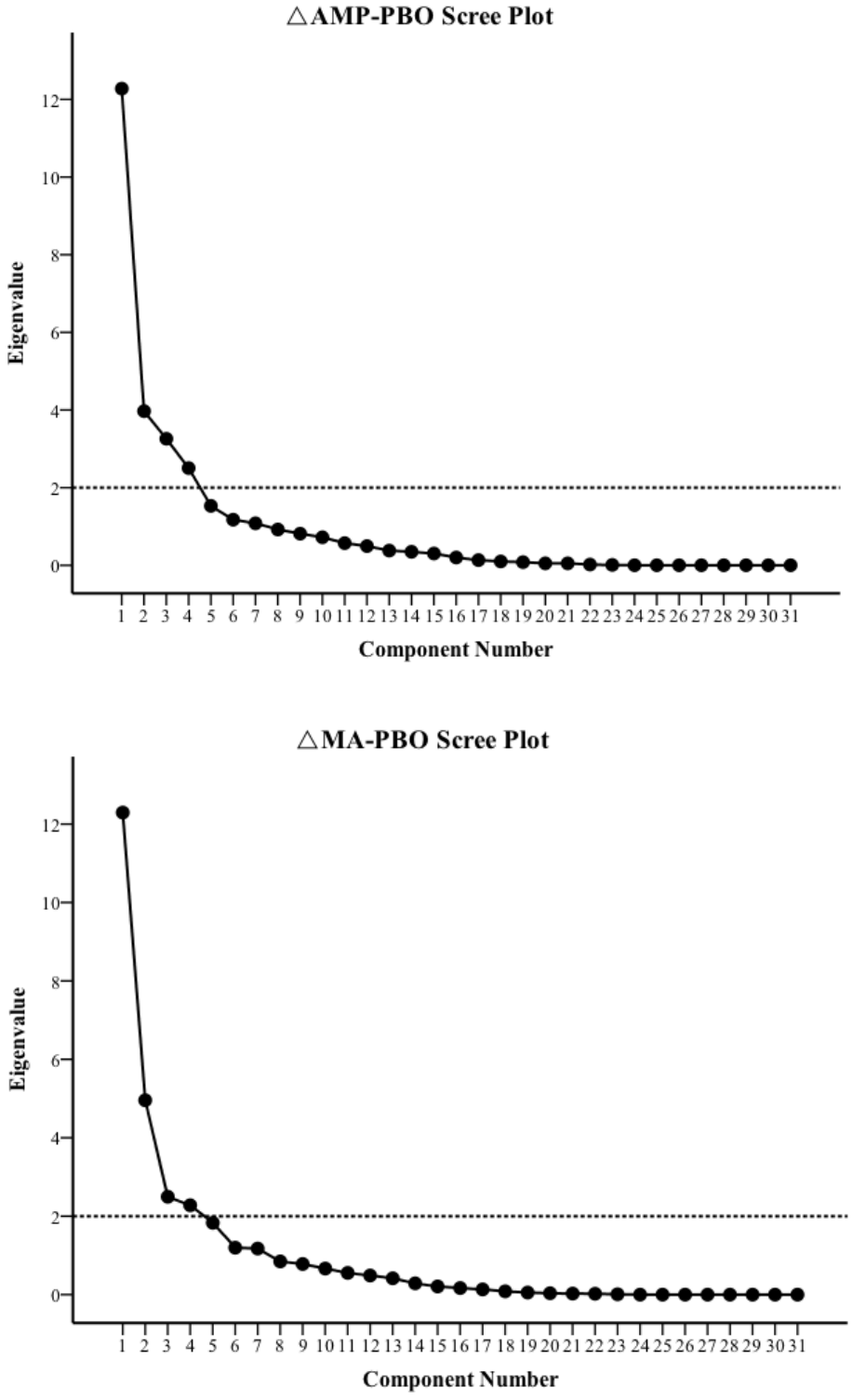
Legend: left: sagittal view; middle: coronal view; right: axial view

Figure 2
Example of dACC MRS Spectra with Labeled Peaks



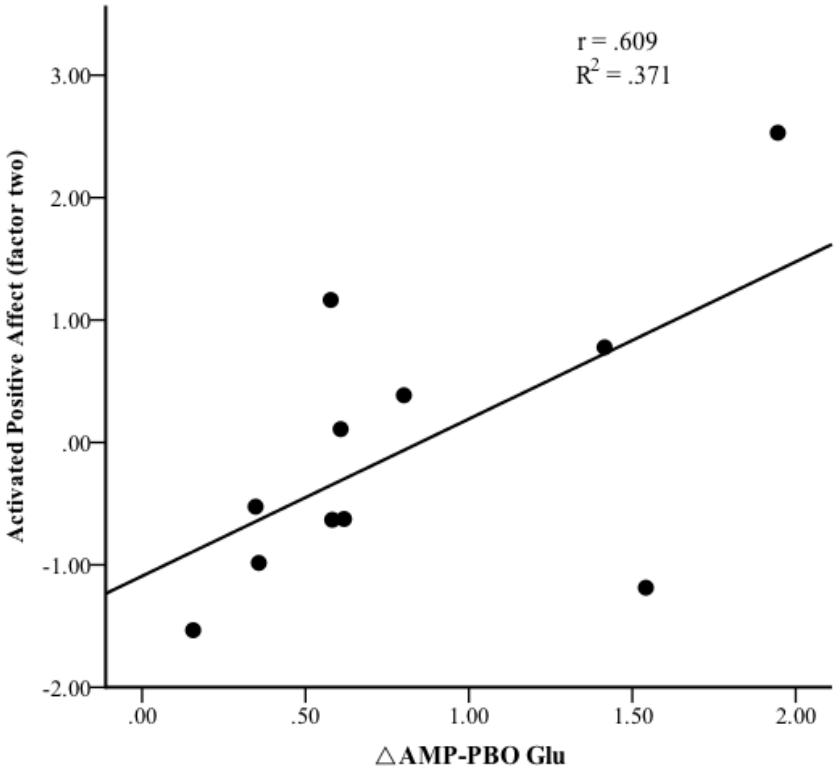
Legend: The dark-gray overlay curve represents the fitted spectra from the LC model, the light gray curve represents the raw data, and baseline is below. The top panel represents the plot of residuals, which appears as noise in the occurrence of a proper fit. Labeled peaks: Cr: creatine; Ins: myo-inositol; Cho: choline; Glx: pooled glutamine and glutamate; NAA: N-acetyl-aspartate.

Figure 3
∆AMP-PBO & ∆MA-PBO Scree Plots



Legend: The dashed-line represents the cut-off for an eigenvalue of 2.

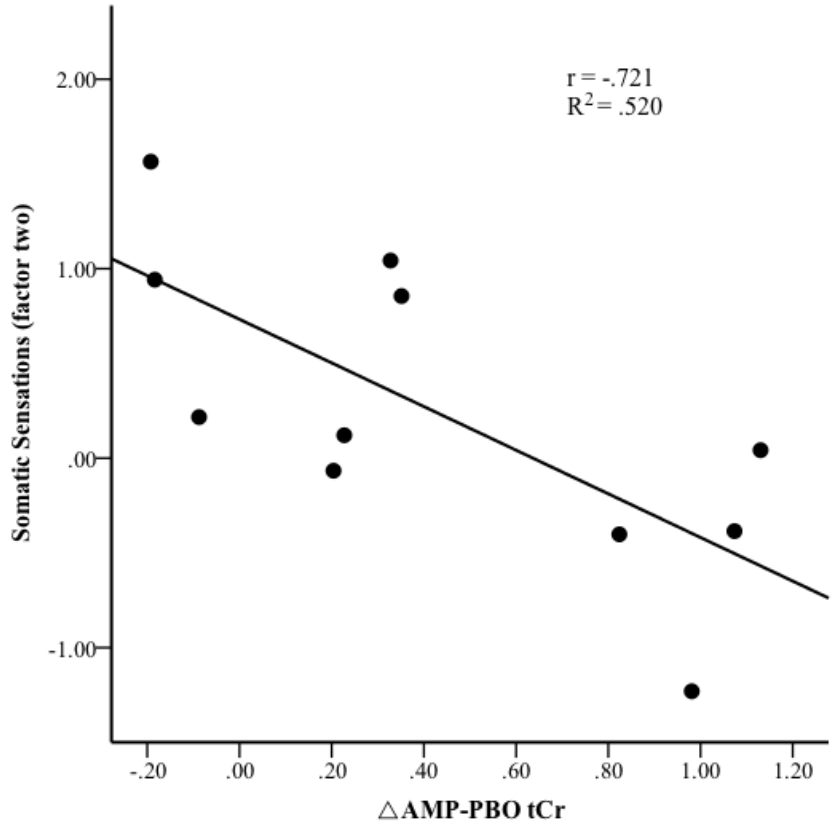
Figure 4
Relationship Between ∆AMP-PBO Activated Positive Affect and Glu in Females



Legend: $p < .05$

Figure 5

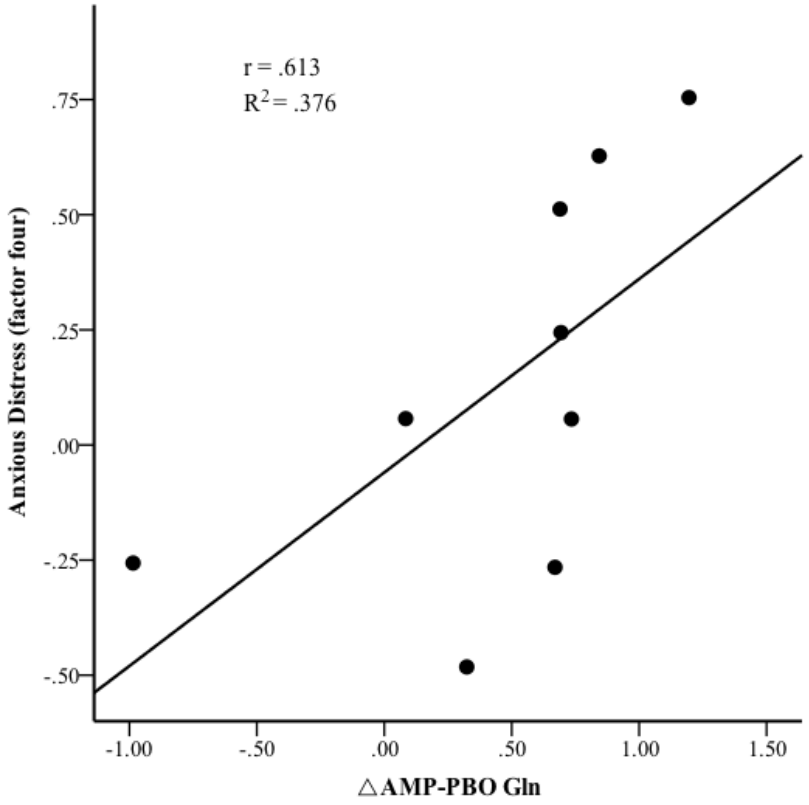
Relationship Between ∆AMP-PBO Aversive Somatic Sensations and tCr in Females



Legend: $p < .01$

Figure 6

Relationship Between ∆AMP-PBO Anxious Distress and Gln in Females

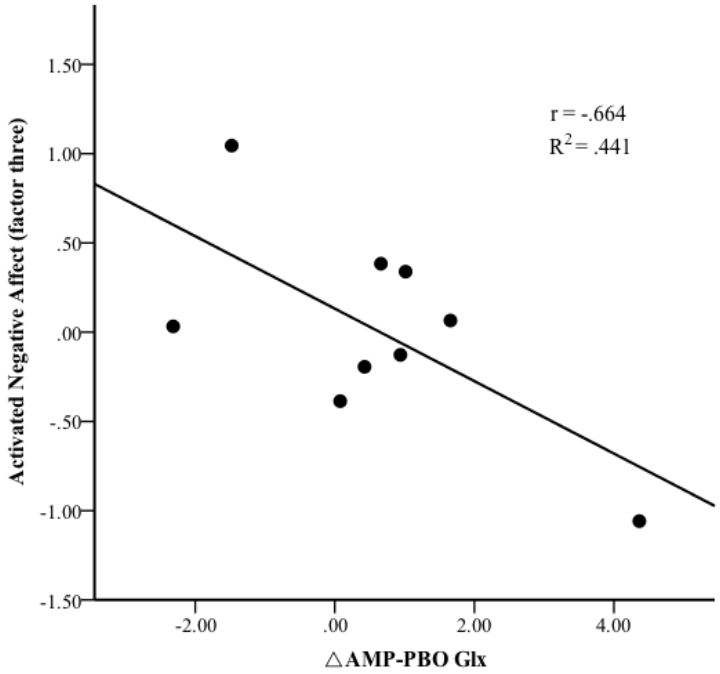


Legend: p < .05

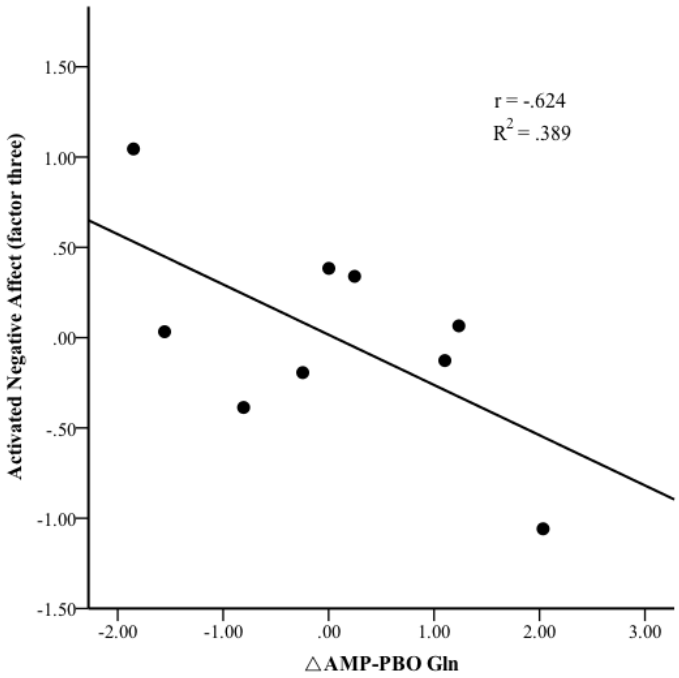
Figure 7

Relationship Between ∆AMP-PBO Activated Negative Affect and Glx & Gln in Males

A.



B.

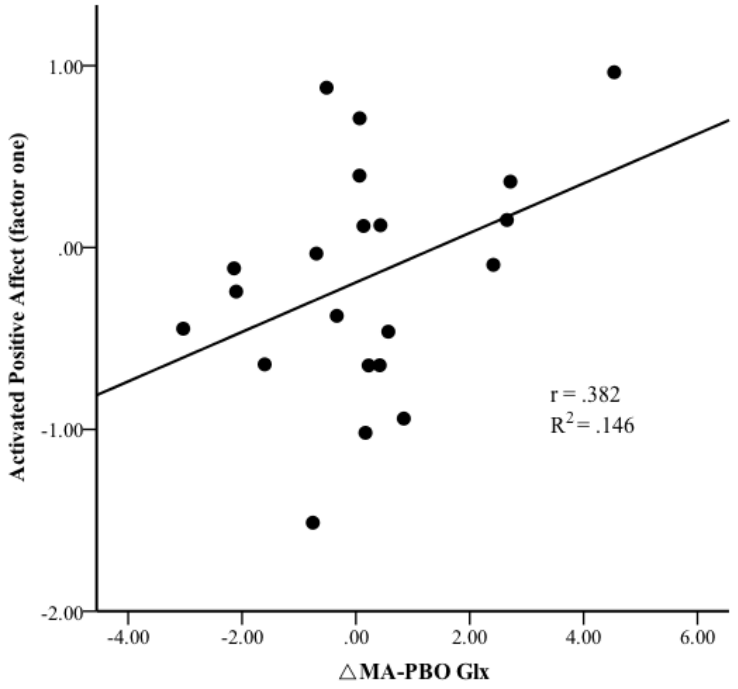


Legend. Panel A illustrates the relationship between AMP-induced changes in Activated Negative Affect and Glx in males; $p < .05$. Panel B indicates the relationship between AMP-induced changes in Activated Negative Affect and Gln in males; $p < .05$.

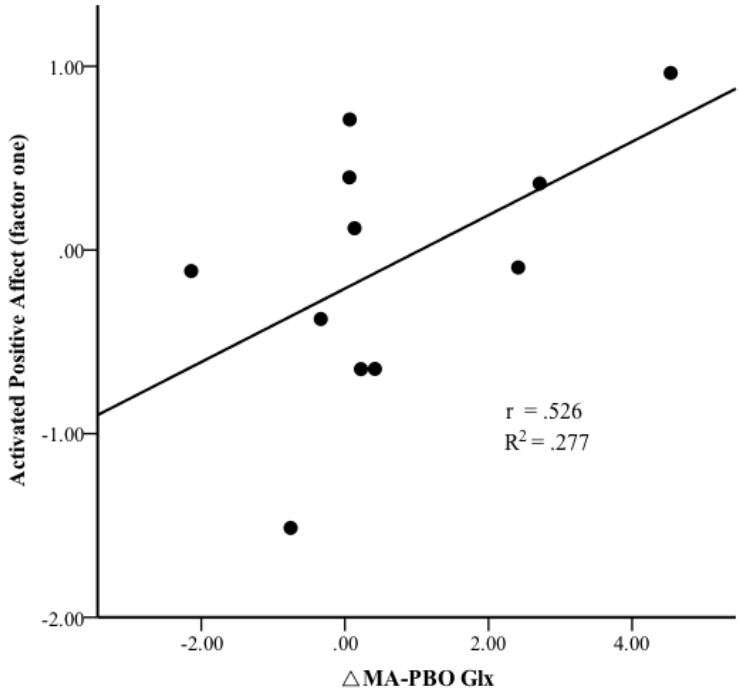
Figure 8

Relationship Between ∆MA-PBO Activated Positive Affect and Glx

A



B

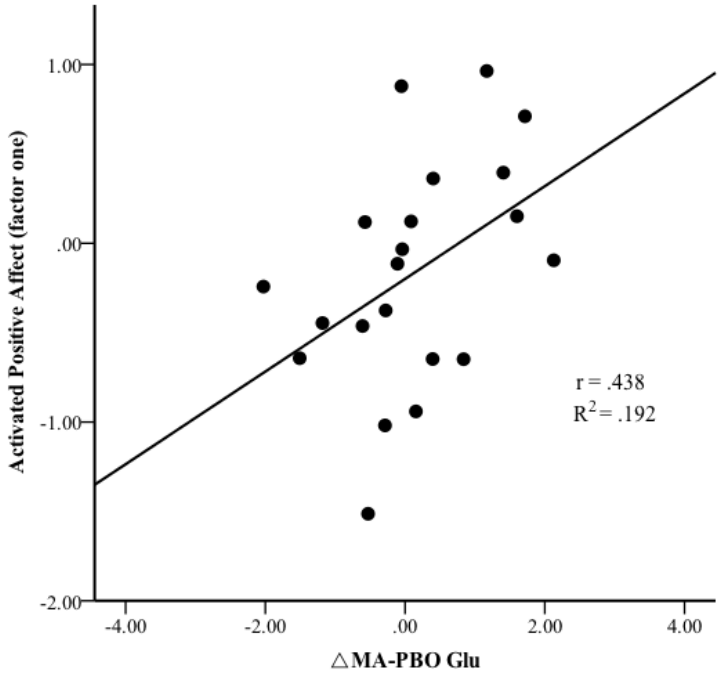


Note: Panel A illustrates the relationship between MA-induced changes in Glx and Activated Positive Affect at the sample level; $p < .05$. Panel B indicates the relationship between MA-induced changes in Glx and Activated Positive Affect in females; $p < .05$.

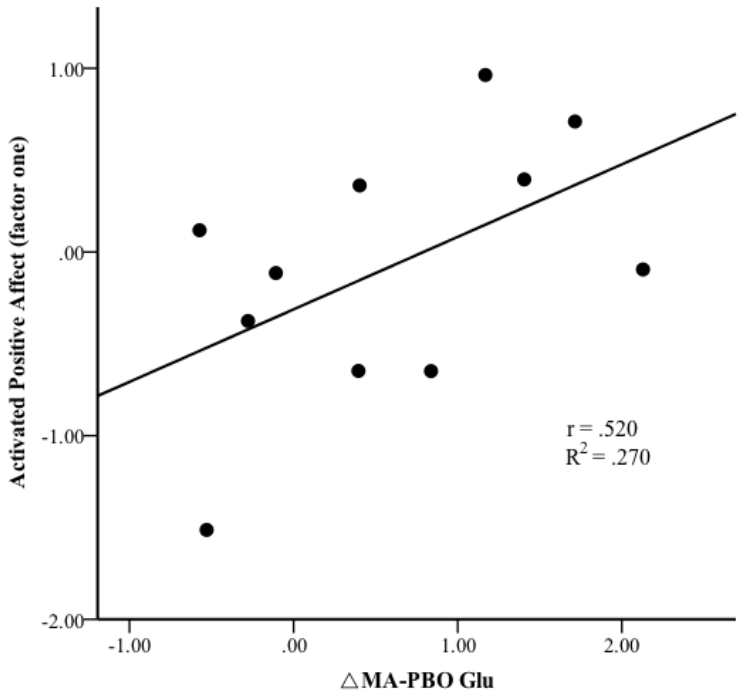
Figure 9

Relationship Between ∆MA-PBO Activated Positive Affect and Glu

A



B

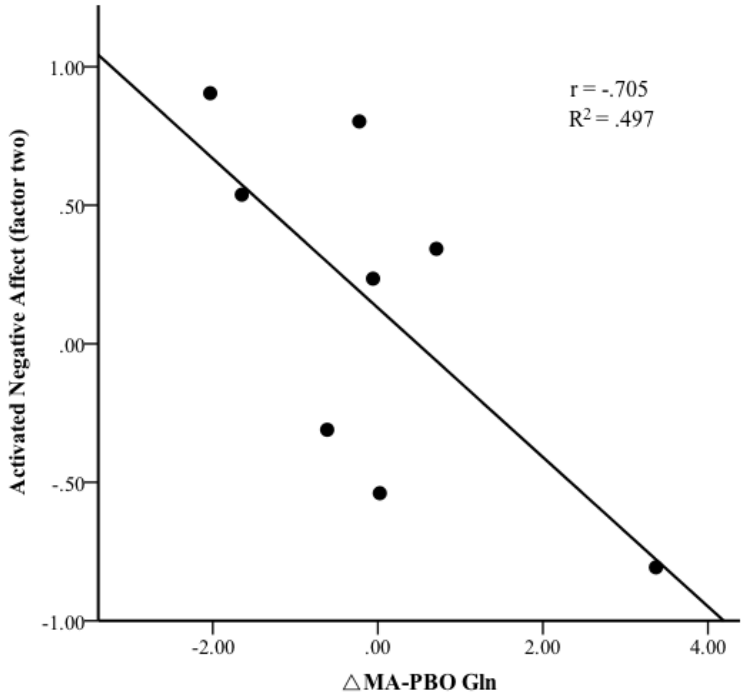


Legend: Panel A illustrates to the relationship between Activated Positive Affect and MA-induced changes in Glu at the sample level; $p < .05$. Panel B refers to the relationship between Activated Positive Affect and MA-induced changes in Glu in females; $p < .05$.

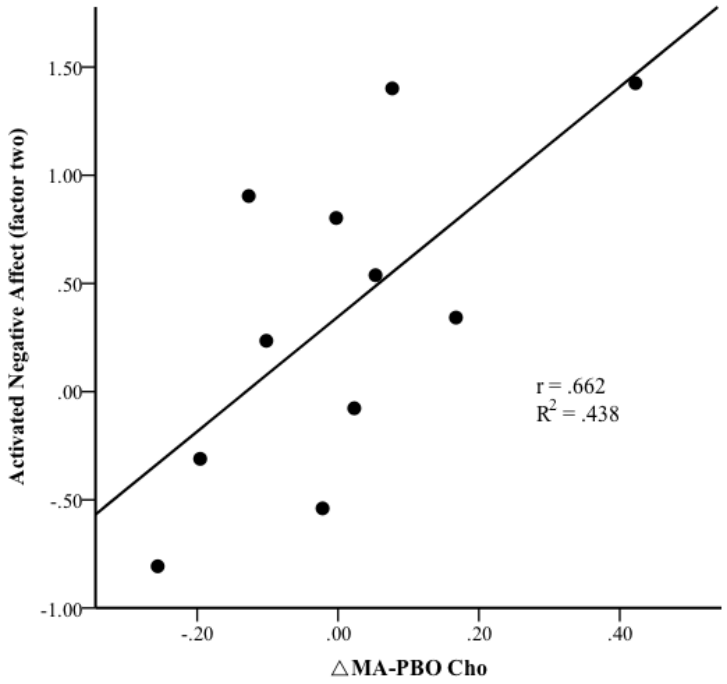
Figure 10

Relationship Between ∆MA-PBO Activated Negative Affect and Gln & Cho in Females

A



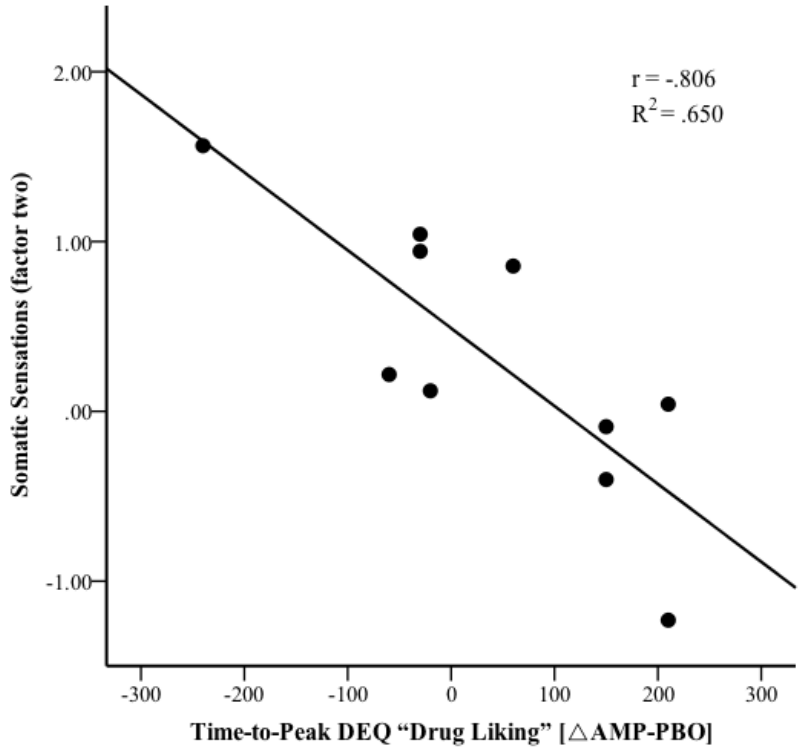
B



Legend: Panel A illustrates the relationship between Activated Negative Affect and MA-induced changes in Gln in females; $p < .05$. Panel B indicates the relationship between Activated Negative Affect and MA-induced changes in Cho in females; $p < .01$.

Figure 11

Relationship ∆AMP-PBO Aversive Somatic Sensations and Duration of Drug Liking in Females



Legend: $p < .05$