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Effect of anticholinergic burden on brain activity during Working Memory and real-world functioning in patients with schizophrenia

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ABSTRACT

Cognitive impairment has been associated with poor real-world functioning in patients with Schizophrenia. Previous studies have shown that pharmacological treatment with anticholinergic properties may contribute to cognitive impairment in Schizophrenia. We investigated the effect of the anticholinergic burden (ACB) on brain activity, cognition, and real-world functioning in Schizophrenia. We hypothesized that greater ACB would be associated with altered brain activity along with poorer cognitive performance and lower real-world functioning. A sample of 100 patients with a diagnosis of schizophrenia or schizoaffective disorder was recruited in the naturalistic multicenter study of the Italian Network for Research on Psychoses (NIRP) across 7 centres. For each participant, ACB was evaluated using the Anticholinergic Cognitive Burden scale. The association of ACB with brain function was assessed using BOLD fMRI during the N-Back Working Memory (WM) task in a nested cohort ($N = 31$). Real-world functioning was assessed using the Specific Level of Functioning (SLOF) scale. Patients with high ACB scores (≥ 3) showed lower brain activity in the WM frontoparietal network (TFCE corrected alpha <0.05) and poorer cognitive performance ($p = 0.05$) than patients with low ACB scores (<3). Both effects were unaffected by demographic characteristics, clinical severity, and antipsychotic dosage. Moreover, patients with high ACB showed poorer real-world functioning than patients with lower ACB ($p = 0.03$). Our results suggest that ACB in Schizophrenia is associated with impaired WM and abnormal underlying brain function along with reduced real-world functioning. Clinical practice should consider the potential adverse cognitive effects of ACB in the treatment decision-making process.

1. Introduction

Cognitive impairment is at the core of schizophrenia psychopathology as demonstrated by a very large body of literature indicating deficits in several domains such as Working Memory (WM), attention, executive function, episodic memory, and social cognition (Antonucci et al., 2020; Barch and Ceaser, 2012; Kahn and Keefe, 2013; McCutcheon et al., 2020; Weinberger and Harrison, 2011). Cognitive deficits impact real-world functioning in patients with schizophrenia contributing to long-term disability and increasing the disease burden (Bowie et al., 2006; Cowman et al., 2021; Green et al., 2004; Mucci et al., 2021). Even though different cognitive remediation strategies have been proposed

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with some currently used in clinical practice (Galderisi et al., 2010; Velligan et al., 2000), the effect on functional outcome is low-to-moderate (Allott et al., 2020; McGurk et al., 2007; Vita et al., 2021; Wykes et al., 2011) and no specific pharmacological treatment is available to modulate cognitive deficits. Cognitive impairment in schizophrenia is observed in the early stages of the disease and is associated with genetic risk and abnormal neurodevelopment (Antonucci et al., 2020; Bertolino and Blasi, 2009; Bora, 2015; Galderisi et al., 2009; Goldberg et al., 1995; Legge et al., 2021); illness duration, number of relapses, comorbidities, and antipsychotic polypharmacy also covary with cognitive impairment (Hagi et al., 2021; Hochberger et al., 2020; Millan et al., 2012). Cognitive deficits in schizophrenia have been associated with altered brain function, including those of the prefrontal cortex (PFC) during WM (Bertolino et al., 2000; Manoach, 2003). In particular, patients with schizophrenia deviate from the physiological inverted-u-shape relationship between PFC activity and WM performance showing either low performance paralleled by increased activity (i.e. inefficiency) (Callicott et al., 2003a; Callicott et al., 2003b) or a flattening of PFC activity associated with reduced WM capacity (i.e. hypo-frontality) (Manoach, 2003; Van Snellenberg et al., 2016). Different neurotransmitters have been implicated in impaired cognitive function in schizophrenia, including dopamine (Pergola et al., 2017; Sakurai et al., 2013), glutamate (Bustillo et al., 2011), serotonin (Blasi et al., 2015; Roth et al., 2004), and acetylcholine (Mulsant et al., 2003), being that latter involved in the physiological modulation of encoding and active maintenance during WM through modulation of neuronal persistent spiking and recurrent feedback circuitry loops (Newman et al., 2012).

Recently, greater attention has been devoted to the negative impact of anticholinergic medication exposure, with evidence supporting an association between high anticholinergic burden, cognitive decline, and increased risk for dementia in the general population (Coupland et al., 2019; Taylor-Rowan et al., 2022). Patients with schizophrenia are exposed to a variety of pharmacological treatments with anticholinergic properties ranging from pure anticholinergic agents used to reduce extrapyramidal symptoms to antipsychotics and antidepressants, some of which also possess an affinity to muscarinic receptors and interact with the acetylcholine system (Chew et al., 2006; Salahudeen et al., 2015). Previous research has shown associations between anticholinergic burden and cognitive function in schizophrenia suggesting that it might contribute to its cognitive impairment (Ang et al., 2017; Eum et al., 2021; Eum et al., 2017; Haddad et al., 2023; Minzenberg et al., 2004; Strauss et al., 1990; Su et al., 2017; Tsoutsoulas et al., 2017). Interestingly, O'Reilly et al. (2016) reported in a longitudinal prospective study that a high anticholinergic burden negatively impacts patients' benefit from psychosocial treatment. Joshi et al. (2021) found in a large cross-sectional study of patients with schizophrenia and schizoaffective disorder that patients with high exposure to anticholinergic medication have poorer cognitive performance (including poorer WM performance) than patients with low anticholinergic exposure. Critically, the effect was robust to several confounding factors such as antipsychotic dosage, number of antipsychotics, positive and negative symptom severity, duration of illness and smoking. Moreover, cross-sectional findings (Khan et al., 2021) suggest that the effect of the anticholinergic burden on cognition mediates the negative association between cognitive performance and functional capacity in patients with Schizophrenia.

Taken together, these findings suggest a deleterious effect of medications with anticholinergic properties on cognition and functional outcome in schizophrenia. However, much less is known about the effect of the anticholinergic burden on brain activity in schizophrenia, which might explain the observed deficit in cognitive performance. In this study, we attempted to fill this gap. Considering the effects of anticholinergic medication on cognition (including WM) (Minzenberg et al., 2004; O'Reilly et al., 2016) and the modulation of WM neurocircuitry by the cholinergic system (Newman et al., 2012), it is reasonable to hypothesize that anticholinergic burden would have an impact on brain activity during WM. Therefore, the anticholinergic burden might contribute to the well-established deficits in WM performance and related brain activity in some patients with schizophrenia. Our primary hypothesis was that patients receiving treatment with a high anticholinergic burden would show either inefficiency or hypo-frontality as compared with patients with a low anticholinergic burden. In addition, we also sought to explore the interplay between anticholinergic burden, brain function and functional capacity.

2. Materials and methods

2.1. Study participants

One hundred patients were recruited as part of the multi-centre study of the Italian Network for Research on Psychoses (NIRP) across 7 Italian centres (Galderisi et al., 2014; Galderisi et al., 2016). A sub-sample of 31 patients completed and had usable functional Magnetic Resonance Imaging (fMRI) scans (see Sections 2.2 and 2.3) and were therefore included in the neuroimaging analysis. Inclusion criteria were diagnosis of schizophrenia or schizoaffective disorder according to DSM-5 criteria as assessed with the Structured Clinical Interview for the DSM (First, 2014) and stable pharmacological treatment for at least 28 days. Exclusion criteria were positive history of head injury with loss of consciousness, moderate or severe mental retardation, neurological diagnoses, history of alcohol or substance abuse or dependence during the past six months, pregnancy, inability to provide informed consent, and contra-indication to MRI scanning. All participants signed a written informed consent to participate after receiving a comprehensive explanation of the study procedures, which were carried out according to the Declaration of Helsinki. Approval of the study protocol was obtained from the local ethics committees of each participating centre. Table 1 reports full details about the demographics and cognitive and clinical characteristics of the sample.

2.2. MRI acquisition and pre-processing

MRI scans were performed at five different sites and with six different 3 Tesla scanners (full details are given in Supplementary Table 1) in 49 patients. For all participants, we collected one structural MRI (sMRI) and one functional fMRI N-Back WM scan. For the sMRI, T1-weighted structural images were acquired using SPGR or MPRAGE sequences. Gradient-echo echo-planar imaging sequence was used to acquire images while subjects performed the N-Back task (240 s, 120 volumes). To compensate for differences between scanners in the MRI acquisition window, individual grey matter images were combined using the ImCalc toolbox in SPM12 with a multiplicative function to obtain a binary mask of voxels acquired only in each scanner. This mask containing only voxels common to all acquisitions (approximately 359,000 isotropic 1 mm voxels) was applied to all individual images. N-back fMRI data were pre-processed with SPM12. For each participant, functional volumes were realigned to correct for head movement. Individual motion parameters were extracted and used to calculate Friston 24 motion parameters. The realigned images were co-registered to T1-weighted structural images, spatially normalized to standard space (MNI 152), and smoothed with an isotropic 8 mm FWHM kernel. Finally, nuisance covariates, including Friston 24 head motion parameters, white matter signals, and cerebrospinal signals, were regressed out. Scans were inspected following the quality-based inclusion criteria: absence of scan artefacts, and low head motion (translation$<$3 mm, rotation$<3^{\circ}$). In the first-level analysis, a convoluted box car model was modelled with the hemodynamic response function in each voxel. Linear contrasts were then calculated, producing a t-statistic map for the 2-Back condition assuming the 0-Back condition as the baseline. Nine subjects were excluded because of scan artefacts, four subjects were excluded because of excessive movement.

Table 1
Summary of demographic, cognitive and clinical characteristic of the sample. PANSS = Positive and Negative Symptom Scale, ACB = anticholinergic burden, SLOF = Specific Level of Functioning, CPZeq = chlorpromazine equivalents, RT = reaction time. Asterisk (*) indicates significant differences between lowACB and highACB groups. Positive effect size indicated highACB>lowACB effects, and negative effect size indicated highACB<lowACB effects.

	All	lowACB	highACB	ACB group difference (effect size)	ACB group difference (p value)
Whole sample					
N	100	66	34		
Age, mean (SD)	36.71 (10.6)	37.81 (11.47)	34.64 (8.58)	$t = -1.42$	0.16
Sex, M (F)	73 (27)	49 (17)	23 (11)	$X^2 = 0.67$	0.41
Education, mean (SD)	12.53 (3.18)	12.26 (3.27)	13.06 (2.98)	$t = 1.18$	0.23
PANSS total, mean (SD)	64.91 (18.39)	62.11 (18.62)	70.17 (16.99)	$t = 2.11$	0.04*
CPZeq, mean (SD)	332.91 (192.70)	310.36 (180.83)	376.03 (209.61)	$t = 1.62$	0.11
SLOF total, mean (SD)	183.21 (19.69)	187.40 (18.93)	175.20 (18.85)	$t = -3.05$	0.003*
ACB score, mean (SD)	1.82 (1.14)	1.09 (0.42)	3.23 (0.65)	–	–
Antipsychotic monotherapy/polytherapy (N)	85/15	57/9	28/6	–	–
fMRI sample					
N	31	19	12		
Age, mean (SD)	35.16 (10.28)	37.26 (11.31)	31.83 (7.69)	$t = -1.46$	0.156
Sex, M (F)	26 (5)	16 (3)	10 (2)	$X^2 = 0.004$	0.948
Education, mean (SD)	13.09 (3.07)	13.11 (3.28)	13.08 (2.84)	$t = -0.02$	0.985
PANSS total, mean (SD)	62.93 (16.75)	58.00 (16.11)	70.75 (15.23)	$t = 2.19$	0.037*
CPZeq, mean (SD)	343.06 (232.81)	273.15 (204.34)	453.75 (240.19)	$t = 2.24$	0.033*
SLOF total, mean (SD)	191.03 (16.4)	198.15 (12.72)	179.75 (15.67)	$t = -3.59$	0.001*
2Back accuracy, mean (SD)	50.03 (22.64)	56.31 (24.64)	40.08 (15.12)	$t = -2.04$	0.050*
2Back RT, mean (SD)	823.06 (229.57)	797.21 (231.37)	864.00 (230.53)	$t = 0.78$	0.439
ACB score, mean (SD)	1.97 (1.97)	1.11 (0.31)	3.33 (0.65)	–	–
Antipsychotic monotherapy/polytherapy (N)	85/15	57/9	28/6	–	–

2.3. fMRI task

During the fMRI scan patients performed the 2-Back task (Fazio et al., 2018; Selvaggi et al., 2019). Stimuli consisted of numbers (from 1 to 4) shown in random sequence and displayed at the points of a diamond-shaped box. The task had 2 conditions: 0-back and 2-back. There was a non-memory-guided control condition (0-back) that required subjects to identify the stimulus currently seen. In the Working Memory condition (2-back), the task required the recollection of two stimuli before, while keeping on encoding incoming stimuli. The stimuli were arranged in a block design, consisting of eight 30-s blocks: four blocks of the control condition alternating with four blocks of each WM. Each block began with task instructions (2 s) and included 14 task trials (duration: 0.5 s, inter-trial interval: 1.5 s). Stimuli were presented via a back-projection system and behavioural responses were recorded through a fibre optic response box which allowed assessment of accuracy (percentage of correct responses) and reaction time for each trial. Participants with below chance accuracy (<25 % of correct responses) were excluded from the analysis (N = 5).

2.4. Anticholinergic burden assessment

For each patient, the anticholinergic burden was assessed using the Anticholinergic Cognitive Burden (ACB) scale (Cai et al., 2013; Salahudeen et al., 2015). The ACB scale is an expert-validated tool that ranks the anticholinergic properties of a medication according to its pharmacological profile. Among the many different tools available to assess the anticholinergic burden, the ACB scale has been evaluated as a tool characterized by high quality, high clinical applicability, strong validation, and high concordance with other clinical scales (Lisibach et al., 2021; Lozano-Ortega et al., 2020). Greater scores on the ACB have been previously associated with a greater risk for dementia in the general population (Coupland et al., 2019; Richardson et al., 2018) and with cognitive deficits in Schizophrenia (Joshi et al., 2019; Khan et al., 2021). The ACB scale ranks each medication according to anticholinergic properties ranging from 0 to 3 for each drug: 0 = no activity, 1 = low activity, 2 = moderate activity, and 3 = strong anticholinergic activity. Supplementary Table 2 reports the ACB score assigned to each medication and their frequency in our sample. ACB score calculation was based on the current medication taken by patients and verified by checking available medical records. For drugs for which ACB scores were not available in the literature, we assigned scores based on known pharmacological similarities with medications with existing ACB scores. In the case of drug for which ACB score was not available in the literature (i.e., amisulpride and zuclopenthixol), we checked affinity to muscarinic receptor using publicly available datasets (e.g., http://www.drugbank.com,). To both amisulpride and zuclopenthixol we assigned a score of 0 given their negligible muscarinic activity (see Supplementary Table 2). For each patient, the total ACB score was calculated by summing ACB values from all medications (Joshi et al., 2019; O'Reilly et al., 2016; Su et al., 2017). As evidence suggests that a total ACB score of three or higher is associated with a clinically significant risk of cognitive impairment (Joshi et al., 2019), we grouped our sample into two categories: low ACB (ACB total score ranging from 0 to 2) and high ACB (ACB total score $\geq$ 3) as in previous investigations (Khan et al., 2021). Table 1 reports the number of patients for each group.

2.5. Assessment of real-world functioning in patients

Real-world functioning in patients with Schizophrenia was assessed using the Italian version of the Specific Level of Function Scale (SLOF) (Mucci et al., 2014). Ratings were based on either direct observation of patients' behaviour or functioning in several domains, combined with the information referred by the caregiver. SLOF self-administration was not employed in this study. The SLOF assesses functional capacity in different domains: i) physical functioning, ii) personal care skills, iii) interpersonal relationship, iv) social acceptability, v) community living, and vi) work (Mucci et al., 2014). We assessed the effect of ACB on both total SLOF and subscales to explore which functional domains were mostly affected by the anticholinergic burden.

2.6. Other clinical assessments

Clinical symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1989). PANSS total score was generated by summing all items from PANSS subscales. Given that illness severity has been associated with both cognitive function and functional outcome in Schizophrenia, the total PANSS score was added

as a covariate of no interest in all statistical models (see Section 2.7). In addition, chlorpromazine equivalents were computed for each patient's treatment using Gardner et al. method (Gardner et al., 2010). Chlorpromazine equivalents were also added as a covariate of no interest in all statistical models to account for the negative effect of high antipsychotic dosage on cognition (see Section 2.7).

2.7. Data analysis

2.7.1. Demographics and clinical variables

In both the whole sample and the fMRI sub-sample between groups (lowACB vs highACB) differences in age, years of education, total PANSS score, and chlorpromazine equivalents were tested using unpaired *t*-tests. Chi-square was used to test sex differences between groups. All analyses were performed in JASP (Version 0.17.1).

2.7.2. Effect of ACB on brain function during the 2-back task

Voxel-wise group difference in brain activity during the 2-Back task was tested using an independent sample t-test in FSL *randomise* (FMRIB software library v6.0) (Winkler et al., 2014). HighACB < lowACB and highACB>lowACB contrasts were evaluated. Age, sex, site of data acquisition, education, chlorpromazine equivalents and total PANSS were added as covariates of no interest. To reduce the effects of spurious activations and to increase SNR, the analysis was restricted within a brain mask obtained by a combination of grey matter and WM group mean activation (Di Giorgio et al., 2014). The resulting brain mask contained 83,916 voxels. The Threshold-Free Cluster Enhancement (TFCE) method was used to correct for multiple comparisons. Ten thousand permutations were used to generate the null distribution to test against. Signal changes from significant clusters were extracted using a binary mask corresponding to the cluster and the *fslmeants* function in FSL.

While other studies using the ACB construct usually assigned a score of 3 to clozapine (as in the present study) (Ang et al., 2017; Cai et al., 2013; Georgiou et al., 2021; Joshi et al., 2021; Joshi et al., 2019; Khan et al., 2021; O'Reilly et al., 2016), one of its primary metabolites (i.e., *N*-desmethylclozapine) has pro-cholinergic effects (Li et al., 2005). Therefore, additional analyses were performed adding clozapine treatment as covariate of no interest (i.e., presence and absence) (Supplementary Material).

2.7.3. Effect of ACB on WM performance

We tested the effect of ACB groups on WM performance using general linear models as implemented in JASP. Two separate models with accuracy (% of correct responses) and reaction time as independent variables were tested. Age, sex, chlorpromazine equivalents, education and total PANSS score entered the model as covariates of no interest. As for imaging analysis, we also performed here additional analysis including clozapine treatment as covariate of no interest.

2.7.4. Effect of ACB on real-world functioning and association with Working Memory

The General Lineal Model (GLM) as implemented in JASP was also used to test group differences (lowACB vs highACB) in total SLOF score in the whole sample. Total SLOF was the independent variable while age, sex, chlorpromazine equivalents, education and total PANSS were added as covariates of no interest. Two-tail significance was tested. To explore the effect of the ACB group on SLOF subscales Multivariate Analysis of Variance (MANOVA, Pillai test) was used with age, sex, chlorpromazine equivalents, education, total PANSS and site as a covariate of no interest. In addition, to explore the association between WM processing and SLOF, we performed a non-parametric Spearman correlation between brain signals extracted from significant clusters in the prefrontal cortex (see Section 2.7.2) and the total SLOF score. As in imaging and behavioural analyses, here we also performed additional analyses using clozapine treatment as covariate of no interest.

3. Results

3.1.1. Demographics and clinical variables

ACB groups did not differ in age, sex, and years of education (all $p > 0.2$). Patients with high ACB had higher PANSS total scores and higher chlorpromazine equivalents as compared with low ACB patients (both $p = 0.04$). Both PANSS total score and chlorpromazine equivalents were therefore included as a covariate of no interest in both voxel-wise and cognitive performance analyses. Full statistics are shown in Table 1.

3.1.2. Effect of ACB on brain function during Working Memory

Voxel-wise analysis revealed significant (TFCE corrected) clusters of reduced brain activation in the high ACB group as compared with the low ACB group in different brain regions within the WM network including the right dorsolateral prefrontal cortex (DLPFC) and the right and left supplementary motor areas. Fig. 1 illustrates the results of the voxel-wise analysis. Table 2 shows full statistics of the voxel-wise analysis. The opposite contrast (high ACB > low ACB) did not reveal any significant cluster. Inclusion of clozapine treatment as covariate of no interest did not significantly affect the results (see Supplementary materials).

3.1.3. Effect of ACB on Working Memory performance

The general linear model revealed a significant main effect of the ACB group on accuracy ($\eta_p^2 = 0.151$, $p = 0.049$, marginal means(standard error): highACB = 30.672(7.805), lowACB = 50.105(6.631)) with patients having high ACB performing worse than patients with low ACB. We did not find any significant effect of age, sex, education, and total PANSS score on accuracy (all $p > 0.06$). Fig. 2 illustrates group differences in accuracy. No statistically significant differences between high ACB vs low ACB groups were found in reaction times ($p = 0.4$). Inclusion of clozapine treatment as covariate of no interest did not significantly affect the results (see Supplementary materials).

3.1.4. Effect of ACB on real-world functioning and association with Working Memory

GLM in the whole sample revealed a significant main effect of the ACB group on total SLOF score, with high ACB patients scoring lower on total SLOF as compared with low ACB patients ($\eta_p^2 = 0.047$, $p = 0.03$). Age, sex, education, and chlorpromazine equivalents did not have a significant effect on the total SLOF score (all $p > 0.2$). As expected, PANSS total score negatively correlated with SLOF total score ($\eta_p^2 = 0.231$, $p < 0.01$) (Rocca et al., 2018). However, we did not find a significant PANSS total score by ACB group interaction ($p = 0.5$). Pillai's test revealed an overall significant multivariate effect of ACB groups on SLOF subscales ($F = 2.7$, $p = 0.02$). Patients with high ACB had significantly lower scores in the interpersonal relationship ($F = 4.3$, $p = 0.04$), community living ($F = 7.1$, $p = 0.01$) and work skills ($F = 14.1$, $p < 0.01$) domains as compared with patients with low ACB. No significant group differences were found for physical functioning, personal care, and social acceptability domains (all $p > 0.06$). Finally, brain signal extracted from the prefrontal cluster in which a significant difference between ACB groups was found, was negatively associated with SLOF total score (rho = 0.35, $p = 0.05$). Fig. 3 summarizes the results of these analyses. Inclusion of clozapine treatment as covariate of no interest did not significantly affect the results (see Supplementary materials).

4. Discussion

In this study, we investigated the effect of the anticholinergic burden on brain function during WM and on real-world functioning in a naturalistic multicentre cohort of patients with schizophrenia. We found that patients exposed to high anticholinergic burden have lower brain

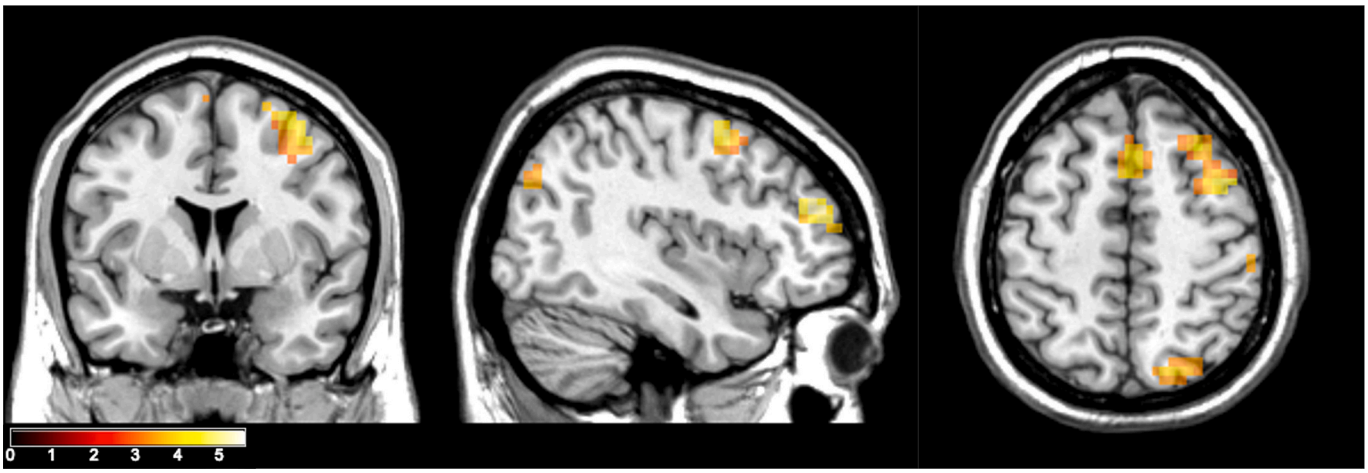

Fig. 1. Brain sections showing significantly lower brain activity during the 2-Back task in patients with high ACB scores relative to patients with low ACB scores in prefrontal, parietal and occipital cortex (TFCE corrected clusters). Color-bar indicates t-statistics. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Voxel-wise analysis results (cluster extent threshold = 10). MNI coordinates (x, y, z) are in mm.

Cluster index	Cluster size (voxels)	t	x	y	z	Brain region
1	11	3.57	34.5	−78.2	32.5	Right angular gyrus
2	18	3.8	53.2	−25.8	47.5	Right supramarginal gyrus
3	22	4.48	19.5	−74.5	55	Right precuneus
4	28	5.5	42	45.5	21.2	Right dorsolateral prefrontal cortex
5	52	4.05	4.5	19.2	51.2	Right medial premotor area
5	52	4	0.75	11.8	55	Right medial premotor area
5	52	3.28	−3	0.5	70	Right medial premotor area
6	111	4.99	27	8	66.2	Right supplementary motor area
6	111	4.53	38.2	8	55	Right supplementary motor area
6	111	4.08	30.8	23	51.2	Right supplementary motor area
6	111	3.99	53.2	11.8	36.2	Right supplementary motor area
6	111	3.97	49.5	11.8	43.8	Right supplementary motor area

activity in large brain areas within the frontoparietal network and lower behavioural performance during WM as compared with patients with low anticholinergic medication exposure. In addition, we found that the high ACB was also associated with low real-world functioning, especially in the interpersonal relationship, community living and work skills domains. Interestingly, SLOF domains also correlate with brain activity in the frontoparietal network where a significant effect of ACB was found, indicating that prefrontal activity during WM is lower in the same patients with high ACB who show lower functioning.

Our results extend previous literature reporting an effect of serum anticholinergic activity on brain volumes and brain activity during cognitive control (Schreiber et al., 2018; Wojtalik et al., 2012) by showing that the anticholinergic burden has a detrimental effect on WM

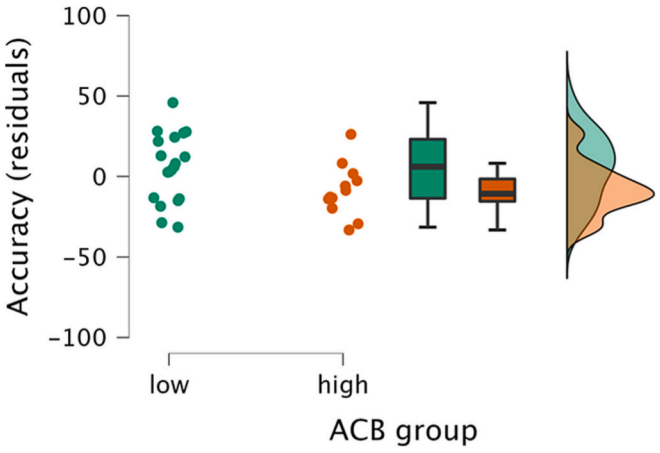

Fig. 2. Raincloud plot showing lower accuracy during the 2-Back task in patients with high ACB scores as compared with patients with low ACB scores. Accuracy is expressed in unstandardized residuals (see main text for statistics).

performance and related brain activity. Deficits in WM performance and related brain function are well-established and replicated findings in schizophrenia (Callicott et al., 2000; Minzenberg et al., 2009) and they have been associated with genetic liability for this disorder (Goghari, 2011; Krug et al., 2018; Pergola et al., 2017). Here we showed that patients receiving medications with high anticholinergic properties display more severe deficits than those with lower exposure to anticholinergic effects. Patients with high ACB scores showed, as compared with patients with low ACB, lower activation in key brain areas within the WM network including the prefrontal cortex paralleled by lower cognitive performance. Taken together, both results suggest that patients exposed to high anticholinergic burden are prone to reduced brain function during WM processing (Callicott et al., 2000) and are consistent with previous findings reporting failure to engage the PFC during WM task in patients with schizophrenia (Van Snellenberg et al., 2016). Remarkably, the effect was robust to several confounding factors such as age, sex, education, illness severity (evaluated as PANSS total score), overall antipsychotic dosage (expressed as chlorpromazine equivalents) and MRI scanner differences across participating centres. Our findings extend evidence of an undesired impact of anticholinergic medication on cognitive function in schizophrenia (Joshi et al., 2021; Khan et al., 2021) to show that also related brain activity is negatively affected by anticholinergic burden. Our imaging results are also in line with animal

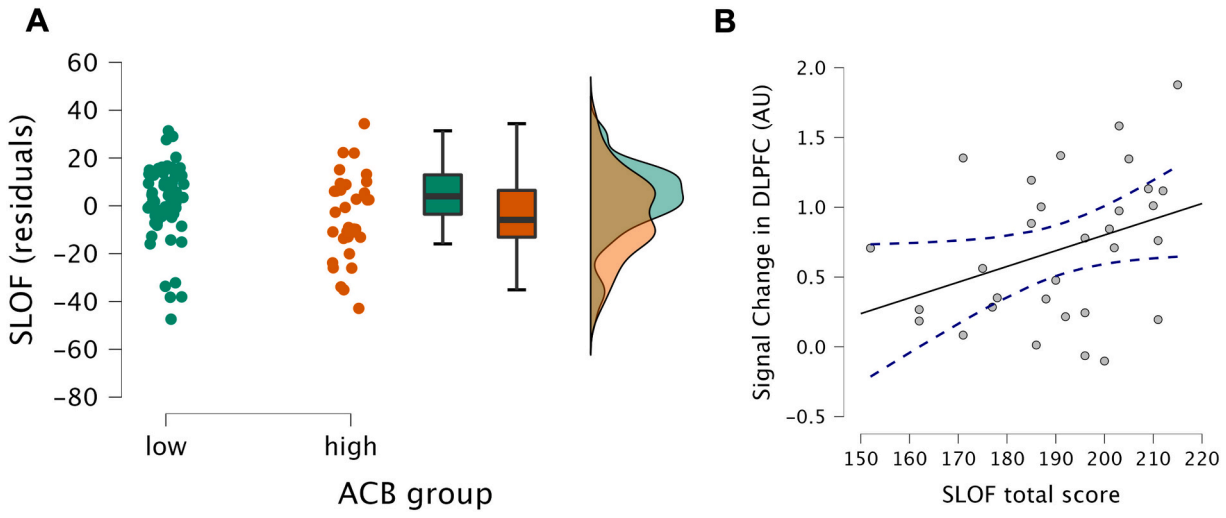

Fig. 3. 3A. Raincloud plot showing lower SLOF total scores in patients with high ACB scores as compared with patients with low ACB scores. SLOF scores are expressed in unstandardized residuals (see main text for statistics). 3B. Scatterplot showing positive correlation between BOLD signal change in the right dorsolateral prefrontal cortex (DLPC) and total SLOF score. AU: arbitrary units.

models (Bang and Brown, 2009; Esclassan et al., 2009) and experimental medicine studies in humans (Green et al., 2004; Koller et al., 2003) indicating that antagonism at muscarinic receptors impairs WM processing. Here we did not find an effect of ACB on reaction time during the N-Back. These negative results appear in contrast with the ones in Joshi et al. (2021) where they reported an effect of ACB on an efficiency index which averages accuracy and reaction times. However, differences between the two studies in task design (letter N-Back vs visuospatial N-Back), in performance indices (absolute vs combined), in the way to operationalize ACB scores (5 groups vs 2 groups), and in sample size might explain this discrepancy.

We also found that the anticholinergic burden affected not only cognition and related brain function but also real-world functioning in patients with schizophrenia. Here, we extend previous evidence of a negative association between ACB and functional capacity in elderly patients with schizophrenia (Khan et al., 2021) by showing that greater anticholinergic burden is associated with reduced real-world functioning not only in old patients with schizophrenia but also in younger patients (mean age in our sample: 36.7 years old). Our results therefore suggest that the impact of ACB on cognitive performance in SCZ might also be independent to neurobiological and neuropsychological alterations associated with normal and pathological aging. Thus, our results suggest that greater attention should be devoted to reducing the exposure to anticholinergic burden also in young patients with schizophrenia to prevent cognitive and functional decline. In addition, the effect of ACB was particularly marked in functional domains such as interpersonal relationships, community living and work skills, which are functional domains highly impaired in schizophrenia and unaffected siblings (Galderisi et al., 2016; Rocca et al., 2018). Interestingly, we found that these functional domains also correlate with brain activity in the prefrontal cortex where a significant effect of ACB was found suggesting that altered prefrontal dynamics may in part explain the link between anticholinergic burden, cognition, and real-world functioning.

4.1. Strengths and limitations

The strengths of this study are the naturalistic design and the multicentre implementation which are known to be associated with higher generalizability of the findings, especially in neuroimaging (Thompson et al., 2022). We must also acknowledge some limitations of our study. First, the sample size of our voxel-wise analysis is small as compared with state-of-the-art fMRI cross-sectional studies in schizophrenia. For this reason, we opted for a permutation-based approach which is less prone to false positives when testing voxel-wise group differences in small samples (Eklund et al., 2016). Nonetheless, further studies are needed to test the generalizability of our findings to larger cohorts and different settings (e.g., longitudinal prospective study in first-episode psychosis patients). Another limitation is that both ACB scores and chlorpromazine equivalents were evaluated considering the medication received by the patients at the time of the assessment. Therefore, our findings did not consider previous longitudinal exposure to anticholinergic burden and antipsychotic treatment. In addition, we did not formally test treatment adherence in patients. Considering that a proportion of patients with schizophrenia tend to discontinue their treatment or are partially adherent (McCutcheon et al., 2018) we cannot rule out that this issue might have biased our results. However, all patients included in our study were clinically stable and with stable treatment for at least 4 weeks. Moreover we investigated the effect of the anticholinergic burden of psychotropic medication, and we did not estimate anticholinergic burden related to non-psychotropic compounds. Given that several non-psychotropic compounds have also anticholinergic properties (Coupland et al., 2019), we might have underestimated the global anticholinergic burden in our sample. Finally, even though in our study we found that ACB was associated with reduced fronto-parietal brain activity during WM, and that brain activity during WM was associated with poor functional outcome we were underpowered to detect an indirect effect of ACB on functional outcome through brain activity. Future, better powered studies should investigate this relationship in greater detail.

4.2. Implication for treatment of schizophrenia

Even though all antipsychotic medications have side effects and concerns have been raised about long-term effects (Correll et al., 2018), it is glaring that they have dramatically changed the course of the illness. Indeed, the evidence supports the efficacy of antipsychotics for the acute treatment of psychosis and the prevention of relapse and suggests that early intervention might improve long-term outcomes (Goff et al., 2017). Notably, in our cohort none of the patients was taking pure anticholinergic agents (e.g., benztropine, diphenhydramine, or trihexyphenidyl) indicating that the effects were attributable to common medication taken by patients with psychosis such as antipsychotics, antidepressants, and mood stabilizers. Our findings should be interpreted with caution in terms of their clinical implications. The results of

this study do not indicate that drugs with high anticholinergic load should not be prescribed for their effects on cognition and functional outcome or massively deprescribed. Unmedicated first-episode psychosis patients and unmedicated chronic schizophrenia patients also show cognitive impairment and low real-world functioning (Solís-Vivanco et al., 2020). Therefore, the anticholinergic burden may be just one among many different genetic (Lencz et al., 2014) or environmental factors (Rabin et al., 2011) contributing to cognitive impairment and decline of functional capacity in schizophrenia. Therefore, a more conservative interpretation of our results can suggest that the anticholinergic burden should be introduced as a variable for the evaluation of the risk-benefit ratio in the clinical decision-making process. For example, a switch to a medication with a low anticholinergic load can be considered as a treatment strategy in elderly patients or in patients with severe cognitive impairment to facilitate outcomes when referred to rehabilitation programs.

5. Conclusion

Our results provide evidence for an association between high anticholinergic burden with reduced behavioural performance and engagement of related brain areas during WM, together with lower real-world functioning in patients with schizophrenia. These findings extend previous literature to suggest a plausible biological mechanism underlying the negative effect of anticholinergic medication on cognition and functioning. The anticholinergic burden should be considered in the treatment decision-making process in real-world clinical practice.

CRediT authorship contribution statement

Pierluigi Selvaggi: conceptualization, writing – original draft, data analysis; **Leonardo Fazio:** writing – review & editing, data acquisition, data preprocessing, data analysis; **Veronica Debora Toro:** writing – review & editing, data preprocessing, data analysis; **Armida Mucci:** data acquisition, data curation, writing – review & editing; **Paola Rocca:** data acquisition, data curation, writing – review & editing; **Giovanni Martinotti:** data acquisition, data curation, writing – review & editing; **Gianmarco Cascino:** data acquisition, data curation, writing – review & editing; **Alberto Siracusano:** data acquisition, data curation, writing – review & editing; **Patrizia Zeppegno:** data acquisition, data curation, writing – review & editing; **Giulio Pergola**: supervision, data curation, writing – review & editing; **Alessandro Bertolino:** conceptualization, funding acquisition, supervision, writing – review & editing; **Mario Maj:** funding acquisition, supervision, writing – review & editing; **Giuseppe Blasi**: conceptualization, supervision, writing – review & editing; **Silvana Galderisi:** funding acquisition, supervision, writing – review & editing; **authors listed in the Italian Network for Research on Psychosis:** data acquisition, data curation, review & editing.

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Declaration of competing interest

Dr. Galderisi reported receiving personal fees from Angelini Pharma, Gedeon Richter-Recordati, Innova Pharma-Recordati Group, Janssen Pharmaceutica NV, Lundbeck Italia, Millennium Pharmaceutical, and Sunovion Pharmaceuticals outside the submitted work. Dr. Bertolino reported receiving grants and personal fees from Lundbeck and receiving personal fees from Janssen and from Otsuka during the conduct of the study. All other authors declare no biomedical financial interests and no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2023.08.015.

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