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The muscarinic M_1 receptor modulates associative learning and memory in psychotic disorders

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A B S T R A C T

Background: Psychotic disorders are characterized by prominent deficits in associative learning and memory for which there are currently no effective treatments. Functional magnetic resonance imaging (fMRI) studies in psychotic disorders have identified deficits in fronto-temporal activation during associative learning and memory. The underlying pathology of these findings remains unclear. Postmortem data have suggested these deficits may be related to loss of muscarinic M_1 receptor mediated signaling. This is supported by an in-vivo study showing improvements in these symptoms after treatment with the experimental $M_{1/4}$ receptor agonist xanomeline. The current study tests whether reported deficits in fronto-temporal activation could be mediated by loss of M_1 receptor signaling in psychotic disorders.
Methods: Twenty-six medication-free subjects diagnosed with a psychotic disorder and 29 age-, gender-, and IQ-matched healthy controls underwent two functional magnetic resonance imaging (fMRI) sessions, one under placebo and one under selective M_1 antagonist biperiden, while performing the paired associated learning task. M_1 binding potentials (BP_{ND}) were measured in the dorsolateral prefrontal cortex (DLPFC) and hippocampus using ^{123}I-IDEX single photon emission computed tomography.
Results: In the subjects with psychotic disorders DLPFC hypoactivation was only found in the memory phase of the task. In both learning and memory phases of the task, M_1 antagonism by biperiden elicited significantly greater hyperactivation of the parahippocampal gyrus and superior temporal gyrus in subjects with a psychotic disorders compared to controls. Greater hyperactivation of these areas after biperiden was associated with greater hippocampal M_1 receptor binding during learning, with no association found with M_1 receptor binding in the DLPFC. M_1 receptor binding in the DLPFC was related to greater functional sensitivity to biperiden of the cingulate gyrus during the memory phase.
Conclusion: The current study is the first to show differences in M_1 receptor mediated functional sensitivity between subjects with a psychotic disorder and controls during a paired associate learning and memory task. Results point to subjects with psychotic disorders having a loss of M_1 receptor reserve in temporal-limbic areas.

1. Introduction

Psychotic disorders affect approximately 3% of the general population (Perälä et al., 2007). Aside from the positive and negative symptoms, psychotic disorders are characterized by cognitive impairments that develop in approximately 80 to 85% of patients. These symptoms often develop before the onset of the other symptoms and have been found to be the strongest predictor of illness progression and relapse (Green et al., 2004). Cognitive symptoms do not respond to currently available antipsychotic treatments and persist even after other symptoms have been treated. Although several domains of cognition are affected, executive functioning and learning and memory processes have been shown to be most impaired (Barch and Ceaser, 2012; Murray et al., 2010; Sheffield et al., 2018). Specifically patients with psychotic disorders have deficits in associative learning and memory. Through associative learning and memory relationships between unrelated items are encoded and retrieved and relies on both executive functioning and learning and memory processes. Severity of these deficits have been shown to be an important predictor of poor functional outcome, and exist independent of poor concentration, administered medication, and presence of positive symptoms (Barch and Sheffield, 2014; Barnett et al., 2005).

Using functional magnetic resonance imaging (fMRI), studies have investigated alterations in underlying brain activation during associative learning and memory in psychotic disorders. Significant hypo-activation of the dorsolateral prefrontal cortex (DLPFC) and increased activation of the parahippocampal gyrus are most consistently reported (Ragland et al., 2012, 2009, 2004). It has been suggested that findings

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of the increased parahippocampal gyrus activation may be a compensatory mechanism. Meta-analytical studies, however, highlight a high level of inconsistencies in reported results (Kraguljac et al., 2013). These inconsistencies have mainly been attributed to the high heterogeneity among patient cohorts studied, with patients in different phases of the disorder (first episode to chronic) and under different antipsychotic treatment regiments (Kraguljac et al., 2013).

Impairments in associative learning and memory processes have shown little to no improvement under antipsychotic treatment and recently it has been proposed that lowered signaling of the muscarinic system, a system not targeted by most antipsychotic treatments, could underlie these abnormalities (Carruthers et al., 2015; Erskine et al., 2019). This neurotransmitter system became implicated after studies showed reversible deficits in associative learning and memory after administration of anti-muscarinic agents in healthy subjects and subjects with neurodegenerative disorders (Mufson et al., 2003). Similar to subjects with psychostic disorders, functional MRI showed attenuation of activation in the DLPFC after administration of anti-muscarinic agents to healthy volunteers (Grasby et al., 1995). Preclinical data identified that these effects were most likely driven by the muscarinic M_1 receptor subtype rather than the other muscarinic receptor subtypes ($M_{2\text{-}5}$). The M_1 receptor subtypes has high expression rates in frontolimbic brain regions which are critically involved in associative learning and memory (Anagnostaras et al., 2003; Carruthers et al., 2015; Levey et al., 1991).

The potential loss of M_1 receptor functioning in psychotic disorders was first supported by human post-mortem studies showing significantly reduced M_1 receptor expression in the DLPFC of schizophrenia patients compared to controls (Dean et al., 2002; Scarr et al., 2013). Empirical in-vivo evidence, however, for lower M_1 receptor functioning in psychotic disorders is more limited. Experimental drug xanomeline, which is a $M_{1/4}$ preferring receptor agonist, has been shown to improve learning and memory scores in schizophrenia patients, and in keeping with these results, we showed lower M_1 receptor binding in-vivo in the DLPFC was associated with worse verbal learning and memory scores in psychotic disorders (Bakker et al., 2018; Shekhar et al., 2008a). However, it is still unclear whether loss of M_1 related modulation of DLPFC and hippocampus activation during associative learning and memory drives deficits in psychotic disorders, as xanomeline's effects on cognition may be related to the drug's affinity for both M_1 and M_4 receptors, which are also highly expressed in the hippocampus and striatum. Recent preclinical data highlights the importance of the balance between M_1 and M_4 signaling for learning and memory, suggesting that it may in fact be the disruption of this balance driving deficits seen in psychotic disorders (Gould et al., 2018; Thorn et al., 2017).

Taken together it is still unknown what role M_1 receptor mediated signaling plays in associative learning and memory deficits of psychotic disorders in-vivo. Although loss of M_1 receptor specific modulation on associative learning and memory is best studied using a selective M_1 agonist drug, these drugs are still in early phases of development and not yet available for research in humans. Selective M_1 antagonists, however, are available and well tolerated, therefore, we choose to investigate differences in M_1 mediated functional reactivity in psychotic disorders and controls using a challenge with the M_1 antagonist biperiden. We were also interested in modeling how loss of M_1 related signaling translated to abnormalities in functional activation underlying associative learning and memory. Therefore, we examined whether functional reactivity to biperiden in subjects with psychotic disorders was related to M_1 receptor binding in the DLPFC and hippocampi using single photon emission computed tomography (SPECT). Based on previous studies on associative learning and memory we anticipate that subjects with psychotic disorders will show (1) hypo-activation of the prefrontal cortex and increased parahippocampal gyrus activation during associative learning and memory compared to controls, and that (2) biperiden will attenuate this task-induced activation in the DLPFC in both groups, with subjects with psychotic disorders showing smaller reduction in activation under biperiden. Biperiden will also lead to task induced increased parahippocampal gyrus activation which will be greater in controls than the subjects with psychotic disorders. Lastly, we anticipate that (3) subjects with psychotic disorders with lower DLPFC and hippocampal M_1 receptor binding will show greater hypo-activation in the prefrontal cortex and increased activation in parahippocampal regions during associative learning and memory.

2. Materials and methods

2.1. Participants

The current study included 26 subjects with a psychotic disorder and 29 healthy control subjects matched for age, gender, and IQ. All subjects with a psychotic disorder were medication-free and were recruited from early detection and intervention programs, the national first episodic psychosis network, and through advertisements in newspapers. The 26 subjects diagnosed with a psychotic disorder also participated in another study (Bakker et al., 2018). In the previous study, 30 psychotic disorders subjects were included, but only 26 of them were willing to also participate in the current fMRI study. Diagnosis of a DSM-IV psychotic disorder was confirmed using the Comprehensive Assessment of Symptoms and History (CASH) semi-structured interview (Andreasen et al., 2000). For an overview of the psychotic disorders included in the study see table 1 of sample demographic variables. Duration of untreated psychosis was not allowed to be longer than 1 year, and both subjects with psychotic disorders and control subjects needed to be 18 years of age or older. Exclusion criteria were contraindications for MRI, severe neurological or endocrine disorders, current use of recreational drugs and pregnancy, which were checked through urine samples. Due to the administration of biperiden subjects with tardive dyskinesia and narrow angle glaucoma were excluded. Psychotic symptom severity at time of scanning was determined using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Ethical approval was obtained from the Amsterdam University Medical Centres Medical Ethical Committee. Written informed consent was obtained from all participants after complete description of the study.

2.2. Procedures

All subjects underwent 2 functional MRI sessions on separate days, one after the oral administration of a placebo and one after the oral administration of 4 mg of biperiden, in a counterbalanced and randomized controlled trial. Biperiden is a well-tolerated muscarinic receptor antagonist with a 10-fold higher binding affinity for the M_1 receptor subtype over the other muscarinic receptor subtypes (Kimura et al., 1999). Scans were conducted 90 min after biperiden administration at which peak plasma levels of biperiden have been reached (Grimaldi et al., 1986). Biperiden (Akineton) instant release tablets were used (Knoll AG, Ludwigshafen, Germany). Only subjects that were diagnosed

Table1
Demographic variables.

	Psychotic Disorder	Controls	stat	p
N	26	29		
Gender (male/female)	19/7	(20/9)	0.12	0.7
Age in years (M/SD)	27.7 (4.9)	25.6 (5.2)	−1.51	0.14
IQ (M/SD)	102 (16)	109 (16)	1.78	0.081
Psychotic disorder subtype				
Schizophrenia	11			
Schizophreniform disorder	2			
Schizoaffective disorder	2			
Psychosis NOS	11			
Number of episodes: 1/2/3	18/6/2			

with a psychotic disorders received an additional single photon emission computed tomography (SPECT) scan to quantify M_1 receptor binding using the M_1 preferring radiotracer ^{123}I-iododexetimide (^{123}I-IDEX) (Bakker et al., 2015). These subjects received two 100 mg iodide tablets the night before and one tablet in the morning of the day of scanning to prevent thyroid uptake of free radioactive iodide. Each subject received a bolus injection of approximately 185 MBq (5 mCi) 6h prior to SPECT scanning. Further details of radiosynthesis and binding characteristics of ^{123}I-IDEX are extensively described elsewhere (Bakker et al., 2015; Lavalaye et al., 2001).

2.3. fMRI task paradigm

In order to assess the modulatory role M_1 mediated signaling on functional response during associative learning and memory we adapted the paired associate learning task (PAL) of the Cambridge Neuropsychological Test Automated Battery (CANTAB) for MRI (De Rover et al., 2011). Similar to the PAL CANTAB version of the task, subjects were presented with a black screen displaying white boxes. Each box opened randomly for 3 s, with four or more containing a pattern. After all boxes had opened and closed, a figure would appear in the center and the subject had to identify the original box it was shown in (learning phase). The same trial would be repeated showing the same figures in the same boxes again after which subjects were asked to identify the box the figure had appeared in (memory phase). Cognitive load was increased as the task progressed with subjects having to learn 4, 6, and 8 figure-place associations. Figure-place associations were presented randomly to control for practice effects between sessions. No figure was be presented twice within the same session. Subjects selected a box by navigating a red square around a box to the correct location using the right index finger and middle finger, and the left index finger was used to confirm the location. The experimental design was an event-related design with a learning and memory phase followed by a control phase in which subjects had to select the box to which the arrow was pointing. The task was programmed using E-prime software. For an overview of the task see Fig. 1.

2.4. Image acquisition and parameters

All functional magnetic resonance (fMRI) images were acquired using a Philips Ingenia 3.0 Tesla system (Phillips, Best, The Netherlands) using gradient echo echo-planar imaging (EPI) with a 32-channel head sense coil. 37 slices were scanned with 3-mm isotropic voxel size with a repetition time of 2s, and an echo time of 27 ms. Additionally, a structural T1 weighted anatomical MPRAGE scan was made (180 slices, voxel size 1 × 1 × 1 mm, TR: 7.0 ms, TE: 3.2 ms) for co-registration of functional images, and SPECT images in the psychotic disorders group. ^{123}I-IDEX SPECT images were obtained using a brain-dedicated camera (inSPira HD, Neurologica, Boston, USA) (Stam et al., 2018). Acquisition, attenuation correction, and reconstruction of the SPECT images was performed as described in Bakker et al., 2018.

2.5. Data analyses

Statistical analysis of group demographic variables was conducted using IBM SPSS release 24 (SPSS Inc. Chicago IL, USA). Normality of distribution was tested for all dependent variables using Kolmogorov-Smirnov and Shapiro-Wilk test. Group differences in gender were assessed using a chi-square test. Group differences in age and IQ were assessed with an independent samples *t*-test, and changes in psychotic symptom severity between placebo and biperiden conditions were assessed using a paired samples *t*-test. Behavioral responses on the PAL task were extracted using E-prime and analyzed using IBM SPSS version 24. A 2 factor (group and medication) by 2 level (group: psychotic disorders/controls and medication: placebo/biperiden) ANCOVA was used to assess main effect of group, medication, and group by medication interactions on performance accuracy for 4, 6, and 8 figure place associations controlling for counterbalance order. Group differences between accuracy for 4, 6, and 8 figure place associations were assessed using a paired samples *t*-test.

All preprocessing and statistical analysis of functional images were done using FEAT (fMRI expert analysis tool) implemented in FSL 6.0.0 (FMRIB's software library). All functional images were skull stripped, realigned, corrected for slice timing, spatially normalized, resampled to the Montreal Neurological Institute (MNI) 2 mm template, and smoothed using a 5 mm full width at half maximum Gaussian kernel. First level contrast images were created by modelling signal changes during learning and memory phases in comparison to the control condition (see Fig. 1), convolved with a canonical hemodynamic response function. Six standard rigid body motion parameters were estimated

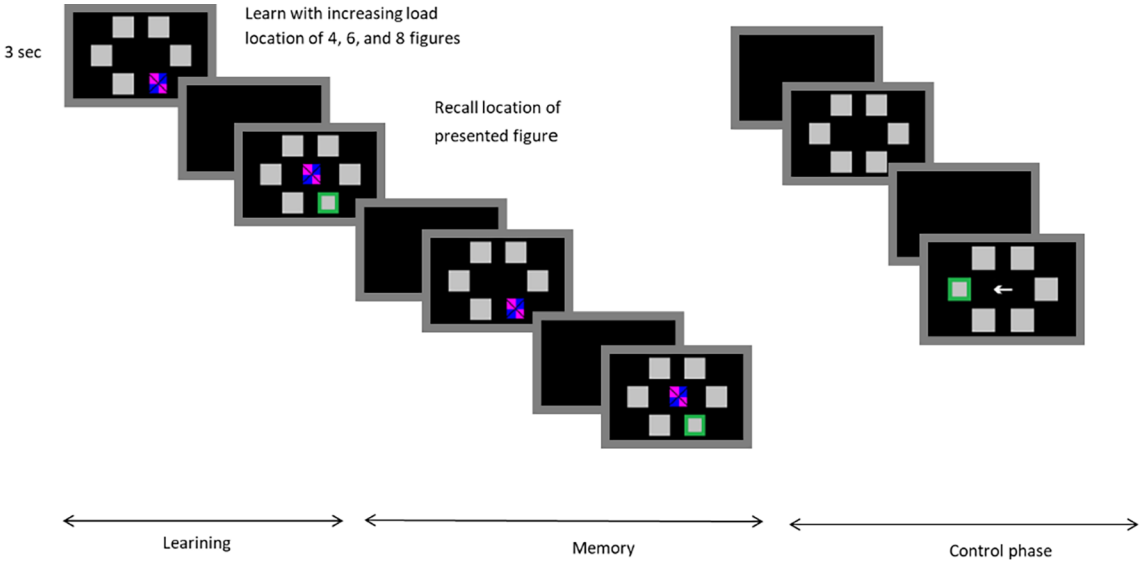

Fig. 1. Overview of the paired associate learning task (PAL) task as performed during scanning. The initial phase of the task was the learning phase. Each white box opened for 3s displaying a figure and subjects had to try to remember the figure and it's location. After all boxes have opened and closed each figure appeared in the center of the screen and subjects had to select the box in which it originally appeared. The same figure place associations were presented twice. During the control phase subjects had to select the box to which the arrow was pointing.

and a confound matrix of volumes corrupted by motion effects greater than two standard deviations was added to the model. A multi-level (hierarchical) statistical analysis was done to identify group differences and effects of medication, and group by medication interaction effects. The group by medication interaction effect was the main analysis of interest. First, the mean response over the three sessions of increasing learning and memory load (4, 6, and 8 figure–place associations) were pooled using a fixed effects analysis using FILM modeling tool (FMRIB's Improved Linear Model). Resulting contrast parameter estimates were assessed in a higher level 2-way mixed effects ANOVA using FLAME 1 + 2 (FMRIB's Local Analysis Local Analysis of Mixed Effect) to assess the main effect of medication (placebo/biperiden) and group (psychosis/controls) by medication interaction effect. To test the main effect of group and group differences a separate higher-level model was run. For this analysis within-subject mean effect of medication was pooled in a fixed effects analysis and resulting contrast parameter estimates were analysed using a 2 group mean comparison controlling for IQ and accuracy. Activity was considered significant if $Z > 3.1$ with a whole brain corrected cluster probability of $p < 0.05$.

Lastly, analysis of the SPECT images was done as earlier described (Bakker et al., 2018), and these data were used to examine how functional response was related to within subject in-vivo M_1 receptor binding in psychotic disorders. M_1 receptor binding was quantified in terms of the binding potential (BP_{ND}) which is a tissue ratio of specific to non-specific binding. Specific binding was measured as ^{123}I-IDEX binding in the M_1-rich hippocampus and DLPFC, and non-specific binding was measured in cerebellar gray matter which is devoid of M_1 receptors. These regions were delineated on subject's own T1 weighted image using Freesurfer (Fischl et al., 2002), as earlier described (Bakker et al., 2018). Relationship between M_1 receptor binding and functional reactivity to biperiden during learning and memory was assessed using a fixed effects analysis with M_1 binding added as covariate regressor, controlling for task performance accuracy.

3. Results

3.1. Demographic variables

Subjects did not differ significantly in age, gender, or IQ. The subjects with psychotic disorders reported mild psychotic symptoms and no difference in psychotic symptom severity was found between scanning sessions. All subjects were antipsychotic medication-free, and 5 subjects were antipsychotic-naive. Two subjects that were being treated with a subtherapeutic dose of a non-cholinergic antipsychotic (haloperidol, 1 mg; quetiapine, 200 mg) underwent a washout (5 times the mean terminal elimination half-life of the specific antipsychotic) prior to inclusion. Sample details and statistics are summarized in Tables 1 and 2.

3.2. Cognitive performance

Estimated IQ was trend significantly ($p = 0.081$) lower in the subjects diagnosed with a psychotic disorder than the control group. Therefore, it was included as covariate in all further analyses.

Table 2
Clinical variables.

Clinical variables	Placebo	Biperiden	statistic	p
Psychotic disorders				
positive symptom severity	9.02 (3.2)	9.13 (4.1)	−0.29	0.77
negative symptom severity	9.41 (3.8)	9.39 (4.0)	0.05	0.91
general psychopathology	19.81 (5.9)	19.39 (5.4)	0.87	0.39
Controls				
positive symptom severity	7.2 (0.8)	7.1 (0.4)	1.01	0.33
negative symptom severity	7.45 (0.3)	7.1 (0.06)	1.05	0.31
general psychopathology	16.9 (0.37)	16.5 (0.31)	0.77	0.45

Correcting for IQ and counterbalance results showed a significant main effect for group on the amount of accurate responses on the PAL during the memory phase ($F = 3.08$ (1,26), $p = 0.027$), but not the learning phase. Patients were significantly worse at recalling 4, 6, and 8 figure-place associations than controls in the memory phase. There was also a significant main effect for medication (placebo vs biperiden condition) on accuracy of recall of 4, 6, and 8 figure-place associations in the memory phase, showing significantly lower accuracy scores after biperiden compared to the placebo condition in both groups ($F = 4.7$ (25,26), $p = 0.035$). No main effect of medication was found on accuracy in the learning phase. No significant group by medication interaction effect was found in either the learning or the memory phase. Post hoc paired samples *t*-test examining the effect of biperiden on accuracy for each cognitive load showed biperiden significantly impaired learning and memory at all cognitive loads except for the highest cognitive load of 8 figure-place associations, and the 6 and 8 figure-place associations in subjects with psychotic disorders. This may be due to a ceiling effect. Group means and standard deviations of repeated measures analyses are displayed in table 3.

3.3. Functional reactivity

3.3.1. Psychosis vs controls

3.3.1.1. Learning phase. During the learning phase no significant main effect of group was found in functional activation between control subjects and subjects with a psychotic disorder. A significant main effect of medication (both groups showing a significant increased activation under biperiden) was found in the right superior temporal gyrus ($p < 0.001$; x: 64 y: −4 z: −2; Z_{max}: 9.28), left parahippocampal gyrus ($p < 0.001$; x: −30 y: −16 z: −28; Z_{max}: 7.58) and left precuneus ($p = 0.03$; x: 2 y: −66 z:60; Z_{max}: 5.67). These clusters were also identified as showing a significant group by medication interaction effect (main comparison of interest), showing both controls and the subjects with a psychotic disorder having increased functional activation in these areas under biperiden, but in the subjects with a psychotic disorder this was significantly greater than in controls. See Fig. 2A. Comparisons were corrected for accuracy, IQ, and multiple comparisons.

3.3.1.2. Memory phase. A significant main effect for group was found in the left middle frontal gyrus ($p < 0.001$; x: −38 y: 18 z: 52; Z_{max}:10.5), left lateral occipital lobe ($p < 0.001$; x: −36 y: −74 z:44; Z_{max}:8.06), and fusiform cortex ($p = 0.025$; x:40 y: −16 z: −26; Z_{max}:5.99). Control subjects showed significantly greater activation in these clusters than the subjects diagnosed with a psychotic disorder. No significant main effect for medication was found. Results did show a significant group by medication interaction effect on functional activation in the right superior temporal gyrus ($p = 0.01$; x: −64 y: −4 z: −2; Z_{max}: 9.36) and left parahippocampal gyrus ($p < 0.001$; x: −30 y: −16 z: −28; Z_{max}: 4.27). The subjects diagnosed with a psychotic disorder showed significant hyperactivation in these regions under biperiden, whereas control subjects showed attenuation of functional response (see Fig. 2B). All comparisons were corrected for IQ, accuracy of performance, and multiple comparisons.

3.4. Relationship between functional response to biperiden and M_1 receptor binding

In the placebo condition a significant positive correlation was found between hippocampal M_1 binding and functional activation in the left middle temporal gyrus ($p = 0.036$, x: −50 y: −6 z: −20; Z_{max}: 5.75) in the subjects diagnosed with a psychotic disorder. Lower hippocampal M_1 binding also significantly predicted a reduced functional response to biperiden during learning (smaller attenuation of activation under biperiden compared to high binders) in the left inferior temporal gyrus ($p = 0.013$; x: −44 y: −2 z: −30; Z_{max}:6.58) with significant local

Table 3
Accuracy of responses on the PAL task.

Accuracy	N (fig)	Psychotic disorder			Controls		
		Placebo (mean/SD)	Biperiden (mean/SD)	P	Placebo (mean/SD)	Biperiden (mean/SD)	p
Phase 1: Learning	4	3.07 (0.2)	2.98 (0.3)	< 0.001	3.24 (0.2)	3.11 (0.3)	< 0.001
	6	3.28 (0.01)	3.09 (0.02)	< 0.001	3.29 (0.02)	3.08(0.01)	< 0.001
	8	4.40 (0.3)	3.98 (0.05)	0.002	4.3(0.05)	3.99 (0.3)	< 0.001
Phase 2: Memory	4	3.34 (0.5)	2.88 (0.6)	< 0.004	4.52 (0.1)	3.28 (0.2)	< 0.001
	6	3.35 (0.2)	3.33 (0.2)	*0.9 ns*	5.34 (1.1)	3.06 (0.09)	< 0.001
	8	3.85 (0.9)	3.76 (0.3)	*0.7 ns*	4.65 (0.6)	4.34 (0.9)	*0.2 ns*

All findings were corrected for IQ and counterbalance.

peak activation within this cluster in the left fusiform gyrus (x: −40 y: −12 z: −22; Z_{max}: 6.32) and left parahippocampal gyrus (x: −30 y: −8 z: −24; Z_{max}: 5.82). In contrast, lower M_1 binding in the DLPFC significantly predicted lower functional reactivity to biperiden (smaller attenuation of activation under biperiden) during the memory phase in the left cingulate gyrus ($p < 0.001$; x: −2 y:34 z: −6; Z_{max}:7.57) and right cingulate gyrus although this finding in the right hemisphere did not survive multiple comparison's correction. See Fig. 3.

3.5. Relationship between functional response to biperiden and psychotic symptom severity

Greater negative symptom severity was associated with significantly lower functional reactivity to biperiden in the right caudate nucleus ($p = 0.023$; x: 14 y:20 z: −2; Z_{max}:4.2) during learning, and in the left parahippocampal gyrus ($p = 0.043$; x: −32 y: 0 z: −24; Z_{max}: 4.73) during the memory phase. Other psychotic symptoms did not show a significant association with functional reactivity to biperiden.

4. Discussion

The current study assessed the modulatory role of the muscarinic M_1 receptor on associative learning and memory in medication-free subjects with a psychotic disorder and matched controls. In contrast to the first hypothesis, no evidence for task induced DLPFC hypoactivation was found in the subjects with a psychotic disorder compared to controls during the learning phase of the task. Biperiden also did not cause DLPFC hypoactivation in controls or further decreased DLPFC hypoactivation in the subjects with psychotic disorders. In similar vein, no evidence for increased parahippocampal gyrus activation was found in the subjects with psychotic disorders compared to controls during learning, but biperiden did induce significantly increased activation in

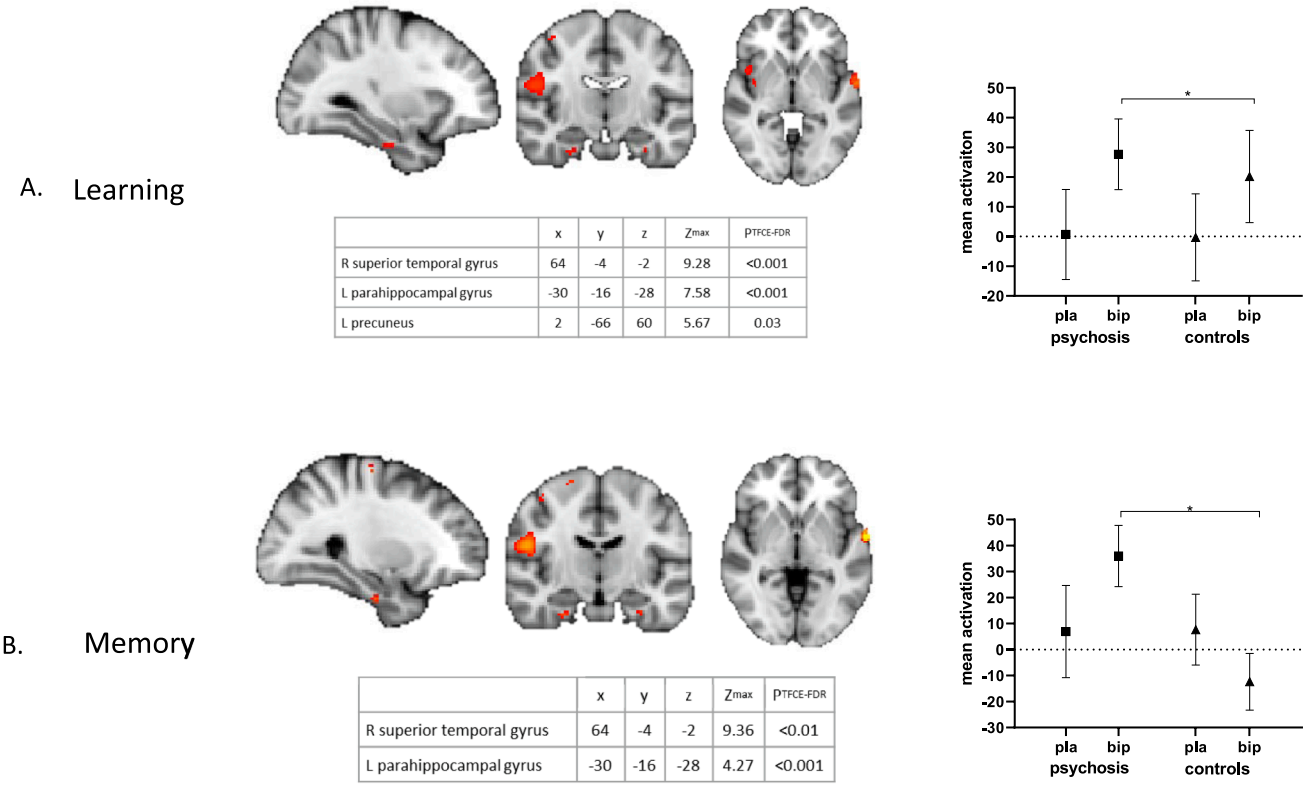

Fig. 2. A. Overview of clusters showing a significant group by drug interaction during the learning phase, with both groups showing significantly increased functional activation in the right superior temporal gyrus, left parahippocampal gyrus, and left precuneus under biperiden compared to placebo. In the subjects diagnosed with a psychotic disorder this hyperactivation was significantly greater compared to controls. B. Overview of clusters showing a significant group by drug interaction effect during the memory phase. Patients diagnosed with a psychotic disorder had significantly increased activation in these clusters whereas controls showed attenuation of activation. All findings were corrected for IQ, accuracy and multiple comparisons. R: right; L: left.

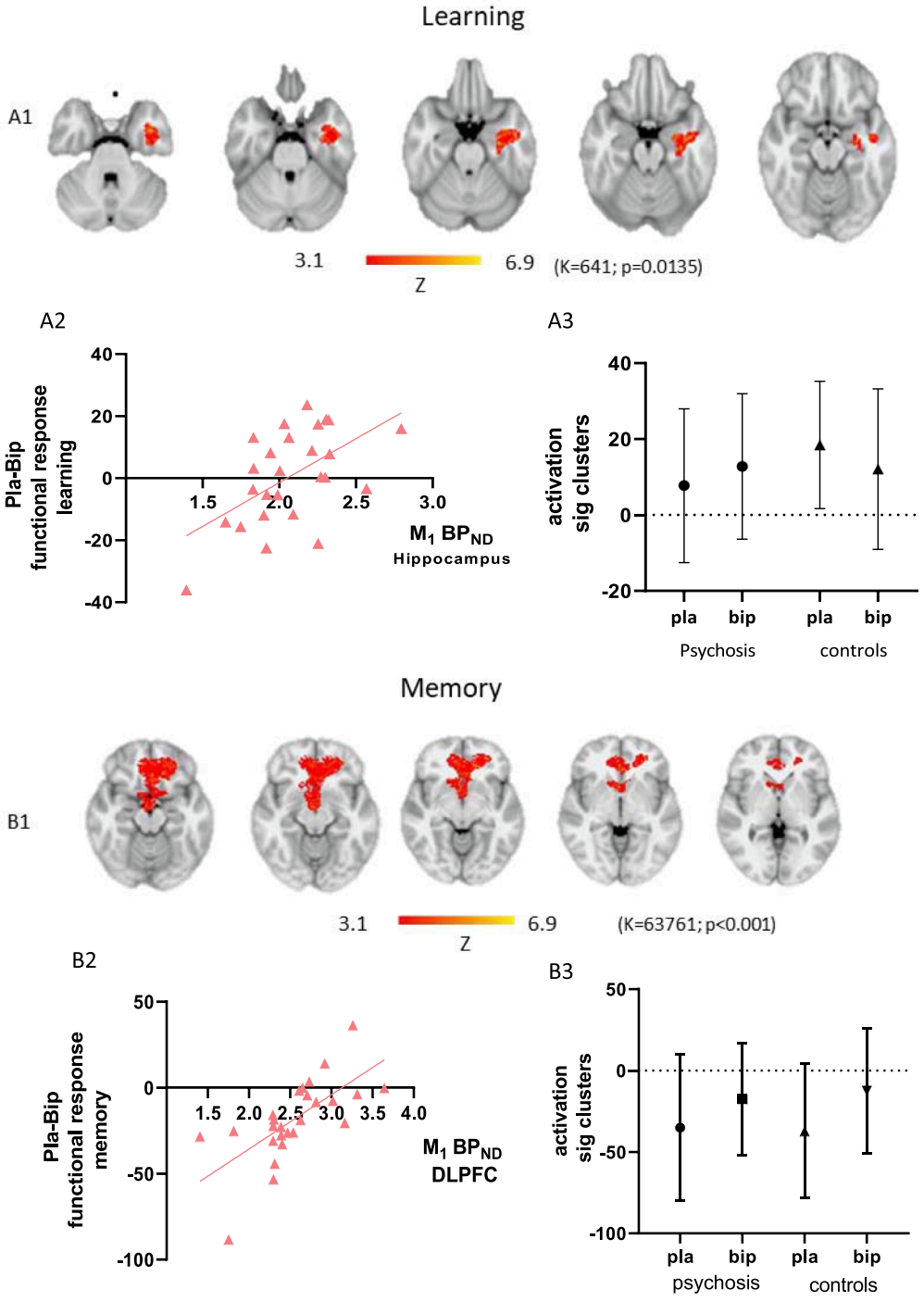

Fig. 3. A: A1&A2: Lower hippocampal M_1 binding significantly predicted a smaller functional response to biperiden in left inferior temporal gyrus, fusiform gyrus, and parahippcampal gyrus during learning suggesting less M_1 reserve to elicit functional response during learning in the subjects with psychotic disorder. A3: Activation in significant clusters associated with hippocampal M_1 binding in the subjects with a psychotic disorder plotted out for both groups. B: B1&B2: Lower M_1 binding in the DLPFC significantly predicted a decreased functional response in the left cingulate gyrus in to biperiden during the memory phase. Lower DLPFC M_1 binding as associated with lower ability to functionally activate this area after biperiden. B3: Activation in significant clusters associated with DLPFC M_1 binding in the subjects diagnosed with a psychotic disorder plotted out for both groups. k: cluster size, pla: placebo bip: biperiden.

both subjects with psychotic disorders and controls of the parahippocampal gyrus, and this increase was significantly greater in the subjects with psychotic disorders.

In line with our hypothesis results did support significant hypoactivation of the DLPFC (left middle frontal gyrus) in the subjects with a psychotic disorder compared to controls during memory, but no evidence for increased parahippocampal gyrus activation was found. Under biperiden however, supporting the hypothesis, significant hyperactivation was found in the subjects with a psychotic disorder controls however, showed significant hypoactivation.

Lastly, no evidence was found for lower M_1 binding and patterns of greater DLPFC hypoactivation and increased parahippocampal cortex activation in the subjects with psychotic disorders during learning or memory. Instead lower hippocampal M_1 binding predicted lower

functional response of the fusiform gyrus and parahippocampal gyrus to biperiden during learning, and lower M_1 binding in the DLPFC predicted lower functional response in the cingulate gyrus during memory.

4.1. Functional activation differences between the subjects with psychotic disorders and controls

In the current study we did not replicate findings of earlier studies in schizophrenia showing significant hypoactivation in the DLPFC and increased parahippocampal gyrus activation during associative learning, but only showed DLPFC (middle frontal gyrus) hypoactivation in the memory phase (Ragland et al., 2012, 2009, 2004). Previous studies concluded that in general the memory phase seemed to be more affected than the learning phase which is supported by our results (Barch and Sheffield, 2014). Lack of DLPFC hypoactivation during the learning phase in our cohort may be attributed to the fact that they were less ill. In the current study subjects were experiencing mild symptoms and were not as cognitively impaired as those investigated in previous studies, which may account for differences in results. In this regard, it is of interest that another previous study also did not find DLPFC hypoactivation in subjects with psychosis that were only mildly impaired (Ragland et al., 2012). Similarly, previous findings may have been confounded by use of antipsychotic medication that can affect cognitive functioning (MacKenzie et al., 2018).

4.2. Group difference in functional sensitivity to biperiden

Contrary to anticipated, biperiden did not attenuate task-induced activation in the DLPFC during associative learning, in neither control subjects, nor those diagnosed with a psychotic disorder, but instead elicited a significantly increased functional response in the parahippocampal gyrus in both groups, with patients having even greater hyperactivation. In the subjects with an psychotic disorder the greater hyperactive response was more widespread and included the superior temporal gyrus. These results suggest that loss of M_1 signalling by biperiden may have a greater impact on functional response in temporal limbic structures rather than the DLPFC. Associative learning depends on glutamate driven longterm potentiation (LTP) in the hippocampus of signals from the environment (Takeuchi et al., 2014). M_1 and M_4 critically modulate the underlying glutamatergic signalling of LTP in the hippocampus. Preclinical data has shown that loss of M_1 mediated signalling leads to loss of the controlled suppression of glutamate underlying LTP leading to a hyperactive response. The significantly greater activation under biperiden in learning and memory in the psychosis patients could be driven by a greater loss in M_1 mediated suppression on glutamate in the hippocampus compared to controls, potentially due to lower reserve of M_1 receptors. Despite the subjects with psychotic disorders having very mild impairments in associative learning and memory they seem to be functionally more sensitive to M_1 antagonism than controls.

These preliminary data suggest that greater functional sensitivity of limbic structures in psychotic disorders in learning and memory may have it's origin in loss of muscarinic M_1 mediated signalling. These findings are important, as the preclinical rodent (prenatal) methylazoxymethanol acetate (MAM) model of schizophrenia has also shown hippocampal hyperactivity from direct cell recordings (Lodge, 2013). In this model the hyperactivity translates to an increased drive on ventral tegmentum firing which results in an increased dopamine synthesis and release in the striatum (Lodge and Grace, 2011). As such, targeting the M_1 mediated signalling upstream may be beneficial to suppressing striatal hyperdopaminergia, in addition to improving cognition. Although more conclusive studies would need to be done, our data suggests that the parahippocampal gyrus hyperactivation reported in previous studies may not be a compensatory strategy as suggested but be disease mechanistic. It may also ultimately explain why hippocampal and limbic structure atrophy is seen in chronic schizophrenia (Lieberman et al., 2018).

4.3. Relationship between functional sensitivity to biperiden and M_1 receptor binding

In this study we also assessed whether differences in functional reactivity to biperiden in the subjects diagnosed with a psychotic disorder showed any direct relationship with M_1 receptor binding (Fig. 3). Results showed that levels of hippocampal M_1 binding specifically drove functional sensitivity to biperiden in limbic areas during the learning phase, with no association found for M_1 binding in the DLPFC. These results add to our previous findings showing an association between lower hippocampal M_1 binding and greater impairments in verbal learning and memory task (Bakker et al., 2018). Current results suggest this may be explained by a lower M_1 mediated functional response in limbic areas in the low binding subjects.

In contrast, M_1 binding in the DLPFC seemed to play more of a role during the memory or recall phase. It was specifically associated with greater functional sensitivity to biperiden in the bilateral cingulate gyrus (Fig. 3). The cingulate gyrus receives processed information from the hippocampus and parahippocampal gyrus to store in the cortex allowing for memory retrieval (Brem et al., 2013). Thus, lowered M_1 mediated signalling in the DLPFC seems to translate to lower activation of regions involved in the retrieval of traces formed in the learning phase. It is still unclear whether this is secondary to functional changes underlying learning processes or exist independently. Patients diagnosed with a psychotic disorder with lower M_1 binding in the DLPFC which has been hypothesised to be present in a subgroup of patients (Dean et al., 2002) may show greater impairments in memory retrieval capacity. More generally, current data show relatively greater degree of abnormalities in functional response to learning compared to memory under biperiden, suggesting psychotic disorders may be more characterised by lim bic changes in muscarinic M_1 receptor sensitivity. This is in line with earlier meta-analytical studies showing greater functional abnormalities in paired associate encoding (learning) than retrieval in schizophrenia (Ragland et al., 2012).

4.4. Relationship between functional sensitivity to biperiden and severity of psychotic symptoms

The present results also showed that some of the abnormalities in functional response to biperiden were tied to negative symptom severity. Reduced functional response to biperiden in the caudate nucleus (during learning) and parahippocampal gryus (during memory) was found in the subjects with greater negative symptoms severity. The caudate nucleus plays an important role in the association of two stimuli and hyperactivation of this area is implicated in abnormal processing of environmental cues giving rise to psychotic symptoms (McCutcheon et al., 2019). The caudate nucleus is an integral part of the fronto-striatal limbic circuitry, and although the genesis of the negative symptoms has been tied loosely to alterations in dopamine, noradrenaline and glutamate in this circuitry, the exact underlying pathology is not understood (Mitra et al., 2016). Current results seem to suggest that altered, or lower M_1 functioning may also play a role in negative symptom severity and linked to fronto-striatal-limbic functioning. This may underlie the efficacy of antipsychotic clozapine treat negative symptoms (Khan and Zaidi, 2017), and the positive effect of $M_{1/4}$ agonist xanomeline on negative symptoms in patients with chronic schizophrenia (Shekhar et al., 2008b). Clozapine's efficacy to improve negative symptoms has been attributed to the formation of its brain penetrant metabolite n-desmethylclozapine that acts a positive allosteric modulator at the M_1 receptor (Sur et al., 2003).

Taken together results from this study point to differences in functional sensitivity of the muscarinic system during learning and memory in patients diagnosed with a psychotic disorder compared to controls. This difference is particularly apparent in temporal- limbic areas and

seemingly already present in early phases of the disorder and in mildly impaired subjects. This increased muscarinic sensitivity in limbic-temporal areas in the subjects with psychotic disorders was related to hippocampal M_1 receptor binding, rather than M_1 receptor binding in the DLPFC.

5. Strengths and limitations

A key strength of the current study was that the subjects diagnosed with a psychotic disorder were medication free, and results did not have confounding effects of medication, or affected by indirect interaction effects between biperiden and antipsychotic medication affecting dopaminergic and serotonergic signalling. As cognitive symptoms often develop prior to the onset of psychotic disorder, alteration in M_1 signaling has been proposed to be an upstream factor in the dysregulation of dopamine and serotonergic systems making it paramount to investigate medication free subjects (Vakalopoulos, 2014).

Another major strength was that differences in functional sensitivity to biperiden between controls and subjects with psychotic disorders was linked to regional M_1 receptor binding in the subjects with psychotic disorders. These findings are critical to ongoing efforts to develop M_1 agonists to improve cognition (Erskine et al., 2019; Melancon et al., 2013). Also both biperiden and ^{123}I-IDEX have been shown to preferentially bind to the M_1 receptor (Bakker et al., 2015; Klinkenberg and Blokland, 2011). Previous studies have used non-selective $M_{1/4}$ tracer IQNB or used scopolamine, an antagonist at the all muscarinic receptor subtypes, to investigate the role of the muscarinic system in schizophrenia or cognition which made it more difficult to attribute finding specifically to the M_1 receptor. Notwithstanding this, we cannot rule out marginal effects on the functional response by biperiden blockade of the M_4 receptor that has high density in the hippocampus. In addition, because both biperiden and ^{123}I-IDEX are antagonists, they sample all M_1 receptors rather than only those in a high-affinity state. The modulatory effects of the M_1 receptor on the functional response will be mediated by those in a high affinity state, which we did not directly assess in this study.

Unfortunately, we were not allowed to acquire in-vivo M_1 receptor data in the control subjects by the medical ethical board. Therefore, it was not possible to determine whether the lower hippocampal M_1 binding that was shown to predict loss of functional activation in striatal-limbic regions during associative learning and memory was significantly reduced compared to controls. In addition, the subjects with a psychotic disorder included in this study displayed mild to moderate symptoms, as such findings may not translate well to more severely affected patients.

6. Conclusion

The current study showed an abnormally high hyperactivation of the parahippocampal gyrus during associative learning and memory in subjects diagnosed with a psychotic disorders in response to biperiden which may be related to loss of M_1 mediated modulation over glutamate in these regions. Results further linked this hyperactivation to lower hippocampal M_1 receptor binding, rather than M_1 binding in the DLPFC. Lastly, subjects with psychotic disorder with greater negative symptom severity and lower hippocampal and DLPFC M_1 binding showed greater loss of functional activation in the striatal-limbic circuitry. Performance on the PAL task together with negative symptom profile may be sensitive marker for greater therapeutic sensitivity to drugs targeting the muscarinic system.

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Disclosure/conflicts of interest

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