



Changes in corticostriatal connectivity and striatal tissue iron associated with efficacy of clozapine for treatment-resistant schizophrenia

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Abstract

Rationale Though numerous studies demonstrate the superiority of clozapine (CLZ) for treatment of persistent psychotic symptoms that are characteristic of treatment-refractory schizophrenia (TRS), what remains unknown are the neural and molecular mechanisms underlying CLZ's efficacy. Recent work implicates increased corticostriatal functional connectivity as a marker of response to non-CLZ, dopamine (DA) D2-receptor blocking antipsychotic drugs. However, it is undetermined whether this connectivity finding also relates to CLZ's unique efficacy, or if response to CLZ is associated with changes in striatal DA functioning.

Objective In a cohort of 22 individuals with TRS, we examined response to CLZ in relation to the following: (1) change in corticostriatal functional connectivity; and (2) change in a magnetic resonance-based measure of striatal tissue iron (R2'), which demonstrates utility as a proxy measure for elements of DA functioning.

Methods Participants underwent scanning while starting CLZ and after 12 weeks of CLZ treatment. We used both cortical and striatal regions of interest to examine changes in corticostriatal interactions and striatal R2' in relation to CLZ response (% reduction of psychotic symptoms).

Results We first found that response to CLZ was associated with an increase in corticostriatal connectivity between the dorsal caudate and regions of the frontoparietal network ($P < 0.05$, corrected). Secondly, we observed no significant changes in striatal R2' across CLZ treatment.

Conclusion Overall, these results indicate that changes in corticostriatal networks without gross shifts in striatal DA functioning underlies CLZ response. Our results provide novel mechanistic insight into response to CLZ treatment.

Keywords Clozapine · Treatment-resistant schizophrenia · Antipsychotic treatment · Treatment response · Striatum · Schizophrenia · Functional connectivity · R2'

Introduction

Over a third of individuals with schizophrenia demonstrate persistent psychotic symptoms despite sequential trials with antipsychotic drugs, accounting for a high proportion of illness burden (Kennedy et al. 2014; Meltzer 1997). Over three

decades of evidence supports the superiority of clozapine (CLZ) for these refractory psychotic symptoms in treatment-resistant schizophrenia (TRS) (Kane et al. 1988; McEvoy et al. 2006; Siskind et al. 2016; Taylor 2017). Thus, CLZ is widely considered to be a model agent for antipsychotic drug discovery (Nucifora et al. 2017). However, the precise mechanism of action underlying CLZ's characteristic antipsychotic efficacy has been difficult to parse secondary to its heterogeneous profile of interactions with receptors and neurotransmitter systems (Coward 1992; Nucifora et al. 2017; Seeman 2014). To progress the development of circuit-based therapeutics that mirror CLZs clinical action, we conducted the following study focused on the largely unknown neural circuitry underlying CLZ's efficacy.

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Studies of non-CLZ, D2-receptor blocking antipsychotic drugs have explored corticostriatal functional connectivity as a marker of treatment. Studies of both first-episode and chronic schizophrenia report an increase in functional connectivity between the striatum and prefrontal cortical and limbic regions corresponding with antipsychotic treatment response (Anticevic et al. 2015; Cadena et al. 2019; Lahti et al. 2009; Li et al. 2020; Sarpal et al. 2015). Abnormal corticostriatal connectivity has also been linked to a longer duration of untreated psychosis and relapse in the context of stable antipsychotic treatment, two clinical scenarios that influence antipsychotic response (Rubio et al. 2021; Sarpal et al. 2017). Additional studies that leverage corticostriatal connectivity of response demonstrate its prognostic associations with successful treatment with non-CLZ antipsychotic agents (Li et al. 2020; Sarpal et al. 2016). Studies of white matter have also linked antipsychotic response with alterations in white matter, including tracts important for functioning of corticostriatal systems (Kochunov et al. 2019; Ochi et al. 2020; Reis Marques et al. 2014). Overall, these findings may reflect downstream action secondary to D2-receptor blockade in the context of abnormal striatal dopamine (DA) functioning. Given evidence suggesting a pathophysiologic inverse relationship between hyperdopaminergic striatal activity and hypo-functioning of cortical systems, hyperdopaminergic activity in the striatum, including the dorsal caudate, which serves as an integrative hub for information processing, is likely related to aberrant interactions across neural systems (Averbeck et al. 2014; Choi et al. 2017; Jarbo and Verstynen 2015; McCutcheon et al. 2019; Tziortzi et al. 2014). In the context of D2 blockade, an increase in functional connectivity between the dorsal striatum and nodes within these systems likely reflect modulation of information and stimulus processing in the context of decreased “noise” within the dorsal caudate (McCutcheon et al. 2019).

Though CLZ also demonstrates moderate occupancy of striatal D2 receptors with rapid dissociation, it remains unknown whether this dopaminergic action underlies its antipsychotic action in TRS (Seeman 2014). Thus, examining whether striatal DA plays a central role in CLZ response represents a critical step in stratifying the pathophysiology of TRS. Recent studies have advanced magnetic resonance-based measures of striatal tissue iron, which has been linked to a variety of physiologic processes, including the modulation of binding affinity at dopaminergic receptors, and DA synthesis and release (Haacke et al. 2005; Youdim et al. 1984; Youdim and Green 1978; Youdim 2018). Located in the dendrites of DA neurons, iron is a cofactor for tyrosine hydroxylase, the rate-limiting step in DA synthesis (Daubner et al. 2011; Molinoff and Axelrod 1971; Ortega et al. 2007; Torres-Vega et al. 2012; Zucca et al. 2017). While tissue iron is found throughout the brain, its highest concentration is in the basal ganglia (Brass et al. 2006; Thomas et al.

1993). Non-invasive MR-based assessments of striatal tissue iron have been utilized as markers of DA-related disorders, including Parkinson’s disease, Huntington’s disease, restless leg syndrome, ADHD, and cocaine use disorders (Adisetiyo et al. 2019; Allen and Earley 2007; Ersche et al. 2017; Piao et al. 2017; Ward et al. 2014; Zucca et al. 2017). Recent work has also demonstrated a strong relationship between ventral striatal tissue iron (R2’) and presynaptic vesicular DA storage (Larsen et al. 2020).

Since it remains unclear whether corticostriatal connectivity or significant changes in DA functioning contribute to CLZ’s antipsychotic efficacy, we conducted the following prospective study in a cohort of individuals with TRS undergoing CLZ treatment. First, we examined changes in corticostriatal connectivity in relation to reductions in psychotic symptoms. A significant relationship would suggest that corticostriatal interactions represent a generalized mechanism of antipsychotic response; a negative finding would implicate an alternative neural system underlying CLZ treatment. Secondly, considering CLZ’s heterogeneous pharmacologic action and the possibility for non-dopaminergic action, we explored changes in striatal R2’ along with symptomatic improvement.

Methods and materials

Participants

This prospective, observational study included a cohort of twenty-two individuals with TRS who were recruited from treatment services of UPMC Western Psychiatric Hospital and affiliated facilities. Participants were undergoing CLZ initiation per routine clinical care by their treatment providers. Clinical characteristics of our cohort were guided by recommendations of the Treatment Response and Resistance in Psychosis working group (Howes et al. 2017). Participants were as follows: (1) between the ages of 18 and 60; (2) presented with a diagnosis of schizophrenia or schizoaffective disorder; (3) exhibited chronic psychotic symptoms with a score of at least a 4 (moderate) on one or more Brief Psychiatric Rating Scale (BPRS) psychosis measures (hallucinatory behavior, unusual thought content, or conceptual disorganization) (Hedlund and Vieweg 1980); (3) had at least two failed trials of non-CLZ antipsychotic drugs for documented periods of at least 6 weeks duration; and (4) no CLZ for at least 4 weeks if prior CLZ treatment occurred. Exclusion criteria included the presence of a substance-induced psychotic disorder, concurrent electroconvulsive therapy, neurologic or medical conditions that could affect brain functioning, significant risk of suicidal or homicidal behavior determined by primary clinicians, or contraindications to magnetic resonance imaging.

Recruitment efforts, summarized in Supplemental Table 1, relied on collaboration between the study team and primary clinicians. Given our observational study design, medication decisions were made by primary clinicians and tracked by the study team. This included the use of non-CLZ antipsychotic drugs, which were converted to chlorpromazine equivalents, and other psychiatric medications. All study participants provided written consent and all procedures were approved by the University of Pittsburgh Institutional Review Board.

Clinical data acquisition

Demographic and clinical information was gathered for all study participants based on clinical interviews, and information from treatment teams and medical records. Diagnoses were confirmed by a Structured Clinical Interview for Axis I Diagnostic and Statistical Manual-IV (First et al. 2015). Symptoms were rated with the BPRS (Hedlund and Vieweg 1980). Cognition was assessed with the Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery (MCCB) (Nuechterlein et al. 2008). Of note, we aimed to collect cognitive assessments at baseline for participants. However, we encountered unforeseen challenges in data collection secondary to halted research activities due to the COVID-19 pandemic or difficulty in coordinating cognitive assessments at baseline across study sites. Thus, cognitive assessments were split between baseline and follow-up with most participants ($N=12$) receiving assessments at follow-up. The Social and Occupational Functioning Assessment Scale (SOFAS) aided in determining the overall functioning status of study participants (Morosini et al. 2000). Finally, we collected levels of CLZ and norclozapine at follow-up to reflect a steady-state level at time of scanning. All assessments were collected by trained psychometricians who collaborated with primary clinical teams.

Given that all participants presented with moderate to severe psychosis at study entry, and our primary analyses included continuous neuroimaging measures, CLZ efficacy was defined by a continuous percent reduction in positive symptoms (hallucinatory behavior, unusual thought content, or conceptual disorganization): % reduction in positive symptoms = (baseline positive symptoms – follow-up positive symptoms)/baseline positive symptoms.

Neuroimaging acquisition

Participants with TRS underwent scanning on a Siemens Prisma 3 Tesla scanner at the Magnetic Resonance Research Center of the University of Pittsburgh prior to or within the first week of CLZ initiation (baseline), and after 12 weeks of CLZ treatment (follow-up). Structural scans were acquired

with a magnetization-prepared rapid gradient-echo sequence with the following parameters: TR = 2400 ms, TE = 2.22 ms, flip angle = 8°, FOV = 240 × 256 mm, voxel size = 1 mm³, total slices = 208. Two resting state images were acquired using a multiband echo-planar sequence sensitive to blood oxygen level dependent (BOLD) signal: TR = 800 ms, TE = 37 ms, flip angle = 52°, FOV = 208 × 208 mm, voxel size = 2 mm³, total slices = 72, and total volumes = 420. Each scan was 5:46 min in duration, for a total of 11:32 min of resting state data. Participants were instructed to keep eyes open and fixated on a “+” sign. Two additional sequences were collected to calculate our R2’ calculation: a multi-echo turbo spin echo (mTSE; effective TE, 12, 98, and 196 ms; TR, 10,680 ms; FoV, 220 × 220 mm²; 40 3 mm transverse slices) to calculate R2, and a multi-echo gradient echo (mGRE; TE, 3.6, 8.6, 16.5, 12.7, and 23 ms; TR, 1090 ms; flip angle, 25°; FoV, 220 × 220 mm²) to estimate R2*.

Preprocessing and functional connectivity analyses

Resting-state data were preprocessed with a pipeline that has been applied to several clinical and developmental cohorts to account for head motion and physiologic confounds (Calabro et al. 2020; Hallquist et al. 2013; Parr et al. 2021; Tarcijonas et al. 2019). Preprocessing was performed in the Neuroimaging in Python (NIPy) software environment, which combines tools primarily from AFNI and FSL packages (Cox 1996; Jenkinson et al. 2012). Preprocessing steps included the following: 4D slice timing and motion correction, skull stripping, co-registration and warping to standard MNI space, spatial smoothing using a 5 mm full width at half maximum Gaussian kernel, band-pass filtering between 0.009 and 0.08 Hz, and grand mean intensity normalization (10,000/global median). Resting-state BOLD images were registered to MNI152 space with via affine and nonlinear transformations. To account for head motion and minimize physiologic confounds, wavelet despiking was performed using the Brain Wavelet Toolbox and the data-driven ICA AROMA correction was applied (Patel et al. 2014; Pruim et al. 2015). In addition to these steps, scans also underwent field unwarping to correct for spatial distortion. Preprocessed scans were concatenated, resulting in one 11:32 min scan with 840 volumes. Consistent with our prior work, we ensured quality of our imaging data through the following steps: (1) visual inspection of anatomic and functional data at multiple stages (before, during, and after preprocessing); (2) examination of motion parameters in functional data across each scan; and (3) inspection of time-series before and after preprocessing for outlying values and slice timing failures (Calabro et al. 2020; Hallquist et al. 2013; Manivannan et al. 2019; Parr et al. 2021; Sarpal et al. 2020; Tarcijonas et al. 2019). One participant was not included in functional

connectivity analyses since only 1 resting state sequence in both pre and post CLZ treatment scans, resulting in partial data, resulting in a total of 21 individuals with usable data.

To examine corticostriatal functional connectivity, we used both a *bottom-up* method, driven by seed regions of interest (ROI), and a *top-down* method that seeded canonical cortical networks to examine connectivity of these networks with the striatum. We generated striatal ROIs based on results of our previous studies of antipsychotic efficacy and corticostriatal connectivity and a widely adopted striatal parcellation (Di Martino et al. 2008; Sarpal et al. 2015). Consistent with these and numerous other prior studies, we generated a priori ROIs with a 3.5 mm radius around central MNI coordinates localized in the left and right dorsal caudate ($x = \pm 13$, $y = 15$, and $z = 9$), nucleus accumbens ($x = 9$, $y = 9$, and $z = -8$), ventral caudate ($x = \pm 10$, $y = 15$, and $z = 0$), and ventral rostral putamen ($x = \pm 20$, $y = 12$, and $z = -3$). Functional connectivity maps were generated for each of these ROIs in pre and post treatment concatenated scans for all participants. Resulting maps were z transformed and a difference map representing change in connectivity of each ROI was calculated with AFNI's 3dcalc tool (difference = follow-up map – baseline map).

We also examined functional connectivity with a top-down approach based on the 7-network parcellation by Yeo et al. Consistent with prior antipsychotic studies that focus on regions the default mode network, frontoparietal network, and/or the salience network, we generated functional connectivity of these 3 a priori cortical ROIs (Han et al. 2020; Smucny et al. 2019; Yang et al. 2021). Connectivity maps were generated all pre and post scans, z -transformed, and difference maps were calculated.

At the group level, consistent with the bottom-up approach difference maps for each striatal and cortical ROIs was examined along with percent reduction of psychotic symptoms (hallucinations, conceptual disorganization, unusual thought content) before and after treatment (% reduction = [(follow-up score – baseline score)/baseline score] * 100). We used AFNI's 3dttest++ tool with the “Clustsim” option, which is AFNI's most accurate method for limiting false positive results (Cox et al. 2017). This method computes cluster-size thresholds using 10,000 simulated noise-only t -tests for an accurate spatial autocorrelation function, estimates the probability of study-specific false positive clusters, and automatically corrects voxel clustering thresholds. We then examined all group-level analyses with a voxel-wise threshold of $p < 0.005$, which exceeds Bonferroni correction for our ROIs ($0.05/8 = 0.00625$) and searched for clusters that survived familywise error correction at $p < 0.05$. For our *bottom-up* striatal ROIs, our search space was voxel-wise. A cluster size of 99 voxels represented a corrected alpha of $p < 0.05$. For our top-down cortical ROIs, our search space was limited to striatal mask derived from the

Harvard–Oxford brain atlas, resulting in a significant cluster size of 17 voxels at $p < 0.05$, corrected (Jenkinson et al. 2012).

For all significant clusters, we extracted functional connectivity data around the central coordinate with a 2-mm radius. These extracted connectivity values were then further examined with general linear models (GLMs) in the R statistical environment (<https://www.r-project.org>) that included percent reduction of positive symptoms, and age, sex, and average framewise displacement (FD) as covariates. To further account for type-1 error, results were Bonferroni-corrected for multiple comparisons based on the number of associated examined.

In secondary exploratory analyses, we also examined whether baseline functional connectivity of any significant findings in our primary set of analyses predicted antipsychotic efficacy. For each significant corticostriatal connectivity result, we extracted functional connectivity from baseline scans at the start of CLZ treatment. In GLMs we examined whether these baseline connectivity values significantly related to percent decrease in psychotic symptoms with age, sex, and FD added as covariates. Post hoc analyses similarly examined relationships between our significant connectivity results with CLZ/norclozapine ratios at follow-up, and with dose reductions in chlorpromazine equivalents of non-CLZ drugs.

R2' preprocessing

For each pre and post CLZ treatment scans, we also calculated R2', an MR-based measure of tissue iron, which represents the reversible transverse relaxation rate ($1/T2'$), based on a difference between effective ($R2^*$; $1/T2^*$) and irreversible ($R2$; $1/T2$) relaxation rates (Haacke et al. 2005; Sedlacik et al. 2014). Consistent with methods described in Larsen et al. and Funai et al. (2008), a quadratic penalized least squares approach was used to estimate $R2^*$ and $R2'$. Both $R2$ and $R2^*$ images were registered to MNI space: for $R2$ registration the affine registration between the first echo of the mTSE and the anatomical image, and the non-linear registration of the anatomical image to MNI space was concatenated, and for the $R2^*$ registration, we added a rigid-body registration between the first echoes of the mGRE and mTSE images to concatenation. Estimates of $R2'$ were derived for each participant by subtracting $R2$ from $R2^*$ estimates, and visually assessed by A.B., W.F, and D.K.S to rule out the presence of motion, shimming, or registration artifacts. A quality control script was also run on these data which confirmed the integrity of processed $R2'$ maps. Data from one participant was not used due to incomplete image acquisition, resulting in a total number of 21 participants in $R2'$ analyses. Images that represent absolute change in $R2'$ (follow-up – baseline) were calculated for group level

analyses. We examined all $\Delta R2'$ images along with percent reductions of positive symptoms for our cohort with 3dttest + + and the Clustsim option. The analytic search space was limited to the striatal mask described above. All significant results were further examined in GLMs with age and sex entered as covariates.

Results

Participant demographics and clinical findings

We included 22 individuals with TRS in this study. Demographic and clinical data for our cohort are displayed in Table 1. Our cohort demonstrated clinical characteristics consistent with TRRIP criteria for TRS: severe impairments in overall functioning reflected a mean SOFAS score of 34.91 ± 12.67 (range: 15 to 55); a mean of 3.56 ± 1.6 failed antipsychotic trials at least 6 weeks duration; mean baseline non-CLZ antipsychotic dose of $550.65 \text{ mg} \pm 479.73 \text{ mg}$ chlorpromazine equivalents; and untreated moderate to severe psychosis at study entry. Out of our 19 participants, 14 received a baseline MRI scan following CLZ initiation, with a mean time between start date and scan of 0.6 days. Across our 12-week study period, CLZ treatment was associated

with a reduction in symptoms, consistent with known clinical effects (24.5% reduction in positive symptoms, 20.6% reduction in total BPRS symptoms). Non-CLZ antipsychotic drugs were prescribed per usual care by prescribing clinicians during the study period. We observed a mean chlorpromazine equivalent of $379.55 \text{ mg} \pm 214.51 \text{ mg}$ across the study period, and, importantly, $288.61 \text{ mg} \pm 371.64 \text{ mg}$ at follow-up, indicating a substantial amount of D2-receptor blockade independent of CLZ use (Supplemental Fig. 1).

Changes in corticostriatal connectivity versus CLZ efficacy

Corticostriatal functional connectivity was first examined with a bottom-up approach that seeded the striatum with a priori ROIs. Significant results were observed with the right dorsal caudate. A higher percent reduction in psychotic symptoms was associated with significant increase in connectivity between the right dorsal caudate and two frontal regions: the right anterior insula (peak MNI coordinate: 38, 24, -2; $p < 0.04$, corrected; $k = 127$; $z = 4.55$) and the right inferior frontal lobe (peak MNI coordinate: 40, 36, -14; $p < 0.05$, corrected; $k = 100$; $z = 3.95$). Results are displayed in Fig. 1. Extracted data from peak regions of these two significant clusters were further examined in GLMs along with

Table 1 Demographics and clinical data

	Patients ($N = 22$)		
	Mean	SD	N
Age (years)	35	9.2	
Sex			
Male			16
Female			6
Race			
White			10
Black or African American			12
Asian			1
Diagnosis			
Schizophrenia			21
Schizoaffective disorder			1
Baseline BPRS total	45.09	7.21	
Baseline BPRS positive symptoms	13.32	2.12	
Follow-up total BPRS	35.77	7.74	
Follow-up BPRS positive symptoms	10.05	3.0	
Number of unsuccessful non-CLZ antipsychotic trials	3.56	1.6	
Baseline equivalent dose of non-CLZ antipsychotic drugs (mg)	550.65	479.73	
Follow-up equivalent dose of non-CLZ antipsychotic drugs (mg)	288.61	371.64	
Baseline SOFAS score	34.91	12.67	
Baseline CLZ dose	69.79	58.39	
Follow-up CLZ dose	337.50	119.21	
Follow-up plasma CLZ/norclozapine level (ng/mL)	2.215	0.688	
Composite MCCB T score	22.53	14.52	

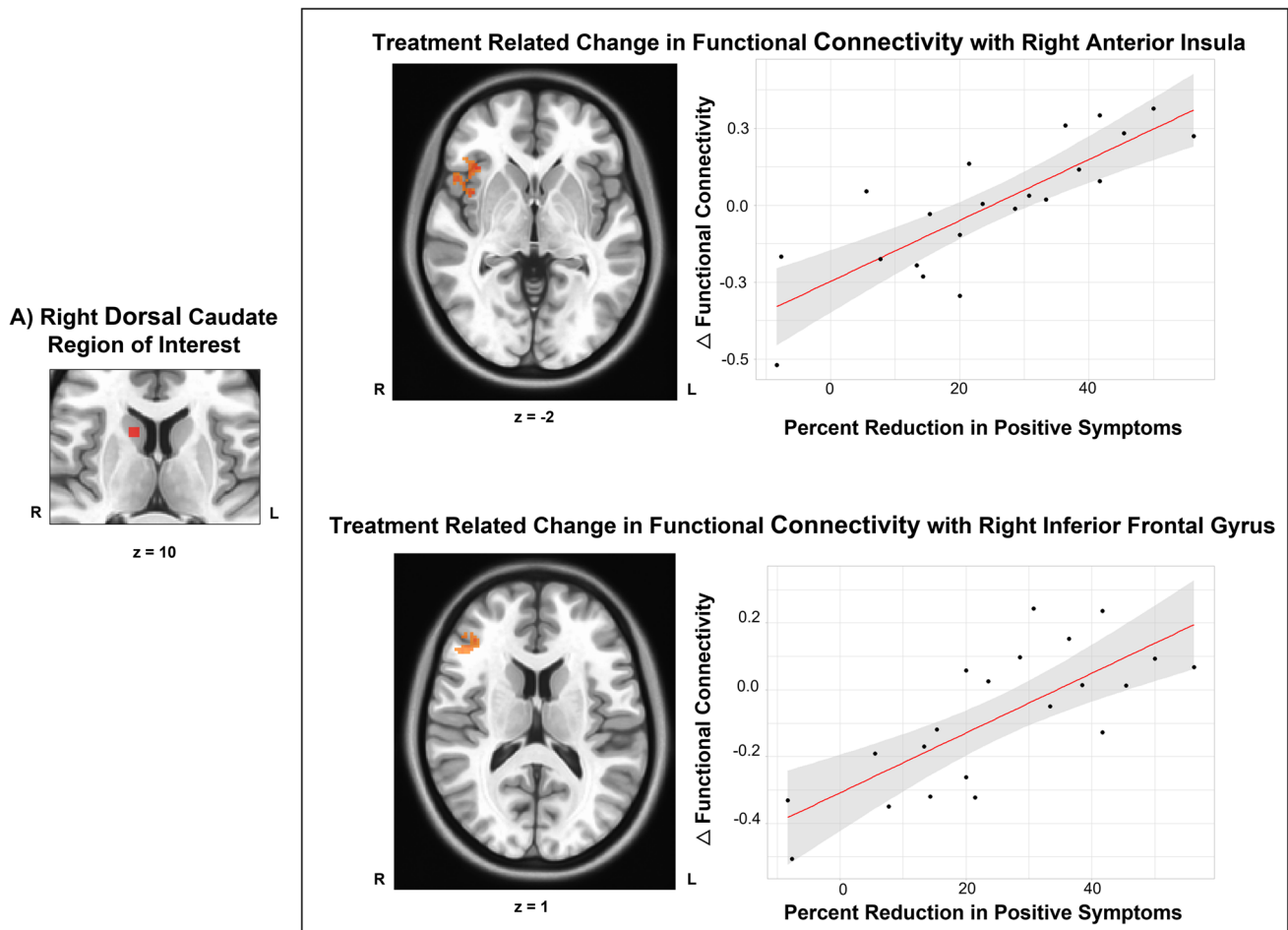


Fig. 1 CLZ efficacy and “bottom-up” striatal connectivity. Results of our striatal connectivity analyses are displayed. In **A**, we display our right dorsal caudate ROI. Increased functional connectivity between

this ROI and clusters within **B** right anterior insula and the right inferior frontal gyrus was observed ($P < 0.05$, corrected)

percent reduction in positive symptoms and age, sex, and FD as covariates. Both findings remained significant with Bonferroni correction ($0.05/2 = 0.025$; insula: $B(\text{se}) = 1.19 (0.20)$, $\beta = 0.82$, $t(20) = 6.03$, $p = 0.000015$; inferior frontal lobe: $B(\text{se}) = 0.9(0.18)$, $\beta = 0.76$, $t(20) = 4.9$, $p = 0.00013$). We also observed a noteworthy trend-level finding that coincided with our previous with in first-episode schizophrenia: a negative relationship between symptomatic improvement in increased connectivity between the ventral caudate and the posterior parietal cortex (Supplemental Fig. 2).

In addition to the bottom-up striatal connectivity, we also examined top-down connections of canonical cortical networks with striatal connectivity. Out of our 3 cortical networks of interest, only the frontoparietal network displayed a significant increase in connectivity with the striatum in the right dorsal caudate in relation to percent reduction of psychotic symptoms (peak MNI coordinate: 12, 20, 10; $p < 0.03$, corrected; $k = 23$; $z = 4.07$; Fig. 2). This finding remained

significant in a GLM with age, sex, and FD as covariates ($B(\text{se}) = 1.33 (0.26)$, $\beta = 0.77$, $t(20) = 5.2$, $p = 0.000072$).

In secondary exploratory analyses, we examined whether baseline connectivity from any of our 3 significant functional connectivity findings predicted symptom reduction. Our bottom-up findings both showed a significant relationship between treatment efficacy and corticostriatal connectivity at baseline: (right dorsal caudate – right anterior insula: $B(\text{se}) = -0.36(0.12)$, $\beta = -0.56$, $t(20) = -3.0$, $p = 0.009$; and right dorsal caudate – right inferior frontal gyrus: $B(\text{se}) = -0.51(0.17)$, $\beta = -0.56$, $t(20) = -2.9$, $p = 0.009$; Supplementary Fig. 3). The top-down finding (frontoparietal network and right dorsal caudate) demonstrated a trend-level relationship: $B(\text{se}) = 0-0.51 (0.26)$, $\beta = -0.43$, $t(20) = -1.97$, $p = 0.07$ (Supplemental Fig. 3). These results suggest that higher corticostriatal connectivity at baseline may predict CLZ efficacy, which coincides with our previous work (Sarpal et al. 2016).

A) Frontoparietal Network Region of Interest

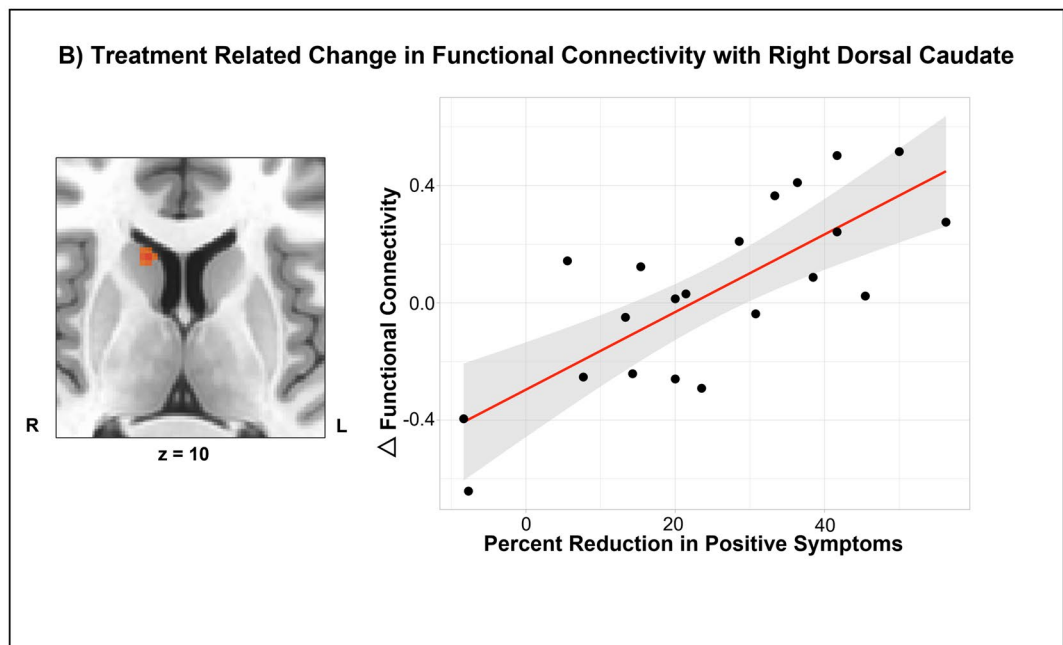


Fig. 2 CLZ efficacy and “top-down” cortical connectivity. Increased frontoparietal connectivity (A) with the right dorsal caudate (B) was significantly associated with positive symptom reduction across CLZ treatment ($P < 0.05$, corrected)

Post hoc GLMs with age, sex, and FD as covariates also examined whether follow-up CLZ/norclozapine ratio or dose reduction of non-CLZ drugs related to our significant corticostriatal functional connectivity results (Supplemental Fig. 1). Neither the ratio (right dorsal caudate- right anterior insula: $\beta = 0.38$, $t(20) = 1.52$, $p = 0.15$; right dorsal caudate- right inferior frontal gyrus: $\beta = 0.307$, $t(20) = 1.19$, $p = 0.25$; frontoparietal network – right dorsal caudate: $\beta = 0.24$, $t(20) = 0.93$, $p = 0.37$) nor dose reduction (right dorsal caudate- right anterior insula: $\beta = -0.07$, $t(20) = 0.48$, $p = 0.64$; right dorsal caudate- right inferior frontal gyrus: $\beta = -0.25$, $t(20) = -1.70$, $p = 0.11$; frontoparietal network – right dorsal caudate: $\beta = 0.22$, $t(20) = 1.50$, $p = 0.15$) showed a significant relationship.

Changes in striatal R2' versus CLZ efficacy

In addition to examining cortical striatal connectivity in relation to percent change in psychotic symptoms, we also examined changes in striatal R2' in relation to symptomatic reduction. In a group level analysis, we did not observe any significant relationship between changes in R2' and CLZ efficacy. Regions of the dorsal caudate from our functional connectivity analyses were further examined in GLMs (Fig. 3). Nonsignificant findings were observed for both our bottom-up seed ROI ($B(\text{se}) = 0.014$ (0.03); $\beta = 0.10$;

$t(20) = -0.47$; $p = 0.65$), and top-down striatal finding ($B(\text{se}) = -0.021$ (0.02); $\beta = -0.22$; $t(20) = -0.96$; $p = 0.35$).

Discussion

We examined CLZ efficacy in relation to changes in corticostriatal functional connectivity and striatal tissue iron in a cohort of individuals with TRS. Response to CLZ was associated with an increase in functional connectivity between the dorsal caudate and prefrontal regions within the frontoparietal network. Secondly, we found that baseline connectivity values from our significant corticostriatal connectivity results predicted reduction in positive symptoms. Meanwhile, no significant relationship between change in striatal tissue iron and CLZ efficacy was noted. To our knowledge, this is the first study focused on CLZ efficacy in relation to longitudinal corticostriatal functional connectivity or striatal tissue iron.

It is important to note that our findings were observed in the real-world context of TRS that included failed antipsychotic trials and concomitant use of non-CLZ, D2 receptor-blocking antipsychotic drugs. TRS may represent a pathophysiologic subtype of schizophrenia characterized by more global disruptions in functional connectivity and a

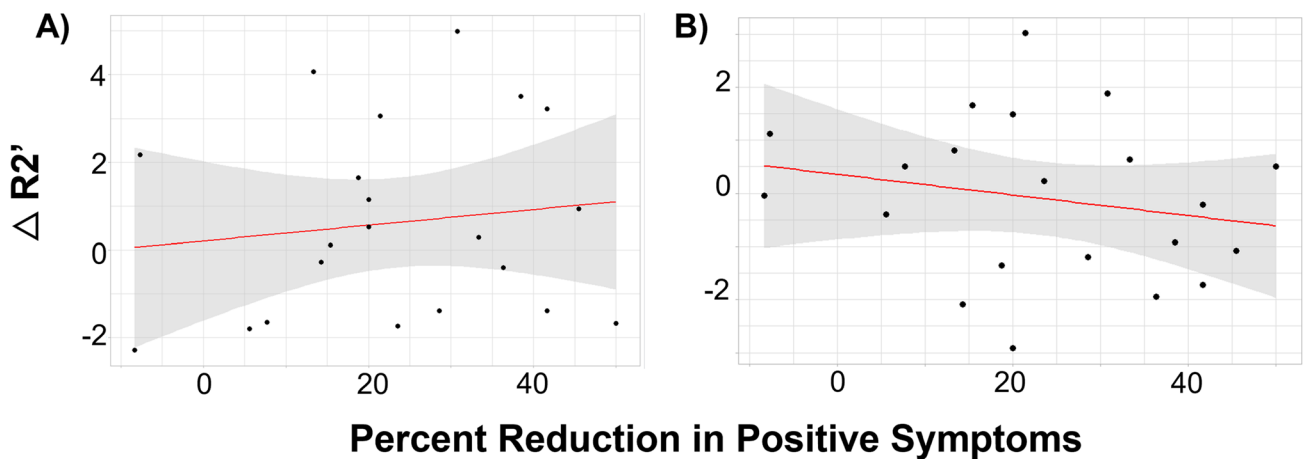


Fig. 3 CLZ efficacy and $R2'$. No significant results were observed in analyses comparing change in $R2'$ versus positive symptom reduction. Results from right dorsal caudate ROIs from our **A** bottom-up seed ROI and **B** top-down striatal findings are displayed

unique pattern of activation of cognitive systems (Ganella et al. 2017; Horne et al. 2021). Furthermore, evidence from positron emission tomography studies implicate a non-DA signature of illness, relative to responders of non-CLZ antipsychotic drugs (Demjaha et al. 2012; Kim et al. 2019). Thus, our results indicate that changes in corticostriatal connectivity may represent a final common neural mechanism underlying antipsychotic efficacy, or positive symptom reduction, and that CLZ response is likely driven by a non-DA mechanism. Our finding showing a significant relationship between baseline corticostriatal connectivity of our striatal ROIs and symptomatic reduction sheds light on the possibility of the development of a prognostic imaging-based assay of CLZ response, consistent with our prior work (Sarpal et al. 2016). Larger studies with cross-validation and more complex analytical methods will be necessary.

Our top-down cortical and bottom-up striatal analyses converged on a pattern of corticostriatal connectivity that implicate the frontoparietal executive network, mirroring our previous work in first-episode schizophrenia (Sarpal et al. 2015). Our findings also support the observation that the anterior insula and inferior frontal gyrus may include regions that contribute to both salience and executive functional systems (Sridharan et al. 2008; Yeo et al. 2011). Similar to our findings in the prefrontal cortex, both of our top-down and bottom-up corticostriatal connectivity analyses converge on parts of the dorsal caudate, a structure with anatomic connections to executive regions of the prefrontal cortex (Choi et al. 2017; Verstynen et al. 2012). The dorsal caudate overlaps with the associate striatum and has pathophysiological linkages with psychotic illness (Kegeles et al. 2010; McCutcheon et al. 2019). In addition, the dorsal caudate supports an array of cognitive processes that include gating of information important for representations during executive cognition (Chatham et al. 2014; Hazy et al. 2006;

Murty et al. 2011). Consistent with these cognitive and pathophysiological findings, the frontoparietal-caudate system has been characterized as specific to humans, relative to non-human primates, which may reflect its particular role in higher-order psychotic phenomenology (Liu et al. 2021). Greater synchronicity between the frontoparietal executive network and the dorsal striatum across CLZ treatment may reflect a gain of top-down control of salience processing (Sridharan et al. 2008; Supekar et al. 2019). The net effect of this circuitry may be associated with changes in information processing that correspond with the resolution of psychotic states.

Given the heterogeneity of interactions between CLZ and pharmacologic targets across neurotransmitter systems, CLZ's mechanism of action is potentially multifactorial (Coward 1992; Nucifora et al. 2017; Seeman 2014). However, our non-significant relationship between CLZ response and changes in striatal tissue iron does not support a primary role for the DA system, consistent with existing interpretations from prior work (Knable and Weinberger 1994). Importantly, there are several caveats related to this. For one, the effect captured by $R2'$, a proxy measure, may be too small for us to capture with our sample size. Alternatively, the DA system might be involved, possibly via D1 or D4 receptor blockade, but changes are not captured by changes in tissue iron. Our interpretation that a significant change in DA functioning does not primarily underlie CLZ efficacy could reflect a role for other systems such as the serotonergic system, or cholinergic action via muscarinic targets of cholinergic interneurons. CLZ's pharmacologically exhibits an idiosyncratic effect on the muscarinic system relative to other antipsychotic drugs (Nucifora et al. 2017). Cholinergic interneurons are robust regulators of striatal DA activity and corticostriatal circuitry more broadly, as well as glutamatergic

functioning, which has been recently been linked to CLZ response and antipsychotic resistance (Abudukeyoumu et al. 2019; Goldberg et al. 2012; Goldstein et al. 2015; Iwata et al. 2019; Mallet et al. 2019; McQueen et al. 2021). In addition, studies have also shown alterations related to antipsychotic response in white matter tracts important for corticostriatal functioning, supporting widespread alterations in neural systems (Kochunov et al. 2019; Ochi et al. 2020; Reis Marques et al. 2014). Future multimodal work with larger sample sizes will be needed to characterize treatment-related changes in striatal tissue iron across in CLZ and non-CLZ antipsychotic drug trials to further contextualize findings from this study. In addition, subsequent studies may link CLZ treatment with molecular and histological assays of the striatum and its broader neural systems (McCutcheon et al. 2021).

Strengths of our work include the focus on individuals with TRS, our within-subject, longitudinal imaging data with over 11 min of resting state at each timepoint, and our multimodal assessment of the mechanisms underlying CLZ efficacy. The observed response to CLZ in our TRS cohort coincides with its established role as a uniquely efficacious antipsychotic and further validates our effort. While a non-CLZ antipsychotic treatment cohort was not feasible given study constraints, and is a limitation of our work, our naturalistic design included concomitant non-CLZ treatment. Thus, our observed connectivity findings emerged in our TRS cohort with a background of clinically significant D2-receptor blockade by non-CLZ antipsychotic drugs.

Our study contains some inherent limitations. Though consistent with recent work, our sample size was low, reflecting the difficulty in examining TRS populations longitudinally (Li et al. 2020; McQueen et al. 2021). In addition, given the observational study design, we did not confirm TRS status, with the use of blood levels, which may have further enriched our study cohort for TRS. The lack of a non-CLZ treatment group did not allow us to explore whether our findings are related to a final common pathway of antipsychotic drug action, or more generalized mechanism underlying positive symptom reduction. Future work with be necessary to compare CLZ response with either nonpharmacologic interventions (e.g., neuromodulation), or pharmacologic agents, including non-D2 drugs, such as xanomeline or trace amine-associated receptor 1 agonists (Brannan et al. 2021; Koblan et al. 2020; Shekhar et al. 2008). Relatedly, the inclusion of a healthy control comparison group would have allowed for the characterization of potential normalizing effects of CLZ treatment. Finally, the reliance on a proxy measure that provides a partial snapshot of DA functioning, limiting the interpretability of our findings. Future work with larger sample sizes and multiple treatment arms are necessary to further contextualize the present results in TRS, a clinically unique population.

Overall, this study dissects the mechanism of response to CLZ with MR-based neuroimaging. Our findings suggest that increased corticostriatal connectivity represents a downstream marker of response to CLZ, and gross changes in DA functioning may not drive CLZ's antipsychotic action. A more comprehensive understanding of the mechanism underlying CLZ treatment may elucidate neural systems that serve as targets for treatment development. Larger studies based on these results may also lead to prognostic measures of CLZ response. Subsequent work will especially be crucial given the high societal cost associated with TRS and limited existing treatment options.

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Declarations

Conflict of interest The authors declare no competing interests.

References

- Abudukeyoumu N, Hernandez-Flores T, Garcia-Munoz M, Arbutnott GW (2019) Cholinergic modulation of striatal microcircuits. *Eur J Neurosci* 49:604–622
- Adisetiyo V, Gray KM, Jensen JH, Helpert JA (2019) Brain iron levels in attention-deficit/hyperactivity disorder normalize as a function of psychostimulant treatment duration. *Neuroimage Clin* 24:101993
- Allen RP, Earley CJ (2007) The role of iron in restless legs syndrome. *Mov Disord* 22(Suppl 18):S440–S448
- Anticevic A, Hu X, Xiao Y, Hu J, Li F, Bi F, Cole MW, Savic A, Yang GJ, Repovs G, Murray JD, Wang XJ, Huang X, Lui S, Krystal JH, Gong Q (2015) Early-course unmedicated schizophrenia patients exhibit elevated prefrontal connectivity associated with longitudinal change. *J Neurosci* 35:267–286
- Averbeck BB, Lehman J, Jacobson M, Haber SN (2014) Estimates of projection overlap and zones of convergence within frontal-striatal circuits. *J Neurosci* 34:9497–9505
- Brannan SK, Sawchak S, Miller AC, Lieberman JA, Paul SM, Breier A (2021) Muscarinic cholinergic receptor agonist and peripheral antagonist for schizophrenia. *N Engl J Med* 384:717–726
- Brass SD, Chen NK, Mulkern RV, Bakshi R (2006) Magnetic resonance imaging of iron deposition in neurological disorders. *Top Magn Reson Imaging* 17:31–40
- Cadena EJ, White DM, Kraguljac NV, Reid MA, Jindal R, Pixley RM, Lahti AC (2019) Cognitive control network dysconnectivity and

- response to antipsychotic treatment in schizophrenia. *Schizophr Res* 204:262–270
- Calabro FJ, Murty VP, Jalbrzikowski M, Tervo-Clemmens B, Luna B (2020) Development of hippocampal-prefrontal cortex interactions through adolescence. *Cereb Cortex* 30:1548–1558
- Chatham CH, Frank MJ, Badre D (2014) Corticostriatal output gating during selection from working memory. *Neuron* 81:930–942
- Choi EY, Tanimura Y, Vage PR, Yates EH, Haber SN (2017) Convergence of prefrontal and parietal anatomical projections in a connectional hub in the striatum. *Neuroimage* 146:821–832
- Coward DM (1992) General pharmacology of clozapine. *Br J Psychiatry Suppl*: 5–11.
- Cox RW (1996) AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 29:162–173
- Cox RW, Chen G, Glen DR, Reynolds RC, Taylor PA (2017) FMRI clustering in AFNI: false-positive rates redux. *Brain Connect* 7:152–171
- Daubner SC, Le T, Wang S (2011) Tyrosine hydroxylase and regulation of dopamine synthesis. *Arch Biochem Biophys* 508:1–12
- Demjaha A, Murray RM, McGuire PK, Kapur S, Howes OD (2012) Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. *Am J Psychiatry* 169:1203–1210
- Di Martino A, Scheres A, Margulies DS, Kelly AM, Uddin LQ, Shehzad Z, Biswal B, Walters JR, Castellanos FX, Milham MP (2008) Functional connectivity of human striatum: a resting state FMRI study. *Cereb Cortex* 18:2735–2747
- Ersche KD, Acosta-Cabronero J, Jones PS, Ziauddeen H, van Swelm RP, Laarakkers CM, Raha-Chowdhury R, Williams GB (2017) Disrupted iron regulation in the brain and periphery in cocaine addiction. *Transl Psychiatry* 7:e1040
- First M, Williams J, Karg R, Spitzer R (2015) Structured clinical interview for DSM-5—research version (SCID-5 for DSM-5, research version; SCID-5-RV). American Psychiatric Association, Arlington, VA
- Funai AK, Fessler JA, Yeo DT, Olafsson VT, Noll DC (2008) Regularized field map estimation in MRI. *IEEE Trans Med Imaging* 27:1484–1494
- Ganella EP, Bartholomeusz CF, Seguin C, Whittle S, Bousman C, Phassouliotis C, Everall I, Pantelis C, Zalesky A (2017) Functional brain networks in treatment-resistant schizophrenia. *Schizophr Res* 184:73–81
- Goldberg JA, Ding JB, Surmeier DJ (2012) Muscarinic modulation of striatal function and circuitry. *Handb Exp Pharmacol*: 223–41.
- Goldstein ME, Anderson VM, Pillai A, Kydd RR, Russell BR (2015) Glutamatergic neurometabolites in clozapine-responsive and -resistant schizophrenia. *Int J Neuropsychopharmacol* 18.
- Haacke EM, Cheng NY, House MJ, Liu Q, Neelavalli J, Ogg RJ, Khan A, Ayaz M, Kirsch W, Obenaus A (2005) Imaging iron stores in the brain using magnetic resonance imaging. *Magn Reson Imaging* 23:1–25
- Hallquist MN, Hwang K, Luna B (2013) The nuisance of nuisance regression: spectral misspecification in a common approach to resting-state fMRI preprocessing reintroduces noise and obscures functional connectivity. *Neuroimage* 82:208–225
- Han S, Becker B, Duan X, Cui Q, Xin F, Zong X, Hu M, Yang M, Li R, Yu Y, Liao W, Chen X, Chen H (2020) Distinct striatum pathways connected to salience network predict symptoms improvement and resilient functioning in schizophrenia following risperidone monotherapy. *Schizophr Res* 215:89–96
- Hazy TE, Frank MJ, O'Reilly RC (2006) Banishing the homunculus: making working memory work. *Neuroscience* 139:105–118
- Hedlund J, Vieweg B (1980) The brief psychiatric rating scale (BPRS): a comprehensive review. *J Oper Psychiatry* 11:48–65
- Horne CM, Vanes LD, Verneuil T, Mouchlianitis E, Szentgyorgyi T, Averbek B, Leech R, Moran RJ, Shergill SS (2021) Cognitive control network connectivity differentially disrupted in treatment resistant schizophrenia. *Neuroimage Clin* 30:102631
- Howes OD, McCutcheon R, Agid O, de Bartolomeis A, van Beveren NJ, Birnbaum ML, Bloomfield MA, Bressan RA, Buchanan RW, Carpenter WT, Castle DJ, Citrome L, Daskalakis ZJ, Davidson M, Drake RJ, Dursun S, Ebdrup BH, Elkis H, Falkai P, Fleischacker WW, Gadelha A, Gaughran F, Glenthøj BY, Graff-Guerrero A, Hallak JE, Honer WG, Kennedy J, Kinon BJ, Lawrie SM, Lee J, Leweke FM, MacCabe JH, McNabb CB, Meltzer H, Moller HJ, Nakajima S, Pantelis C, Reis Marques T, Remington G, Russell SL, Russell BR, Siu CO, Suzuki T, Sommer IE, Taylor D, Thomas N, Uçok A, Umbricht D, Walters JT, Kane J, Correll CU (2017) Treatment-resistant schizophrenia: treatment response and resistance in psychosis (TRRIP) working group consensus guidelines on diagnosis and terminology. *Am J Psychiatry* 174:216–229
- Iwata Y, Nakajima S, Plitman E, Caravaggio F, Kim J, Shah P, Mar W, Chavez S, De Luca V, Mimura M, Remington G, Gerretsen P, Graff-Guerrero A (2019) Glutamatergic neurometabolite levels in patients with ultra-treatment-resistant schizophrenia: a cross-sectional 3T proton magnetic resonance spectroscopy study. *Biol Psychiatry* 85:596–605
- Jarbo K, Verstynen TD (2015) Converging structural and functional connectivity of orbitofrontal, dorsolateral prefrontal, and posterior parietal cortex in the human striatum. *J Neurosci* 35:3865–3878
- Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM (2012) Fsl *Neuroimage* 62:782–790
- Kane J, Honigfeld G, Singer J, Meltzer H (1988) Clozapine for the treatment-resistant schizophrenic. a double-blind comparison with chlorpromazine. *Arch Gen Psychiatry* 45:789–796
- Kegeles LS, Abi-Dargham A, Frankle WG, Gil R, Cooper TB, Slifstein M, Hwang DR, Huang Y, Haber SN, Laruelle M (2010) Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. *Arch Gen Psychiatry* 67:231–239
- Kennedy JL, Altar CA, Taylor DL, Degtjar I, Hornberger JC (2014) The social and economic burden of treatment-resistant schizophrenia: a systematic literature review. *Int Clin Psychopharmacol* 29:63–76
- Kim S, Jung WH, Howes OD, Veronese M, Turkheimer FE, Lee YS, Lee JS, Kim E, Kwon JS (2019) Frontostriatal functional connectivity and striatal dopamine synthesis capacity in schizophrenia in terms of antipsychotic responsiveness: an [(18F)]DOPA PET and fMRI study. *Psychol Med* 49:2533–2542
- Knable MB, Weinberger DR (1994) Limbic-prefrontal connectivity and clozapine. *J Clin Psychiatry* 55(Suppl B):70–3
- Koblan KS, Kent J, Hopkins SC, Krystal JH, Cheng H, Goldman R, Loebel A (2020) A non-D2-receptor-binding drug for the treatment of schizophrenia. *N Engl J Med* 382:1497–1506
- Kochunov P, Huang J, Chen S, Li Y, Tan S, Fan F, Feng W, Wang Y, Rowland LM, Savransky A, Du X, Chiappelli J, Chen S, Jahanshad N, Thompson PM, Ryan MC, Adhikari B, Sampath H, Cui Y, Wang Z, Yang F, Tan Y, Hong LE (2019) White matter in schizophrenia treatment resistance. *Am J Psychiatry* 176:829–838
- Lahti AC, Weiler MA, Holcomb HH, Tamminga CA, Cropsey KL (2009) Modulation of limbic circuitry predicts treatment response to antipsychotic medication: a functional imaging study in schizophrenia. *Neuropsychopharmacology* 34:2675–2690
- Larsen B, Bourque J, Moore TM, Adebimpe A, Calkins ME, Elliott MA, Gur RC, Gur RE, Moberg PJ, Roalf DR, Ruparel K, Turetsky BI, Vandekar SN, Wolf DH, Shinohara RT, Satterthwaite TD (2020) Longitudinal development of brain iron is linked to cognition in youth. *J Neurosci* 40:1810–1818
- Li A, Zalesky A, Yue W, Howes O, Yan H, Liu Y, Fan L, Whitaker KJ, Xu K, Rao G, Li J, Liu S, Wang M, Sun Y, Song M, Li P, Chen J, Chen Y, Wang H, Liu W, Li Z, Yang Y, Guo H, Wan P, Lv L, Lu L, Yan J, Song Y, Wang H, Zhang H, Wu H, Ning Y, Du Y, Cheng Y, Xu J, Xu X, Zhang D, Wang X, Jiang T, Liu B (2020) A

- neuroimaging biomarker for striatal dysfunction in schizophrenia. *Nat Med* 26:558–565
- Liu X, Eickhoff SB, Caspers S, Wu J, Genov S, Hoffstaedter F, Mars RB, Sommer IE, Eickhoff CR, Chen J, Jardri R, Reetz K, Dogan I, Aleman A, Kogler L, Gruber O, Caspers J, Mathys C, Patil KR (2021) Functional parcellation of human and macaque striatum reveals human-specific connectivity in the dorsal caudate. *Neuroimage* 235:118006
- Mallet N, Leblois A, Maurice N, Beurrier C (2019) Striatal cholinergic interneurons: how to elucidate their function in health and disease. *Front Pharmacol* 10:1488
- Manivannan A, Foran W, Jalbrzikowski M, Murty VP, Haas GL, Tarcijonas G, Luna B, Sarpal DK (2019) Association between duration of untreated psychosis and frontostriatal connectivity during maintenance of visuospatial working memory. *Biol Psychiatry Cogn Neurosci Neuroimaging* 4:454–461
- McCutcheon RA, Abi-Dargham A, Howes OD (2019) Schizophrenia, dopamine and the striatum: from biology to symptoms. *Trends Neurosci* 42:205–220
- McCutcheon RA, Brown K, Nour MM, Smith SM, Veronese M, Zelaya F, Osugo M, Jauhar S, Hallett W, Mehta MM, Howes OD (2021) Dopaminergic organization of striatum is linked to cortical activity and brain expression of genes associated with psychiatric illness. *Sci Adv* 7.
- McEvoy JP, Lieberman JA, Stroup TS, Davis SM, Meltzer HY, Rosenheck RA, Swartz MS, Perkins DO, Keefe RS, Davis CE, Severe J, Hsiao JK, Investigators C (2006) Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. *Am J Psychiatry* 163:600–610
- McQueen G, Sendt KV, Gillespie A, Avila A, Lally J, Vallianatou K, Chang N, Ferreira D, Borgan F, Howes OD, Barker GJ, Lythgoe DJ, Stone JM, McGuire P, MacCabe JH, Egerton A (2021) Changes in brain glutamate on switching to clozapine in treatment-resistant schizophrenia. *Schizophr Bull* 47:662–671
- Meltzer HY (1997) Treatment-resistant schizophrenia—the role of clozapine. *Curr Med Res Opin* 14:1–20
- Molinoff PB, Axelrod J (1971) Biochemistry of catecholamines. *Annu Rev Biochem* 40:465–500
- Morosini PL, Magliano L, Brambilla L, Ugolini S, Pioli R (2000) Development, reliability and acceptability of a new version of the DSM-IV social and occupational functioning assessment scale (SOFAS) to assess routine social functioning. *Acta Psychiatr Scand* 101:323–329
- Murty VP, Sambataro F, Radulescu E, Altamura M, Iudicello J, Zolnick B, Weinberger DR, Goldberg TE, Mattay VS (2011) Selective updating of working memory content modulates mesocortico-striatal activity. *Neuroimage* 57:1264–1272
- Nucifora FC Jr, Mihaljevic M, Lee BJ, Sawa A (2017) Clozapine as a model for antipsychotic development. *Neurotherapeutics* 14:750–761
- Nuechterlein KH, Green MF, Kern RS, Baade LE, Barch DM, Cohen JD, Essock S, Fenton WS, Frese FJ 3rd, Gold JM, Goldberg T, Heaton RK, Keefe RS, Kraemer H, Mesholam-Gately R, Seidman LJ, Stover E, Weinberger DR, Young AS, Zaleman S, Marder SR (2008) The MATRICS consensus cognitive battery, part 1: test selection, reliability, and validity. *Am J Psychiatry* 165:203–213
- Ochi R, Noda Y, Tsuchimoto S, Tarumi R, Honda S, Matsushita K, Tsugawa S, Plitman E, Masuda F, Ogyu K, Wada M, Miyazaki T, Fujii S, Chakravarty MM, Graff-Guerrero A, Uchida H, Mimura M, Nakajima S (2020) White matter microstructural organizations in patients with severe treatment-resistant schizophrenia: a diffusion tensor imaging study. *Prog Neuropsychopharmacol Biol Psychiatry* 100:109871
- Ortega R, Cloetens P, Deves G, Carmona A, Bohic S (2007) Iron storage within dopamine neurovesicles revealed by chemical nano-imaging. *PLoS One* 2:e925
- Parr AC, Calabro F, Larsen B, Tervo-Clemmens B, Elliot S, Foran W, Olafsson V, Luna B (2021) Dopamine-related striatal neurophysiology is associated with specialization of frontostriatal reward circuitry through adolescence. *Prog Neurobiol* 201:101997
- Patel AX, Kundu P, Rubinov M, Jones PS, Vertes PE, Ersche KD, Suckling J, Bullmore ET (2014) A wavelet method for modeling and despiking motion artifacts from resting-state fMRI time series. *Neuroimage* 95:287–304
- Piao YS, Lian TH, Hu Y, Zuo LJ, Guo P, Yu SY, Liu L, Jin Z, Zhao H, Li LX, Yu QJ, Wang RD, Chen SD, Chan P, Wang XM, Zhang W (2017) Restless legs syndrome in Parkinson disease: clinical characteristics, abnormal iron metabolism and altered neurotransmitters. *Sci Rep* 7:10547
- Pruim RHR, Mennes M, van Rooij D, Llera A, Buitelaar JK, Beckmann CF (2015) ICA-AROMA: a robust ICA-based strategy for removing motion artifacts from fMRI data. *Neuroimage* 112:267–277
- Reis Marques T, Taylor H, Chaddock C, Dell'acqua F, Handley R, Reinders AA, Mondelli V, Bonaccorso S, Diforti M, Simmons A, David AS, Murray RM, Pariante CM, Kapur S, Dazzan P (2014) White matter integrity as a predictor of response to treatment in first episode psychosis. *Brain* 137:172–182
- Rubio JM, Lencz T, Barber A, Moyett A, Ali S, Bassaw F, Ventura G, Germano N, Malhotra AK, Kane JM (2021) Striatal functional connectivity in psychosis relapse: a hypothesis generating study. *Schizophr Res*.
- Sarpal DK, Robinson DG, Lencz T, Argyelan M, Ikuta T, Karlsgodt K, Gallego JA, Kane JM, Szeszko PR, Malhotra AK (2015) Antipsychotic treatment and functional connectivity of the striatum in first-episode schizophrenia. *Jama Psychiatr* 72:5–13
- Sarpal DK, Argyelan M, Robinson DG, Szeszko PR, Karlsgodt KH, John M, Weissman N, Gallego JA, Kane JM, Lencz T, Malhotra AK (2016) Baseline striatal functional connectivity as a predictor of response to antipsychotic drug treatment. *Am J Psychiatry* 173:69–77
- Sarpal DK, Robinson DG, Fales C, Lencz T, Argyelan M, Karlsgodt KH, Gallego JA, John M, Kane JM, Szeszko PR, Malhotra AK (2017) Relationship between duration of untreated psychosis and intrinsic corticostriatal connectivity in patients with early phase schizophrenia. *Neuropsychopharmacology* 42:2214–2221
- Sarpal DK, Tarcijonas G, Calabro FJ, Foran W, Haas GL, Luna B, Murty VP (2020) Context-specific abnormalities of the central executive network in first-episode psychosis: relationship with cognition. *Psychol Med*: 1–10.
- Sedlacik J, Boelmans K, Lobel U, Holst B, Siemonsen S, Fiehler J (2014) Reversible, irreversible and effective transverse relaxation rates in normal aging brain at 3T. *Neuroimage* 84:1032–1041
- Seeman P (2014) Clozapine, a fast-off-D2 antipsychotic. *ACS Chem Neurosci* 5:24–29
- Shekhar A, Potter WZ, Lightfoot J, Lienemann J, Dube S, Mallinckrodt C, Bymaster FP, McKinzie DL, Felder CC (2008) Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. *Am J Psychiatry* 165:1033–1039
- Siskind D, McCartney L, Goldschlager R, Kisely S (2016) Clozapine v. first- and second-generation antipsychotics in treatment-refractory schizophrenia: systematic review and meta-analysis. *Br J Psychiatry* 209:385–392
- Smucny J, Lesh TA, Carter CS (2019) Baseline frontoparietal task-related BOLD activity as a predictor of improvement in clinical symptoms at 1-year follow-up in recent-onset psychosis. *Am J Psychiatry* 176:839–845
- Sridharan D, Levitin DJ, Menon V (2008) A critical role for the right fronto-insular cortex in switching between

- central-executive and default-mode networks. *Proc Natl Acad Sci U S A* 105:12569–12574
- Supekar K, Cai W, Krishnadas R, Palaniyappan L, Menon V (2019) Dysregulated brain dynamics in a triple-network saliency model of schizophrenia and its relation to psychosis. *Biol Psychiatry* 85:60–69
- Tarcijonas G, Foran W, Haas GL, Luna B, Sarpal DK (2019) Intrinsic connectivity of the Globus pallidus: an uncharted marker of functional prognosis in people with first-episode schizophrenia. *Schizophr Bull*.
- Taylor DM (2017) Clozapine for treatment-resistant schizophrenia: still the gold standard? *Cns Drugs* 31:177–180
- Thomas LO, Boyko OB, Anthony DC, Burger PC (1993) MR detection of brain iron. *AJNR Am J Neuroradiol* 14:1043–1048
- Torres-Vega A, Pliego-Rivero BF, Otero-Ojeda GA, Gomez-Olivan LM, Vieyra-Reyes P (2012) Limbic system pathologies associated with deficiencies and excesses of the trace elements iron, zinc, copper, and selenium. *Nutr Rev* 70:679–692
- Tziortzi AC, Haber SN, Searle GE, Tsoumpas C, Long CJ, Shotbolt P, Douaud G, Jbabdi S, Behrens TE, Rabiner EA, Jenkinson M, Gunn RN (2014) Connectivity-based functional analysis of dopamine release in the striatum using diffusion-weighted MRI and positron emission tomography. *Cereb Cortex* 24:1165–1177
- Verstynen TD, Badre D, Jarbo K, Schneider W (2012) Microstructural organizational patterns in the human corticostriatal system. *J Neurophysiol* 107:2984–2995
- Ward RJ, Zucca FA, Duyn JH, Crichton RR, Zecca L (2014) The role of iron in brain ageing and neurodegenerative disorders. *Lancet Neurol* 13:1045–1060
- Yang C, Tang J, Liu N, Yao L, Xu M, Sun H, Tao B, Gong Q, Cao H, Zhang W, Lui S (2021) The effects of antipsychotic treatment on the brain of patients with first-episode schizophrenia: a selective review of longitudinal MRI studies. *Front Psychiatry* 12:593703
- Yeo BT, Krienen FM, Sepulcre J, Sabuncu MR, Lashkari D, Hollinshead M, Roffman JL, Smoller JW, Zollei L, Polimeni JR, Fischl B, Liu H, Buckner RL (2011) The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J Neurophysiol* 106:1125–1165
- Youdim MBH (2018) Monoamine oxidase inhibitors, and iron chelators in depressive illness and neurodegenerative diseases. *J Neural Transm vienna* 125:1719–1733
- Youdim MB, Green AR (1978) Iron deficiency and neurotransmitter synthesis and function. *Proc Nutr Soc* 37:173–179
- Youdim MB, Ashkenazi R, Ben-Shachar D, Yehuda S (1984) Modulation of dopamine receptor in the striatum by iron: behavioral and biochemical correlates. *Adv Neurol* 40:159–170
- Zucca FA, Segura-Aguilar J, Ferrari E, Munoz P, Paris I, Sulzer D, Sarna T, Casella L, Zecca L (2017) Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease. *Prog Neurobiol* 155:96–119

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