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Influence of muscarinic M_1 receptor antagonism on brain choline levels and functional connectivity in medication-free subjects with psychosis: A placebo controlled, cross-over study

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ABSTRACT

An increasing number of studies implicate the muscarinic cholinergic system in cognitive dysfunction associated with psychosis. This study examined the effect of muscarinic M_1 receptor modulation on anterior cingulate cortex (ACC) and striatal choline concentrations and the relation with cognitive performance, as well as functional connectivity of cognitive networks. Thirty medication-free subjects with a psychosis spectrum disorder and 30 gender, age and IQ-matched healthy control subjects underwent ^{1}H-proton magnetic resonance spectroscopy (^{1}H-MRS) twice, once after placebo and once after a single dose of biperiden (M_1 receptor antagonist, 4 mg). A subset of 19 psychotic subjects and 28 controls underwent resting-state functional magnetic resonance imaging (rs-fMRI) as well. No significant differences were found in ACC and striatal choline levels, nor in functional connectivity, between the two groups after placebo. Moreover, M_1 antagonism did not significantly affect choline levels or functional connectivity. No correlations were found between choline levels and cognition as well as psychotic symptoms. Our findings do not support an association between the cholinergic system and cognition and psychotic symptoms. However, the lack of group differences in choline concentrations and functional connectivity, both after biperiden and placebo, may indicate that there were no severe cholinergic abnormalities present in our sample.

1. Introduction

Psychotic disorders, including schizophrenia, are disabling mental disorders characterized by positive and negative symptoms. In addition, the majority of patients also report substantial cognitive impairments. Effective treatment is currently unavailable as the mechanisms underlying cognitive impairments in these patients are largely unknown.

A growing number of studies implicate the cholinergic muscarinic system in both cognitive function and psychosis (Carruthers et al., 2015; Raedler et al., 2007). The neurotransmitter acetylcholine not only plays an important role in motor function, but also in various domains of cognition and binds to the muscarinic and nicotinic receptors in the brain (Friedman, 2004; Klinkenberg et al., 2011). The muscarinic M_1 receptor (M_1R) in particular may be involved in cognition as this receptor subtype is most abundantly expressed in brain regions indispensable for cognition such as the dorsolateral prefrontal cortex (DLPFC), striatum and hippocampus. Post-mortem studies reported decreased expression of both nicotinic (Court et al., 1999; Freedman et al., 1995; Marutle et al., 2001) and muscarinic receptors in patients with schizophrenia (Crook et al., 2001, 2000; Dean et al., 2002, 1996; Gibbons et al., 2013). A hallmark study by Scarr et al. (2009) even reports a 75% decrease of M_1Rs in the DLPFC of a subgroup of schizophrenia patients, further implicating the muscarinic cholinergic system in schizophrenia.

In-vivo studies also suggest a role for the cholinergic system in psychosis. Previous magnetic resonance spectroscopy (^{1}H-MRS) studies

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in psychosis demonstrated elevated choline levels in the DLPFC of medicated first-episode psychosis and chronic schizophrenia patients (Kirtaş et al., 2016), the dorsal anterior cingulate in medicated schizophrenia patients (Bustillo et al., 2014) and the caudate nucleus of medication-free first-episode schizophrenia patients (Bustillo et al., 2002; Plitman et al., 2016). However, not all ^{1}H-MRS studies replicate these differences in choline concentrations between patients and controls (Block et al., 2000; Galińska et al., 2009; Kalaycı et al., 2012; Uhl et al., 2011; Zabala et al., 2007), possibly due to differences in methodological and sample characteristics. Nevertheless, brain choline levels may represent a non-invasive proxy for cholinergic neurotransmission as it is not possible to measure acetylcholine concentration directly non-invasively. Acetylcholine synthesis strongly depends on the availability of intraneuronal choline (Sarter and Parikh, 2005) as it is synthesized from a reaction between acetylco-enzyme A and choline (cho) and then catalyzed by cho acetyltransferase and stored in presynaptic vesicles (Sarter and Parikh, 2005). Studies in rodents showed that a single dose of the non-selective muscarinic receptor antagonist scopolamine led to a marked increase in acetylcholine concentrations in the striatum, frontal cortex and hippocampus (Day et al., 1991; Durkin et al., 1992; Watanabe and Shimizu, 1989). Moreover, striatal choline concentrations increased after scopolamine injections in rodents (Day et al., 1991).

Altered cholinergic neurotransmission may influence functional connectivity of networks with a high density of cholinergic receptors in the brain. Previous research reported a relationship between metabolic activity and functional connectivity in healthy individuals (Passow et al., 2015). In patients with schizophrenia, abnormal functional connectivity has been reported. Although results vary across studies, a substantial number of studies report hyperconnectivity of the default mode network (DNM) (Hu et al., 2017; Karbasforoushan and Woodward, 2013) and increased functional connectivity in cortical and parietal clusters, both regions with a high density of M_1Rs (Skåtun et al., 2016). Scopolamine increased functional connectivity in both young and elderly healthy subjects between the frontal cortex and hippocampus (Wink et al., 2006). Nevertheless, it is unknown whether functional connectivity of cholinergic / cognitive networks is modulated by the M_1R in psychotic disorders.

With this study we aimed to explore the effect of M_1R modulated cholinergic neurotransmission on choline concentrations in subjects with a psychotic disorder and healthy controls. Furthermore, we correlated choline concentrations with cognitive functioning and psychotic symptom severity. In addition, we explored the effect of M_1R modulated neurotransmission on functional connectivity in networks associated with cognition and / or the cholinergic system. For this purpose, we obtained measurements of choline (as a proxy for cholinergic neurotransmission) in the anterior cingulate cortex (ACC) and striatum using ^{1}H-MRS and functional connectivity using resting state functional magnetic resonance imaging (rs-fMRI) (as a proxy for inter-region cholinergic drive).

2. Methods

This study was approved by the Medical Ethical Committee of the Academic Medical Center (AMC), Amsterdam. All participants gave written informed consent prior to participation.

2.1. Participants

30 medication-free subjects with a psychosis spectrum disorder (PSD) and 30 gender, age and IQ-matched healthy controls were included in the study. Sample characteristics (Table 2) are described elsewhere (Bakker et al., 2018; Vingerhoets et al., 2017). Eight patients had never used antipsychotics. Past antipsychotic use of the other patients is displayed in supplementary Table 1. Due to technical difficulties (broken head coil), only a subgroup of 28 healthy controls and 19 subjects with a PSD had both a placebo and biperiden rs-fMRI scan. Demographics of this subsample are displayed in supplementary Table 2. Subjects with a PSD were recruited via specialized mental health care institutions and advertisement in national newspapers. Healthy controls were recruited via advertisement on the internet and in newspapers. For subjects with a PSD inclusion criteria were: a confirmed diagnosis of a psychotic disorder (bipolar disorder or psychotic depression were excluded), aged between 18 and 40 years, no use of antipsychotics or anticholinergics and onset of psychosis no longer than 10 years prior to participation. Two patients using low-dose antipsychotic medication who were willing to participate underwent a wash-out period of 5 times the mean terminal elimination half-life of the specific antipsychotic prior to participation. Olanzapine and clozapine use was an exclusion criterion for wash-out because of their affinity for the muscarinic system. One participant was using quetiapine (200 mg) and one used haloperidol (1 mg). Exclusion criteria for both groups were: contra-indications for biperiden or MRI, severe neurological or comorbid psychiatric disorders, pregnancy and illicit drug use 4 weeks prior to participation. In addition, healthy controls with past or present psychiatric illness or a first degree relative with a psychotic disorder were also excluded from participation.

2.2. Instruments

Diagnosis of a psychotic disorder was validated with The Comprehensive Assessment of Symptoms and History (CASH, Andreasen, 1992) in subjects with a psychotic disorder and to exclude such a diagnosis in the healthy control group. The Positive and Negative Syndrome Scale (PANSS, Kay and Qpjer, 1987) was conducted to measure psychotic symptoms severity. On both occasions, potential side effects of biperiden were measured by means of a 31-items self-report questionnaire which included items as nausea, headache and dry mouth. Participants were asked to report to what extent they experienced these symptoms on a 4-point scale with 0 indicating not affected and 3 very affected (Klinkenberg et al., 2012). A shortened version of the Wechsler Adult Intelligence Scale (WAIS III, Velthorst et al., 2013) was used to determine the Intelligence quotient (IQ) of the participants.

Several cognitive domains were measured with the Cambridge Neuropsychological Automated Test Battery (CANTAB Levaux et al., 2007). We used the schizophrenia test battery (extended with a social cognition task) as this battery covers all cognitive domains that have been found to be significantly impaired in schizophrenia (Nuechterlein et al., 2004). The CANTAB is a well validated, automated test battery, sensitive for effects of pharmaceutical agents (Barnett et al., 2010). A brief description of the different subtests and outcome measures is displayed in Table 1.

2.3. Biperiden

Biperiden (Akineton®, instant release, Knoll AG) belongs to the class of anticholinergic medication and is a muscarinic receptor antagonist. Clinically, biperiden is used for treatment of Parkinson's disease and extrapyramidal side effects induced by (first generation) antipsychotics. It is the most selective M_1 receptor antagonist available for use in humans (Bolden et al., 1992; Sambeth et al., 2015) given its 10-fold higher affinity for M_1 receptors compared to the M_2–M_5 receptors. The elimination constant of a single dose is initially steep and after approximately 6 h it slowly levels off. Plasma levels are clear of biperiden 18 h after administration (Hollmann et al., 1984). The dose of 4 mg given in this study, lies within the interval of therapeutically recommended daily doses (1–4 mg).

2.4. Procedure

This study had a single blind, placebo-controlled, cross-over design. All participants were scanned twice on separate occasions, once after

Table 1
Overview of CANTAB tasks and outcome measures.

Domain	Task	Task description	Outcome measures	Direction
Visual learning & Memory	Paired Associate Learning (PAL)	Boxes are displayed on a screen and open in a randomized order. The boxes contain patterns. Subjects must remember and identify the correct locations of these patterns.	Total errors (adjusted for completed stages) First trial memory score	Lower is better Higher is better
Verbal learning & Memory	Verbal Recognition & Memory (VRM)	Subjects are shown 12 words and afterwards have to reproduce as many words as possible as well as recognizing these words from a list of 24 words. Recognition was repeated after 20 min.	Free recall total words correct Immediate recognition total words correct Delayed recognition total words correct	Higher is better Higher is better Higher is better
Working memory	Spatial Working Memory (SWM)	A self-ordered task which test a person's ability to retain spatial information and to manipulate this information.	Total errors Strategy	Lower is better Lower is better
Attention & Vigilance	Rapid Visual Processing (RVP)	Subjects have to detect a target sequence of digits.	A'prime (measure of how good subjects detect a target sequence using total hits and false alarms) Mean latency	Higher is better Lower is better
Processing speed	Reaction Time (RTI)	Subjects have to response to a visual target.	Simple reaction (response to a stimulus in a single, predictable location) 5-choice reaction (response to a stimulus in any of 5 locations)	Lower is better Lower is better
Reasoning & Problem solving	One Touch Stockings of Cambridge (OTS)	Spatial planning task based on the Tower of London test. Subjects have to identify how many moves are required to solve a problem.	Total number of problems solved on first choice	Higher is better
Social cognition	Emotion Recognition (ERT)	Subjects are shown a series of faces showing specific emotions and have to select the emotion that most closely corresponds to the emotion depicted on the face.	Total number correctly identified emotions	Higher is better

placebo (cellulose, corn starch, Placebo Wit tablet) and once after oral administration of biperiden. The order of drug administration was counterbalanced so that in each group half of the participant received placebo first and the other half biperiden. To ensure complete drug wash out, the time interval between both test days was at least one week. The scan protocol was similar for both test days. All participants were instructed to abstain from alcohol and nicotine 24 h prior to testing. On both test days, prior to scanning a urine drugs screening was conducted to verify abstinence of recreational drugs of abuse. After drug screening, oral placebo or biperiden was administered. Drug intake was at all times supervised to make sure the participant indeed swallowed the pills. To measure choline concentrations and functional connectivity, participants underwent two ^{1}H-MRS and resting state functional magnetic resonance imaging (rs-fMRI) measurements respectively on each occasion. Central pharmacodynamic effects of biperiden peak around 90 min after oral administration (Hollmann et al., 1984). Therefore, MRI scanning commenced 90 min after administration of placebo/biperiden. During this 90-min waiting period, the CASH and the PANSS were conducted. The questionnaire assessing side-effects was always conducted at T_{max} of biperiden shortly before scanning. After scanning the CANTAB was conducted. The shortened version of the WAIS-III was always conducted before administration of medication to eliminate confounding effects.

2.5. Image acquisition, pre-processing and analyses

^{1}H-MRS and resting state fMRI (RS-fMRI) data were acquired on a 3-Tesla Ingenia MRI system (Philips, Best, The Netherlands), equipped with a 32 channel head-coil. A transversal high-resolution structural T1-weighted volumetric image, with full head coverage (180 slices; TR = 7.0 ms; TE = 3.2 ms; FOV =256 × 240 mm; voxel size: 1.0 × 1.0 × 1.0 mm, 180 slices) was acquired for each participant for registration and voxel planning purposes.

2.6. ^{1}H-MRS

Metabolite concentrations were obtained from a (20 × 20 × 20 mm) voxel of interest, manually positioned in the ACC and striatum (Fig. 1), using the T1-weighted structural image for anatomical localization. Spectra were acquired using a point-resolved spatially localized spectroscopy sequence (PRESS, TE = 45 ms, TR = 2000 ms, 96 averages), and analyzed using the Linear Combination of Model spectra (LCModel) commercial spectral-fitting package (Provencher, 2001) with a standard basis set. To estimate choline concentrations, we analyzed levels of the choline containing compounds glycerophosphocholine + phosphocholine (choline). Choline concentration was expressed as the ratio of choline by creatine (Cho/Cr), to reduce intra-subject variance as creatine is considered a stable metabolite in the brain. Spectra with a Cramer–Rao Lower Bound (CRLB) higher than 20% were considered poor quality and were excluded from further analyses (1 healthy control subject). The T1-weighted image was segmented into percentage grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) within the voxel of interest using Statistical Parametric Mapping (SPM8, Wellcome Department of Imaging Neurosciences, University College London, UK) to examine group difference in fractions and to correct for the amount of CSF within the spectroscopy voxel as described in Quadrelli et al. (2016).

2.7. Resting state fMRI

Resting state functional MRI scans were acquired for approximately 7 min after structural and ^{1}H-MRS images during the same scanning session using the following parameters: echo-planar imaging (EPI) sequence, TR = 2 s, TE = 27 ms, 206 whole brain volumes, slice spacing 0.3 mm, 37 transverse slices, voxel size 3 × 3 × 3 mm, FOV

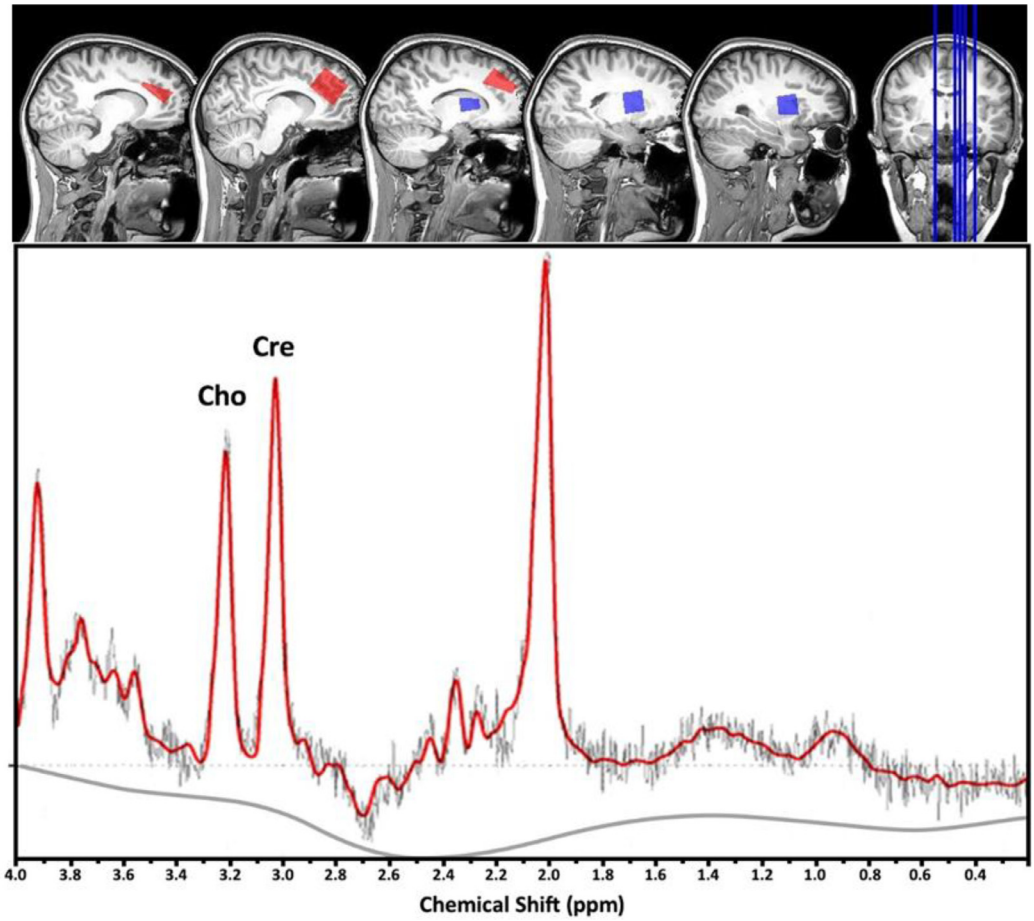

Fig. 1. (A) Voxel placement. The red box indicates the voxel placement in the anterior cingulate cortex. The blue box indicates the voxel placement in the striatum. (B) Example of a striatal spectrum derived from a healthy control subject. Choline resonates at 3.2 chemical shift. Creatine resonates at 3.0 chemical shift.

240 × 240 mm, flip angle 76°, All subjects were instructed to keep their eyes open, let their minds wander and not to fall asleep. RS-fMRI data were preprocessed using tools from the FMRIB Software Library (FSL), version 5.0.10 (Jenkinson et al., 2012; Smith et al., 2004). All time series were visually inspected, brain extracted, motion corrected, high-pass temporal filter (100s), and normalized to MNI space using subjects' individual T1-weighted image. To detect and remove motion related artifacts, Automatic Removal Of Motion Artifacts based on independent component analysis (ICA-AROMA version 0.3-Beta) was used (Pruim et al., 2015). Then, data were spatially smoothed using a 6 mm kernel. Subsequently, dual-regression with variance normalization was applied using predefined masks of the default-mode, sensorimotor, executive control and frontoparietal network (left and right) (Smith et al., 2009), with additional masks for white matter and CSF to regress out residual physiological noise components (Beckmann et al., 2009).

2.8. Data-analyses

All statistical analyses were performed with IBM SPSS Statistics, version 24. Differences in demographic variables (gender, age, smoking and IQ) and in PANSS scores and side-effects were analyzed by means of independent samples t-tests and chi-square tests. A univariate analysis of variance (ANOVA) was conducted to examine differences in ACC and striatal choline levels after placebo between subjects with a psychotic disorder and healthy controls. To examine the effects of biperiden on choline levels in both the ACC and striatum (main effect) as well as differential effects of biperiden on choline levels (medication x group interaction) between subjects with a psychotic disorder and healthy controls, a repeated measures ANOVA was performed. Cognitive domain scores were computed by converting the raw scores into standardized *Z*-scores. Positive *Z*-scores larger than 3 and negative scores larger than −3 were considered outliers and removed from further (cognitive) analyses. Outcome measures where a lower score represents better performance were reversed so that a higher score represents better performance for all outcome measures. Subsequently, cognitive domain scores were computed by adding the standardized *Z*-scores of each domain. Pearson's correlation coefficient was computed to examine the relation between choline concentration and separate cognitive domains and psychotic symptom severity (PANSS scores). Bonferroni correction was applied correcting for the number of cognitive domains. Therefore, *p*-values of <0.007 were considered significant.

Functional connectivity in the predefined networks of interest after placebo was compared between both groups using Randomise (5000 permutations) with a two sample *t*-test (Winkler et al., 2014) across all participants. Analyses were initially thresholded at *p*-value <0.05 with a family wise error (FWE) correction using threshold free cluster enhancement (TFCE) (Smith and Nichols, 2009). To examine the effect of biperiden and the interaction effect of drug and group on functional connectivity, a one-sample t-test and two-sample t-test were performed, again using Randomise. Bonferroni correction was applied to correct for the number of brain networks. Consequently, clusters with a *p*-value <0.01 were considered significant (0.05/5 networks).

3. Results

3.1. Demographics

The two groups did not significantly differ in terms of gender, age, IQ and cigarette smoking (Table 2). As expected, subjects with a psychotic disorder scored higher on all subscales of the PANSS (Table 2). Subjects with a psychotic disorder reported significantly more unwanted effects in both the placebo ($t(1,61) = -3.29$, $p = 0.002$) and the biperiden condition ($t(55) = -2.08$, $p = 0.042$). However, within subjects difference of unwanted effects (total score biperiden − total score placebo) did not differ significantly between groups ($t(55) = 0.13$, $p = 0.488$), indicating that biperiden did not cause significantly more unwanted effects in either group.

3.2. ^{1}H-MRS

No significant differences were found in voxel composition (percentage GM, WM and CSF) between groups both after placebo and biperiden (supplementary Table 3). No differences were found between healthy controls and subjects with a psychotic disorder in both ACC and striatal choline concentrations after placebo (Fig. 2(A), (B)). Moreover, biperiden had no significant influence on choline concentrations in both brain regions (main effect). No group x medication interaction effect was found (Fig. 2(C), (D))[1].

3.3. Relation between choline concentrations, cognition and psychotic symptoms

Striatal choline concentration was positively associated with attention in the psychotic subjects ($r = 0.406$, $n = 28$, $p = 0.032$); higher choline concentration was related to better attention (Fig. 3(A)), although not significant after correcting for multiple comparisons. No such correlation was found in the healthy control group. Choline concentration in the ACC was not significantly related to cognition. With regard to psychotic symptom severity, a significant correlation was found between severity of negative symptoms and striatal choline concentration ($r = 0.405$, $n = 29$, $p = 0.029$). Higher striatal choline concentration was associated with higher negative symptom severity (Fig. 3(B)). However, after Bonferroni correction, this was not significant. None of the other PANSS subscales correlated significantly with striatal choline concentration. Moreover, no significant correlation was found between choline concentration in the ACC and the psychotic symptoms severity.

3.4. Resting-state functional connectivity

After placebo, no between group differences in functional connectivity in any of the networks were found. Although *not* significant after Bonferroni correction, we found increased functional connectivity in the default-mode network within the frontal pole and left hippocampus in the psychosis group, (Fig. 4(A)). A main effect of biperiden was found for the frontoparietal network, including clusters in the middle frontal gyrus, precentral gyrus and insular cortex. Functional connectivity increased in the frontoparietal network after biperiden administration in both groups (Fig. 4(B)), although this finding did not survive after correcting for multiple network comparison using a Bonferroni correction.

[1] Analyses were repeated including smoking (y/n) as covariate. Smoking did not significantly influence placebo ACC or striatal choline levels ($p = 0.687$ and $p = 0.444$, respectively). Moreover, we did not find a main ($p = 0.744$) or interaction effect of smoking on the effect of biperiden on ACC and striatal choline levels ($p = 0.843$).

Table 2
Sample demographics.

	Psychosis Mean (SD)	Healthy controls Mean (SD)	Statistic	*p*
Gender (male/female)	21/9	20/10	0.08	0.781
Age	27.33 (4.66)	25.40 (5.08)	−1.54	0.130
IQ	101.10 (15.12)	105.93 (14.76)	1.24	0.219
Cigarette smoking (y/n)	6/23	4/26	0.57	0.451
Side effect severity placebo	13.36 (11.29)	5.66 (7.27)	−3.05	**0.004**
Side effect severity biperiden	13.43 (12.34)	8.04 (8.17)	−1.92	0.061
ΔSide effect severity (b-p)	0.07 (14.52)	2.11(8.54)	0.64	0.530
PANSS				
Positive symptoms	12.00 (4.21)	7.10 (0.31)	−6.15	<**0.001**
Negative symptoms	11.71 (5.33)	7.43 (1.36)	−4.13	<**0.001**
General psychiatry	23.43 (6.41)	16.87 (3.33)	−4.84	<**0.001**
Total score	47.21 (13.66)	31.10 (4.59)	−5.83	<**0.001**
Diagnosis				
Schizophrenia	13			
Schizophreniform disorder	2			
Schizoaffective disorder	2			
Psychotic disorder NOS	13			
Time since last use of AP (months)	38.68 (37.02)			

NB: IQ: Intelligence Quotient; Δ Side effect severity (b-p): total score on the side effects scale biperiden – total score on the side effects scale placebo; PANSS: Positive and Negative Syndrome Scale; Psychotic disorder NOS: psychotic disorder not otherwise specified; AP: antipsychotic.

4. Discussion

With this study we aimed to explore differences in ACC and striatal choline concentrations and functional connectivity between healthy controls and medication-free subjects with a psychotic disorder and how they are modulated by M_1 antagonism. In addition, we examined if ACC and striatal concentrations were related to cognitive functioning. No significant differences in striatal and ACC choline concentrations and functional connectivity between subjects with a psychotic disorder and healthy controls after placebo and biperiden were found. After Bonferroni correction, no significant correlations were found between choline levels and cognition and psychotic symptoms.

4.1. Choline concentrations (^{1}H-MRS)

^{1}H-MRS studies examining differences in choline concentrations between psychotic subjects and healthy controls to date are inconclusive. This discrepancy in findings may be related to differences in methods, such as field strength, post-processing steps, and sample size and characteristics. Studies that did not find a difference in choline concentrations, generally used lower MRI field strength and smaller samples which could suggest that possible differences in choline concentrations are subtle and bigger samples would be needed to detect hypothesized differences between patients and controls. Subsequently, our study may have been underpowered to detect subtle differences in choline concentrations. In addition, our psychotic subjects were relatively well functioning and had a relatively short illness duration. This may be inherent to our inclusion criterion of medication-free subjects. Despite significant differences in PANSS scores between patients and controls, psychotic symptom severity was relatively low in our patient sample. This could indicate that cholinergic abnormalities are only present in more severely impaired schizophrenia patients. Indeed, the positive correlation between duration of untreated psychosis and choline levels reported by Théberge et al. (2004), suggests that choline concentrations may increase as the disease progresses. Unfortunately, our sample size did not allow for a subgroup analysis to further examine

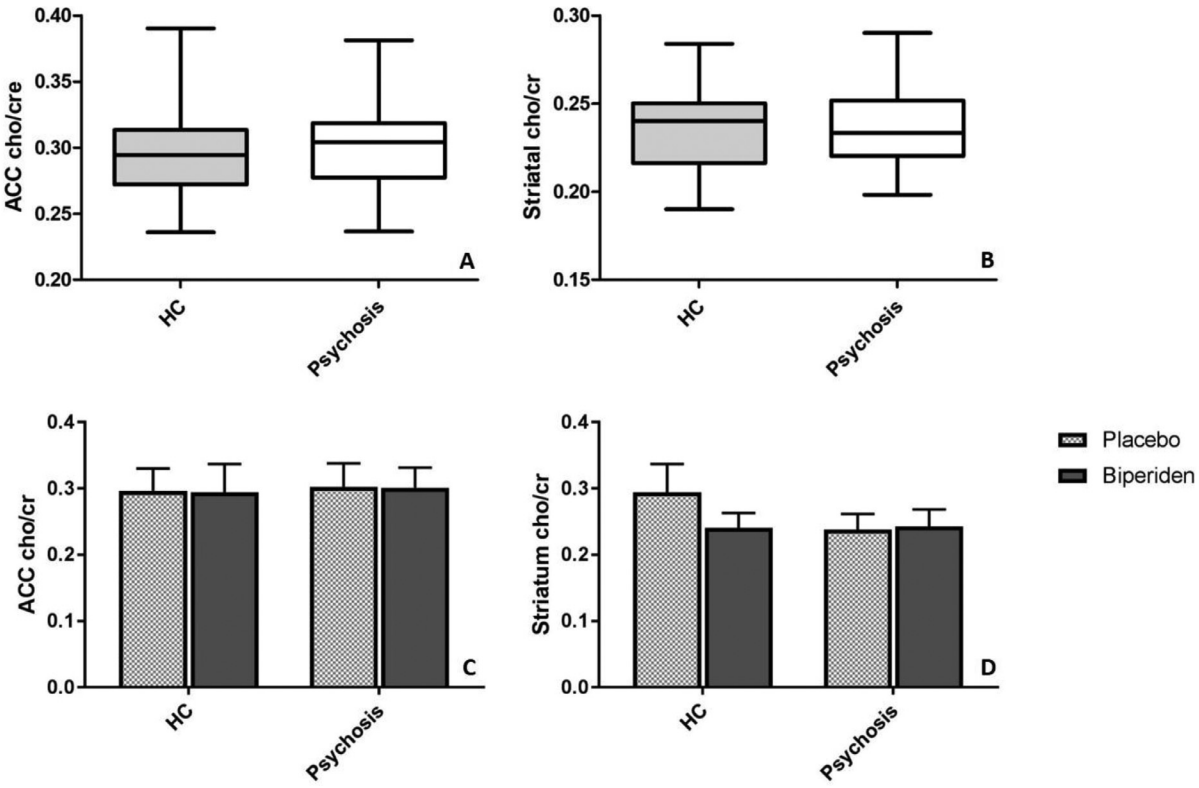

Fig. 2. Choline concentrations in the anterior cingulate cortex (ACC) and striatum. (A) Choline concentrations (ratio choline / creatine) in the anterior cingulate cortex did not differ between groups after placebo $t(57) = -0.655$, $p = 0.515$. (B) Choline concentrations (ratio choline / creatine) in the striatum after placebo $t(57) = -0.148$, $p = 0.883$. (C) Biperiden did not significantly affect choline concentrations in the ACC in both groups ($F(1,55) = 0.607$, $p = 0.439$). No significant interaction effect was found ($F(1,55) = 0.122$, $p = 0.729$). (D) Biperiden did not significantly affect choline concentrations in the striatum in both groups ($F(1,54) = 1.954$, $p = 0.168$). No significant interaction effect was found ($F(1,54) = 0.102$, $p = 0.751$). Psychosis: subjects with a psychotic disorder; HC: healthy controls.

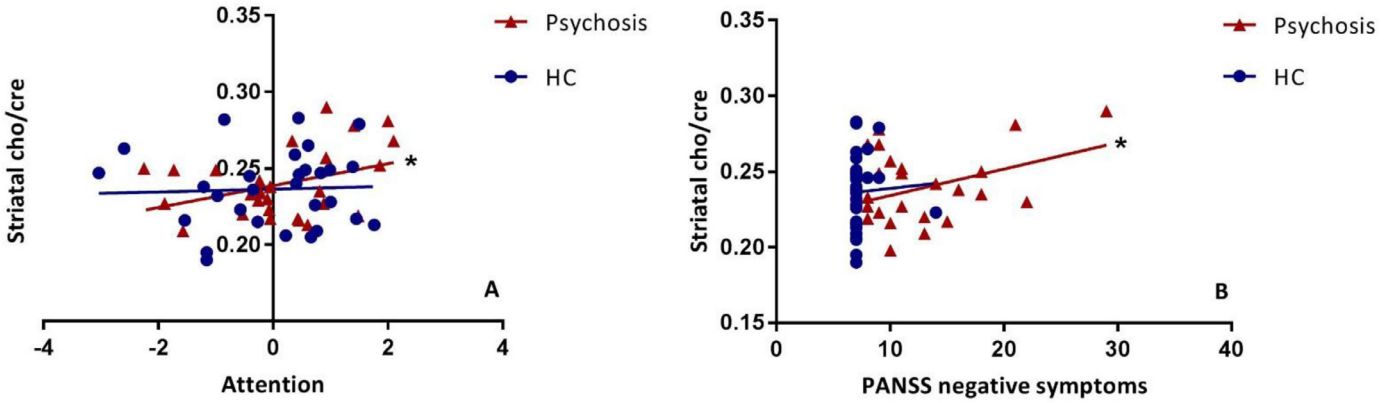

Fig. 3. (A) Relationship between striatal choline concentration and sustained attention. A significant relation was found within the group of patients with a psychotic disorder ($r = -0.406$, $p = 0.032$). (B) Relationship between striatal choline concentration and negative symptoms severity. A significant relation was found within the group of patients with a psychotic disorder ($r = 0.405$, $p = 0.029$). Psychosis: subjects with a psychotic disorder; HC: healthy controls.

this theory.

Although findings in rodents showed increases in both free acetylcholine and choline after blockade of the muscarinic receptors, our results did not show a significant effect of biperiden on human brain choline concentrations, both in controls and psychotic subjects. The lack of a biperiden times group interaction effect could also indicate that there were no (severe) muscarinic abnormalities present in our psychosis sample. Contrary, given the lack of effect of biperiden on choline levels in both groups, it could also indicate that choline when measured with ^{1}H-MRS is not sensitive enough to examine M_1R modulated cholinergic transmission in humans. However, we only measured choline in the ACC and striatum after a single dose of biperiden. Therefore, we cannot rule out long term effects of M_1 blockade nor differences in choline concentrations in other brain regions.

Although not significant after correction for multiple comparisons, striatal choline concentrations correlated with attention in psychotic subjects. This suggests that the cholinergic system may play a role in attention in psychosis. Possibly, attention is primarily modulated by other cholinergic receptors. Future research should elaborate on this. Finally, striatal choline levels were positively associated with negative symptom severity. Although this relation was also not significant after Bonferroni correction, these results are in line with our previous

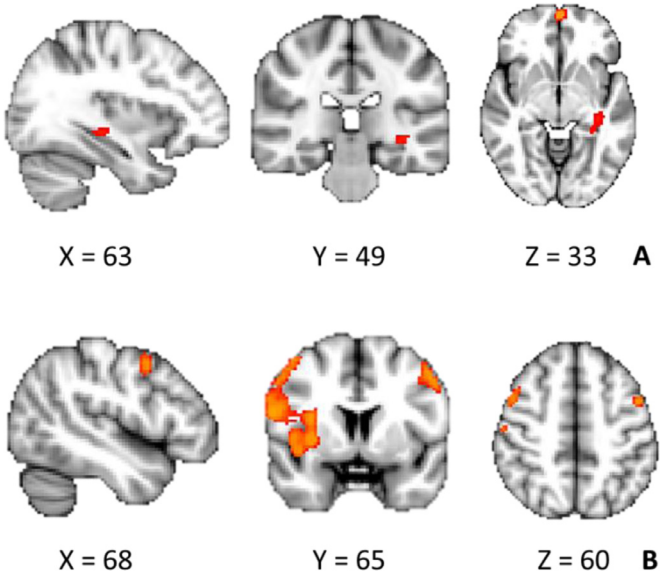

Fig. 4. (A) Increase in functional connectivity after biperiden in subjects with a psychotic disorder of the default mode network. Significant clusters were found in the frontal pole ($x = 46, y = 95, z = 33$) and left hippocampus ($x = 61, y = 44, z = 33$). (B) Increase in functional connectivity of the frontoparietal network after biperiden in the total sample (psychotic subjects and controls). Significant clusters were found in the: middle frontal gyrus ($x = 24, y = 65, z = 64$), precentral gyrus ($x = 14, y = 65, z = 49$), the insular cortex ($x = 25, y = 65, z = 32$) and the central opercular cortex ($x = 22, y = 65, z = 38$). All results depicted were thresholded at $p < 0.05$, FWE-corrected with threshold-free cluster enhancement. Results were not significant after Bonferroni correction for the number of networks.

findings (Bakker et al., 2018) of a significant association between greater negative symptom severity and lower M_1 receptor binding potential in subjects with a psychotic disorder. Lower M_1 binding potential could be indicative of more free acetylcholine and thus, theoretically, higher choline concentration. Improvement of both positive and negative symptoms by $M_1/_4$ agonist xanomeline (Shekhar et al., 2008) further implicates the muscarinic cholinergic system in the pathophysiology of psychotic disorders.

4.2. Functional connectivity (resting state fMRI)

Contrary to previous studies, we did not observe differences in functional connectivity in brain networks associated with cognition between subjects with a psychotic disorder and controls after placebo. This unexpected finding may be explained by our relatively small sample of patients. Due to technical issues (broken coil) we only had resting state fMRI data of 19 patients resulting in limited power. As mentioned previously, we included a relatively well-functioning sample of psychotic subjects with low symptom severity. Most studies reporting altered functional connectivity included schizophrenia patients or schizophrenia spectrum patients with higher symptom severity. Indeed, altered frontoparietal connectivity has been associated with symptom severity in chronic schizophrenia patients (Venkataraman et al., 2012). The sample size and characteristics of the psychosis group could also explain the lack of a differential effect of biperiden on functional connectivity on the analyzed networks. Alternatively, since scopolamine, a non-selective muscarinic receptor antagonist, increased functional connectivity in healthy controls, our results may suggest that functional connectivity of cholinergic / cognitive networks is not primarily modulated by the M_1R. However, prior to Bonferroni correction, we did find increased functional connectivity of the DMN, in patients but not controls after biperiden. Furthermore, a main effect of biperiden on the frontoparietal network was found. This may suggest that connectivity of the frontoparietal network is to some extent modulated by M_1Rs regardless of group status whereas M_1R modulation of the DMN may be patient specific. However, results should be interpreted with caution given the small sample size. More studies examining M_1R modulation of functional connectivity in psychosis are needed.

4.3. Strengths and limitations

An important strength of this study is that, unlike most other studies, the results are not confounded by acute effects of medication use since we only included medication-free subjects with a psychotic disorder. As outlined above, this may however have caused a selection bias of participants with less severe psychotic symptoms. When interpreting these findings some limitations have to be taken into account as well. First, the sample size, in particular for the functional connectivity analyses was relatively small, resulting in limited power and an increase chance of a type II error. In addition, a substantial number of participants was not diagnosed with schizophrenia but with schizoaffective, schizophreniform disorder or psychotic disorder not otherwise specified, most often because their psychotic episode lasted shorter than 6 months. This may explain why severity of psychotic symptoms was relatively low in this sample which may partly explain the lack of significant differences. Second, we did not obtain blood samples from the participants to verify effects of biperiden in plasma. Although subjects were scanned at the theoretical T_{max} of biperiden, when the strongest effects of M_1 blockade are expected to occur, we cannot rule out inter-subject differences on the T_{max}. The tablets were at all times administered under supervision to ensure that participants took the medication. Finally, we used a relatively high dose of biperiden which may have 'masked' potential differential between group effects of biperiden. However, given the lack of effect of biperiden on choline concentrations as well as functional connectivity, it is not likely that a lower dose would have yielded significant results.

In conclusion, this study did not provide evidence for altered M_1 modulated cholinergic neurotransmission in terms of choline concentrations and functional connectivity in subjects with a psychotic disorder. More research in bigger samples is necessary to replicate these findings.

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CRediT authorship contribution statement

Claudia Vingerhoets: Formal analysis, Data curation, Writing - original draft, Writing - review & editing. **Geor Bakker:** Data curation, Writing - review & editing. **Anouk Schrantee:** Formal analysis, Writing - review & editing. **Marieke van der Pluijm:** Data curation, Writing - review & editing. **Oswald J.N. Bloemen:** Writing - review & editing. **Liesbeth Reneman:** Writing - review & editing. **Matthan Caan:** Writing - review & editing. **Jan Booij:** Conceptualization, Writing - review & editing. **Therese A.M.J. van Amelsvoort:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that, except for income received from their primary employer, no other financial support or compensation has been received from any individual or corporate entity for research or professional service and there are no personal financial holdings that could

be perceived as constituting a potential conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2019.06.005.

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