Neurochemical effects of oxytocin in people at clinical high risk for psychosis

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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 min 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 ~75-99 min 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.

1. 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 (Fig. 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).

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
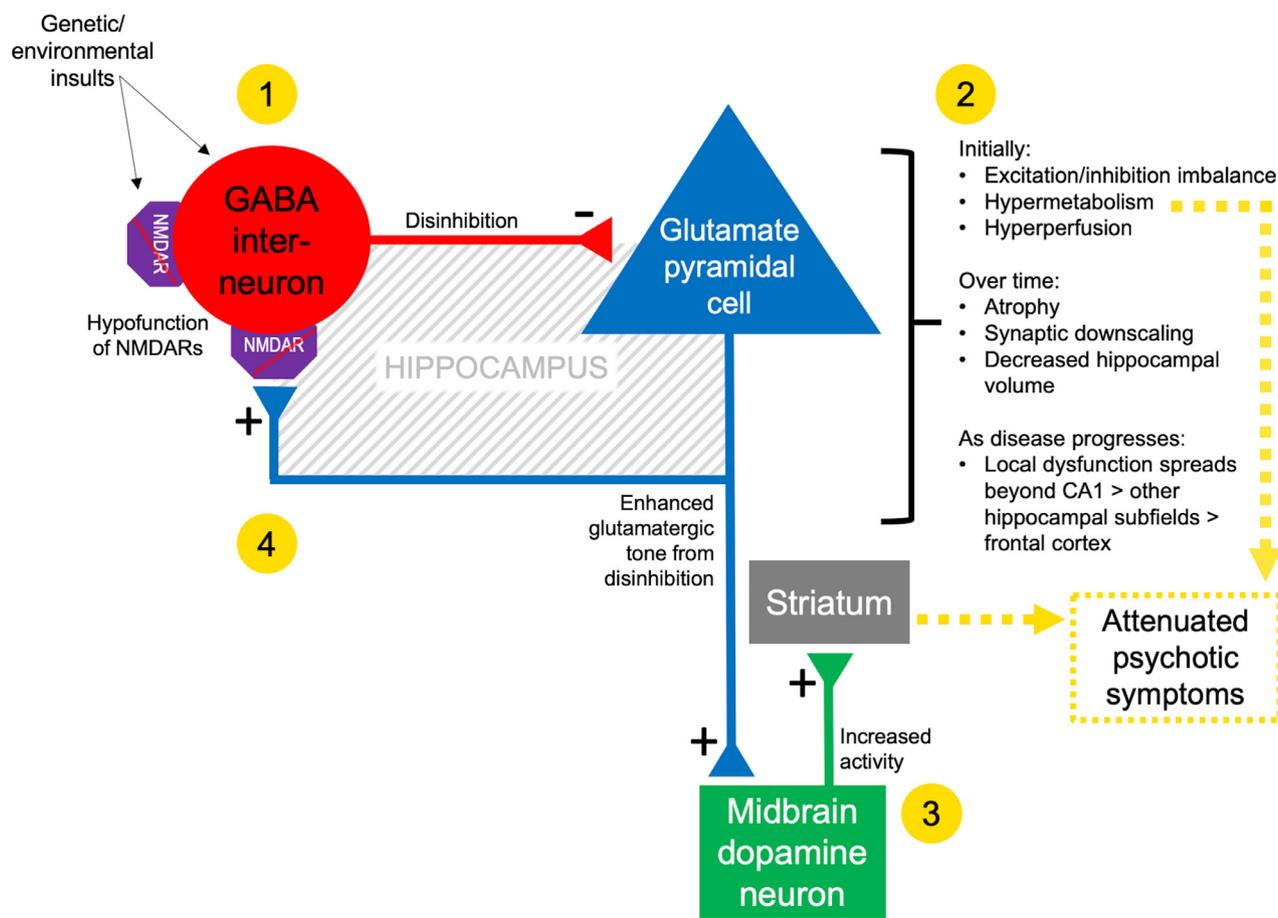
Fig. 1 *Simplified schematic of proposed neural circuit mechanisms of glutamatergic dysfunction and CHR-P pathophysiology.* In (1), low glutamate signal/input from hypofunctioning NMDARs (akin to 'faulty homeostatic sensors') leads GABAergic interneurons to homeostatically increase excitation by reducing 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 (CCBY 4.0)[1] 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.

are currently no licensed pharmacological treatments for this patient group, which remains an unmet clinical need (Davies et al., 2018a, b). 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 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 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 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 ^{1}H-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 enhances 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 (e.g., using n-acetyl-cysteine), increases and decreases (respectively) in the concentrations of regional glutamate/Glx levels as measured by ^{1}H-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 ^{1}H-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 double-blind, oxytocin vs. placebo single-dose challenge crossover ^{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 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.

2. Experimental procedures

2.1. 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, 30 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 (Fusar-Poli et al., 2016), some of whom may meet Acute and Transient Psychotic Disorder criteria (Fusar-Poli et al., 2017; Fusar-Poli et al., 2018)) 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.

2.2. Procedure

We used a 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 11:30 AM 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 s, 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).

2.3. 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=3.016 ms; TR=7.31 ms; TI=400 ms; voxel size=$1.1 \times 1.1 \times 1.2$ mm^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 (Fig. 2) using conventional Point-Resolved Spectroscopy acquisition (PRESS; TR = 3000 ms; TE = 30 ms; 96 averages) in three separate 6-min 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) $20 \times 20 \times 15$ mm^3 in the hippocampus, $20 \times 20 \times 20$ mm^3 in the ACC, and $15 \times 20 \times 20$ mm^3 in the thalamus. The mean $\pm$ SD of the time from dosing offset (end of intranasal administration) for each region was: thalamus (75 ± 4 min), ACC (84 ± 5 min), and hippocampus (93 ± 6 min). These equate to approximate (mean) post-dosing sampling periods of: thalamus (75–81 min), ACC (84–90 min), and hippocampus (93–99 min).

2.4. 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 T), 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: $M_{corr}= M \times ([GM \times 1.21] +WM+ [CSF \times 1.55]) / (WM+GM)$, 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.

2.5. 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).

3. Results

3.1. 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 ~75min 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.

3.2. ^{1}H-MRS data quality

Representative spectra for all regions (left hippocampus, ACC, and left thalamus) are provided in Fig. 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).

3.3. 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
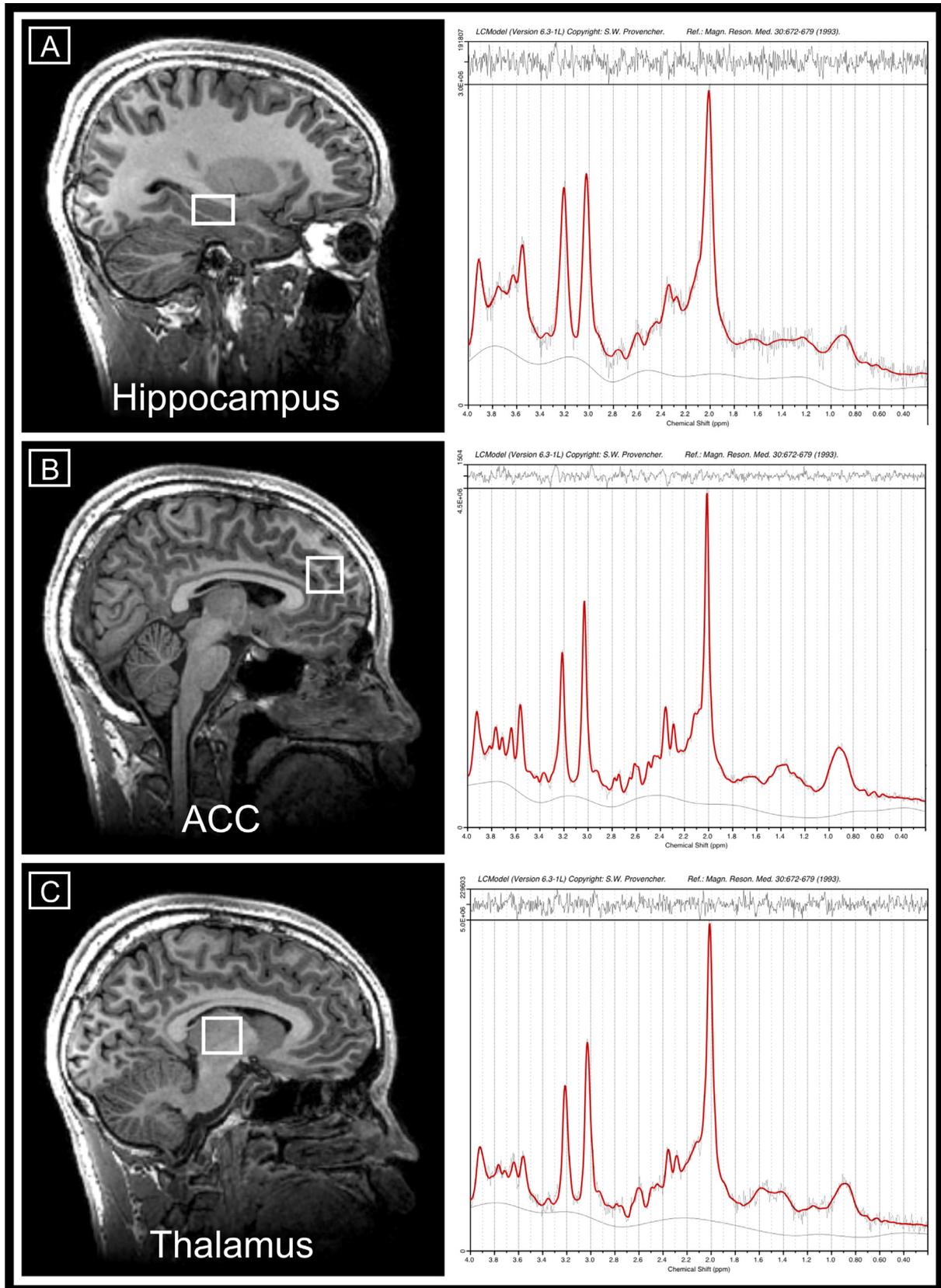
Fig. 2 Example ^{1}H-MRS voxel positioning and spectra in (A) left hippocampus, (B) anterior cingulate cortex, and (C) left thalamus.

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)
	GF social score; mean (SD)	6.8 (1.5)
	GF 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. GF – Global Functioning (role and social) scale.

to creatine (nor in voxel tissue-corrected glutamate or Glx levels) in the hippocampus, ACC or thalamus (Table 3).

3.4. Effects of oxytocin on other metabolite levels

Analysis of the secondary outcomes revealed no significant effects on any other metabolites quantifiable from the ¹H-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 $d = 0.54$; Fig. 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).

3.5. 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 ($t(20) = 3.11$, $p = .006$).

3.6. Exploratory correlations

3.6.1. 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 = -0.546, $p = .005$) and Glx scaled to creatine (rho = -0.509, $p = .009$) 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.

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.

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

Scaled to creatine

Metabolite	Hippocampus (N = 26)			Anterior cingulate cortex (N = 28)			Thalamus (N = 28)		
	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	$\mathbf{t(27)=2.88, p=.008}$*	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

Metabolite	Hippocampus (N = 26)			Anterior cingulate cortex (N = 28)			Thalamus (N = 28)		
	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	$\mathbf{t(27)=2.57, p=.02}$**	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.

* Significant at the Bonferroni-corrected $p<.01$ threshold.

** Although significant at the $p<.05$ level, this does not meet the Bonferroni-corrected $p<.01$ threshold.
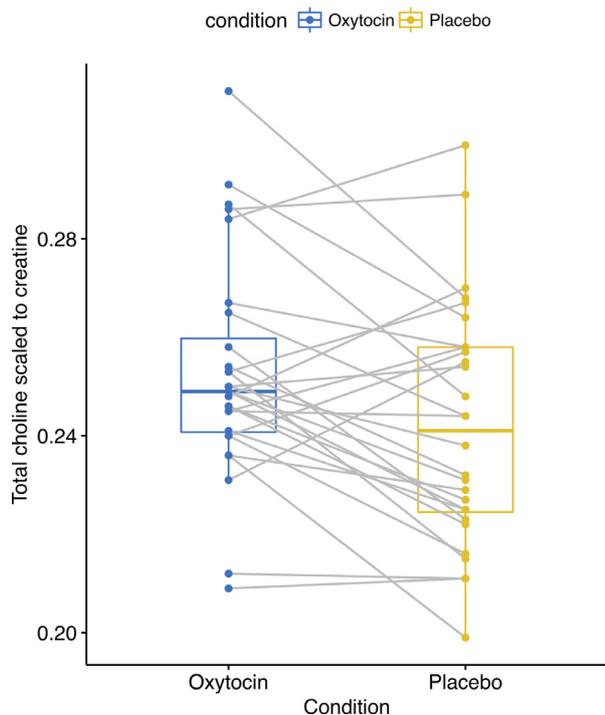
Fig. 3 Choline levels (scaled to creatine) in the anterior cingulate cortex in oxytocin and placebo conditions. Individual data points are presented (overlaid) on standard boxplots, with grey lines connecting paired values.

3.6.2. 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).

4.   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 75–99 min 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 min), ACC (84 ± 5 min), and hippocampus (93 ± 6 min). 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 min post-dosing (Striepens et al., 2013). Another study found that while the effects of oxytocin on human cerebral blood flow peak at 39-51 min post-administration (with a gradual diminution thereafter), they observed sustained perfusion effects over the entire post-treatment interval (up to 78 min) (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., 2019). For example, in one study of healthy males, oxytocin's effects on amygdala inhibition were observed only between 45-70 min post-administration, and not before or after (Spengler et al., 2017). A forthcoming study (Martins et al., 2019) 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 min post-dosing. Furthermore, we recently showed that in the same CHR-P sample used in the current

study, the effects of oxytocin on hippocampal perfusion were more robust in the earlier (22-28 min) vs the later (30-36 min) 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. repeated (longer-term) oxytocin dosing regimen. 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 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 (glutamate or Glx) 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 ~75-99 min 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 effects on choline in the hippocampus and thalamus, but did have an effect 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 effects on choline were not within our primary hypothesis and these findings should, therefore, 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 ~75-99 min 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. 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.

5. Conclusions

This study suggests that at ∼75-99 min 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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Author contributions

All authors made substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data. Authors PFP, PMcG, DT, SW, PMo, SS, PA, FZ, YP designed the study, wrote the protocol, and/or obtained funding. Authors CD, GR, ADM, VRC, UP, MC, ES, DO, SM, DJL conducted study recruitment, data collection, or provided administrative or clinical support. Author CD conducted the analysis in consultation with PFP and JMS. Author CD wrote the first draft of the manuscript which was revised by PFP. All authors contributed to and have approved the final manuscript.

Conflict of interest

PFP has received advisory consultancy fees from Lundbeck outside of this work. The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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Supplementary materials

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