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NEUROCHEMICAL EFFECTS OF OXYTOCIN IN PEOPLE AT CLINICAL HIGH RISK FOR PSYCHOSIS

Running title: Neurochemical effects of oxytocin in psychosis risk

Authors

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ABSTRACT

Alterations in neurochemical metabolites are thought to play a role in the pathophysiology of psychosis onset. Oxytocin, a neuropeptide with prosocial and anxiolytic properties, modulates glutamate neurotransmission in preclinical models but its neurochemical effects in people at high risk for psychosis are unknown. We used proton magnetic resonance spectroscopy (^{1}H-MRS) to examine the effects of intranasal oxytocin on glutamate and other metabolites in people at Clinical High Risk for Psychosis (CHR-P) in a double-blind, placebo-controlled, crossover design. 30 CHR-P males were studied on two occasions, once after 40IU intranasal oxytocin and once after placebo. The effects of oxytocin on the concentration of glutamate, glutamate+glutamine and other metabolites (choline, N-acetylaspartate, myo-inositol) scaled to creatine were examined in the left thalamus, anterior cingulate cortex (ACC) and left hippocampus, starting approximately 75, 84 and 93 minutes post-dosing, respectively. Relative to placebo, administration of oxytocin was associated with an increase in choline levels in the ACC ($p=.008$, Cohen's d =0.54). There were no other significant effects on metabolite concentrations (all $p>.05$). Our findings suggest that, at ~75-93 minutes post-dosing, a single dose of intranasal oxytocin does not alter levels of neurochemical metabolites in the thalamus, ACC, or hippocampus in those at CHR-P, aside from potential effects on choline in the ACC.

Keywords: glutamate, oxytocin, magnetic resonance spectroscopy, psychosis risk, schizophrenia, neuroimaging

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INTRODUCTION

The onset of psychosis is usually preceded by attenuated psychotic symptoms (Fusar-Poli *et al*, 2016b) and a clinical presentation that can include social, cognitive and functional impairment (Fusar-Poli *et al*, 2012a, 2013a, 2015). Such individuals are said to be at Clinical High-Risk for Psychosis (CHR-P)(Fusar-Poli, 2017) and have approximately 20% risk of developing a first-episode psychosis over the following two years (Fusar-Poli *et al*, 2016a). The neurobiological mechanisms underlying psychosis onset remain incompletely understood, but alterations in regional brain structure, function and neurochemical composition have been observed in CHR-P samples (Allen *et al*, 2016; Bartholomeusz *et al*, 2017; Fusar-Poli *et al*, 2012b; Modinos *et al*, 2018a).

In particular, accumulating evidence suggests that the pathophysiological processes underlying psychosis onset may be driven by dysregulated glutamate neurotransmission (Lieberman *et al*, 2018), with hypofunction of N-methyl-D-aspartate receptors (NMDAR) on γ-aminobutyric acid (GABA)-ergic interneurons leading to disinhibition of pyramidal cells, and via polysynaptic projection pathways from the hippocampus to the midbrain/striatum, to midbrain hyperdopaminergia (Figure 1) (Lisman *et al*, 2008; Modinos *et al*, 2015). Glutamate is an ubiquitous neurotransmitter with excitotoxic potential (Farber *et al*, 1995), and animal models have demonstrated that excess extracellular glutamate is sufficient to induce hippocampal hypermetabolism and volume loss (Lieberman *et al*, 2018; Schobel *et al*, 2013) comparable to that observed as the CHR-P state progresses to psychosis (Allen *et al*, 2016; Ho *et al*, 2017; Schobel *et al*, 2013). Novel pharmacotherapies that can modulate the glutamate system—or associated neural circuits—have therefore become a target for drug development (Lieberman *et al*, 2018; Millan *et al*, 2016).

<Figure 1>

In humans, metabolite levels can be quantified in vivo using proton magnetic resonance spectroscopy (^{1}H-MRS)(Provencher, 1993). Using this technique, numerous (but not all (Merritt *et al*, 2016)) studies have reported alterations in glutamate (and/or glutamate plus glutamine (Glx)) levels in people at high risk of psychosis vs controls, particularly in the hippocampus (Bloemen *et al*, 2011; Shakory *et al*, 2018), frontal (Merritt *et al*, 2016) or anterior cingulate cortex (ACC)(Stone *et al*, 2009; Tibbo *et al*, 2004) and thalamus (Stone *et al*, 2009). Moreover, within CHR-P samples, altered metabolite levels at baseline appear related to clinical outcomes; thalamic glutamate is lower at baseline in those who do not remit by follow up (Egerton *et al*, 2014), and baseline hippocampal glutamate is elevated in

those who transition (vs those who do not), and in those with poor (vs good) functional outcomes (Bossong *et al*, 2018). There is also evidence that neurochemical alterations in CHR-P are not restricted to glutamate or Glx; higher levels of hippocampal myo-inositol at first presentation have been reported in CHR-P patients with poor functional and clinical outcomes (with effect sizes surpassing that of glutamate), which suggests that hippocampal integrity may be compromised more generally (Bossong *et al*, 2018). Metabolite alterations have also been observed in other brain regions in CHR-P and other (i.e. familial) high-risk groups, such as reduced N-acetylaspartate in the thalamus (Stone *et al*, 2009; Tandon *et al*, 2013; Yoo *et al*, 2009), ACC (Capizzano *et al*, 2011; Jessen *et al*, 2006), and caudate (Keshavan *et al*, 2009)—although increases are also observed in the caudate (de la Fuente-Sandoval *et al*, 2011). Increased Glx has been reported in the thalamus and caudate, as well as increased (de la Fuente-Sandoval *et al*, 2016) or decreased (Menschikov *et al*, 2016) medial prefrontal GABA—although differences are not always found (Modinos *et al*, 2018b; Wang *et al*, 2016). Finally, other studies have reported increased choline in the ACC (Tandon *et al*, 2013), prefrontal cortex (Wood *et al*, 2003) and hippocampus (Capizzano *et al*, 2011) in populations at risk for psychosis.

Prevention of psychosis in patients at CHR-P and amelioration of symptoms are clinical priorities. However, there are currently no licensed pharmacological treatments for this patient group, which remains an unmet clinical need (Davies *et al*, 2018a, 2018b). One potential novel treatment is the neuropeptide oxytocin, which is currently under investigation in relation to a number of neuropsychiatric disorders characterised by social dysfunction, such as autism spectrum disorder and schizophrenia. Oxytocin is a key modulator of social and emotional processes and possesses anxiolytic, prosocial, and potential anticonvulsant properties (Domes *et al*, 2007; Guastella *et al*, 2008; Kanat *et al*, 2015; Kirsch, 2005; Meyer-Lindenberg *et al*, 2011). Intranasal oxytocin is also safe and has a benign side effect profile (MacDonald *et al*, 2011), which is particularly important in CHR-P groups because many will not go on to develop psychosis. The effects of oxytocin on brain metabolites are not completely clear, but available evidence implicates some of the metabolites that have been found to be abnormal in CHR-P individuals: glutamate, Glx, N-acetylaspartate, choline and GABA. Specifically, in a previous randomised, double-blind, placebo-controlled crossover trial of a single acute dose of 24IU intranasal oxytocin in people with autism spectrum disorder, oxytocin's normalisation of brain activation during a social cognition task and improvement in social-communication symptoms was found to be directly related to its effects on N-acetylaspartate levels (Aoki *et al*, 2015). Choline levels in the ACC also appeared to be decreased by oxytocin, albeit at a relaxed significance threshold (p=.02, uncorrected for multiple comparisons; see supplementary material in (Aoki *et al*, 2015)). In a

separate randomised, double-blind, placebo-controlled, crossover trial of 48IU/day oxytocin, which was administered repeatedly over 6 weeks in people with autism spectrum disorder, oxytocin significantly reduced levels of Glx and N-acetylaspartate in the medial prefrontal cortex/ACC relative to placebo (Benner *et al*, 2018). This prior work demonstrates that exogenously administered oxytocin can alter levels of metabolites as measured by 1H-MRS. Evidence for effects of oxytocin on glutamate and GABA comes from preclinical studies. For instance, oxytocin dose-dependently restores social and sensorimotor gating deficits in NMDAR antagonist-treated rats (Feifel and Reza, 1999; Lee *et al*, 2005). Mice lacking the oxytocin receptor (through gene knock-out) are more vulnerable to NMDAR antagonist-induced sensorimotor gating deficits (but not those induced by amphetamine or apomorphine), which suggests that it is the glutamatergic (rather than dopaminergic) component of sensorimotor gating that is protected by oxytocin (Caldwell *et al*, 2009). In mice, oxytocin attenuates methamphetamine-induced changes in glutamatergic neurotransmission in prefrontal cortex and hippocampus via regulation of NMDAR subunit (NR1) and glutamate transporter (GLT1) expression (Qi *et al*, 2012). Furthermore, in animals, oxytocin has been found to enhance the signal-to-noise ratio of pyramidal cell firing by targeting fast-spiking GABAergic interneuron function (Owen *et al*, 2013; Zaninetti and Raggenbass, 2000)—part of the neural circuit implicated in psychosis onset (Lieberman *et al*, 2018; Lisman *et al*, 2008; Modinos *et al*, 2015). When this circuit is acutely manipulated in humans, for example, by inducing NMDAR hypofunction using NMDAR antagonists, or by reducing glutamate release itself (e.g. using n-acetyl-cysteine), increases and decreases (respectively) in the concentrations of regional glutamate/Glx levels as measured by 1H-MRS can be observed (Kegeles *et al*, 2014; Kraguljac *et al*, 2017; McQueen *et al*, 2018; Schmaal *et al*, 2012). This demonstrates that the acute modulation of microcircuit function can alter metabolite concentrations, and that this can be observed experimentally using 1H-MRS.

Despite these initial findings, no studies have yet examined the effects of intranasal oxytocin on neurochemical metabolites in CHR-P individuals, which would further our understanding of its neurophysiological mechanism of action and potential disease-engaging effects. To fill this gap in knowledge, we investigated the effects of intranasal oxytocin on several neurochemical metabolites that are thought to be altered in CHR-P individuals in a randomised, double-blind, oxytocin vs placebo single-dose challenge ^{1}H-MRS study. In line with previous findings in CHR-P individuals, our primary aim was to assess the effects of oxytocin on levels of glutamate, and glutamate plus glutamine (Glx), in the left hippocampus, ACC and left thalamus (Bloemen *et al*, 2011; Shakory *et al*, 2018; Stone *et al*, 2009; Tibbo *et al*, 2004). In view of recent literature suggesting that N-acetylaspartate, myo-inositol and

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choline may be altered in populations at risk of psychosis (Bossong *et al*, 2018; Tandon *et al*, 2013), and that oxytocin's effects may involve several neurochemical pathways (Aoki *et al*, 2015; Benner *et al*, 2018; Ninan, 2011; Qi *et al*, 2012), our secondary aim was to examine the effects of oxytocin on these metabolite levels.

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EXPERIMENTAL PROCEDURES

Participants

The study received National Research Ethics Service approval (14/LO/1692) and all subjects gave written informed consent. Using data from a previous ^{1}H-MRS study (Stone *et al*, 2009) we conducted a power calculation using G*Power 3, which indicated that a sample size of 30 was sufficient to detect a medium within-subject effect size ($d_{z=}$0.53), when alpha = 0.05 and power = 80%. Accordingly, thirty male, help-seeking CHR-P individuals aged 18-35 were recruited from two specialist early detection services—the Outreach and Support in South London (OASIS) (Fusar-Poli *et al*, 2013b) and Tower Hamlets Early Detection Service (THEDS). A CHR-P status was determined using the Comprehensive Assessment of At-Risk Mental States (CAARMS) 12/2006 criteria (Yung *et al*, 2005). The CHR-P state is heterogeneous (Fusar-Poli *et al*, 2016a) because subjects can meet one or more of the following subgroup criteria: (a) attenuated psychotic symptoms, (b) brief limited intermittent psychotic symptoms (BLIPS, psychotic episode lasting <1 week, remitting without treatment), or (c) either schizotypal personality disorder or first-degree relative with psychosis (Yung *et al*, 2005), all coupled with functional decline. Individuals were excluded if there was a history of previous psychotic disorder (with the exception of BLIPS, some of whom may meet Acute and Transient Psychotic Disorder criteria (Fusar-Poli *et al*, 2017)) or manic episode, exposure to antipsychotics, neurological disorder or current substance-use disorder, estimated IQ <70, acute intoxication on the day of scanning, and any contraindications to magnetic resonance imaging (MRI) or intranasal oxytocin or placebo.

Procedure

We used a randomised, double-blind, 40IU intranasal oxytocin vs placebo single-dose challenge in a crossover design (one-week wash out). During each challenge, subjects underwent an MRI scan which started at 11:30AM to minimise potential effects of diurnal variation in oxytocin or vasopressin (Paloyelis *et al*, 2016). Prior to the first scan, subjects completed a computerised Reading the Mind in the Eyes (RMET) task (Baron-Cohen *et al*, 2001), which indexes mentalising (theory of mind) ability, for use in correlation analyses. The RMET requires participants to match the mental state of a person (as shown in a photograph of the eye region only) with one of four possible mental state words. Higher scores (out of a maximum of 36) index better mentalising ability. For descriptive purposes, we also collected information on baseline anxiety (State-Trait Anxiety Inventory [STAI]), medication history, use of alcohol (Alcohol Use Disorders Identification Test [AUDIT]), tobacco and cannabis, and functioning using the Global Functioning (GF) Role and Social scales (Cornblatt *et al*, 2007). Intranasal administration followed recommended guidelines and a protocol adopted

by a previous study conducted at our institute (Paloyelis *et al*, 2016). Briefly, participants self-administered one puff (4IU) of intranasal oxytocin or matched placebo every 30 seconds, alternating between nostrils, until 40IU had been administered (Supplementary Material). Although not presented here, the MRI scan included arterial spin labelling, a functional MRI (fMRI) task, various structural scans and resting state fMRI, followed by the three ^{1}H-MRS sequences (detailed below).

Magnetic Resonance Imaging

All scans were conducted on a General Electric Discovery MR750 3 Tesla system (General Electric, Chicago, USA) using a 32-channel head coil. A three-dimensional sagittal high-spatial-resolution Inversion Recovery Spoiled Gradient Echo (IR-SPGR) T1-weighted scan (TE=3.016ms; TR=7.31ms; TI=400ms; voxel size=1.1x1.1x1.2mm^3) was acquired for voxel planning and calculation of ^{1}H-MRS voxel tissue content. ^{1}H-MRS spectra were acquired in the left hippocampus, ACC, and left thalamus (Figure 2) using conventional Point-Resolved Spectroscopy acquisition (PRESS; TR = 3000ms; TE = 30ms; 96 averages) in three separate 6-minute scans. We employed the standard GE PROBE (Proton Brain Examination) sequence, which uses a standardised chemically selective suppression (CHESS) water suppression routine. Unsuppressed water reference spectra (16 averages) were also acquired as part of the standard acquisition for subsequent eddy current correction and water scaling. Shimming was optimised, with auto-prescan performed twice before each scan. The structural T1-weighted scan was used to plan the voxel placement as per standardised protocols, with voxel sizes of (right-left, anterior-posterior, superior-inferior) 20x20x15mm^3 in the hippocampus, 20x20x20mm^3 in the ACC, and 15x20x20mm^3 in the thalamus. The mean ± SD of the time from dosing offset (end of intranasal administration) for each region was: thalamus (75 ± 4 mins), ACC (84 ± 5 mins), and hippocampus (93 ± 6 mins). These equate to approximate (mean) post-dosing sampling periods of: thalamus (75-81 mins), ACC (84-90 mins), and hippocampus (93-99 mins).

Data Processing

Spectra were analysed using LCModel (Provencher, 1993) version 6.3-1L using the standard basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, glutamine, glutamate, glycerophosphocholine, glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, and taurine) acquired at the same field strength (3 Tesla), localisation sequence (PRESS), and echo time (30 ms). Model metabolites and concentrations used in the basis set are fully detailed in the LCModel manual (http://s-provencher.com/pub/LCModel/manual/manual.pdf). Poorly fitted metabolite peaks (Cramer-Rao minimum variance bounds [CRLB] of >20% as reported by

LCModel) were excluded from further analysis. Spectral quality was further assessed using signal-to-noise ratio (SNR) and spectral linewidths (full width at half-maximum; FWHM).

Our primary analysis used metabolite levels scaled to creatine. However, because creatine may be influenced by the experimental condition (oxytocin vs placebo), we also report water-scaled metabolite levels corrected for voxel tissue content. To calculate and correct for ^{1}H-MRS voxel tissue content, an in-house script was used to (a) segment the T1-weighted structural images into grey matter, white matter, and cerebrospinal fluid (CSF) using Statistical Parametric Mapping 12 (SPM12; Wellcome Trust Centre for Neuroimaging, London, UK) running in Matlab R2017a, (b) locate and map the coordinates of each voxel to the segmented T1 images, and (c) provide the tissue content proportions. Metabolite values were corrected for voxel tissue content using the formula: M_{corr}= M×([GM×1.21] +WM+ [CSF×1.55]) / (WM+GM), where M is the uncorrected metabolite value and GM/WM/CSF are proportions of grey matter, white matter and CSF, respectively. The formula assumes a CSF water concentration of 55,556 mol/m^3 and the LCModel default brain water concentration of 35,880 mol/m^3 (Gasparovic *et al*, 2006; Kreis *et al*, 1993). Apart from assuming T_2 = 80 ms for tissue water, no corrections were applied for metabolite and water relaxation times.

Statistical Analysis

Statistical analyses were performed in SPSS version 24 (IBM Corp., Armonk, NY). Paired t-tests were used to examine differences in data quality (i.e. in linewidths, signal-to-noise ratio, CRLB, and voxel tissue proportions) between conditions. The effects of oxytocin vs placebo on regional brain ^{1}H-MRS metabolite levels were assessed using paired t-tests. Our primary hypothesis tested for effects on glutamate and Glx scaled to creatine, and our secondary hypothesis tested for effects on other metabolites (myo-inositol, N-acetylaspartate and choline) scaled to creatine. Choline (or “total choline”) was the sum of glycerophosphocholine and phosphocholine. The alpha level was Bonferroni corrected (thresholded $p<.05$ / 5 metabolites= $p<.01$, two-tailed) for multiple comparisons per region. For both primary and secondary analyses, boxplots were used to identify potential outliers and paired t-tests were repeated after their exclusion in sensitivity analyses. We also repeated the primary and secondary analyses after excluding those participants taking antidepressants (N=8) or benzodiazepines (N=1). The same procedures as detailed above were used in the analysis of water-scaled metabolite levels corrected for voxel tissue content (which also included analysis of creatine itself), which we conducted to ensure that our results were not driven by the scaling to creatine. Exploratory Spearman’s correlations were then used to examine whether the effects of oxytocin on the primary outcome (change in glutamate and Glx levels—scaled to creatine—in the hippocampus, ACC and thalamus in

the oxytocin vs placebo condition) were predicted by the baseline level of behavioural measures that characterise CHR-P patients. These included attenuated positive psychotic symptoms (sum of severity scores for items P1-P4 of the CAARMS) and social cognition, measured through the Reading the Mind in the Eyes task (RMET) scores. Since these correlations were exploratory in nature they were not corrected for multiple comparisons. Finally, given some attrition in study participation, we updated our power calculation for the primary hypotheses (glutamate and Glx) to assess the actual power of our analyses (see Supplementary Material).

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RESULTS

Sample Characteristics

Demographic and clinical characteristics of the sample are presented in Table 1. One subject was removed due to protocol violations, and one further subject did not complete the scanning session due to experiencing attenuated psychotic symptoms (at ~75mins during the placebo condition), leaving a sample of N=28 for the ACC and thalamus. Two further subjects did not complete the hippocampal ^{1}H-MRS scan, leaving N=26 for this region. No adverse side effects of oxytocin were clinically observed.

<Table 1>

<Figure 2>

^{1}H-MRS data quality

Representative spectra for all regions (left hippocampus, ACC, and left thalamus) are provided in Figure 2. Spectra were of good quality in all regions—no data were excluded due to Cramer-Rao minimum variance bounds >20% and no significant differences in spectral quality or voxel tissue content were observed between oxytocin and placebo conditions (Table 2).

<Table 2>

Effects of oxytocin on glutamate and Glx levels

The analysis of the primary outcome revealed no significant effects of oxytocin vs placebo on glutamate or Glx scaled to creatine (nor in voxel tissue-corrected glutamate or Glx levels) in the hippocampus, ACC or thalamus (Table 3).

Effects of oxytocin on other metabolite levels

Analysis of the secondary outcomes revealed no significant effects on any other metabolites quantifiable from the ^{1}H-MRS spectra (myo-inositol, choline and N-acetylaspartate) in any region, with the exception of choline scaled to creatine in the ACC, which was significantly increased in the oxytocin (mean ± SD; 0.25 ± 0.02) relative to the placebo (mean ± SD; 0.24 ± 0.02) condition ($t(27)=2.88$, $p=.008$; Cohen's d =0.54; Figure 3)(Table 3). When using ACC choline levels corrected for voxel tissue content, we observed a numerical increase in the oxytocin (mean ± SD; 2.93 ± 0.36) relative to the placebo (mean ± SD; 2.77 ± 0.41) condition ($t(27)=2.57$, $p=.02$; Table 3), but this effect was not significant at the Bonferroni-corrected significance threshold of $p<.01$ (Table 3).

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<Table 3>

<Figure 3>

Sensitivity analysis

Removal of outliers made no material change to the results of any statistical test (Table 3). Removal of subjects taking antidepressants and benzodiazepines meant that choline scaled to creatine was now significantly increased in the thalamus in the oxytocin (mean ± SD; 0.30 ± 0.03) relative to the placebo (mean ± SD; 0.28 ± 0.03) condition ($t(20)=3.11$, $p=.006$).

Exploratory correlations

Social Cognition

One subject did not complete the Reading the Mind in the Eyes task (RMET), leaving N=25 for the hippocampus, and N=27 for the ACC and thalamus. RMET (theory of mind) scores were negatively associated with absolute change in glutamate scaled to creatine (rho= -.546, p=.005) and Glx scaled to creatine (rho= -.509, p=.009) in the hippocampus (Supplementary Table S1). That is, those with better theory of mind scores tended to have the biggest decreases in hippocampal glutamate after oxytocin, while those with lower scores (where oxytocin is presumed to have the greatest effect (Feeser *et al*, 2015; Spengler *et al*, 2017)) tended to have an increase in hippocampal glutamate after oxytocin (Supplementary Figure S1). There were no significant correlations between RMET scores and glutamate or Glx levels in the ACC or thalamus (all p>.05; Table S1). It should be noted that exploratory correlations were not corrected for multiple comparisons.

Attenuated Psychotic Symptoms

One subject had one item missing out of the four items that constitute the positive subscale of the CAARMS, leaving N=25 for the hippocampus, N=27 for the ACC and N=27 for the thalamus. There were no significant correlations between CAARMS attenuated psychotic symptom scores and change in glutamate or Glx scaled to creatine in the hippocampus, ACC or thalamus (Supplementary Table S2).

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DISCUSSION

This is the first study to investigate the neurochemical effects of a single dose of oxytocin in CHR-P individuals. The key finding was that oxytocin did not modulate glutamate (or glutamate plus glutamine; Glx) levels in the hippocampus, ACC or thalamus in the time interval examined (approximately 75-93 minutes post-dosing). There were also no effects on other metabolites, with the exception of choline in the ACC, concentrations of which were significantly increased after oxytocin.

Our primary hypothesis, that an acute dose of oxytocin would modulate glutamate or Glx levels in CHR-P individuals, was not verified. One implication of these findings relates to the potential mechanism of oxytocin's effects. A previous study found that an acute dose of intranasal oxytocin had marked effects on cerebral perfusion (Paloyelis *et al*, 2016) across regions that show neurochemical alterations in CHR-P groups (Merritt *et al*, 2016; Poels *et al*, 2014; Tandon *et al*, 2013). Our own work has also revealed that acute oxytocin modulates hippocampal perfusion in those at CHR-P (Davies *et al*, 2019). Given these demonstrable neural effects, in this study we sought to test the hypothesis that oxytocin also modulates metabolite concentrations (especially glutamate and Glx) in CHR-P individuals. Negative findings are underreported in the scientific literature and can be difficult to interpret, but three different possibilities are presented below. First, while we did not find any effect on glutamate or Glx levels in the hippocampus, ACC or thalamus, it is still possible that oxytocin mediates its effects via modulation of glutamatergic neurotransmission either (a) directly, but we are not able to detect it using ^{1}H-MRS (e.g. due to its lack of specificity for neuronal glutamate/Glx and large voxel size), or (b) indirectly via modulation of GABA and associated neural microcircuits, which was not directly quantifiable in the current study. Indeed, some preclinical studies have shown that exogenous oxytocin administration does not alter basal glutamate levels per se, but blocks or attenuates changes in glutamate induced by deleterious manipulations such as drug-induced deficits and restraint stress (Qi *et al*, 2009, 2012)—while it can directly alter basal GABA levels (Qi *et al*, 2012). It may also be the case that the heterogeneity inherent in CHR-P samples, both clinically and in relation to regional metabolite concentrations at the specific time of scanning (which may differ between those who will and will not later transition, and those with good vs poor functional outcomes (Bossong *et al*, 2018)), may dilute and obscure observable oxytocin effects on glutamate when measured at group level. Stratification of CHR-P samples in future studies would—in theory—allow exploration of this possibility.

A second possible explanation for these negative findings may be that the time interval between intranasal administration of oxytocin and the onset of the ^{1}H-MRS acquisition was too long and potential effects on glutamate/Glx were missed. The mean ± SD of the time since intranasal administration for each region in the current study was: thalamus (75 ± 4 mins), ACC (84 ± 5 mins), and hippocampus (93 ± 6 mins). Early—albeit indirect—evidence suggested that the neural effects of intranasal oxytocin would last at least as long as our data sampling intervals. For example, notwithstanding the extremely small sample, previous work reported that following intranasal administration, the oxytocin signal in cerebrospinal fluid only begins to significantly increase at 75 minutes post-dosing (Striepens *et al*, 2013). Another study found that while the effects of oxytocin on human cerebral blood flow peak at 39-51 minutes post-administration (with a gradual diminution thereafter), they observed sustained perfusion effects over the entire post-treatment interval (up to 78 minutes) (Paloyelis *et al*, 2016). However, more recent work suggests that the effects of oxytocin vary as a function of method of administration, brain region, dosage and time since dosing (Martins *et al*, under review). For example, in one study of healthy males, oxytocin's effects on amygdala inhibition were observed only between 45-70 minutes post-administration, and not before or after (Spengler *et al*, 2017). A forthcoming study (Martins *et al*, under review) using a double-blind, placebo-controlled, crossover procedure and comparing various methods of oxytocin administration, indicates that oxytocin-induced decreases in amygdala perfusion are present only within the first 15-30 minutes post-dosing. Furthermore, we recently showed that in the same CHR-P sample as used in the current study, the effects of oxytocin on hippocampal perfusion were more robust in the earlier (22-28 minutes) vs the later (30-36 minutes) post-dosing interval (Davies *et al*, 2019). It may therefore be that in the hippocampus, thalamus and ACC, changes in glutamate and Glx are occurring closer to the time of administration.

Third, the observed lack of effects (especially on glutamate and Glx) may be related to the acute vs long-term effects of repeated oxytocin administration. A recent study found that repeated administration of 48IU/day intranasal oxytocin over 6 weeks in people with autism spectrum disorder significantly decreased N-acetylaspartate and Glx levels in the medial prefrontal cortex (Benner *et al*, 2018), effects that were not observed after 24IU acute challenge (Aoki *et al*, 2015). Akin to the current work, both of the aforementioned studies were randomised, double-blind, placebo-controlled, crossover designs (Aoki *et al*, 2015; Benner *et al*, 2018). In addition, their mouse model showed that repeated—but not acute—oxytocin administration significantly downregulated Nr2b (a subunit of the glutamate NMDA receptor) transcript expression, while acute administration reduced levels of molecules associated with the oxytocinergic system (oxytocin mRNA) and with neural activity

(immediate early genes; cFos and Arc), but not Nr2b (Benner *et al*, 2018). In light of these findings, it is possible that longer-term treatment with oxytocin would have had significant effects on the glutamate system, with altered metabolite levels detectable using ^{1}H-MRS.

A final potential contributing factor for our lack of observed effects on glutamate and Glx relates to statistical power. The current study had a relatively large sample size for a neuroimaging-based, within-subject drug challenge study. However, we cannot rule out the possibility that oxytocin had effects of smaller magnitude (e.g. Cohen's D less than ~0.56), which corresponds to a maximum percent change in metabolite levels of between ~13-20% (depending on the brain region).

Our secondary hypothesis was that oxytocin would modulate metabolites that have been found to be altered in CHR-P and other high-risk groups (Bossong *et al*, 2018; Stone *et al*, 2009; Tandon *et al*, 2013). We found that, at ~75-93 minutes post-dosing, oxytocin had no effects on concentrations of N-acetylaspartate, myo-inositol or creatine in the hippocampus, ACC or thalamus. The poor availability of previous literature hinders interpretation of these results, and as discussed above, we may have had insufficient power to test for effects on these metabolites. We also found that oxytocin had no effect on choline in the hippocampus and thalamus, but had significant effects on choline in the ACC, which was significantly increased after oxytocin. Choline has a two-fold greater concentration in glial cells compared to neurons (Urenjak *et al*, 1993), is a precursor and metabolite of acetylcholine, and as an essential component of membrane phospholipids is considered a marker of membrane turnover and integrity (Bertolino and Weinberger, 1999). Previous evidence has implicated altered choline in the early phases of psychosis. Increased regional choline levels have been found in patients with first-episode psychosis (Plitman *et al*, 2016) and schizophrenia (Bustillo *et al*, 2014) and is associated with a longer duration of untreated illness (Théberge *et al*, 2004), potentially due to glutamatergic excitotoxicity causing cell damage (Gasull *et al*, 2000), resulting in elevated choline from increased cell membrane turnover (Bertolino and Weinberger, 1999; Théberge *et al*, 2004), or potentially due to increased astrocytic turnover of glutamatergic compounds (Bustillo *et al*, 2014; Plitman *et al*, 2016). There is also specific evidence suggesting that abnormal choline levels characterise populations at risk of psychosis. Earlier work showed that choline levels are altered in familial high risk individuals vs low risk controls in the hippocampus (Capizzano *et al*, 2011) and in the ACC (Tandon *et al*, 2013), with ACC increases correlating with attenuated psychotic symptom severity and schizotypy (Tandon *et al*, 2013). Within CHR-P individuals, ACC choline levels are increased in those who go on to transition vs those who do not (Jessen *et al*, 2006)—although CHR-P differences are not always found (Stone *et al*, 2009; Uhl *et al*, 2011). There is also evidence

demonstrating an effect of oxytocin on choline in the ACC. A previous study conducted in males with autism spectrum disorder found that acute administration of oxytocin modulated levels of choline—but not any other metabolite—in the medial prefrontal cortex/ACC, albeit at a relaxed statistical theshold (Aoki *et al*, 2015). However, it is important to note that the oxytocin effects on choline were not within our primary hypothesis and therefore these findings should be interpreted with caution. Despite similar results in other neurodevelopmental disorders (Aoki *et al*, 2015), further independent replication studies are needed to validate this finding. The stringent correction for multiple comparisons (implemented because our primary hypothesis was for glutamate and Glx only) may also account for the (statistically) discordant choline results from the creatine-scaled vs the voxel tissue content-corrected methods.

This study has some limitations. First, ^{1}H-MRS is not able to distinguish between intracellular vs extracellular (or neuronal vs non-neuronal) metabolites, and rather represents a whole tissue measure within the specified voxel (Jelen *et al*, 2018). This leaves the possibility that oxytocin modulated glutamate/Glx specifically in the neuronal component (i.e. related to neurotransmission/pyramidal neurons—which represents a very small proportion of the total glutamate in brain tissue—in the region of uM concentrations) vs the non-neuronal component (i.e. within glia), which cannot be discriminated. Nevertheless, ^{1}H-MRS at 3 Tesla is widely used to quantify glutamate and Glx and the data acquired in the current study were of high quality. We therefore think it unlikely that acute oxytocin has effects on neurochemical metabolite concentrations other than choline in CHR-P individuals (at least at ~75-93 minutes post-dosing) and propose that this absence of effects is not attributable to methodological shortcomings. Scanning at higher field strengths (e.g. 7 Tesla) and advanced techniques (such as GluCEST or ^{1}H-fMRS) are now becoming available, and will enable future research to reliably separate spectral components and investigate dynamic changes in metabolite levels (Jelen *et al*, 2018; Roalf *et al*, 2017; Thakkar *et al*, 2017). Our use of metabolite values scaled to creatine could also have meant that the results were caused by changes in voxel creatine levels. We overcame this limitation by also presenting water-scaled metabolite levels corrected for voxel tissue content, which were concordant with those scaled to creatine. Furthermore, while we recruited a representative sample of CHR-P males as typically found in specialist CHR-P services (Fusar-Poli *et al*, 2013b), none of which were taking antipsychotics, a number of participants were taking antidepressants and benzodiazepines. However, we found no material change in the results after exclusion of these cases, aside from an increase in thalamic choline after oxytocin. Future oxytocin studies should also investigate effects in females (which we excluded due to the known sexual dimorphism in oxytocinergic function (Rilling *et al*, 2014)) and may want to explore

variation in the oxytocin receptor gene, which is likely to modulate response to intranasal oxytocin (Tost *et al*, 2010). Finally, an interesting caveat is that while previous studies in autism spectrum disorder found no absolute differences in metabolite levels after acute oxytocin beyond choline in the ACC, oxytocin's effects on medial prefrontal N-acetylaspartate levels were related to the oxytocin-induced recovery in functional MRI signal in the same region during a social cognition fMRI task, and improvement in social-communication related symptoms (Aoki *et al*, 2015). Examining whether changes in brain activation or functional connectivity after oxytocin are associated with oxytocin's effects on metabolites in CHR-P patients remains an avenue for future research.

Conclusions

This study suggests that at ~75-93 minutes post-dosing, acute administration of oxytocin does not alter levels of glutamate, glutamate+glutamine, N-acetylaspartate, myo-inositol or choline in the hippocampus, ACC, or thalamus in those at CHR-P, aside from potential effects on choline in the ACC.

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TABLES

TABLE 1. Participant demographic and clinical characteristics

	Variable	Total sample (N=30)
Demographic	Age, years; mean (SD)	23.2 (4.7)
	Age range, years	18–35
	Sex, male/female	30/0
	Ethnicity (White/Black/Asian/Mixed)	16/6/4/4
	Handedness, right/left	26/4
	Education, years; mean (SD)	13.2 (1.9)
Clinical	CHR-P Subtype[a] (BLIPS/APS/GRD)	6/23/1
	CAARMS attenuated positive symptoms[b]; mean (SD)	11.7 (3.3)
	Baseline anxiety score[c]; mean (SD)	35.6 (8.7)
	GAF social score; mean (SD)	6.8 (1.5)
	GAF role score; mean (SD)	7.0 (1.7)
	RMET score[d]; mean (SD)	24.7 (5.6)
	Current antidepressant medication (yes/no)	8/22
	Current antipsychotic medication (yes/no)	0/30
	Current benzodiazepine medication (yes/no)	1/29
Substance Use	Current smoker (yes/no)	17/13
	Cigarettes/day; mean (SD)	9.8 (6.0)
	Cannabis use[e]; median (range)	2 (0–4)
	Alcohol, AUDIT total; mean (SD)	7.2 (7.7)

[a]Comprehensive Assessment of At-Risk Mental States (CAARMS) subgroup, BLIPS – Brief Limited Intermittent Psychotic Symptoms; APS – Attenuated Psychotic Symptoms; GRD – Genetic Risk and Deterioration. [b]Sum of the global (severity) ratings for positive subscale items (P1-P4) of the CAARMS. [c]Mean of pre-scan anxiety scores across conditions as measured by the State Trait Anxiety Inventory (STAI), with 4 subjects missing 1/20 items that constitute the total score, these were thus imputed using next-observation-carried-backwards. [d]Reading the Mind in the Eyes (RMET) social cognition task - two subjects did not complete the RMET, leaving N=28. [e]Cannabis use: 0 = never, 1 = experimental use (tried occasionally), 2 = occasional use (small quantities from time to time), 3 = moderate use (moderate quantities regularly / large amounts occasionally), 4 = severe use (frequently used large quantities, often to intoxication/debilitation). AUDIT – Alcohol Use Disorders Identification Test. CHR-P – Clinical High Risk for Psychosis.

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TABLE 2. Spectral and structural voxel data. Mean ± SD estimates of linewidths, signal-to-noise ratios, CRLB, and voxel proportions of white matter, grey matter and CSF in the hippocampus, anterior cingulate cortex and thalamus in oxytocin and placebo conditions.

Spectral and structural voxel data									
	Hippocampus (N=26)			**Anterior Cingulate Cortex (N=28)**			**Thalamus (N=28)**		
	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic
FWHM	0.07 ± 0.02	0.07 ± 0.03	t(25)=-1.12, p=.27	0.04 ± 0.01	0.04 ± 0.01	t(27)=-0.37, p=.71	0.05 ± 0.02	0.05 ± 0.01	t(27)=-0.63, p=.53
SNR	15.54 ± 3.40	15.15 ± 2.59	t(25)=0.60, p=.56	36.07 ± 6.09	34.79 ± 5.72	t(27)=1.21, p=.24	21.0 ± 3.21	20.64 ± 2.83	t(27)=0.82, p=.42
WM	0.36 ± 0.07	0.36 ± 0.09	t(25)=-0.25, p=.81	0.08 ± 0.02	0.09 ± 0.06	t(27)=-1.15, p=.26	0.78 ± 0.08	0.76 ± 0.08	t(27)=0.71, p=.48
GM	0.60 ± 0.06	0.60 ± 0.07	t(25)=0.17, p=.87	0.65 ± 0.05	0.64 ± 0.08	t(27)=0.85, p=.40	0.22 ± 0.07	0.23 ± 0.08	t(27)=-0.80, p=.43
CSF	0.04 ± 0.02	0.04 ± 0.02	t(25)=0.41, p=.69	0.27 ± 0.05	0.27 ± 0.06	t(27)=0.51, p=.62	0.005 ± 0.007	0.004 ± 0.005	t(27)=0.58, p=.57
Cramér Rao Lower Bounds (CRLB)									
	Hippocampus (N=26)			**Anterior Cingulate Cortex (N=28)**			**Thalamus (N=28)**		
Metabolite	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic
Glu	9.62 ± 2.17	8.92 ± 1.62	t(25)=1.79, p=.09	5.04 ± 0.43	5.18 ± 0.43	t(27)=-1.07, p=.29	9.25 ± 2.27	9.21 ± 2.11	t(27)=0.06, p=.96
Glx	10.65 ± 2.73	10.15 ± 2.22	t(25)=0.78, p=.44	5.54 ± 0.79	5.82 ± 0.61	t(27)=-1.49, p=.15	10.61 ± 2.47	11.18 ± 3.54	t(27)=-0.68, p=.50
NAA	3.62 ± 0.98	3.88 ± 1.61	t(25)=-0.70, p=.49	2.21 ± 0.42	2.39 ± 0.50	t(27)=-1.72, p=.10	2.93 ± 0.90	2.93 ± 0.60	t(27)=0.00, p=1.00
TCho	3.54 ± 0.76	3.65 ± 0.89	t(25)=-0.68, p=.50	2.71 ± 0.53	2.82 ± 0.61	t(27)=-0.83, p=.42	3.14 ± 0.45	3.25 ± 0.44	t(27)=-1.14, p=.26
ml	5.04 ± 0.82	5.46 ± 1.61	t(25)=-1.49, p=.15	4.14 ± 0.76	4.11 ± 0.88	t(27)=0.19, p=.85	6.50 ± 1.43	7.29 ± 3.23	t(27)=-1.25, p=.22
Cre	3.50 ± 0.65	3.73 ± 0.83	t(25)=-1.36, p=.18	2.07 ± 0.38	2.07 ± 0.26	t(27)=0.00, p=1.00	3.14 ± 0.45	3.18 ± 0.39	t(27)=-0.37, p=.71

Abbreviations: FWHM, full width at half-maximum (linewidth) in ppm (parts per million); WM, white matter; GM, grey matter; CSF, cerebrospinal fluid; CRLB, Cramér Rao Lower Bounds; SNR, signal-to-noise ratio; Glu, Glutamate; Glx, Glutamate + Glutamine; NAA, N-acetylaspartate; TCho, Total Choline; ml, myo-Inositol; Cre, Creatine.

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TABLE 3. Creatine-scaled and tissue-corrected metabolite values in the hippocampus, anterior cingulate cortex and thalamus (mean ± SD)

Scaled to creatine									
	Hippocampus (N=26)			**Anterior Cingulate Cortex (N=28)**			**Thalamus (N=28)**		
Metabolite	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic
Glu/Cre	1.01 ± 0.16	1.07 ± 0.17	t(25)=-1.47, p=.15	1.33 ± 0.11	1.36 ± 0.16	t(27)=-0.74, p=.46	0.98 ± 0.18	0.98 ± 0.16	t(27)=-0.01, p=1.00[a]
Glx/Cre	1.40 ± 0.24	1.42 ± 0.24	t(25)=-0.31, p=.76	1.70 ± 0.17	1.72 ± 0.23	t(27)=-0.45, p=.66[a]	1.19 ± 0.28	1.24 ± 0.27	t(27)=-0.54, p=.59
NAA/Cre	1.24 ± 0.19	1.23 ± 0.16	t(25)=0.03, p=.98[a]	1.24 ± 0.09	1.24 ± 0.08	t(27)=-0.09, p=.93	1.59 ± 0.15	1.58 ± 0.19	t(27)=0.25, p=.81
TCho/Cre	0.32 ± 0.03	0.33 ± 0.02	t(25)=-1.11, p=.28	0.25 ± 0.02	0.24 ± 0.02	**t(27)=2.88, p=.008****	0.30 ± 0.03	0.29 ± 0.02	t(27)=1.63, p=.12
ml/Cre	0.90 ± 0.12	0.86 ± 0.11	t(25)=1.35, p=.19[a]	0.74 ± 0.07	0.75 ± 0.06	t(27)=-0.82, p=.42[a]	0.58 ± 0.09	0.56 ± 0.11	t(27)=0.76, p=.45[a]
Corrected for voxel tissue content									
	Hippocampus (N=26)			**Anterior Cingulate Cortex (N=28)**			**Thalamus (N=28)**		
Metabolite	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic	Oxytocin	Placebo	Statistic
Glu	7.44 ± 1.33	7.66 ± 1.04	t(25)=-0.69, p=.50	15.43 ± 1.62	15.43 ± 1.74	t(27)=0.02, p=.99	7.30 ± 1.39	7.28 ± 1.12	t(27)=0.05, p=.96[a]
Glx	10.37 ± 2.08	10.19 ± 1.81	t(25)=0.31, p=.76	19.65 ± 2.51	19.51 ± 2.53	t(27)=0.27, p=.79[a]	8.88 ± 2.06	9.16 ± 1.96	t(27)=-0.48, p=.64
NAA	9.05 ± 0.90	8.80 ± 0.84	t(25)=1.18, p=.25[a]	14.33 ± 1.12	14.15 ± 1.23	t(27)=0.85, p=.40[a]	11.75 ± 0.87	11.62 ± 0.69	t(27)=0.76, p=.46
TCho	2.38 ± 0.29	2.37 ± 0.41	t(25)=0.12, p=.90[a]	2.93 ± 0.36	2.77 ± 0.41	**t(27)=2.57, p=.02***	2.19 ± 0.22	2.13 ± 0.20	t(27)=1.38, p=.18[a]
ml	6.68 ± 1.28	6.21 ± 1.12	t(25)=1.57, p=.13	8.57 ± 1.11	8.57 ± 1.05	t(27)=-0.01, p=.99	4.29 ± 0.62	4.20 ± 0.93	t(27)=0.46, p=.65[a]
Cre	7.42 ± 0.86	7.25 ± 1.13	t(25)=0.73, p=.47[a]	11.60 ± 1.05	11.42 ± 0.96	t(27)=1.15, p=.26[a]	7.44 ± 0.44	7.44 ± 0.72	t(27)=-0.05, p=.96

Abbreviations: Glu, Glutamate; Glx, Glutamate + Glutamine; NAA, N-acetylaspartate; TCho, Total Choline; ml, myo-Inositol; /Cre, scaled to Creatine.

[a]removal of outliers made no material change to the results or conclusions

*although significant at the p<.05 level, this does not meet the Bonferroni-corrected p<.01 threshold

**significant at the Bonferroni-corrected p<.01 threshold

FIGURES

FIGURE 1. Simplified schematic of proposed neural circuit mechanisms of glutamatergic dysfunction and CHR-P pathophysiology. In **(1)**, low glutamate signal/input from hypofunctioning NMDARs (akin to 'faulty homeostatic sensors') leads GABAergic interneurons to homeostatically increase excitation by reducing inhibition (disinhibition) of glutamatergic pyramidal cells. However, by disinhibiting pyramidal cells (and thus increasing glutamate signalling) in this dysfunctional neural environment, the potential homeostatic adaptation becomes allostatic **(2)**. In **(3)**, enhanced excitation leads to an overdrive in the responsivity of midbrain dopamine neurons which project to the associative striatum (note that the connection between hippocampal pyramidal cells and midbrain dopamine neurons is presented as monosynaptic but is actually polysynaptic via the ventral striatum and ventral pallidum). Completing the (simplified) circuit, local glutamatergic tone is increased in **(4)** but is not detected as such by hypofunctioning NMDARs on GABAergic interneurons. Figure reproduced and adapted with permission from (Davies *et al*, 2019). For original diagrams and discussion of evidence for this proposed circuit, see (Krystal *et al*, 2017; Krystal and Anticevic, 2015; Lieberman *et al*, 2018; Lisman *et al*, 2008; Modinos *et al*, 2015). *Abbreviations:* Glu, glutamate; NMDAR, N-methyl-D-aspartate receptor; CA1, Cornu Ammonis 1.

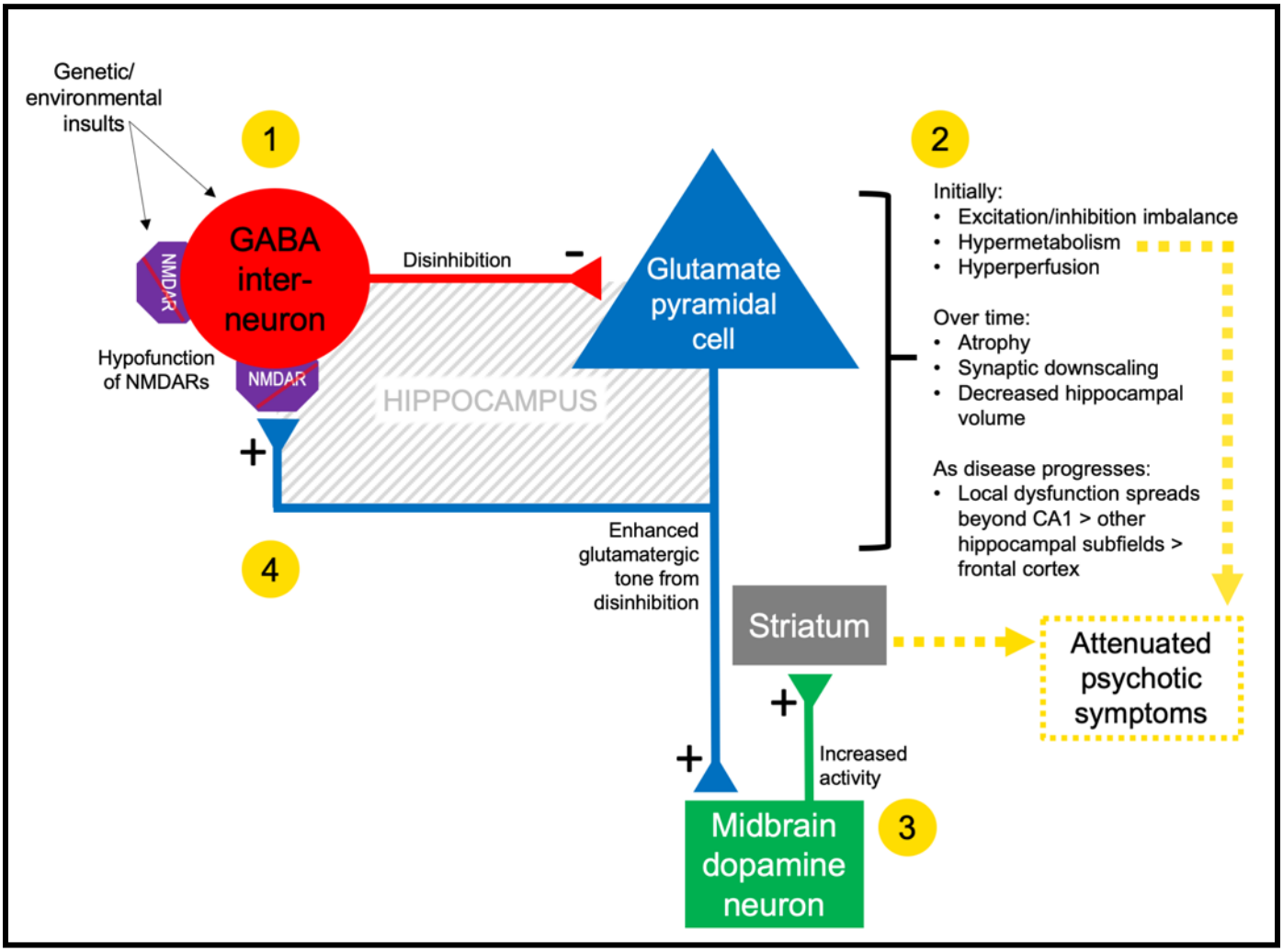

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FIGURE 2. Example ^{1}H-MRS voxel positioning and spectra in (A) left hippocampus, (B) anterior cingulate cortex, and (C) left thalamus.

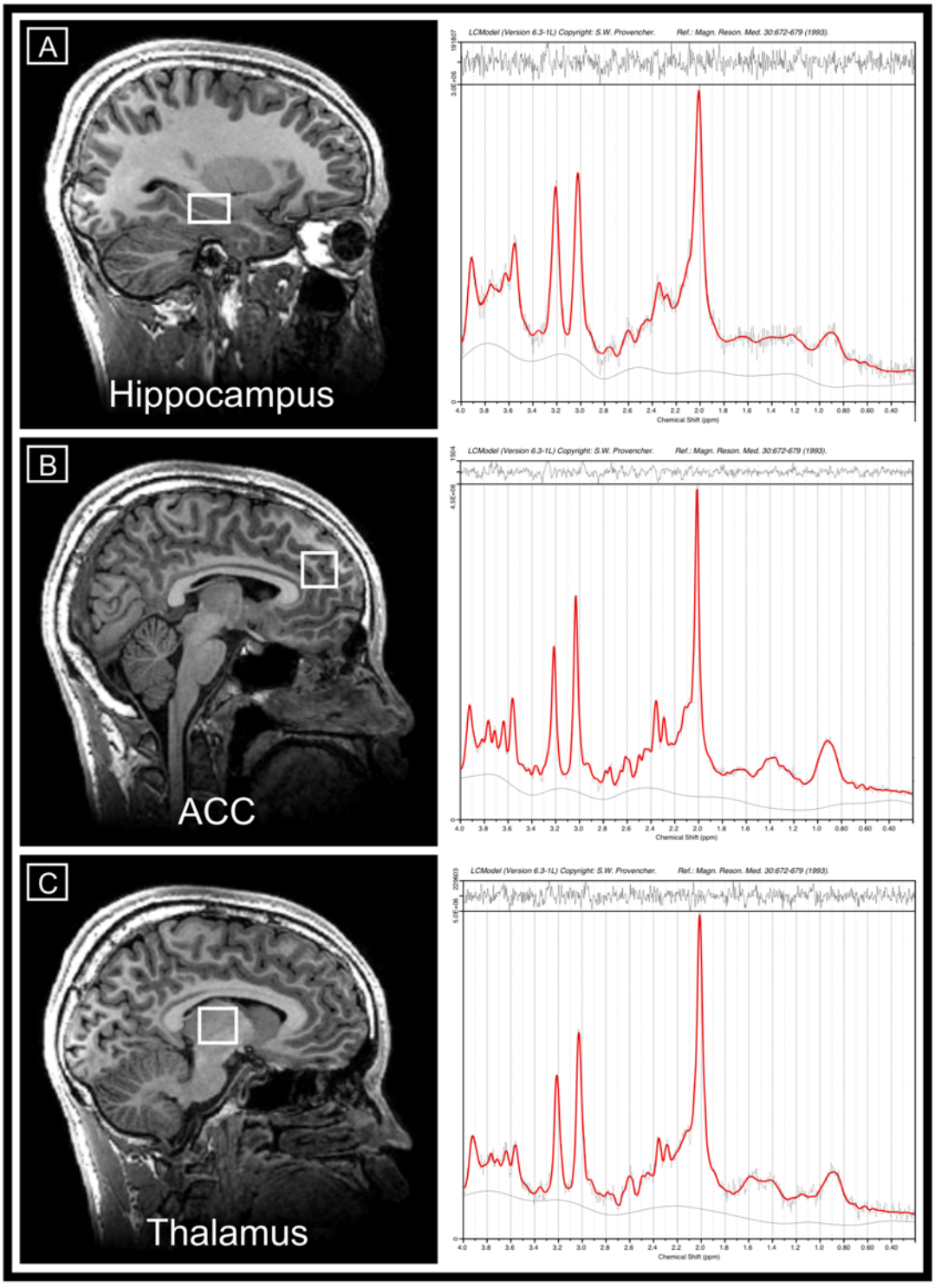
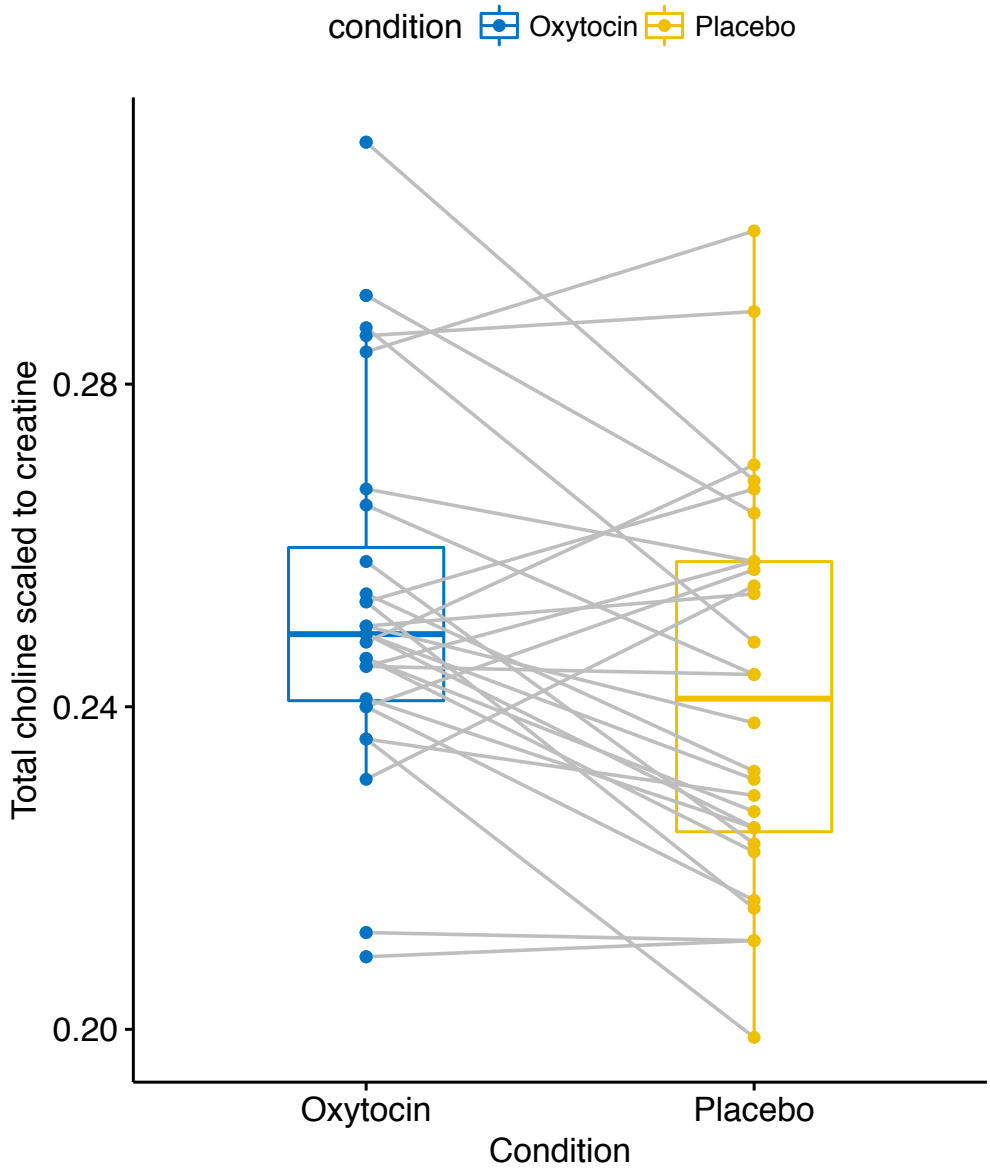


FIGURE 3. Choline levels (scaled to creatine) in the anterior cingulate cortex in oxytocin and placebo conditions. Individual data points are presented (overlaid) on standard boxplots, with grey lines connecting paired values.



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NEUROCHEMICAL EFFECTS OF OXYTOCIN IN PEOPLE AT CLINICAL HIGH RISK FOR PSYCHOSIS

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– SUPPLEMENTARY MATERIAL –

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Supplementary Experimental Procedures

Subjects were asked to abstain from using recreational drugs for at least one week prior to each MRI scan, and alcohol for at least 24 hours prior to each MRI scan. Urine screening was conducted before the scan for each participant.

The blinded spray bottles used for each session (containing oxytocin or matched placebo; see section below for formulation details) were visually identical and dispensed by the Maudsley Hospital Pharmacy. Intranasal administration followed recommended guidelines (Guastella *et al*, 2013) and a protocol adopted by a previous study conducted at our institute (Paloyelis *et al*, 2016). After a demonstration of the intranasal administration from a researcher using a spray bottle containing water, participants self-administered (in the presence of and with feedback from a researcher) one puff (4IU) of intranasal oxytocin or placebo every 30 seconds, alternating between nostrils, until 40IU (10 puffs) had been administered. The administration phase lasted approximately 4.5 minutes.

Both the participants and researchers were blind to the (crossover) treatment sequence (AB or BA) allocation. A randomisation list was generated by the Maudsley Hospital Pharmacy, which determined whether a participant received oxytocin or placebo in their first study visit and vice versa for the second study visit. On recruitment of a study participant, an unblinded clinical trial pharmacist, who was not involved with the rest of the study, allocated the participant to one of the two sequences (AB, BA) based on the randomisation list. Allocation information was kept concealed in the Maudsley Hospital Pharmacy.

Oxytocin and Placebo Formulation

The finished intranasal product was manufactured by the Pharmacy Manufacturing Unit, Guy's and St Thomas' NHS Foundation Trust. Active (Oxytocin): Syntocinon was obtained as Syntocinon Spray, marketed by Hersteller, 68330 Huningue, France. This was decanted into a 10ml amber dropper bottle and capped with a 10IU white nasal atomiser with 69mm dip and overcap. Placebo was placed in the same containers as above and contained excipients matching the active formulation.

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Exploratory correlations

Social Cognition

Table S1. Spearman's correlation coefficients for change in metabolites (oxytocin – placebo) in the hippocampus, ACC and thalamus vs RMET scores

Metabolite	Spearman's Rho for RMET	Sig. (2-tailed)	N
Hippocampus			
Change in Glu/Cre	-.546**	.005	25
Change in Glx/Cre	-.509**	.009	25
ACC			
Change in Glu/Cre	.288	.145	27
Change in Glx/Cre	-.035	.861	27
Thalamus			
Change in Glu/Cre	.120	.551	27
Change in Glx/Cre	.232	.245	27

** Correlation is significant at the 0.01 level (2-tailed).

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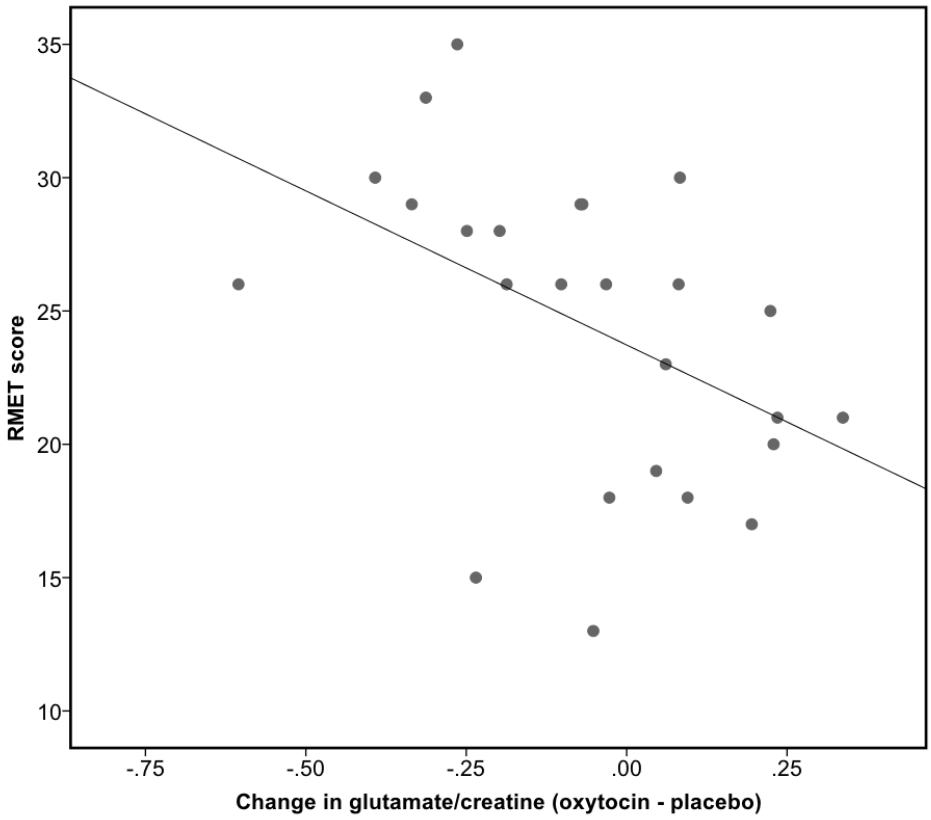
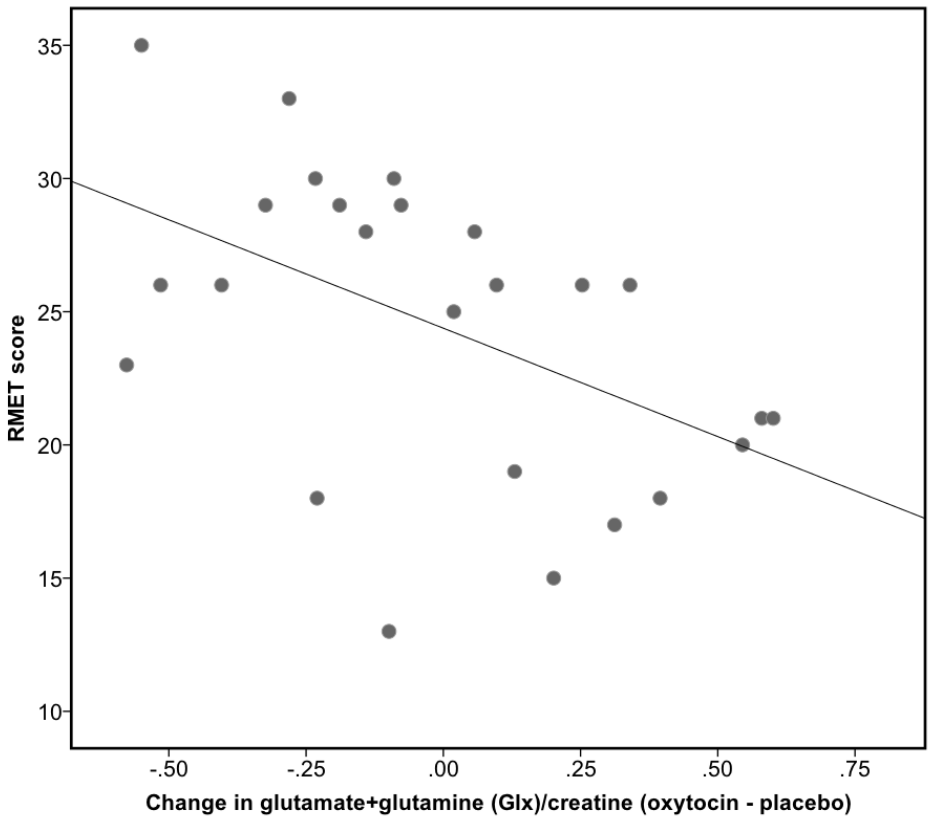
Figure S1. Scatterplots depicting relationship between change in glutamate and Glx scaled to creatine (oxytocin – placebo) in the hippocampus vs RMET scores





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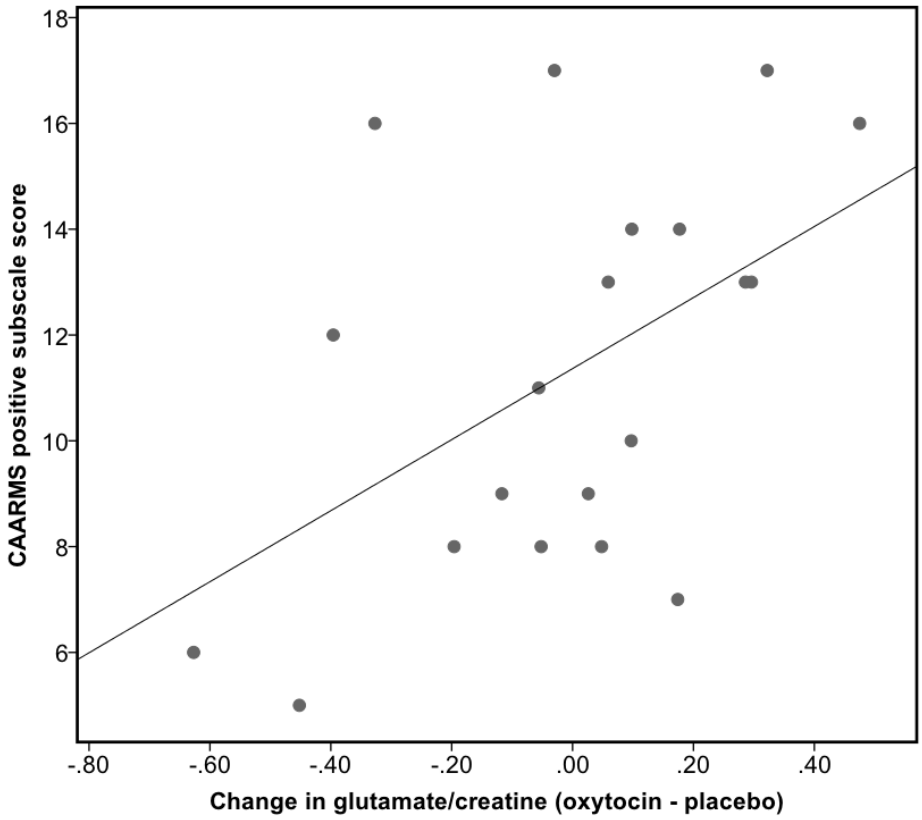
Attenuated Psychotic Symptoms

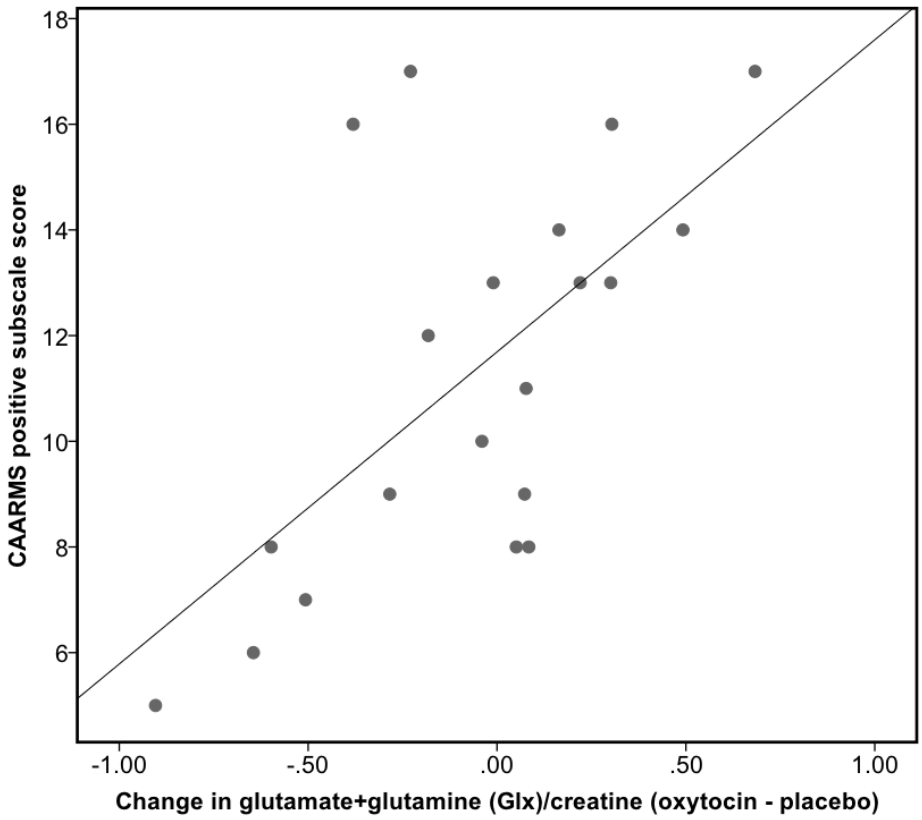
Table S2. Spearman's correlation coefficients for change in metabolites (oxytocin – placebo) in the hippocampus, ACC and thalamus vs CAARMS positive subscale severity scores

Metabolite	Spearman's Rho for CAARMS	Sig. (2-tailed)	N
Hippocampus			
Change in Glu/Cre	-.326	.111	25
Change in Glx/Cre	-.211	.311	25
ACC			
Change in Glu/Cre	.043	.831	27
Change in Glx/Cre	.175	.383	27
Thalamus			
Change in Glu/Cre	.344	.079	27
Change in Glx/Cre	.336	.087	27

There were no significant correlations between CAARMS positive scores and change in glutamate or Glx scaled to creatine in the hippocampus, ACC or thalamus (Table S2). However, after removing those subjects taking antidepressants and benzodiazepines in sensitivity analyses, there was a significant positive association between CAARMS positive scores and change in thalamic glutamate scaled to creatine (rho= .523, p=.018; N=20) and change in thalamic Glx scaled to creatine (rho= .606, p=.005; N=20)(Figure S2). That is, higher levels of positive symptoms were associated with increased glutamate (and Glx) after oxytocin, while lower levels of positive symptoms were associated with a reduction of thalamic glutamate (and Glx) after oxytocin vs placebo (Figure S2).

Figure S2. Scatterplots depicting relationship between change in glutamate and Glx scaled to creatine (oxytocin – placebo) in the thalamus vs CAARMS positive subscale severity scores [after excluding those subjects taking antidepressants or benzodiazepines in sensitivity analyses]





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Updated Power Calculations

Updated power calculations were computed using the mean and SD of control metabolite values from a previous study (Stone *et al*, 2009). All calculations are for paired t-tests (two-tailed; 80% power; a=.05), where the assumed SD is the same for both sessions: $SD_{diff} = \sqrt{2}$ x SD. The final sample sizes in the current study were hippocampus=26, ACC=28, thalamus=28.

Overall, the updated power calculations indicated that the minimum effect size (Cohen's D) for within-subject change in glutamate (detectable at 80% power when a=.05), was d=0.57 (~18% change in glutamate levels) in the hippocampus, d=0.55 (~13% change) in the ACC, and d=0.55 (~13% change) in the thalamus. The same values for Glx were: d=0.57 (~20% change in Glx) in the hippocampus, d=0.55 (~14.5% change) in the ACC, and d=0.55 (~19% change) in the thalamus. Therefore, it is possible that oxytocin induced changes of smaller magnitude but we were unable to detect them with our final sample sizes due to low statistical power. Detailed power calculations are presented in Table S3 below.

Table S3. For each Cohen's D effect size (small, medium, large), showing the associated % change in metabolite, the power we had in the current study to detect this % change (or D) [given the sample size for each region], and the *required* sample size to detect such an effect size

Cohen's D	Glutamate			Glx		
	% change in metabolite	Power in **this study** to detect D	Required N	% change in metabolite	Power in **this study** to detect D	Required N
Hippocampus (this study N=26)						
Small (d=0.2)	7	18%	176	7	16%	210
Medium (d=0.5)	16	66%	36	18	69%	34
Large (d=0.8)	26	97%	15	29	98%	15
ACC (this study N=28)						
Small (d=0.2)	5	19%	180	5	16%	223
Medium (d=0.5)	12	73%	33	13	71%	35
Large (d=0.8)	19	98%	15	21	98%	15
Thalamus (this study N=28)						
Small (d=0.2)	5	18%	186	7	18%	195
Medium (d=0.5)	12	72%	34	17	71%	35
Large (d=0.8)	19	98%	15	28	98%	15

From Table S3 it becomes clear that effects of oxytocin would have to be of medium to large magnitude (as defined by Cohen's D) for us to detect them with our final sample sizes.

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