NEUROBIOLOGY, CLINICAL COURSE, AND THERAPEUTIC APPROACHES OF TREATMENT RESISTANT SCHIZOPHRENIA: TOWARD AN INTEGRATED VIEW

EDITED BY: Felice Iasevoli, Vincenzo De Luca and Frederick Charles Nucifora
PUBLISHED IN: Frontiers in Psychiatry

frontiers Research Topics

Frontiers eBook Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-88963-331-9
DOI 10.3389/978-2-88963-331-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

NEUROBIOLOGY, CLINICAL COURSE, AND THERAPEUTIC APPROACHES OF TREATMENT RESISTANT SCHIZOPHRENIA: TOWARD AN INTEGRATED VIEW

Topic Editors:
Felice Iasevoli, University of Naples Federico II, Italy
Vincenzo De Luca, University of Toronto, Canada
Frederick Charles Nucifora, Johns Hopkins Medicine, United States

Citation: Iasevoli, F., De Luca, V., Nucifora, F. C., eds. (2020). Neurobiology, Clinical Course, and Therapeutic Approaches of Treatment Resistant Schizophrenia: Toward an Integrated View. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-331-9

Table of Contents

frontiers in Psychiatry

EDITORIAL
published: 21 November 2019
doi: 10.3389/fpsyt.2019.00870

Editorial: Neurobiology, Clinical Course, and Therapeutic Approaches of Treatment-Resistant Schizophrenia: Toward an Integrated View

Felice Iasevoli[1], Vincenzo De Luca[2] and Frederick Charles Nucifora[3]*

[1] Department of Neuroscience, University of Naples Federico II, Naples, Italy, [2] Molecular Brain Science Department, Centre for Addiction and Mental Health,Toronto, ON, Canada, [3] The Department of Psychiatry and Behavioral Sciences, School of Medicine, Johns Hopkins University, Baltimore, MD, United States

Keywords: psychosis, antipsychotic, clozapine, schizophrenia, treatment refractory

Editorial on the Research Topic

Neurobiology, Clinical Course, and Therapeutic Approaches of Treatment-Resistant Schizophrenia: Toward an Integrated View

Treatment-resistant schizophrenia (TRS) is a disease entity whose tracts are yet to be fully deciphered. The characterization of effective therapeutic strategies for this severe condition represents one of the more relevant unmet need of contemporary psychiatry. Nonetheless, investigations on therapeutic strategies are strictly intermingled with the characterization of clinical determinants and diagnostic boundaries of the disease, and with the elucidation of its biological underpinnings. These elements cannot be separated from each other and their combined evaluation has been the objective of this Research Topic.

As an ideal introduction to the Topic, Leung et al. provide a thorough summary of the current knowledge on TRS. These Authors make an excellent overview of the current challenges with the definition and neurobiology of TRS, pointing out the heterogeneity of clinical course, the difficulty with an optimal characterization of predictors, and the lack of evidence based standard of care in TRS.

The idea that schizophrenia and TRS may be categorically distinct is tackled in the contribution by Kinon, as the Author critically discusses the issue of TRS heterogeneity. Recalling the classical definition of Bleuler for schizophrenia, Kinon proposes to refer to TRS as *The Group of Treatment Resistant Schizophrenias*, due to the patent heterogeneity in the trajectory of non-response to antipsychotics. This heterogeneity depends on multiple factors and mostly on inconsistency in defining TRS, preventing the possibility to understand whether TRS may be a distinct disease category or on a diagnostic continuum with schizophrenia. Parsing patient segments to achieve more homogenous ones sharing common pathophysiology may allow moving from more broadly to more targeted segments, paving the way to data or at least hypothesis-driven novel drug strategies for TRS.

In agreement with these reports, Iasevoli et al. has attempted to delineate the distinctive features and determinants of disease severity in TRS vs. non-TRS patients. We find that disease severity is higher in TRS patients and mostly associated with negative symptoms. In turn, negative symptoms mediate the effects of cognitive dysfunctions and are likely related to neurodevelopmental alterations in TRS patients. Despite this contribution appears to support the idea of a categorical distinction between TRS and non-TRS patients, it also enlightens one of the limitations of current operational criteria: the most relevant factor driving disease severity in TRS patients is the extent of negative symptoms, that are notoriously not targeted by current antipsychotics. A dog chasing its own tail.

OPEN ACCESS

Edited and reviewed by:
Philip D. Harvey,
University of Miami,
United States

****Correspondence:***
Felice Iasevoli
felice.iasevoli@unina.it

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *14 September 2019*
Accepted: *04 November 2019*
Published: *21 November 2019*

Citation:
Iasevoli F, De Luca V and Nucifora FC (2019) Editorial: Neurobiology, Clinical Course, and Therapeutic Approaches of Treatment-Resistant Schizophrenia: Toward an Integrated View. Front. Psychiatry 10:870. doi: 10.3389/fpsyt.2019.00870

These challenges and uncertainties strongly illustrate the urge to achieve pathophysiological models and neurobiological markers of TRS to develop targeted therapies. As reported in Leung et al. article, the traditional model of dopamine dysfunction for the pathogenesis of schizophrenia seems not to be applicable to explain TRS, and other neurochemical dysfunctions (e.g., cortical hyper-glutamatergy) may play a role in the disease.

In partial agreement with this consideration, the contribution of Amato et al. depicts an intriguing novel theoretical model to explain some forms of TRS. Based on previous experimental studies (1), Amato et al. suggest that response to antipsychotics may stem from an imbalance between D2 receptor blockade and dopamine transporter (DAT) blockade to achieve adequate extracellular dopamine levels to trigger presynaptic dopaminergic neuron autoinhibition. Presynaptic autoinhibition alleviates psychotic symptoms by reducing dopamine release and post-synaptic neuron activation. A failure of this mechanism, due to multiple factors (e.g., reduced DAT expression as a consequence of genetic factors, prior exposure to psychostimulants, or aging), may lead to treatment resistance.

Another remarkable contribution, by Mostaid et al., describes an overall upregulation of transcripts within the Neuregulin-ErbB signaling pathway among individuals with schizophrenia. Indeed, Authors investigated Neuregulin signaling pathway mRNA transcripts in whole blood of 71 TRS patients and 57 healthy controls and found upregulated levels in TRS patients for five transcripts, although only one surviving correction for multiple testing.

Still on neurobiological markers of TRS, the excellent review by MacKay et al. summarizes current findings on system and circuit-level brain dysconnectivity in treatment-resistant schizophrenia based on neuroimaging studies. As described in this report, a clear-cut separation at multiple levels of connectivity emerges between TRS and non-TRS patients, opening the way to circuit-based interventions.

The issue of therapeutic strategies has been addressed in multiple articles. An intriguing contribution is given by Miyaoka et al. These authors describe the case of a schizophrenia patient with predominant severe hallucinations and delusions non-responsive to antipsychotics, who showed a reduction of psychotic symptoms and improvement in social functioning after receiving bone marrow transplantation for acute myeloid leukemia. This case report has a place into the current debate on immune pathogenesis of schizophrenia (2).

Unfortunately, TRS patients are exposed to high doses of antipsychotics, causing severe undesirable effects. The contribution by Eriksson et al. deals with impaired bone mineral status, which was investigated in obese non-diabetic antipsychotic-treated patients, showing a reduction of bone mineral density in 23% of the subjects.

The search for strategies beyond mere pharmacological interventions is the object of the meta-analysis conducted by Polese et al. These authors focused on psycho-social interventions in TRS patients, either in augmentation or in substitution of antipsychotics. Psychological interventions showed a therapeutic effect in 40 of 42 selected studies. The most improvement was found in positive symptoms for cognitive behavioral therapy, as well as for other psychological interventions (albeit with different degrees). This contribution strongly encourages psychological interventions in TRS.

The contribution of Souto et al. illustrates the results of a randomized controlled trial for an online emotional training devoted to social cognition rehabilitation in schizophrenia patients. The authors found significant improvement in emotion recognition and multiple theory-of-mind tasks. Although to date impairment of social cognition has been only limitedly studied in TRS, it is presumable that social cognition-oriented interventions may soon become indicated in these patients.

However, literature on severe mental illness should face relevant methodological limitations, as illustrated in the contribution by Lally et al. The group found that psychotic participants in a large trial of psychosocial interventions to improve physical health in severe mental illness had a lower degree of overall illness severity and functional impairment than eligible non-participant psychotic individuals, therefore challenging representativeness of participants to the trial and concluding that more severe patients may tendentially be not predisposed to be enrolled. Although a generalization of these results to other kinds of trials (e.g., pharmacological or psychological) is beyond the authors' scope, more focused recruitment efforts should be considered when carrying out trials on severely ill patients. This recommendation should be specifically applied to TRS patients since they exhibit more severe psychopathology and more impaired social functioning even when compared to non-TRS patients (3).

AUTHOR CONTRIBUTIONS

The Editorial has been written by FI, VD, and FN.

REFERENCES

1. Amato D, Canneva F, Cumming P, Maschauer S, Groos D, Dahlmanns JK. A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failurereversal: the role of the dopamine transporter. *Mol Psychiatry* (2018). doi: 10.1038/s41380-018-0114-5
2. van Kesteren CF, Gremmels H, de Witte LD, Hol EM, Van Gool AR, Falkai PG. Immune involvement in the pathogenesis of schizophrenia: a meta-analysis on postmortem brain studies. *Transl Psychiatry* (2017) 7(3):e1075. doi: 10.1038/tp.2017.4
3. Iasevoli F, Giordano S, Balletta R, Latte G, Formato MV, Prinzivalli E. Treatment resistant schizophrenia is associated with the worst community functioning among severely-ill highly-disabling psychiatric conditions and is the most relevant predictor of poorer achievements in functional milestones. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 65:34–48. doi: 10.1016/j.pnpbp.2015.08.010

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Iasevoli, De Luca and Nucifora. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

CASE REPORT
published: 21 September 2017
doi: 10.3389/fpsyt.2017.00174

Remission of Psychosis in Treatment-Resistant Schizophrenia following Bone Marrow Transplantation: A Case Report

Tsuyoshi Miyaoka, Rei Wake, Sadayuki Hashioka, Maiko Hayashida, Arata Oh-Nishi, Ilhamuddin Abdul Azis, Muneto Izuhara, Keiko Tsuchie, Tomoko Araki, Ryosuke Arauchi, Rostia Arianna Abdullah and Jun Horiguchi*

Department of Psychiatry, School of Medicine, Shimane University, Izumo, Japan

The authors present the case of a 24-year-old male with treatment-resistant schizophrenia, with predominant severe delusion and hallucination, who received bone marrow transplantation (BMT) for acute myeloid leukemia. After BMT, he showed a remarkable reduction in psychotic symptoms without administration of neuroleptics. He also showed drastic improvement in social functioning. Follow-up evaluations 2 and 4 years after BMT showed persistent significant improvement of the psychotic state and social functioning. Recent findings show that the major underlying pathogenic mechanism of schizophrenia is immune dysregulation. Thus, conceptually, BMT, a cellular therapy, that facilitates the counteractive processes of balancing inflammation by immune regulation, could produce beneficial clinical effects in patients with treatment-resistant schizophrenia. Further studies are required to define the true benefits of BMT for the possible curative treatment of schizophrenia.

Keywords: schizophrenia, bone marrow transplantation, acute myeloid leukemia, curative treatment, immune alterations, cellular therapy, maternal immune activation

OPEN ACCESS

Edited by:
Felice Iasevoli,
University of Naples Federico II, Italy

Reviewed by:
Belinda Lennox,
University of Oxford,
United Kingdom
Robert McCutcheon,
Institute of Psychiatry, Psychology & Neuroscience (IoPPN),
United Kingdom

****Correspondence:***
Tsuyoshi Miyaoka
miyanyan@med.shimane-u.ac.jp

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *21 July 2017*
Accepted: *05 September 2017*
Published: *21 September 2017*

Citation:
Miyaoka T, Wake R, Hashioka S, Hayashida M, Oh-Nishi A, Azis IA, Izuhara M, Tsuchie K, Araki T, Arauchi R, Abdullah RA and Horiguchi J (2017) Remission of Psychosis in Treatment-Resistant Schizophrenia following Bone Marrow Transplantation: A Case Report. Front. Psychiatry 8:174. doi: 10.3389/fpsyt.2017.00174

BACKGROUND

Increasing evidence suggests a correlation between schizophrenia and immune system disturbances. Genome-wide association studies for linkages with schizophrenia have revealed that the odds ratio is frequently high in immune-related regions among many schizophrenia-related genome loci of patients (1–3). Although schizophrenia is regarded as a syndrome with different biological backgrounds, involvement of immune system disturbances could be one of the common mechanisms.

The association between maternal infection and neurodevelopmental disorders is long standing but not without controversy. After the 1964 rubella pandemic, the incidence of schizophrenia rose from less than 1% in the unexposed population to about 20% in the exposed population (4). Subsequent studies charting historic outbreaks of flu, measles, mumps, chickenpox, and polio have revealed an association with schizophrenia (5). However, not all ecological studies have replicated these associations (6). The differing conclusions may stem from differences in estimating the exposed population (6). Nevertheless, several prospective studies following birth cohorts (7, 8) have consistently revealed an association between maternal viral infection and psychiatric disorders in offspring and added other classes of pathogens to the list: namely, bacterial infections—including pneumonia, sinusitis, and tonsillitis—and the parasite *Toxoplasma gondii* (7, 9).

How can such a diverse group of pathogens confer similar risks of psychotic disorder? Common to the implicated pathogens is the maternal immune response. In support of this possibility, enduring fevers above a certain threshold pose the greatest risk (10). It follows that immune system activation above that threshold due to any environmental insult or genetic predisposition would also increase the risk. Indeed, maternal autoimmune disorders, allergies, asthma, acute stress, and exposure to environmental pollutants—all of which lead to elevated immune responses—have been linked to an enhanced risk of schizophrenia (7, 8). These findings may help to contextualize two recent prospective studies that failed to find a significant association between prenatal infection and schizophrenia after adjusting for parental infection in general, parental psychiatric disorder, and socioeconomic status (11, 12).

An accumulative evidence points to the significant role of neuroinflammation and the immune system in the pathophysiology of schizophrenia (13). There are also numerous reports that support the hypothesis that immune activation is a risk for onset of schizophrenia at adulthood (14, 15). Moreover, evidence from genomic (16), blood (17), postmortem (18), and *in vivo* imaging (19) investigations suggests that immune activation is concerned in the pathophysiology of schizophrenia.

In almost all cases, autoimmune diseases are their favorable reaction to immunoablation and saved by bone marrow transplantation (BMT) (20). Investigation in radiation chimeras established that the immunological and hematological systems possess a mutual stem cell (20).

Knowledge of the clinical observation of schizophrenia after BMT would significantly improve our comprehension of the importance of immune system in schizophrenia. Sommer and van Bekkum requested hematologists and psychiatrists to notify them their case reports, and they submitted this request to the relevant expert journals (20).

In this case report, we show that BMT was effective in treatment of treatment-resistant schizophrenia with predominantly delusion and hallucination symptoms. To the best of our knowledge, this is the primary case observation of successful therapy of treatment-resistant schizophrenia with BMT.

CASE PRESENTATION

The patient was a 24-year-old male. His birth was ordinary, and he grew as normal. After he had graduated from university, he labored in a corporation. His level of social skill was standard. There was not any description of alcohol or drugs use or seizures of epilepsy. In his family, there is nobody with psychiatric and developmental disorders. When the patient was 23 years old, he suffered from insomnia, irritability, and anxiety. In addition, he developed into agitated and spoke incoherently, and persecutory delusions and paranoid ideation arose. Problems of consciousness and convulsions were not detected. He visited the Department of Psychiatry of Shimane University Hospital. Assessment of his psychiatric status confirmed auditory hallucination, suspiciousness, active social avoidance, persecutory delusion, and decline in the social function. His diagnosis was "paranoid schizophrenia" according to DSM-IV-TR (21). Physical and neurological examinations revealed no marked abnormalities. There is no abnormal finding in routine laboratory investigations of serum and urine. In electric encephalography, computed tomography, and magnetic resonance imaging of the brain, there is no abnormality. Administration of quetiapine (QTP) (300 mg/day) was started. One week later, his auditory hallucinations, suspiciousness, active social avoidance, persecutory delusion, and deterioration in the level of social functioning continued. Because he refused to take neuroleptics, the patient's family managed antipsychotics for him and confirmed that he took antipsychotics. However, significant worsening of his psychiatric symptoms followed. Administration of risperidone (RIS) (12 mg/day) and olanzapine (20 mg/day) was added to QTP. However, his psychotic symptoms were not improved at all. His social functioning also deteriorated. Treatment-resistant schizophrenia was classified as little or no response to treatment from at least two adequately dosed antipsychotic trials for least 4 weeks including at least one second-generation antipsychotic (22). As result, he was diagnosed with treatment-resistant schizophrenia (23).

When he was 24 years old, he experienced severe tiredness, continuing elevation of fever, pain of general joint, gingival bleeding, and shortness of breath. As result of further examinations, he was diagnosed with acute myeloid leukemia at the Department of Hematology of Shimane University Hospital. His willingness to receive BMT was confirmed; however, the problem of whether he could stand the considerable psychological pressure of BMT, particularly throughout the isolation phase, was not obvious. To elucidate this, a test isolation was performed for 7 days. While his severe auditory hallucinations, suspiciousness, and persecutory delusion continued, severe psychomotor excitement was not recognized. Moreover, the hospital staff could communicate with him with no difficulty. So that hematologists and we judged that, he would be able to tolerate the stress during the isolation period. All neuroleptics were stopped during the test isolation in the germ-free unit.

One week later, BMT was performed. He was treated in isolation room at germ-free unit for 34 days. We met him three times a week throughout the isolation phase to assessment his psychiatric status and necessity of administering additional therapy. No neuroleptics were administered because of his refusal to take them; however, his psychotic status maintained with stable condition. Moreover, the BMT isolation was accomplished with no trouble. After he underwent BMT, administration of methotrexate and cyclosporin A was begun to avoid graft versus host disease (GVHD). Three weeks after BMT, early symptoms of GVHD were recognized, and hematologists administered tacrolimus in place of cyclosporin A.

Thirty days later, his psychotic symptom had almost disappeared. He was sustained without any neuroleptic treatment and need for any other administration. His psychiatric status was assessed by the Positive and Negative Symptom Scale (24). Social functioning was assessed using the Global Assessment of Functioning Scale of the DSM-IV-TR (21). The treatment and clinical course are shown in **Figure 1**. In 2017, 8 years after BMT, the improvements of somatic and psychiatric symptoms are continued, and the patient is very well and there are no residual psychiatric symptoms. Moreover, his social functioning was drastically recovered, and he continues to work at a famous company.

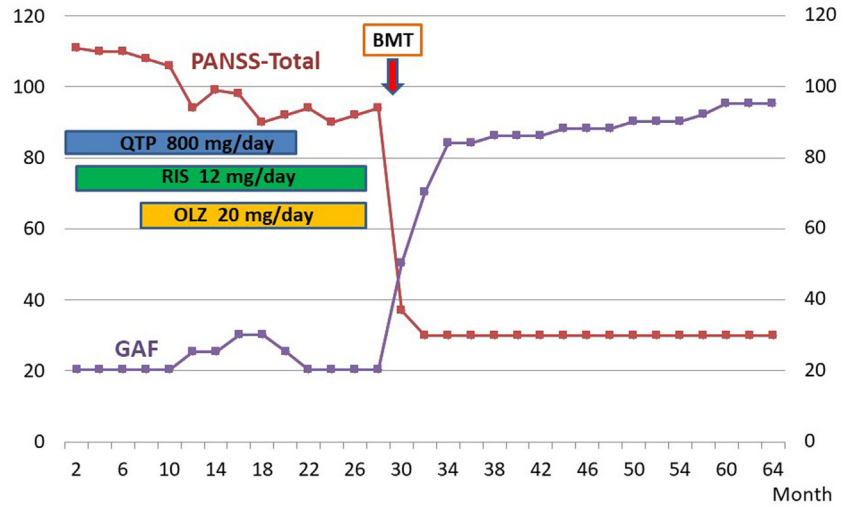


FIGURE 1 | Treatment and clinical course of the case. Their psychiatric symptoms were evaluated by PANSS (24). Functioning was assessed using the GAF of the DSM-IV-TR (21). BMT, bone marrow transplantation; QTP, quetiapine; RIS, risperidone; OLZ, olanzapine; PANSS, Positive and Negative Symptom Scale; GAF, Global Assessment of Functioning Scale.

DISCUSSION

Bone marrow transplantation might be effective in treatment of this patient's acute and treatment-resistant schizophrenia characterized predominantly by delusion and hallucination. During the remission of psychosis, this patient did not experience any infection by BMT. To the best of our knowledge, this is the primary case observation of successful therapy of schizophrenia with BMT. In limitation, we could not exclude the possibilities of spontaneous improvement without any treatment, paradoxical improvement following cessation of neuroleptics, and the curative effect of multiple immune modulating drugs.

In consideration of single case report, we apparently cannot confirm an immune pathogenesis of schizophrenia. However, several reports support the theory that immunological system is one of key factor of pathogenesis of schizophrenia (25, 26), and we suggest that physicians and patients involved in BMT consider the possibility that schizophrenia may be treated successfully by BMT.

In an animal study using maternal immune activation (MIA) offspring, Hsiao et al. identified distinction in immune activation in a mouse model of autism and schizophrenia (27). MIA in pregnant rodents can be produced by immunological activation by polyriboinosinic-polyribocytidilic acid (Poly I:C), which causes the offspring to have enduring immune system abnormalities and behavioral abnormalities (9, 27–30). Moreover, it was reported that Poly I:C-induced MIA leads to permanently hyperresponsive CD4+ T cells and a hypersensitive immune system in offspring, and further, that behavioral abnormalities of the rodents could in part be recovered by BMT (27).

Our findings may be contributed to several number of animal model studies reporting the efficacy of BMT on improving symptoms of neurological disorders (31–33). Derecki et al. identified microglia normalized by BMT contributed to recover behavioral abnormalities in a mouse model of Rett syndrome. The findings suggest that BMT normalizes microglia impairments in the brain. Microglia impairment seems to be one important neurological pathology in schizophrenia patient brain (34, 35). However, if only microglia-mediated mechanism is being considered, it is difficult to explain the mechanism *via* which BMT would lead to sudden reversal of symptoms in this case.

In a human clinical case report, Sommer et al. reported the clinical course of a patient who showed severe psychosis after BMT from schizophrenic patients (36). This report also supports the possibility that BMT might be an effective treatment for schizophrenia (37).

Additional research with added subjects is obviously necessary because the association of both schizophrenia and the contribution of BMT in CNS are not comprehended at all.

CONCLUDING REMARKS

In this patient, BMT was effective in treatment of acute and treatment-resistant schizophrenia with predominant delusion and hallucination. During the remission of psychosis, this patient did not experience any infection-associated BMT. In consideration of single case report, we apparently cannot confirm an immune pathogenesis of schizophrenia. However, several reports support the theory that immunological system is one of key factor of pathogenesis of schizophrenia (26), and we suggest that physicians and patients involved in BMT consider the possibility that treatment-resistant schizophrenia may be treated curatively by BMT. Though BMT may not be a cure for all cases of schizophrenia, it definitely possesses the potential to manage overall disease

severity and improve the quality of life, and this case report is a preliminary demonstration of the safety and efficacy of BMT in treatment-resistant schizophrenia. Additional research with added subjects is obviously necessary because the association of both schizophrenia and the contribution of BMT in CNS are not comprehended at all.

ETHICS STATEMENT

This case study was carried out in accordance with the recommendations of the Ethical Committee of Shimane University Faculty of Medicine with written informed consent from the subject. The subject gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

Substantial contributions to the conception or design of the work: TM, JH, RW, SH, MH, and MI. The acquisition, analysis, or interpretation of data for the work: TM, IA, KT, TA, RA, and RAA. Drafting the work or revising it critically for important intellectual content: TM, RW, SH, AO-N, and JH. Final approval of the version to be published: TM, JH, RW, SH, AO-N, IA, KT, TA, RA, and RAA.

REFERENCES

1. DeLisi LE, Shaw SH, Crow TJ, Shields G, Smith AB, Larach VW, et al. A genome-wide scan for linkage to chromosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. *Am J Psychiatry* (2002) 159:803–12. doi:10.1176/appi.ajp.159.5.803
2. Irish Schizophrenia Genomics Consortium and the Wellcome Trust Case Control Consortium 2. Genome-wide association study implicates HLA-C*01:02 as a risk factor at the major histocompatibility complex locus in schizophrenia. *Biol Psychiatry* (2012) 72:620–8. doi:10.1016/j.biopsych.2012.05.035
3. Corvin A, Morris DW. Genome-wide association studies: findings at the major histocompatibility complex locus in psychosis. *Biol Psychiatry* (2014) 75:276–83. doi:10.1016/j.biopsych.2013.09.018
4. Brown AS, Cohen P, Harkavy-Friedman J, Babulas V, Malaspina D, Gorman JM, et al. A.E. Bennett Research Award. Prenatal rubella, premorbid abnormalities, and adult schizophrenia. *Biol Psychiatry* (2001) 49:473–86. doi:10.1016/S0006-3223(01)01068-X
5. Reisinger S, Khan D, Kong E, Berger A, Pollak A, Pollak DD. The poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. *Pharmacol Ther* (2015) 149:213–26. doi:10.1016/j.pharmthera.2015.01.001
6. Selten JP, Frissen A, Lensvelt-Mulders G, Morgan VA. Schizophrenia and 1957 pandemic of influenza: meta-analysis. *Schizophr Bull* (2010) 36:219–28. doi:10.1093/schbul/sbp147
7. Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, et al. Maternal immune activation and abnormal brain development across CNS disorders. *Nat Rev Neurol* (2014) 10:643–60. doi:10.1038/nrneurol.2014.187
8. Estes ML, McAllister AK. Immune mediators in the brain and peripheral tissues in autism spectrum disorder. *Nat Rev Neurosci* (2015) 16:469–86. doi:10.1038/nrn3978
9. Patterson PH. Immune involvement in schizophrenia and autism: etiology, pathology and animal models. *Behav Brain Res* (2009) 204:313–21. doi:10.1016/j.bbr.2008.12.016
10. Bergink V, Gilbney SM, Drexhage HA. Autoimmunity, inflammation, and psychosis: a search for peripheral markers. *Biol Psychiatry* (2014) 75:324–31. doi:10.1016/j.biopsych.2013.09.037
11. Blomström A, Karlsson H, Gardner R, Jorgensen L, Magnusson C, Dalman C. Associations between maternal infection during pregnancy, childhood infections, and the risk of subsequent psychotic disorder – a Swedish Cohort Study of nearly 2 million individuals. *Schizophr Bull* (2016) 42:125–33. doi:10.1093/schbul/sbv112
12. Nielsen PR, Meyer U, Mortensen PB. Individual and combined effects of maternal anemia and prenatal infection on risk for schizophrenia in offspring. *Schizophr Res* (2016) 172:35–40. doi:10.1016/j.schres.2016.02.025
13. Howes OD, McCutcheon R. Inflammation and the neural diathesis-stress hypothesis of schizophrenia: a reconceptualization. *Transl Psychiatry* (2017) 7:e1024. doi:10.1038/tp.2016.278
14. Kirkpatrick B, Miller BJ. Inflammation and schizophrenia. *Schzophr Bull* (2013) 39:1174–9. doi:10.1093/schbul/sbt141
15. Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R, Jones PB. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *Lancet Psychiatry* (2015) 2:197–9. doi:10.1016/S2215-0366(14)00122-9
16. Ripke S, Sanders AR, Kendler KS, Levinson DF, Sklar P, Holmans PA, et al. Genome-wide association study identifies five new schizophrenia loci. *Nat Genet* (2011) 43:969–76. doi:10.1038/ng.940
17. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biol Psychiatry* (2011) 70:663–71. doi:10.1016/j.biopsych.2011.04.013
18. Steiner J, Bielau H, Brisch R, Danos P, Ullrich O, Mawrin C. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J Psychiatr Res* (2008) 42:151–7. doi:10.1016/j.jpsychires.2006.10.013
19. Doorduin J, de Vries EF, Willemsen AT, de Groot JC, Dierckx RA, Klein HC. Neuroinflammation in schizophrenia-related psychosis: a PET study. *J Nucl Med* (2009) 50:1801–7. doi:10.2967/jnumed.109.066647
20. Sommer IE, van Bekkum DW. Call for case histories of BMT in patients with coincident schizophrenia. *Bone Marrow Transplant* (2013) 48:880. doi:10.1038/bmt.2013.30
21. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed. Washington, DC: APA (2000).
22. Kane JM, Monigled G, Singer J, Melzer H. Clozapine in treatment-resistant schizophrenia. *Psychopharmacol Bull* (1988) 24:62–7.
23. Conley RR, Buchanan RW. Evaluation of treatment-resistant schizophrenia. *Schizophr Bull* (1997) 23:663–74. doi:10.1093/schbul/23.4.663
24. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* (1987) 13:261–76. doi:10.1093/schbul/13.2.261
25. Benros ME, Mortensen PB, Eaton WW. Autoimmune diseases and infections as risk factors for schizophrenia. *Ann N Y Acad Sci* (2012) 1262:56–66. doi:10.1111/j.1749-6632.2012.06638.x
26. Estes ML, McAllister AK. Maternal immune activation: implications for neuropsychiatric disorders. *Science* (2016) 353:772–7. doi:10.1126/science.aag3194
27. Hsiao EY, Mcbride SW, Chow J, Mazmanian SK, Patterson PH. Modeling an autism risk factor in mice leads to permanent immune dysregulation. *Proc Natl Acad Sci U S A* (2012) 109:12776–81. doi:10.1073/pnas.1202556109
28. Hsiao EY. Immune dysregulation in autism spectrum disorder. *Int Rev Neurobiol* (2013) 113:269–302. doi:10.1016/B978-0-12-418700-9.00009-5
29. Meyer U. Developmental neuroinflammation and schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* (2013) 42:20–34. doi:10.1016/j.pnpbp.2011.11.003
30. Meyer U. Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems. *Biol Psychiatry* (2014) 75:307–15. doi:10.1016/j.biopsych.2013.07.011
31. Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, et al. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell* (2010) 141:775–85. doi:10.1016/j.cell.2010.03.055
32. Derecki NC, Cronk JC, Lu Z, Xu E, Abbott SBG, Guyenet PG, et al. Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* (2012) 484:105–9. doi:10.1038/nature10907

33. Kwan W, Magnusson A, Chou A, Adame A, Carson MJ, Kohsaka S, et al. Bone marrow transplantation confers modest benefits in mouse models of Huntington's disease. *J Neurosci* (2012) 32:133–42. doi:10.1523/JNEUROSCI.4846-11.2012
34. Laskaris LE, Di Biase MA, Everall I, Chana G, Christopoulos A, Skafidas E, et al. Microglial activation and progressive brain changes in schizophrenia. *Br J Pharmacol* (2016) 173:666–80. doi:10.1111/bph.13364
35. van Kesteren CF, Gremmels H, De Witte LD, Hol EM, Van Gool AR, Falkai PG, et al. Immune involvement in the pathogenesis of schizophrenia: a meta-analysis on postmortem brain studies. *Transl Psychiatry* (2017) 7:e1075. doi:10.1038/tp.2017.4
36. Sommer IE, van Bekkum DW, Klein H, Yolken R, Witte L, Talamo G. Severe chronic psychosis after allogeneic SCT from a schizophrenic sibling. *Bone Marrow Transplant* (2015) 50:153–4. doi:10.1038/bmt.2014.221
37. Gibney SM, Drexhage HA. Evidence for a dysregulated immune system in the etiology of psychiatric disorders. *J Neuroimmune Pharmacol* (2013) 8:900–20. doi:10.1007/s11481-013-9462-8

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2017 Miyaoka, Wake, Hashioka, Hayashida, Oh-Nishi, Azis, Izuhara, Tsuchie, Araki, Arauchi, Abdullah and Horiguchi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 06 November 2017
doi: 10.3389/fpsyt.2017.00225

Peripheral Transcription of *NRG-ErbB* Pathway Genes Are Upregulated in Treatment-Resistant Schizophrenia

*Md Shaki Mostaid[1,2], Ting Ting Lee[3], Gursharan Chana[3,4,5], Suresh Sundram[4,6,7], Cynthia Shannon Weickert[8,9,10], Christos Pantelis[1,2,3,4,6], Ian Everall[1,2,3,4,6,11] and Chad Bousman[1,2,12,13,14]**

[1] Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne and Melbourne Health, Parkville, VIC, Australia, [2] The Cooperative Research Centre (CRC) for Mental Health, Melbourne, VIC, Australia, [3] Centre for Neural Engineering, The University of Melbourne, Carlton, VIC, Australia, [4] Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC, Australia, [5] Department of Medicine, Royal Melbourne Hospital, Parkville, VIC, Australia, [6] NorthWestern Mental Health, Melbourne, VIC, Australia, [7] Department of Psychiatry, School of Clinical Sciences, Monash University and Monash Health, Clayton, VIC, Australia, [8] Schizophrenia Research Institute, Sydney, NSW, Australia, [9] Schizophrenia Research Laboratory, Neuroscience Research Australia, Sydney, NSW, Australia, [10] Faculty of Medicine, School of Psychiatry, University of New South Wales, Sydney, NSW, Australia, [11] Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, [12] Department of Medical Genetics, University of Calgary, Calgary, AB, Canada, [13] Department of Psychiatry, University of Calgary, Calgary, AB, Canada, [14] Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, Canada

OPEN ACCESS

Edited by:
Felice Iasevoli, University of Naples Federico II, Italy

Reviewed by:
Mirko Manchia, Dalhousie University, Canada
Jie Ma, Xi'an Jiaotong University, China

***Correspondence:**
Chad Bousman
chad.bousman@ucalgary.ca

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *05 September 2017*
Accepted: *23 October 2017*
Published: *06 November 2017*

Citation:
Mostaid MS, Lee TT, Chana G, Sundram S, Shannon Weickert C, Pantelis C, Everall I and Bousman C (2017) Peripheral Transcription of NRG-ErbB Pathway Genes Are Upregulated in Treatment-Resistant Schizophrenia. Front. Psychiatry 8:225. doi: 10.3389/fpsyt.2017.00225

Investigation of peripheral gene expression patterns of transcripts within the *NRG–ErbB* signaling pathway, other than neuregulin-1 (*NRG1*), among patients with schizophrenia and more specifically treatment-resistant schizophrenia (TRS) is limited. The present study built on our previous work demonstrating elevated levels of *NRG1* EGFα, EGFβ, and type $I_{(Ig2)}$ containing transcripts in TRS by investigating 11 *NRG–ErbB* signaling pathway mRNA transcripts (*NRG2*, *ErbB1*, *ErbB2*, *ErbB3*, *ErbB4*, *PIK3CD*, *PIK3R3*, *AKT1*, *mTOR*, *P70S6K*, *eIF4EBP1*) in whole blood of TRS patients ($N = 71$) and healthy controls ($N = 57$). We also examined the effect of clozapine exposure on transcript levels using cultured peripheral blood mononuclear cells (PBMCs) from 15 healthy individuals. Five transcripts (*ErbB3*, *PIK3CD*, *AKT1*, *P70S6K*, *eIF4EBP1*) were significantly elevated in TRS patients compared to healthy controls but only expression of *P70S6K* ($P_{corrected} = 0.018$), a protein kinase linked to protein synthesis, cell growth, and cell proliferation, survived correction for multiple testing using the Benjamini–Hochberg method. Investigation of clinical factors revealed that *ErbB2*, *PIK3CD*, *PIK3R3*, *AKT1*, *mTOR*, and *P70S6K* expression were negatively correlated with duration of illness. However, no transcript was associated with chlorpromazine equivalent dose or clozapine plasma levels, the latter supported by our *in vitro* PBMC clozapine exposure experiment. Taken together with previously published *NRG1* results, our findings suggest an overall upregulation of transcripts within the *NRG–ErbB* signaling pathway among individuals with schizophrenia some of which attenuate over duration of illness. Follow-up studies are needed to determine if the observed peripheral upregulation of transcripts within the *NRG–ErbB* signaling pathway are specific to TRS or are a general blood-based marker of schizophrenia.

Keywords: treatment-resistant schizophrenia, *NRG–ErbB* pathway, gene expression, symptom severity, schizophrenia

INTRODUCTION

Intracellular signaling initiated by neuregulins (NRGs) and their cognate receptors (ErbBs) are vital for the assembly of neuronal circuitry (1, 2), including myelination of axonal processes (3, 4), neurotransmission (5), and synaptic plasticity (6–8). Abnormalities in *NRG–ErbB* signaling have been implicated in schizophrenia, with the majority of evidence linked to neuregulin-1 (*NRG1*) and *ErbB4* (5, 9–11).

Neuregulin-1 and *ErbB4*, together, initiate signaling *via* the *PI3K-AKT* signaling pathway, which results in activation of *mTOR* and in turn stimulates protein synthesis (**Figure 1**). Several human postmortem brain studies have shown dysregulation of gene expression of *NRG1*, ErbB4 or down-stream targets among individuals with schizophrenia (12–17). Likewise, evidence of dysregulated gene expression of *NRG1* (18–20), *ErbB1*/*ErbB4* (21), and *PI3K*/AKT (22, 23) in peripheral tissues [i.e., whole blood, peripheral blood mononuclear cells (PBMCs), monocytes] in schizophrenia has also been shown in people with chronic schizophrenia. Treatment-resistant schizophrenia (TRS) patients represent a considerable subgroup who have significant increases in multiple *NRG1* splice variants in peripheral blood (24). Thus, we may expect the biological interactors (receptors) and mediators (kinase) of this pathway to also be changed. However, peripheral examination of gene expression within this pathway among individuals with TRS has yet to be completed. Moreover, the impact of medication, lifestyle (e.g., smoking, alcohol use), and/or symptom severity on *NRG1*-related mRNA expression is largely unknown.

The present investigation, therefore, quantiatively compared (i) whole blood mRNA levels of 11 *NRG–ErbB* signaling receptors and pathway genes (*NRG2*, *ErbB1*, *ErbB2*, *ErbB3*, *ErbB4*, *PIK3CD*, *PIK3R3*, *AKT1*, *mTOR*, *P70S6K*, *eIF4EBP1*) among individuals with TRS and healthy controls, (ii) associations between mRNA levels and symptom severity, age of onset, duration of illness, clozapine plasma level, and chlorpromazine equivalent dosage, and (iii) the effect of clozapine exposure on mRNA expression in PBMCs from healthy controls. We expected that there would be multiple molecular changes in TRS compared to controls that may contribute to the amplification of *NRG1* signaling in perhiperal blood in support of a widespread gain of function model of *NRG1* in the pathophysiology of schizophrenia.

MATERIALS AND METHODS

Participants

Clinical Samples

Seventy-one participants aged 18–65 with schizophrenia who were treated with clozapine were recruited from inpatient and outpatient clinics in Melbourne, Australia. As these individuals failed to respond to two or more previous trials of antipsychotics, had poor functioning, and persistent symptoms, they were considered "treatment-resistant," consistent with current criteria

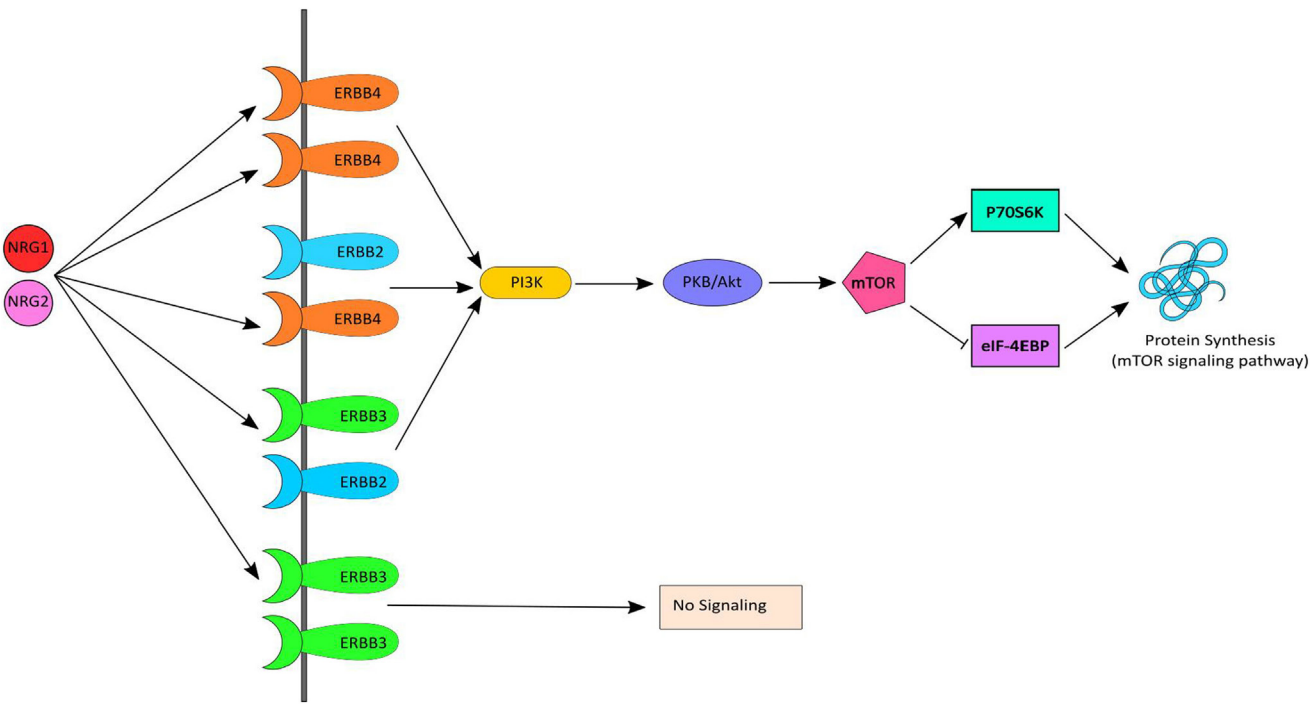


FIGURE 1 | *NRG–ErbB* signaling pathway. Neuregulin-1 (*NRG1*) and *NRG2* bind to *ErbB3* and/or *ErbB4,* which in turn undergoes homo or heterodimerization and activates *PI3K*. *PI3K* then activates AKT and subsequently *mTOR* causing initiation of protein synthesis *via* the *mTOR* signaling pathway. mTOR phosphorylates and activates *P70S6K* which facilitates phosphorylation of small ribosomal protein 6 (*S6*) and eukaryotic translation initiation factor 4B (*eIF4B*) and leads to initiation of protein synthesis. Activated *mTOR* also causes phosphorylation and inactivation of *eIF4EBP1*, which release *eIF4E* and facilitates translation.

(25). In addition, 57 age-, sex-, and socioeconomic-matched unrelated healthy controls were recruited from the general community. Controls with a first-degree family history of psychiatric illness, prior or current use of antipsychotic medication, head injury, seizure, neurological disease, impaired thyroid function, and/or substance abuse/dependence were excluded. Detailed demographic characteristics of all participants are presented in **Table 1**.

Mini International Neuropsychiatric Interview (26) was administered to all participants to confirm the diagnosis of schizophrenia as well as to rule out the presence of psychiatric disorders in healthy controls. The Positive and Negative Syndrome Scale (PANSS) (27) was used to assess the clinical symptoms and the patients were scored in accordance with the consensus five-factor (i.e., positive, negative, disorganized/concrete, excited, depressed) PANSS model (28). Information on tobacco, alcohol, and illicit drug use in the past 3 months was collected using a substance use questionnaire. Whole blood samples were collected after overnight fasting and processed according to standardized blood collection and processing protocol (see supplementary methods for more details). Plasma levels of clozapine were measured and chlorpromazine equivalent dosage (excluding clozapine) were calculated for the 31% ($n = 22$) of participants with schizophrenia who were taking concomitant antipsychotic medication in accordance with published guidelines (29, 30). All the participants provided written informed consent and the study protocol was approved by the Melbourne Health Human Research Ethics Committee (MHREC ID 2012.069). The study complied with the Declaration of Helsinki and its subsequent revisions (31).

TABLE 1 | Demographic data and clinical characteristics of participants.

Characteristic	Schizophrenia ($n = 71$)	Controls ($n = 57$)	*P*-value
Age, mean (SD) years	40 (10)	40 (11)	0.702[a]
Gender, *n* (%) males	53 (75)	35 (61)	0.108[b]
RIN, mean (SD)	8.4 (0.9)	8.7 (0.3)	0.006[a*]
Ancestry, *n* (%) CEU	62 (90)	50 (88)	0.742[b]
Substance use in past 3 months, *n* (%)			
Tobacco (smoked)	33 (47)	12 (21)	0.003[b*]
Alcohol	59 (83)	55 (97)	0.016[b*]
Cannabis	11 (15)	7 (12)	0.385[b]
Amphetamine	4 (6)	2 (4)	0.439[b]
Cocaine	0 (0)	2 (4)	0.137[b]
Opiates	1 (1)	1 (2)	0.990[b]
Clozapine plasma level, mean (SD) µg/L	432 (234)	–	–
Chlorpromazine equivalent (excluding clozapine) dosage mean (SD) mg/day	142 (286)	–	–
Age of onset, mean (SD) years	22.5 (6)	–	–
Duration of illness, mean (SD) years	17 (8)	–	–
PANSS scores, mean (SD)			
Positive	10 (6)	–	–
Negative	15 (5)	–	–
Disorganized	8 (3)	–	–
Excitement	6 (2)	–	–
Depression	6 (3)	–	–
Total	62 (14)	–	–

CEU, Northern and Western European ancestry; TRS, treatment-resistant schizophrenia; RIN, RNA integrity number; PANSS, Positive and Negative Syndrome Scale.

[a]*Independent sample t-test.*

[b]*Chi-square (χ^2) test.*

**P < 0.05.*

In Vitro Clozapine Exposure Samples

To assess the effect of clozapine exposure on gene expression of our candidate transcripts, fresh frozen PBMCs from 15 healthy individuals (8 males and 7 females) of European ancestry with a mean age of 35 (SD = 13.5; range 20–54 years) were purchased from STEMCELL™ Technologies, Inc. (Vancouver, BC, Canada). A sample size of 15 was sufficient to detect a large effect (Cohen's $d = 0.80$) between exposed and unexposed conditions at $\alpha = 0.05$ and power $(1 - \beta) = 0.80$. The percentage of current smokers among the donors was 33.3% ($n = 5$). All the donors were tested for HIV-1, HIV-2, hepatitis B and hepatitis C prior to blood collection.

Peripheral blood mononuclear cells isolated from whole blood were supplied as vials containing 100 million cells. PBMCs were rapid-thawed from liquid nitrogen and seeded in six-well plates in triplicates at a concentration of 2 million cells per well (1×10^6 cells/mL) in RPMI-1640 medium (Sigma-Aldrich; St. Louis, MO, USA) supplemented with L-glutamine (0.3 g/L) and sodium bicarbonate (2 g/L), penicillin (100 U/mL), streptomycin (100 µg/mL), and 10% fetal bovine serum for 24 h. Cells were then exposed to clozapine (Sigma-Aldrich, St. Louis, MO, USA) for 24 h and 7 days, at a concentration of 1.2 µM (control cells were exposed to vehicle only, see supplementary methods for details) and incubated at 37°C in 5% CO_2. Clozapine was initially dissolved in absolute ethanol and media was used for dilution. The final concentration of ethanol on each well was 1 in 8,000. The concentration of clozapine used was determined from the mean plasma concentration of clozapine found in the first 48 recruited clinical samples (1.2 µM or 384 ng/mL). Toxicity assays (CytoTox 96® Non-Radioactive Cytotoxicity Assay; Promega Corporation, Madison, WI, USA) were performed at baseline, 24 h and 7-day time points after clozapine exposure to measure the production of lactate dehydrogenase within the media (see Figure S1 in Supplementary Material for more details).

RNA Extraction, Complementary DNA (cDNA) Synthesis, and Quantitative Real-time PCR

PureLink RNA Mini Kit (ThermoFisher scientific, Waltham, MA, USA) was used to extract total RNA from both clinical and *in vitro* samples following standard manufacturer's instructions. The RNA integrity number (RIN) range was 3.60–9.50 (mean = 8.59, SD = 0.79). Total RNA was reverse transcribed to complementary DNA (cDNA) using SuperScript® IV First-Strand Synthesis System (Invitrogen, Foster city, CA, USA) using random hexamers. cDNA (10.25 ng) was used as a template for real-time PCR (RT-qPCR) using master-mix and gene specific validated Taqman assays from Applied Biosystems, Foster City, CA, USA. Inventoried assays (TaqMan®, Invitrogen, USA) were used for all the genes of interest as well as for four reference genes (beta-actin, ACTB; ubiquitin C, UBC; ABL proto-oncogene 1,

ABL1; Succinate Dehydrogenase Complex Flavoprotein Subunit A, SDHA). See Table S1 in Supplementary Material for a list of each of the probes and primers.

Complementary DNA (10.25 ng) was subjected to quantitative real-time PCR in duplicate using FAM-MGB TaqMan® gene expression probes (Invitrogen, Foster city, CA, USA) in 192 × 24 Dynamic Arrays IFC in Fluidigm® BioMark™ HD system (South San Francisco, CA, USA) at the Monash Health Translation Precinct Medical Genomics Facility (Hudson Institute of Medical Research, Clayton, VIC, Australia). In addition, no reverse transcriptase controls and no template controls were included to rule out genomic DNA contamination and reagent contamination, respectively. Adhering to minimum information for publication of RT-qPCR (MIQE) guidelines (32), normalized relative quantities (NRQ), i.e., $2^{-\Delta Ct}$ where $\Delta C_t = [C_{t(\text{candidate gene})} - C_{t\ (\text{geometric mean of reference genes})}]$ of each mRNA isoform was calculated using the geometric mean expression of two reference genes (UBC and ACTB) that did not differ between groups in the clinical cohort. ABL-1 and SDHA were not used as reference genes because their expression differed significantly by group in the clinical cohort (Figures S2–S4 in Supplementary Material). In the *in vitro* cohort only, ABL-1 was stable after 24 h clozapine exposure and ACTB was stable after 7 days clozapine exposure and were used for normalization and subsequent analysis at specific time points.

Statistical Analysis

Two-sided tests were used for all statistical analyses. Shapiro–Wilk test and quantile–quantile (Q–Q) plots were used to assess normality of variable distributions. Student's *t*-tests were used to test differences for continuous variables between schizophrenia patients and healthy controls, while chi-squared (χ^2) tests were used for categorical variables. The Benjamini and Hochberg (B–H) step-up procedure (33) was used to adjust for multiple comparisons for all analyses. Effect sizes were calculated using the Hedges' *g* method (34).

Prior to analysis, the NRQ values for all the mRNA transcripts were checked for normality using Q–Q plots (Figure S5 in Supplementary Material) and as required were $\log_{10}$ transformed for subsequent analysis. In addition, we assessed the following variables as potential confounders: age, sex, RIN, alcohol use, and smoking status. A variable was considered a confounder and included in our statistical models only when it was significantly different between groups ($P < 0.05$) and was significantly associated with gene expression. The log-transformed NRQ values were compared among groups using general or generalized linear models based on their distribution and adjusted for appropriate covariates. Outliers were identified using the Grubbs' test for outliers and removed from further analysis.

Within the schizophrenia group, Pearson or Spearman correlations, depending on data distribution, were calculated between gene transcript levels and symptom severity, age of onset, illness duration, current chlorpromazine equivalent dose, and clozapine plasma levels. In addition, mRNA transcript levels between participants in positive symptom remission and non-remission were assessed using a *t*-test or Mann–Whitney *U* test. Positive symptom remission was defined as a PANSS score of ≤3 on delusions, hallucinations, grandiosity, and unusual thought content (28).

To assess differences in gene expression between clozapine exposed and unexposed PBMCs at both time points (24 h and 7 days), Wilcoxon matched paired *t*-test were used, adjusting for age, gender, and RIN.

RESULTS

NRG–ErbB Signaling Pathway Transcripts Are Upregulated in TRS

Two (*ErbB1*, *ErbB4*) of the 11 *NRG–ErbB* pathway mRNA transcripts interrogated, were not detectable in more than 80% of the full cohort and so were removed from further analysis. The rates of non-detects were not significantly different between groups (*ErbB1*: case 95%, control: 97%; *ErbB4*: case 81%, control 85%). Analysis on the remaining nine transcripts showed significantly elevated levels of five transcripts: *ErbB3* ($P = 0.046$), *PIK3CD* ($P_{\text{raw}} = 0.035$), *AKT1* ($P_{\text{raw}} = 0.018$), *P70S6K* ($P_{\text{raw}} = 0.002$), and *eIF4EBP1* ($P_{\text{raw}} = 0.013$) in TRS patients compared to healthy controls after adjustment for covariates. However, only *P70S6K* ($P_{\text{B–H}} = 0.018$) remained significant after correction for multiple comparisons (**Figure 2**). Importantly, transcript levels were not correlated with clozapine plasma levels or chlorpromazine equivalent antipsychotic exposure (excluding clozapine) (Table S2 in Supplementary Material). The lack of relationship between mRNA levels and clozapine levels were further corroborated by our *in vitro* analysis that showed no difference in mRNA levels of detectable transcripts ($n = 9$) in clozapine exposed compared to unexposed PBMCs, except *mTOR* mRNA which showed decreased expression levels in clozapine exposed cells at both 24 h ($P = 0.001$) and 7-day ($P = 0.05$) time points (Figures S6 and S7 in Supplementary Material).

NRG–ErbB Signaling Pathway Transcripts Are Associated with Duration of Illness but Not Age of Onset or Symptom Severity

Among individuals with TRS, significant negative correlations between duration of illness and *ErbB2* ($r = -0.293$, $P_{\text{raw}} = 0.016$, $P_{\text{B-H}} = 0.031$), *PIK3CD* ($r = -0.303$, $P_{\text{raw}} = 0.013$, $P_{\text{B-H}} = 0.031$), *PIK3R3* ($r = -0.275$, $P_{\text{raw}} = 0.025$, $P_{\text{B-H}} = 0.038$), *AKT1* ($r = -0.290$, $P_{\text{raw}} = 0.017$, $P_{\text{B-H}} = 0.031$), *mTOR* ($r = -0.339$, $P_{\text{raw}} = 0.005$, $P_{\text{B-H}} = 0.023$), and *P70S6K* ($r = -0.347$, $P_{\text{raw}} = 0.005$, $P_{\text{B-H}} = 0.023$) expression were detected (**Figure 3**). None of the reference genes were significantly correlated with duration of illness, *UBC* ($r = -0.139$, $P_{\text{raw}} = 0.263$), *ACTB* ($r = 0.232$, $P_{\text{raw}} = 0.59$). No significant correlations were observed between any of the transcripts and age of onset (Table S2 in Supplementary Material).

A significant positive correlation between *ErbB2* expression and PANSS excitement score ($r = 0.289$, $P_{\text{raw}} = 0.014$, $P_{\text{B–H}} = 0.667$) was observed but did not survive correction for multiple comparisons (Table S3 in Supplementary Material). An exploratory examination of TRS patients in positive symptom remission versus non-remission revealed no statistically significant differences in levels of any of the gene mRNA

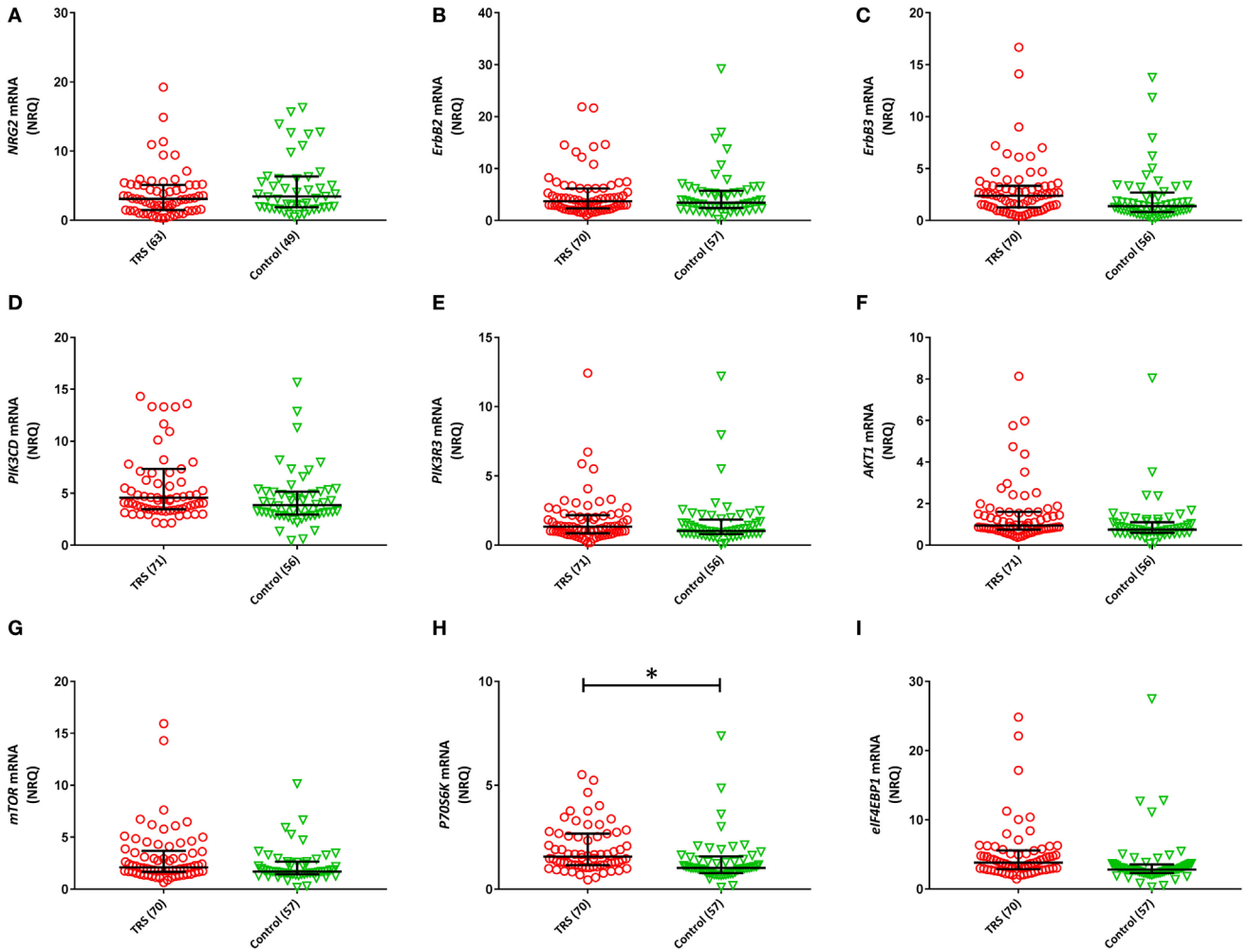


FIGURE 2 | Normalized relative quantities (NRQ) of the gene transcripts: **(A)** *NRG2* [treatment-resistant schizophrenia (TRS): 3.11, interquartile range (IQR) = 1.5–5.12, controls: 3.44, IQR = 1.89–6.34; $F_{1,111} = 0.524$, $P = 0.113$]; **(B)** *ErbB2* (TRS: 3.72, IQR = 2.39–6.19, controls: 3.44, IQR = 2.42–5.73; Wald $\chi^2 = 0.029$, $P = 0.864$); **(C)** *ErbB3* (TRS: 2.39, IQR = 1.26–3.35, controls: 1.38, IQR = 0.82–2.70; $F_{1,126} = 4.071$, $P = 0.083$); **(D)** *PIK3CD* (TRS: 4.57, IRQ = 3.45–7.34, controls: 3.86, IQR = 2.94–5.16; Wald $\chi^2 = 4.464$, $P = 0.079$); **(E)** *PIK3R3* (TRS: 1.34, IQR = 0.86–2.17, controls: 1.02, IQR = 0.8–1.85; Wald $\chi^2 = 0.104$, $P = 0.84$); **(F)** *AKT1* (TRS: 0.94, IQR = 0.75–1.61, controls: 0.75, IQR = 0.59–1.11; Wald $\chi^2 = 5.605$, $P = 0.054$); **(G)** *mTOR* (TRS: 2.10, IQR = 1.66–3.69, controls: 1.44, IQR = 1.44–2.65; Wald $\chi^2 = 4.746$, $P = 0.20$); **(H)** *P70S6K* (TRS: 1.57, IQR = 1.16–2.68, controls: 1.02, IQR = 0.77–1.58; Wald $\chi^2 = 13.90$, $P = 0.018$); **(I)** *eIF4EBP1* (TRS: 3.81, IQR = 2.88–5.58, controls: 2.80, IQR = 2.30–3.55; Wald $\chi^2 = 8.71$, $P = 0.054$). Error bars represent median with interquartile range. Benjamini–Hochberg adjusted *P*-values are shown (*$P < 0.05$).

transcripts after correction for multiple comparisons (Table S4 in Supplementary Material).

DISCUSSION

Our findings suggest transcription in the *NRG–ErbB* signaling pathway is upregulated in the whole blood of individuals with TRS and is negatively correlated with duration of illness. Among the nine detectable *NRG–ErbB* pathway transcripts we examined, five (*ErbB3*, *PIK3CD*, *AKT1*, *P70S6K*, and *eIF4EBP1*) were elevated and, of these, *P70S6K* survived correction for multiple comparisons. Importantly, we could not attribute this upregulation of peripheral transcription in the *NRG–ErbB* pathway to age, sex, or medication. In fact, results from our *in vitro* clozapine exposure experiment suggested clozapine might reduce rather than increase transcription of genes within the *NRG–ErbB* signaling pathway, particularly *mTOR* expression. Overall, our findings support our hypothesis that there is a generalized increase in *NRG1* signaling in people with TRS.

Previous findings by us and others support the notion of increased transcription of genes within the *NRG–ErbB* signaling pathway in schizophrenia. We recently showed in the same cohort used in the current study, an increased expression of three *NRG1* transcripts [i.e., *NRG1*-EGFα, *NRG1*-EGFβ, and *NRG1*-type$I_{(Ig2)}$] in TRS compared to controls (24). In addition, several studies by others have reported increased expression of specific isoforms of *NRG1* (18) and mRNA of down-stream signaling molecules, including *PIK3CD*, *PIK3CB* (16, 22), and *AKT1*

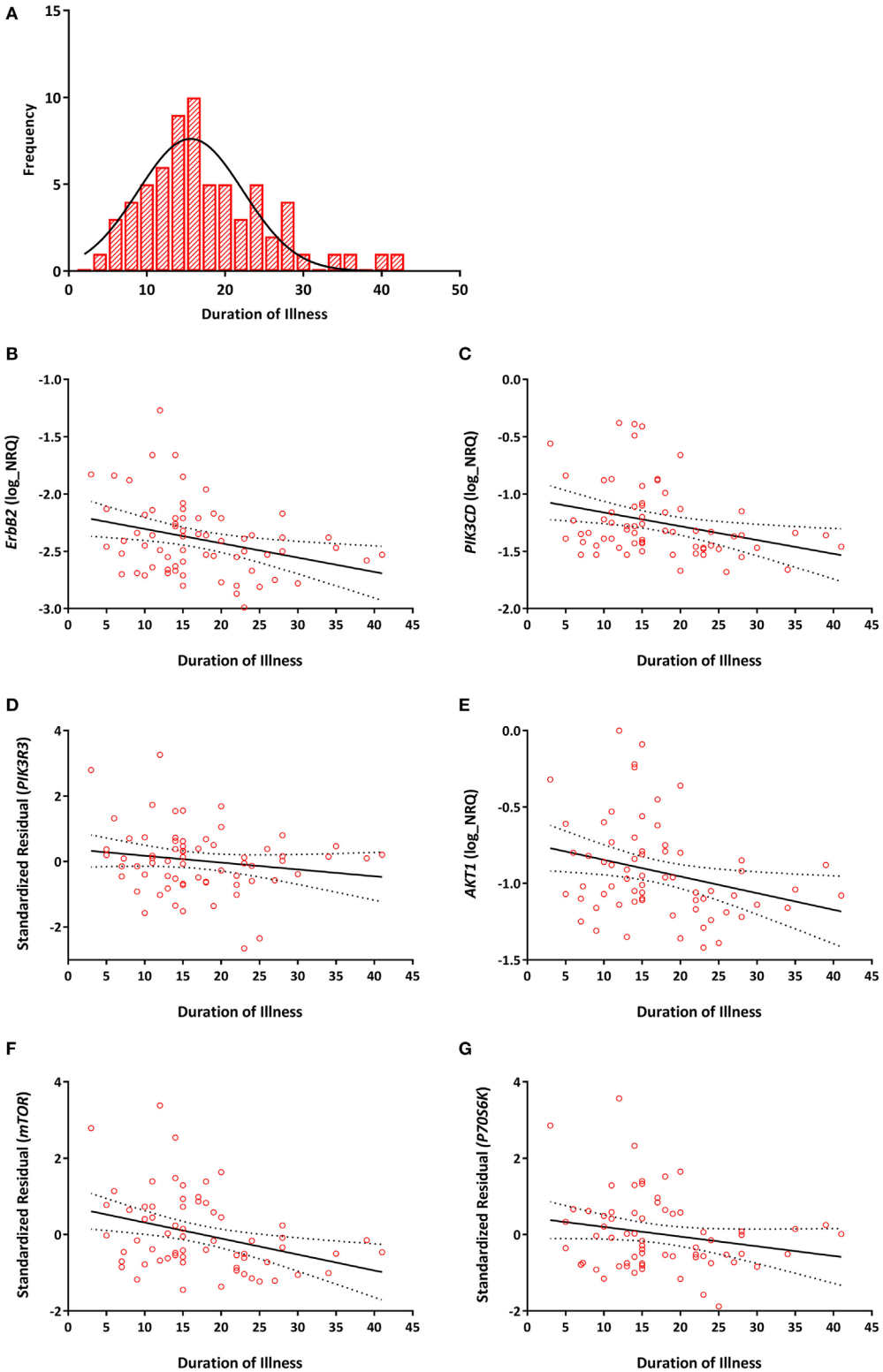


FIGURE 3 | **(A)** Distribution of duration of illness in years (mean = 17, SD = 8). Correlations between duration of illness and **(B)** *ErbB2* ($r = -0.293$, $P_{B\text{-}H} = 0.031$); **(C)** *PIK3CD* ($r = -0.303$, $P_{B\text{-}H} = 0.031$); **(D)** *PIK3R3* ($r = -0.275$, $P_{B\text{-}H} = 0.038$); **(E)** *AKT1* ($r = -0.290$, $P_{B\text{-}H} = 0.031$); **(F)** *mTOR* ($r = -0.339$, $P_{B\text{-}H} = 0.023$); **(G)** *P70S6K* ($r = -0.347$, $P_{B\text{-}H} = 0.023$) mRNA expression. Expression of *PIK3R3*, *mTOR*, and *P70S6K* are represented as the standardized residual from a linear regression model after adjusting for potential confounds [i.e., age for *PIK3R3*, RNA integrity number (RIN) and smoking for *mTOR*, age, RIN and smoking for *P70S6K*]. Solid lines represent the line of best fit and dotted lines represents 95% confidence intervals for the line of best fit.

(22, 23) in schizophrenia patients. Furthermore, other downstream signaling molecules, such as *mTOR*, *P70S6K*, and *eIF4B*, have been shown to be increased in major depressive disorder (35). However, as we are not aware of any human studies that have interrogated *P70S6K*, in schizophrenia, we are the first to report increased mRNA of *P70S6K* in TRS.

P70S6K encodes for a vital kinase in the mTOR signaling pathway (36–38) that when phosphorylated by mTOR results in phosphorylation and activation of translation elongation factors *eIF4B* and *eEF2K*, thereby promoting protein translation (39, 40). Our findings suggest upregulation of *P70S6K*, in part, may result from an increase in transcription of several genes upstream of *P70S6K* within the *NRG–ErbB* signaling pathway. However, other genes (i.e., *BDNF*, *DISC1*) as well as neurotransmitters (i.e., glutamate, serotonin) and hormones (e.g., insulin) have also been shown to activate the *PI3K–AKT–mTOR* signaling pathway (41–43) and as such may contribute or confound the increase in *P70S6K* expression we have observed. However, most studies find decreased BDNF levels in the blood of people with schizophrenia (44) and suggest some degree of insulin resistance in clozapine-treated patients (45). Future investigations should attempt to account for these other signaling factors and the potential confounders of metabolic changes in people with schizophrenia being treated with clozapine, as doing so will further elucidate the suitability of *P70S6K* as a peripheral biomarker of over-activity in the *NRG1* pathway in schizophrenia.

We also detected trend-level increases in three transcripts (*ErbB3*, *PIK3CD*, and *AKT1)* upstream of *mTOR*, within the *NRG–ErbB* signaling pathway among those with TRS. These increases in whole blood expression are, in part, supported by previous studies that have shown an increased *AKT1* mRNA expression in PBMCs from individuals with early-onset (23) and treatment-naïve schizophrenia (46), suggesting peripheral upregulation of *NRG–ErbB* pathway transcripts may not be specific to the stage of illness and may occur during the first phases of schizophrenia and continue during the chronic phases. However, six of the mRNA transcripts (*ErbB2*, *PIK3CD*, *PIK3R3*, *AKT1*, *mTOR*, and *P70S6K*) we examined were negatively correlated with duration of illness, suggesting that as the illness progresses the upregulation of transcription within the *NRG–ErbB* signaling pathway might become less apparent. However, it is not clear whether this correlation represents a potential disease process and/or a compensatory response in an effort to maintain signaling homeostasis. Studies examining patterns of *NRG–ErbB* signaling pathway transcripts over the course of the illness are required to confirm this notion and determine the underlying mechanism.

We did not find differences in the peripheral expression of *NRG2* between TRS patients and controls. To our knowledge, we are the first to examine *NRG2* mRNA in the blood in schizophrenia or other psychiatric disorder. However, a recent study showed that ablation of *NRG2* in the adult mouse brain mimicked dopaminergic imbalance seen in schizophrenia (i.e., high subcortical dopamine, low cortical dopamine) and resulted in severe behavioral phenotypes relevant to psychiatric disorders (47). Thus, *NRG2* may play a role in the pathophysiology of schizophrenia but based on our results seems less likely to serve as a peripheral marker of neurobiological changes found in schizophrenia. Likewise, *ErbB2* mRNA expression seems an unlikely peripheral marker of schizophrenia based on our null findings as well as findings from others that reported no difference in *ErbB2* mRNA expression in monocytes of first-episode, drug-naive patients with schizophrenia compared to healthy controls (48). However, this same study suggested that there may be an exaggerated *NRG1* stimulated cytokine response from PBMC in people with schizophrenia compared to controls (48), suggesting a link between overactive *NRG1* signaling and inflammation.

Our study has notable limitations. First, we were unable to compare affected individuals with and without TRS and as such the specificity of our results to TRS patients remains to be confirmed. Second, we analyzed cross-sectional data, which makes it complicated to predict how gene expression patterns might change with disease progression and their possible relation to clinical symptoms. Third, we measured gene expression in whole blood, as this tissue is clinically accessible and commonly used in biomarker research. However, it is unclear how our findings will relate to other peripheral (PBMCs or lymphocytes) or central tissues (e.g., brain) despite some suggestion for their relevance in schizophrenia (49). Fourth, we did not investigate all transcripts within the *NRG–ErbB* pathway (i.e., *PIK3CA-B*, *PIK3R1-2*, *eIF4B*, *eEF2*, and *eIF4E*). We instead, chose transcripts based on evidence from the current literature in schizophrenia. Furthermore, we only interrogated mRNA levels of our candidate genes within the *NRG–ErbB* pathway and as such cannot rule out the potential that genetic, protein, and/or epigenetic markers in this pathway may differ in those with schizophrenia. Fifth, our sample size was relatively small and as such requires independent validation. Finally, our *in vitro* clozapine exposure experiments examined a single clozapine concentration (1.2 μM) that was guided by pilot data from our study population. While this concentration of clozapine does reflect steady state plasma concentrations (50–52), future work with PBMCs should examine multiple concentrations that reflect the range of clozapine blood levels observed in the clinic together with interrogating a greater number of candidates at both genetic, gene expression and protein levels.

In summary, our results provide the first peripheral gene expression profile of the major *NRG–ErbB* pathway genes among individuals with TRS. We detected an overall upregulation of *NRG–ErbB* pathway transcripts among those with TRS, most robustly for *P70S6K*. We further showed that most of the transcripts we examined were negatively correlated with duration of illness, suggesting the upregulation of *NRG–ErbB* pathway transcripts we observed in the current chronic schizophrenia cohort may be more easily detectable among individuals at earlier stages of the illness relative to healthy individuals. If this notion is substantiated by future research, *NRG–ErbB* pathway gene expression may serve, in part, as a useful peripheral biomarker for staging of the illness and possibly assist in the identification of those at greatest risk for TRS.

ETHICS STATEMENT

All the participants provided written informed consent and the study protocol was approved by the Melbourne Health Human

Research Ethics Committee (MHREC ID 2012.069). The study complied with the Declaration of Helsinki and its subsequent revisions.

AUTHOR CONTRIBUTIONS

MSM, CB, IE, GC, and SS designed the study and wrote the protocol. MM, TL, and GC conducted the lab experiments. MM managed the literature searches and analyses and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

FUNDING

The authors acknowledge the financial support of the CRC for Mental Health. The Cooperative Research Centre (CRC) programme is an Australian Government Initiative. The authors also wish to acknowledge the CRC Scientific Advisory Committee, in addition to the contributions of study participants, clinicians at recruitment services, staff at the Murdoch Children's Research Institute, staff at the Australian Imaging, Biomarkers and Lifestyle Flagship Study of Aging, and research staff at the Melbourne Neuropsychiatry Centre, including coordinators, C. Phassouliotis, A. Merritt, and research assistants, A. Burnside, H. Cross, S. Gale, and S. Tahtalian. Participants for this study were sourced, in part, through the Australian Schizophrenia Research Bank (ASRB), which is supported by the National Health and Medical Research Council of Australia (Enabling Grant N. 386500), the Pratt Foundation, Ramsay Health Care, the Viertel Charitable Foundation, and the Schizophrenia Research Institute. The authors thank the Chief Investigators and ASRB Manager, V. Carr, U. Schall, R. Scott, A. Jablensky, B. Mowry, P. Michie, S. Catts, F. Henskens, C. Pantelis, C. Loughland. The authors acknowledge the help of Jason Bridge for ASRB database queries. MM was supported by a Cooperative Research Centre for Mental Health Top-up Scholarship. SS was supported by One-in-Five Association Incorporated. CSW is supported by Schizophrenia Research Institute (utilizing infrastructure funding from the NSW Ministry of Health and the Macquarie Group Foundation), the University of New South Wales, and Neuroscience Research Australia. CSW is a recipient of a National Health and Medical Research Council (Australia) Principal Research Fellowship (PRF) (#1117079). CP was supported by an NHMRC Senior Principal Research Fellowship (628386 and 1105825), and a Brain and Behavior Research Foundation (NARSAD) Distinguished Investigator Award. CB was supported by a NHMRC Career Development Fellowship (1127700) and Brain and Behavior Research Foundation (NARSAD) Young Investigator Award (20526). None of the funding sources played any role in the study design; collection, analysis, or interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/article/10.3389/fpsyt.2017.00225/full#supplementary-material.

REFERENCES

1. Fazzari P, Paternain AV, Valiente M, Pla R, Lujan R, Lloyd K, et al. Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. *Nature* (2010) 464(7293):1376–80. doi:10.1038/nature08928
2. Barros CS, Calabrese B, Chamero P, Roberts AJ, Korzus E, Lloyd K, et al. Impaired maturation of dendritic spines without disorganization of cortical cell layers in mice lacking NRG1/ErbB signaling in the central nervous system. *Proc Natl Acad Sci U S A* (2009) 106(11):4507–12. doi:10.1073/pnas.0900355106
3. Nave KA. Myelination and support of axonal integrity by glia. *Nature* (2010) 468(7321):244–52. doi:10.1038/nature09614
4. Snaidero N, Mobius W, Czopka T, Hekking LH, Mathisen C, Verkleij D, et al. Myelin membrane wrapping of CNS axons by PI(3,4,5)P3-dependent polarized growth at the inner tongue. *Cell* (2014) 156(1–2):277–90. doi:10.1016/j.cell.2013.11.044
5. Mei L, Nave KA. Neuregulin-ERBB signaling in the nervous system and neuropsychiatric diseases. *Neuron* (2014) 83(1):27–49. doi:10.1016/j.neuron.2014.06.007
6. Mei L, Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat Rev Neurosci* (2008) 9(6):437–52. doi:10.1038/nrn2392
7. Chen YJ, Zhang M, Yin DM, Wen L, Ting A, Wang P, et al. ErbB4 in parvalbumin-positive interneurons is critical for neuregulin 1 regulation of long-term potentiation. *Proc Natl Acad Sci U S A* (2010) 107(50):21818–23. doi:10.1073/pnas.1010669107
8. Li B, Woo RS, Mei L, Malinow R. The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity. *Neuron* (2007) 54(4):583–97. doi:10.1016/j.neuron.2007.03.028
9. Buonanno A. The neuregulin signaling pathway and schizophrenia: from genes to synapses and neural circuits. *Brain Res Bull* (2010) 83(3–4):122–31. doi:10.1016/j.brainresbull.2010.07.012
10. Mostaid MS, Lloyd D, Liberg B, Sundram S, Pereira A, Pantelis C, et al. Neuregulin-1 and schizophrenia in the genome-wide association study era. *Neurosci Biobehav Rev* (2016) 68:387–409. doi:10.1016/j.neubiorev.2016.06.001
11. Iwakura Y, Nawa H. ErbB1-4-dependent EGF/neuregulin signals and their cross talk in the central nervous system: pathological implications in schizophrenia and Parkinson's disease. *Front Cell Neurosci* (2013) 7:4. doi:10.3389/fncel.2013.00004
12. Aston C, Jiang L, Sokolov BP. Microarray analysis of postmortem temporal cortex from patients with schizophrenia. *J Neurosci Res* (2004) 77(6):858–66. doi:10.1002/jnr.20208
13. Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR. Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Mol Psychiatry* (2004) 9(3):299–307. doi:10.1038/sj.mp.4001434
14. Silberberg G, Darvasi A, Pinkas-Kramarski R, Navon R. The involvement of ErbB4 with schizophrenia: association and expression studies. *Am J Med Genet B Neuropsychiatr Genet* (2006) 141B(2):142–8. doi:10.1002/ajmg.b.30275
15. Law AJ, Kleinman JE, Weinberger DR, Weickert CS. Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. *Hum Mol Genet* (2007) 16(2):129–41. doi:10.1093/hmg/ddl449
16. Law AJ, Wang Y, Sei Y, O'Donnell P, Piantadosi P, Papaleo F, et al. Neuregulin 1-ErbB4-PI3K signaling in schizophrenia and phosphoinositide 3-kinase-p110delta inhibition as a potential therapeutic strategy. *Proc Natl Acad Sci U S A* (2012) 109(30):12165–70. doi:10.1073/pnas.1206118109
17. Joshi D, Fullerton JM, Weickert CS. Elevated ErbB4 mRNA is related to interneuron deficit in prefrontal cortex in schizophrenia. *J Psychiatr Res* (2014) 53:125–32. doi:10.1016/j.jpsychires.2014.02.014
18. Petryshen TL, Middleton FA, Kirby A, Aldinger KA, Purcell S, Tahl AR, et al. Support for involvement of neuregulin 1 in schizophrenia pathophysiology. *Mol Psychiatry* (2005) 10(4):366–74, 28. doi:10.1038/sj.mp.4001608

19. Zhang HX, Li WQ, Zhang HS, Zhang Y, Zhao JP, Lv LX, et al. [Expressional changes of neuregulin-1 gene mRNA in peripheral blood from schizophrenia patients]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* (2011) 28(6):620–4. doi:10.3760/cma.j.issn.1003-9406.2011.06.005
20. Zhang HX, Zhao JP, Lv LX, Li WQ, Xu L, Ouyang X, et al. Explorative study on the expression of neuregulin-1 gene in peripheral blood of schizophrenia. *Neurosci Lett* (2008) 438(1):1–5. doi:10.1016/j.neulet.2007.09.051
21. Futamura T, Toyooka K, Iritani S, Niizato K, Nakamura R, Tsuchiya K, et al. Abnormal expression of epidermal growth factor and its receptor in the forebrain and serum of schizophrenic patients. *Mol Psychiatry* (2002) 7(7):673–82. doi:10.1038/sj.mp.4001081
22. Liu L, Luo Y, Zhang G, Jin C, Zhou Z, Cheng Z, et al. The mRNA expression of DRD2, PI3KCB, and AKT1 in the blood of acute schizophrenia patients. *Psychiatry Res* (2016) 243:397–402. doi:10.1016/j.psychres.2016.07.010
23. Xu Y, Yao Shugart Y, Wang G, Cheng Z, Jin C, Zhang K, et al. Altered expression of mRNA profiles in blood of early-onset schizophrenia. *Sci Rep* (2016) 6: 16767. doi:10.1038/srep16767
24. Mostaid MS, Lee T, Chana G, Sundram S, Weickert CS, Pantelis CP, et al. Elevated peripheral expression of neuregulin-1 (NRG1) mRNA isoforms in clozapine-treated schizophrenia patients. *Transl Psychiatry* (2017).
25. Howes OD, McCutcheon R, Agid O, de Bartolomeis A, van Beveren NJ, Birnbaum ML, et al. Treatment-resistant schizophrenia: treatment response and resistance in psychosis (TRRIP) working group consensus guidelines on diagnosis and terminology. *Am J Psychiatry* (2017) 174(3):216–29. doi:10.1176/appi.ajp.2016.16050503
26. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* (1998) 59(Suppl 20):22–33; quiz 4–57.
27. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* (1987) 13(2):261–76. doi:10.1093/schbul/13.2.261
28. Wallwork RS, Fortgang R, Hashimoto R, Weinberger DR, Dickinson D. Searching for a consensus five-factor model of the positive and negative syndrome scale for schizophrenia. *Schizophr Res* (2012) 137(1–3):246–50. doi:10.1016/j.schres.2012.01.031
29. American Psychiatric Association. Practice guideline for the treatment of patients with schizophrenia. American Psychiatric Association. *Am J Psychiatry* (1997) 154(4 Suppl):1–63. doi:10.1176/ajp.154.4.1
30. Woods SW. Chlorpromazine equivalent doses for the newer atypical antipsychotics. *J Clin Psychiatry* (2003) 64(6):663–7. doi:10.4088/JCP.v64n0607
31. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* (2013) 310(20):2191–4. doi:10.1001/jama.2013.281053
32. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* (2009) 55(4):611–22. doi:10.1373/clinchem.2008.112797
33. Benjamini Y, Hochberg Y. Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J Roy Stat Soc B Met* (1995) 57(1):289–300.
34. Hedges LV, Olkin I. *Statistical Methods for Meta-Analysis*. (Vol. xxii). Orlando: Academic Press (1985). 369 p.
35. Jernigan CS, Goswami DB, Austin MC, Iyo AH, Chandran A, Stockmeier CA, et al. The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* (2011) 35(7):1774–9. doi:10.1016/j.pnpbp.2011.05.010
36. Hoeffer CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci* (2010) 33(2):67–75. doi:10.1016/j.tins.2009.11.003
37. Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev* (2004) 18(16):1926–45. doi:10.1101/gad.1212704
38. Gong R, Park CS, Abbassi NR, Tang SJ. Roles of glutamate receptors and the mammalian target of rapamycin (mTOR) signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. *J Biol Chem* (2006) 281(27):18802–15. doi:10.1074/jbc.M512524200
39. Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, Mayeur GL, et al. Phosphorylation of eucaryotic translation initiation factor 4B Ser422 is modulated by S6 kinases. *EMBO J* (2004) 23(8):1761–9. doi:10.1038/sj.emboj.7600193
40. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* (2009) 10(5):307–18. doi:10.1038/nrm2672
41. Bradshaw NJ, Porteous DJ. DISC1-binding proteins in neural development, signalling and schizophrenia. *Neuropharmacology* (2012) 62(3):1230–41. doi:10.1016/j.neuropharm.2010.12.027
42. Abi-Dargham A, Laruelle M, Aghajanian GK, Charney D, Krystal J. The role of serotonin in the pathophysiology and treatment of schizophrenia. *J Neuropsychiatry Clin Neurosci* (1997) 9(1):1–17. doi:10.1176/jnp.9.1.1
43. Gururajan A, van den Buuse M. Is the mTOR-signalling cascade disrupted in schizophrenia? *J Neurochem* (2014) 129(3):377–87. doi:10.1111/jnc.12622
44. Green MJ, Matheson SL, Shepherd A, Weickert CS, Carr VJ. Brain-derived neurotrophic factor levels in schizophrenia: a systematic review with meta-analysis. *Mol Psychiatry* (2011) 16(9):960–72. doi:10.1038/mp.2010.88
45. Lu ML, Chen TT, Kuo PH, Hsu CC, Chen CH. Effects of adjunctive fluvoxamine on metabolic parameters and psychopathology in clozapine-treated patients with schizophrenia: a 12-week, randomized, double-blind, placebo-controlled study. *Schizophr Res* (2017). doi:10.1016/j.schres.2017.06.030
46. Kumarasinghe N, Beveridge NJ, Gardiner E, Scott RJ, Yasawardene S, Perera A, et al. Gene expression profiling in treatment-naive schizophrenia patients identifies abnormalities in biological pathways involving AKT1 that are corrected by antipsychotic medication. *Int J Neuropsychopharmacol* (2013) 16(7):1483–503. doi:10.1017/S1461145713000035
47. Yan L, Shamir A, Skirzewski M, Leiva-Salcedo E, Kwon OB, Karavanova I, et al. Neuregulin-2 ablation results in dopamine dysregulation and severe behavioral phenotypes relevant to psychiatric disorders. *Mol Psychiatry* (2017). doi:10.1038/mp.2017.22
48. Keri S, Szabo C, Kelemen O. Uniting the neuro developmental and immunological hypotheses: neuregulin 1 receptor ErbB and toll-like receptor activation in first-episode schizophrenia. *Sci Rep* (2017) 7(1):4147. doi:10.1038/s41598-017-03736-3
49. Harris LW, Pietsch S, Cheng TM, Schwarz E, Guest PC, Bahn S. Comparison of peripheral and central schizophrenia biomarker profiles. *PLoS One* (2012) 7(10):e46368. doi:10.1371/journal.pone.0046368
50. Facciola G, Avenoso A, Scordo MG, Madia AG, Ventimiglia A, Perucca E, et al. Small effects of valproic acid on the plasma concentrations of clozapine and its major metabolites in patients with schizophrenic or affective disorders. *Ther Drug Monit* (1999) 21(3):341–5. doi:10.1097/00007691-199906000-00017
51. Zoccali R, Muscatello MR, Torre DL, Malara G, Canale A, Crucitti D, et al. Lack of a pharmacokinetic interaction between mirtazapine and the newer antipsychotics clozapine, risperidone and olanzapine in patients with chronic schizophrenia. *Pharmacol Res* (2003) 48(4):411–4. doi:10.1016/S1043-6618(03)00178-6
52. Hiemke C, Dragicevic A, Grunder G, Hatter S, Sachse J, Vernaleken I, et al. Therapeutic monitoring of new antipsychotic drugs. *Ther Drug Monit* (2004) 26(2):156–60. doi:10.1097/00007691-200404000-00012

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2017 Mostaid, Lee, Chana, Sundram, Shannon Weickert, Pantelis, Everall and Bousman. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

CLINICAL TRIAL
published: 26 February 2018
doi: 10.3389/fpsyt.2018.00040

Randomized Clinical Trial with e-MotionalTraining® 1.0 for Social Cognition Rehabilitation in Schizophrenia

*Yolanda Maroño Souto[1], Miriam Vázquez Campo[2], Francisco Díaz Llenderrozas[3], Marina Rodríguez Álvarez[1], Raimundo Mateos[4] and Alejandro García Caballero[2,4]**

[1]Department of Psychobiology and Clinical Psychology, Psychology School, University of Santiago de Compostela, Santiago de Compostela, Spain, [2]Department of Psychiatry, Instituto Biomédico Galicia Sur, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Complexo Hospitalario Universitario de Ourense, Ourense, Spain, [3]Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain, [4]Department of Psychiatry, School of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain

OPEN ACCESS

Edited by:
Felice Iasevoli, University of Naples Federico II, Italy

Reviewed by:
William Horan, University of California, Los Angeles, United States
Philip D. Harvey, Leonard M. Miller School of Medicine, United States

***Correspondence:**
Alejandro García Caballero
alejandro.alberto.garcia.caballero@sergas.es

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *29 August 2017*
Accepted: *30 January 2018*
Published: *26 February 2018*

Citation:
Maroño Souto Y, Vázquez Campo M, Díaz Llenderrozas F, Rodríguez Álvarez M, Mateos R and García Caballero A (2018) Randomized Clinical Trial with e-MotionalTraining® 1.0 for Social Cognition Rehabilitation in Schizophrenia. Front. Psychiatry 9:40. doi: 10.3389/fpsyt.2018.00040

Background: Schizophrenia patients present deficits in social cognition (SC), emotion and social perception, theory of mind (ToM), and attributional style. This study tested the efficacy, in real clinical conditions, of a online self-training program in SC, e-Motional Training®, in comparison with treatment as usual.

Method: A randomized single-blinded multicenter clinical trial was conducted with 60 schizophrenia stable outpatients. All patients (control and intervention) were treated with drug therapy, case management, and individual and group psychotherapy (not focused on SC). Intervention group was treated with e-Motional Training®, an online program devised for SC rehabilitation.

Statistical analysis: A descriptive analysis and parametric/non-parametric tests were used to compare both groups at baseline. Analysis of covariance was used to compared post–pre changes in SC between the two interventions. If the group effect was significant, follow-up univariate test (*t*-test for dependent samples) was carried out in each group to verify whether the effect was due to improvement in the intervention group or deterioration in the control group. We considered statistically significant differences with $P < 0.05$.

Results: Significant improvements were obtained in the intervention group in emotion recognition and most ToM variables in comparison with the control group.

Discussion: *e-Motional Training®* seems to be a promising online training tool for SC deficits in schizophrenia, covering the lack of similar intervention instruments in our community.

Keywords: cognition, emotional adjustment, theory of mind, schizophrenia, emocional perception

BACKGROUND

Social cognition (SC) is defined as the mental operations that underpin perceiving, interpreting, and generating responses during social interactions, including the intentions, dispositions, and behaviors of others (1). The Social Cognition Psychometric Evaluation study identified four core domains of SC, namely emotion recognition (ER), social perception, theory of mind (ToM)/mental state attribution, and attributional style (AS)/bias (2).

In schizophrenia, negative symptoms have been associated with poor performance in SC (3). In particular, individuals with schizophrenia show deficiencies in ER compared with non-clinical participants (4, 5), and these difficulties are significantly associated with symptom severity (6). These limitations are primarily manifested in the identification of negative valence emotions, especially the emotion of fear (7–10). Longitudinal studies have shown that these difficulties are stable over the course of the disease (11, 12) although there is evidence that individuals in the remission phase perform better on ER tests than individuals who are in the acute phase of the disorder (6). These difficulties are also considered to have a moderate association with social functioning of hospitalized patients (13) in comparison with outpatients (14).

Moreover, difficulties in ToM have been associated with negative symptoms, passivity, behavioral disorders, and paranoid symptoms (3, 15, 16). Studies have found that greater hostile attributions (e.g., increased tendency to report guilt/hostility/aggression in response to ambiguous social situations) correlate with higher levels of positive symptoms, anxiety, depression, and general emotional discomfort (17, 18).

In schizophrenia, these difficulties are associated with poorer social functioning (19), fewer social relationships, and poorer quality of life (5, 20). Various research studies have found that SC serves as a mediator between neurocognition and functional results (21) and determines the quality of interpersonal interactions, which facilitates the enjoyment of recreational activities for individuals with schizophrenia (22–25). SC is considered a predictor of social functioning even more relevant than neurocognition (19). However, these difficulties are not restricted to schizophrenia but are also observed in other severe mental disorders (26–28).

Patients with schizophrenia often report these difficulties. Therefore, there is a urgent need to find new treatment strategies to enable individuals with schizophrenia to improve these skills (29), given that drug treatments (typical and atypical antipsychotics) generally only have a marginal impact on the domains that constitute SC and social functioning (30). Conversely, there is evidence that SC in schizophrenia can be improved through psychosocial intervention (29, 31–34).

In view of the significant impact of social cognitive deficits on daily functioning, many interventions have been developed over the past decade to ameliorate social cognitive deficits. Some interventions using virtual reality, cognitive behavioral techniques, and errorless learning in social skills training show positive results in social functioning, but without specifically targeting SC (35–38). Targeted interventions hold much promise for improving SC, particularly ER and ToM. Improvement in ER has been reported, particularly in facial affect recognition. Most of these targeted interventions, such as Training of Affect Recognition (39), Attention Shaping, or MicroExpression Training Tool (40), focus primarily on training affect recognition with good outcomes. ToM is the second most commonly targeted domain, with Mental-State Reasoning Training for Social Cognitive Impairment (SoCog-MSRT) (41), Mary Eddie Bill (MEB) (42), Emotion and ToM Imitation (43), and Theory of Mind Intervention (44) developed to provide effective in-depth training, but with contradictory results in this domain (45). AS is only specifically targeted in SoCog-MSRT and MEB. Social perception and AS appear to be more difficult to measure and train, as evidenced by a meta-analysis that showed no significant effects on these two domains after social cognitive training (46).

Besides video clips, cartoon comic strips, and photographs, computerized online social cognitive games and virtual reality have recently been utilized with high patient satisfaction (36, 37, 47). Specifically, virtual reality has been used for social skills training, but its application has not been yet oriented toward SC training.

e-Motional Training® 1.0 (ET) allows online self-training and stores the data of each individual session. ET is designed following the basic principles of neuropsychological rehabilitation in this domain (48–50). The program aims to deliver realistic and natural but attractive exercises of short duration without irrelevant stimuli or distractions, while offering continuous feedback. ER tasks are designed with increasing difficulty, starting with tutorials, following with eyes and mouths recognition and finally scaling to microexpression training. An animated short film with 33 scenes is the vehicle for ToM, social perception, and AS stories. After each scene, a series of questions including ToM, AS, and control questions are posed. When the answer is incorrect, the patient receives metacognitive suggestions, which lead the user to think about the situation from a different perspective or prompts the user to pay attention to specific aspects of the film.

The program was composed of 12 1-h sessions (the minimum number of face-to-face sessions reported in previous studies).

Our hypothesis was that intervention with treatment as usual (TAU) + ET results in greater improvements in the main domains of SC and the measures of social functioning compared with TAU.

The aim of this study is therefore to assess the possible effects of a new SC training program, *e-Motional Training*® 1.0 (ET), in ER, ToM, AS, and social functioning.

METHOD

A randomized, multicenter, single-blind clinical trial was performed. Sixty patients with schizophrenia were recruited in Psychiatric Day Hospitals at Ourense, Coruña and Vigo and in Associations of Persons and Families with Mental Illness at Vigo, Santiago de Compostela, Coruña and Ourense. After recruitment, the sample was randomized in each center into two balanced groups.

Inclusion and Exclusion Criteria

We included patients who voluntarily agreed to participate in the study, aged 18–50 years with a diagnosis of schizophrenia (DSM-IV TR), who were clinically stable (no acute psychotic symptoms and not hospitalized during the last 3 months), and who had no comorbidity with other psychiatric or neurological diseases (International Neuropsychiatric Interview-MINI) and excluding current substance abuse (except nicotine).

Treatment Conditions

Control Group (TAU)

All patients received drug therapy, case management, and individual and group psychotherapy not focused on social cognitive rehabilitation.

Intervention Group (TAU + ET)

The intervention group received the same intervention of control group plus 12 sessions (1 h per week) with ET®. All participants in the intervention group completed the same number of sessions. To start the intervention, the patient accessed the website www.e-motionaltraining.com (version 1.0) and registered with a username and password. The first four meetings (1 h each session) were dedicated to recognizing facial emotions. This section included a pretest and posttest, tutorials, and scaling minigames starting with eyes and mouths and finally microexpression (<250 ms) training. The next eight sessions (1 h each) include watching a short, interactive animated cartoon in which a couple invites their friends to their home for a party. As the story unfolds, instances of miscommunication occur among the actors, causing various emotions and mental conditions such as anger, affection, appreciation, and jealousy. After each scene, the user is queried about what happened, with questions about ToM (interpreting irony, insinuations, faux pas, second-order false beliefs, etc.), social perception (interpretation and analysis of the social situation through the visual content of each scene), and AS (the individuals' attributions to the events, and questions such as, "What kind of thinking would result in Cristina getting better results in this situation?"), as well as control questions. The game provides user feedback and, in the event of errors, can display a hyperlink with information and metacognitive strategies, whose objective is to help users understand the scene that they just watched.

Supervision of the ET group was conducted by the center's staff as a routine activity, and evaluators were blind to the assignment. No help or guidance regarding social cognitive issues was given, and only advice regarding computer use was provided.

Measurements

Symptoms and Cognitive Ability

Positive and Negative Symptom Scale (PANSS)

Positive and Negative Symptom Scale assesses positive and negative symptom severity (51). The scale consists of 30 items (symptoms) that are scored from 1 (absent) to 7 (extreme). The scale has three subscales: *positive* (PANSS-P), *negative* (PANSS-N), and *general psychopathology* (PANSS-GP).

Kaufman Brief Intelligence Test (K-BIT)

Kaufman Brief Intelligence Test provides a verbal intelligence quotient (IQ), a non-verbal IQ, and a compound IQ that summarizes the total performance on the test (52).

Social Cognition

Ekman 60 Faces Test

The test contains 60 photographs of faces with expressions of the 6 basic emotions: anger, disgust, sadness, fear, surprise, and happiness (53). An overall score of 60 indicates the best possible performance, and each basic emotion also has a maximum score of 10 points.

Hinting Task

Ten stories are presented to the patient who must infer the characters actual intention when using indirect speech (54). The total score on the test ranges from 0 to 20 (55).

Recognition of Faux Pas

The participant must recognize the embarrassing situations in the 10 *faux pas*' stories, while correctly rejecting misinterpretation of the 10 control situations (56). The test provides scores for five variables: *faux pas* detection, understanding inappropriateness, intentions, and belief and empathy (57).

F. Happé's Strange Stories

F. Happé's Strange Stories include stories containing irony and white lies utterances (58). In each of the stories, the character says something that should not be interpreted literally. The participant is asked to explain why the characters said what they said.

Movie for the Assessment of Social Cognition (MASC)

A short film is shown to the participant who must answer a series of questions regarding the ToM and emotional content depicted in social interactions (59).

Ambiguous Intentions Hostility Questionnaire (AIHQ)

The AIHQ is an AS questionnaire to measure the biases of hostility perception, composite blame, and aggressive response (60). The AIHQ is composed of 15 hypothetical negative situations. Each situation was varied in intentionality: five scenarios are accidental (e.g., "You're dancing at a club and someone bumps into you from behind."), five scenarios are ambiguous (e.g., "You walk past a bunch of teenagers at a mall and your hear them start to laugh."), and five scenarios are intentional (e.g., "Your neighbors are playing loud music. You knock on the door and ask them to turn it down. Fifteen minutes later, the music is loud again."). First, participants are prompted to imagine the scenario happening to them. Then, they are asked to write down what is the reason they think that other person (or persons) acted that way. The AIHQ yielded hostility perception and aggressive response bias scores and a composite blame bias score. The scales for the hostility perception and aggressive response indices were rated by rater from 1 ("not at all hostile") to 5 ("very hostile") and 1 ("not at all aggressive") to 5 ("very aggressive"), respectively. The composite blame score (range, 1–5.3) is an average score of subjects' ratings of intent (range, 1–6; rating about the degree to which the other person committed the act on purpose), anger (range, 1–5; rating about how angry the situation would make subject feel), and blame (range, 1–5; rating about how much subjects blame the other person for the outcome).

Emotional Intelligence

Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT)

This test is composed of 141 items and provides a score for emotional intelligence (EIQ), which in turn can be divided into two domains: experiential (EEIQ) and strategic emotional intelligence (SEIQ) (61). The test also provides scores for four areas of emotional intelligence: the ability to perceive emotions accurately (PEIQ), using emotions to facilitate thought (emotional facilitation, FEIQ), understanding emotions (UEIQ), and managing emotions (MEIQ).

Social Functioning

Social Functioning Scale (SFS)

This scale is specifically designed to assess the social functioning of individuals with schizophrenia (62). The scale consists of seven

subscales: social isolation/interaction, interpersonal communication, independence-execution, independence-competence, free time, prosocial activities, and employment/occupation. We applied the self-reported version (SFS-SR).

Sample Size

In our pilot study (63), the measure with most reduced differences pre–post intervention was Happé's Strange Stories with an initial mean (±SD) of 8.20 (±3.58) that increased to 11.20 (±4.68) after intervention. By using these measures for a power of 80% and a confidence level of 95%, the required sample size, assuming 5% of losses, was 30 patients in each group.

Ethical Aspects

This study has been carried out in accordance with national and European legislation on clinical research, following international ethical recommendations, the Declaration of Helsinki, and the Council of Europe with regard to the Convention on Human Rights and Biomedicine. The study has complied at all times with the requirements established in the Spanish legislation in the field of biomedical research, personal data protection, and bioethics. This study was approved by the local ethics committee (Comité de Etica e Investigación Clínica de Galicia) (Registration code: 2014/459) and registered in an international RCT database (BioMed Center: ISRCTN83459317).

Statistical Analysis

Quantitative Gaussian variables were described by mean, SD, and not Gaussian variables as median (range). The qualitative variables were described by frequencies and percentages (%). Parametric/non-parametric tests (Chi square for categorical variables and Student's *t*-test and Mann–Whitney *U* test for continuous variables) were used to compare both groups at baseline.

We compared post–pre changes in SC between the two interventions (TAU + e-Motional Training® vs. TAU) with an analysis of covariance (ANCOVA), entering the change scores on each test (Ekman, Faux Pas, Happé, Hinting, MASC, MSCEIT, and AIHQ) as the dependent variable, treatment as the fixed group effect, and K-BIT score as the covariate.

If the group effect was significant, follow-up univariate test (*t*-test for dependent samples) was carried out in each group to verify whether the effect was due to improvement in the intervention group or deterioration in the control group.

We considered statistically significant differences with $P < 0.05$. The sample size was calculated using the Epidat 4.1, and the analyses were performed with SPSS 22.0 and R (http://www.r-project.org).

RESULTS

A total of 77 participants were selected, 15 patients did not meet inclusion criteria, and 1 suffered a relapse prior to randomization. Finally, 61 patients were assigned to the control group (TAU) or to the intervention group (TAU + ET) between January and November 2015 (**Figure 1**). Prior to retest one patient in the control group abandoned the study and was excluded for further analysis.

Most of the patients recruited were men 47 (78.3%), with a mean (±SD) global age of 39.17 years (±7.03).

There were no significant differences between the two groups at baseline in sociodemographic variables (age, gender, and education) compound IQ ($P = 0.385$) and non-verbal IQ ($P = 0.143$) measured with K-BIT. However, significant differences were observed in the verbal IQ ($P = 0.042$) being the scores in both groups within the normality range (**Table 1**).

All participants were treated with antipsychotics, with a mean chlorpromazine dose 634.82 (±513.01) in control group and 564.74 (±340.19) in the intervention group. There were no significant differences between them ($P = 0.807$).

To demonstrate the existence of differences in SC variables after treatment, we compared post–pre changes between the two interventions (TAU + e-Motional Training® vs. TAU) with an ANCOVA, entering the change scores on each test (Ekman, Faux Pas, Happé, Hinting, MASC, MSCEIT, and AIHQ) as the dependent variable, treatment as the fixed group effect, and K-Bit score as the covariate. ANCOVA results are displayed in **Table 2**. These results indicate that there were statistically significant differences in change scores between e-Motional Training® and TAU group in Ekman's ($F = 48.805$, $P < 0.001$) with a large effect size ($\eta_p^2 = 0.461$), Faux Pas ($F = 9.728$; $P = 0.003$) with a large size effect ($\eta_p^2 = 0.146$), Happé ToM ($F = 9.447$; $P = 0.003$) with a large effect size ($\eta_p^2 = 0.142$), Hinting ($F = 14.286$; $P < 0.001$) with a large effect size ($\eta_p^2 = 0.200$), MASC change score ($F = 12.466$; $P = 0.001$) with a large size effect ($\eta_p^2 = 0.179$), and PANSS negative change score ($F = 5.169$; $P = 0.027$) with a moderate size effect ($\eta_p^2 = 0.083$). No differences were found in Faux Pas and Happé control stories change scores nor in MSCEIT or PANSS positive change scores. Finally, regarding the Ambiguous Stories of AIHQ, only differences in aggressive bias ($F = 4.405$; $P = 0.04$) were significant with a moderate size effect ($\eta_p^2 = 0.072$).

Subsequently follow-up univariate tests (*t*-test for dependent samples) were carried out in ANCOVA's significant variables confirming that the effect was due to improvement in the intervention group and not to deterioration in the control group ($P < 0.001$). Changes in PANSS negative ($P = 0.001$) and AIHQ aggressive bias ($P = 0.018$) were also due to improvement in the intervention group.

There were no differences in the seven variables of the SFS-SR.

DISCUSSION

One of the main objectives of cognitive therapy in schizophrenia is to improve social functioning. In this regard, SC programs seem more promising than those directed at neurocognition (29). However, during the last decade, SC rehabilitation has been delivered in group format requiring a significant number of sessions and specialized training for the therapists, therefore limiting its accessibility (64). Bearing these questions in mind, our team designed ET showing its feasibility in a pilot study (32). This study is the first randomized controlled trial conducted with this program.

After treatment, the intervention group showed a significant improvement in ER (**Table 2**) reaching scores posttreatment within the normal range (65), this result is consistent with other interventions (40, 66–68).

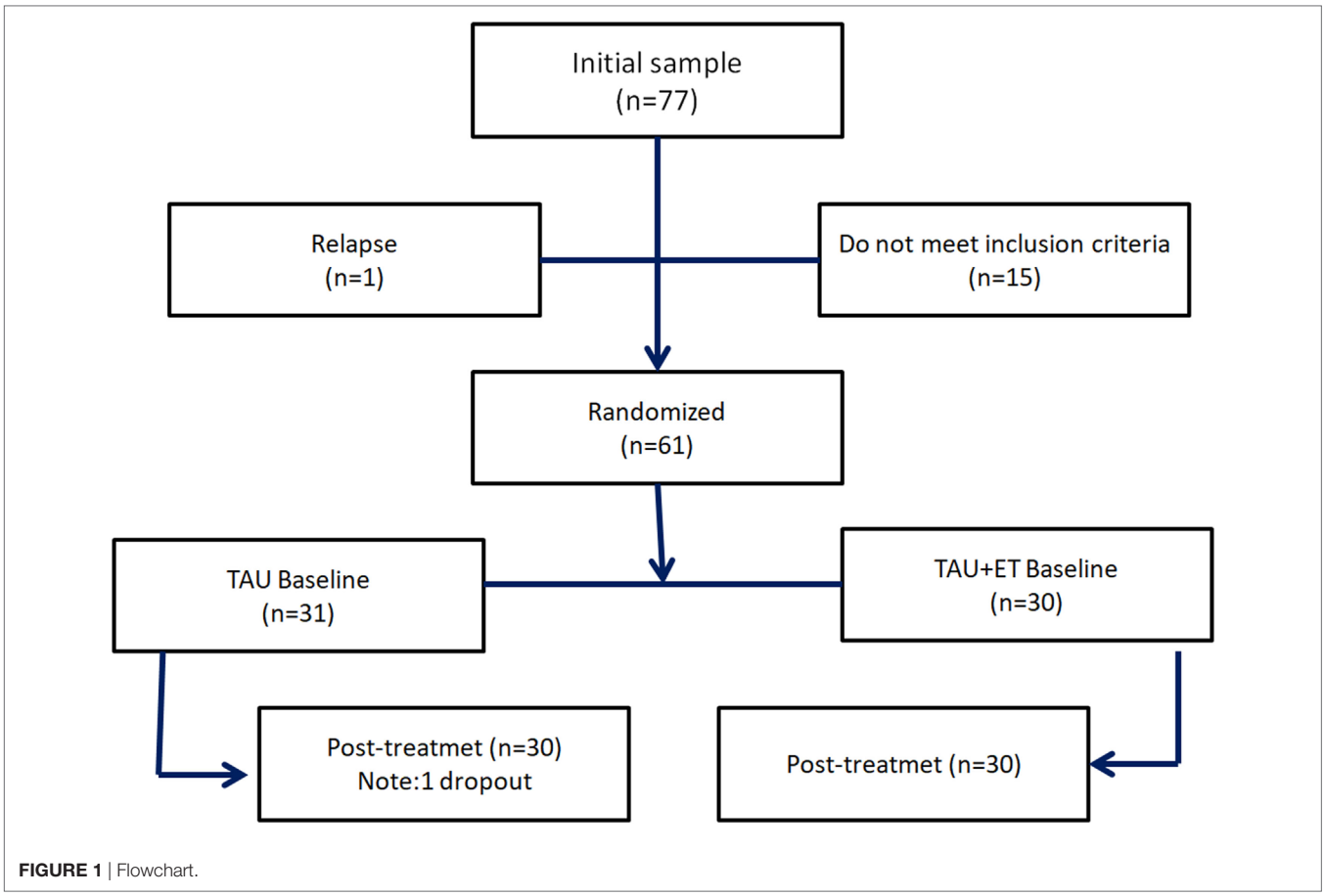


FIGURE 1 | Flowchart.

TABLE 1 | Demographic and clinical characteristics of sample ($N = 60$).

	TAU	TAU + ET	Global	*P* value
Gender, *n* (%)				
Male	23 (76.7)	24 (80.0)	47 (78.3)	0.754
Female	7 (23.3)	6 (20.0)	13 (21.7)	
Education, *n* (%)				
Primary	15 (50.0)	11 (36.7)	26 (43.3)	0.297
Secondary	15 (50.0)	19 (63.3)	34 (56.07)	
Continuous variables, mean (±SD)				
Age (years)	39.87 (±6.12)	38.47 (±7.88)	39.17 (±7.03)	0.445[a]
Age at first hospitalization (years)	24.93 (±7.20)	23.48 (±7.20)	24.22 (±7.18)	0.443[a]
Medication dose (chlorpromazine)	634.82 (±513.01)	564.74 (±340.19)	599.78 (±433.00)	0.807[a]
Lifetime number of hospitalizations, median (range)	2 (0–11)	1 (0–12)	1 (0–12)	0.331[b]
PANSS				
PANSS-P	13.13 (±5.43)	15.83 (±6.74)	14.48 (±6.22)	0.093[a]
PANSS-N	18.60 (±8.11)	17.67 (±9.12)	18.13 (±8.57)	0.677[a]
IQ (K-BIT)				
Overall IQ	95.30 (±12.80)	101.60 (±37.30)	98.45 (±27.83)	0.385[a]
Verbal	95.47 (±20.28)	104.50 (±12.16)	99.98 (±17.19)	0.042[a]
Non-verbal	83.17 (±24.45)	91.20 (±16.76)	87.18 (±21.17)	0.143[a]

Data are represented as n (%) for categorical variables, mean (±SD), and median (range). P value: Chi square test.
[a]*Student's t-test.*
[b]*Mann–Whitney U test.*

Regarding ToM, the intervention group showed significant improvements at Faux Pas, Happé's Strange Stories, Hinting Task, and MASC (**Table 2**). However, even with this improvement, our intervention group did not achieve the level of competence of the healthy population, as was found in other studies (32, 33, 69). Nevertheless, our study indicates that online rehabilitation of

TABLE 2 | Results in social cognition variables.

	TAU		TAU + ET		*F*	*P* value	Effect size η_p^2
	Mean (IC 95%)	SD	Mean (IC 95%)	SD			
Ekman pre	41.23 (38.66 to 43.80)	6.89	42.77 (40.76 to 44.77)	5.36			
Ekman post	41.03 (38.17 to 43.90)	7.68	50.57 (48.15 to 52.99)	6.48			
Ekman change	−0.20 (−1.87 to 1.47)	4.48	7.80 (6.12 to 9.48)	4.49	48.805	<0.001	0.461
Faux Pas HC pre	18.53 (17.86 to 19.21)	1.81	16.53 (15.19 to 17.88)	3.60			
Faux Pas HC post	18.47 (17.62 to 19.31)	2.27	17.60 (16.55 to 18.65)	2.80			
Faux Pas HC Change	−0.07 (−0.81 to 0.68)	2.00	1.07 (0.29 to 1.84)	2.08	4.022	0.050	0.066
Faux Pas pre	27.37 (21.50 to 33.23)	15.71	28.97 (23.72 to 34.21)	14.05			
Faux Pas post	28.47 (22.61 to 34.32)	15.69	37.10 (31.63 to 42.57)	14.64			
Faux Pas change	1.10 (−1.51 to 3.71)	6.98	8.13 (4.60 to 11.67)	9.47	9.728	0.003	0.146
Happè TOM pre	8.63 (7.10 to 10.17)	4.12	8.13 (6.86 to 9.40)	3.40			
Happè TOM post	9.20 (7.64 to 10.76)	4.17	10.80 (9.38 to 12.22)	3.81			
Happè TOM change	0.57 (−0.36 to 1.49)	2.47	2.67 (1.58 to 3.75)	2.90	9.447	0.003	0.142
Happè HC pre	9.07 (7.65 to 10.48)	3.79	8.77 (7.56 to 9.97)	3.22			
Happè HC post	10.00 (8.66 to 11.34)	3.59	10.60 (9.42 to 11.78)	3.16			
Happè HC change	0.93 (−0.07 to 1.94)	2.69	1.83 (0.75 to 2.92)	2.90	1.703	0.197	0.029
Hinting pre	15.23 (14.12 to 16.35)	2.98	13.60 (11.80 to 15.40)	4.83			
Hinting post	15.60 (14.43 to 16.77)	3.14	16.63 (15.15 to 18.12)	3.98			
Hinting change	0.37 (−0.32 to 1.06)	1.85	3.03 (1.79 to 4.28)	3.34	14.286	<0.001	0.200
MASC pre	21.97 (19.71 to 24.23)	6.05	23.17 (21.47 to 24.87)	4.55			
MASC post	21.97 (19.81 to 24.12)	5.77	26.23 (24.28 to 28.19)	5.23			
MASC change	0.00 (−1.22 to 1.22)	3.26	3.07 (1.84 to 4.30)	3.29	12.466	0.001	0.179
MSCIT pre	91.80 (86.76 to 96.84)	13.50	94.83 (90.07 to 99.58)	12.50			
MSCIT post	90.80 (86.06 to 95.54)	12.69	95.60 (91.14 to 100.06)	11.94			
MSCIT change	−1.00 (−3.95 to 1.95)	7.91	0.03 (−2.55 to 2.62)	6.80	0.315	0.577	0.006
PANSS-P pre	13.13 (11.11 to 15.16)	5.42	15.83 (13.32 to 18.35)	6.74			
PANSS-P post	12.03 (10.18 to 13.88)	4.95	14.77 (12.28 to 17.25)	6.65			
PANSS-P change	−1.10 (−1.63 to−0.57)	1.42	−1.07 (−2.56 to 0.42)	3.99	0.002	0.967	<0.001
PANSS-N pre	18.60 (15.57 to 21.63)	8.11	17.67 (14.26 to 21.07)	9.12			
PANSS-N post	17.50 (14.65 to 20.35)	7.63	13.87 (11.39 to 16.34)	6.63			
PANSS-N change	−1.10 (−2.47 to 0.27)	3.67	−3.80 (−5.85 to −1.75)	5.49	5.169	0.027	0.083

complex domains of ToM is possible and also that our training strategies are in the correct path.

Unfortunately, the ANCOVA results in AS only show changes in the Aggressive bias of ambiguous scenes with a reduced effect size. However, this is no surprising because the metacognitive instructions delivered with our ToM short film are not focused on AS and should perhaps deserve a specific module. Nevertheless, the absence of positive results in this domain is consistent with other studies (28, 70).

Furthermore, there were no differences in terms of emotional intelligence assessed with MSCEIT after the intervention, as we can see in **Table 2**, the pretests in both groups were in the normal range [on the MSCEIT's IQ-like scale with a mean of 100 and a SD of 15, a respondent would have to get a score higher than 116 or lower than 84 to be statistically significantly ($P < 0.05$) above or below average]; therefore, the instrument seemed unable to detect impairments in ER or ToM nor changes after treatment, a finding also consistent with previous studies (33, 47, 70). For a more detailed review on the concerns over the MSCEIT's validity, see Maul (2012) (71).

Finally, there was a reduction in PANSS-negative change score (**Table 2**) in the intervention group, suggesting an eventual effect of the intervention in reducing negative symptoms (3).

In conclusion, *e-Motional Training*® is one of the first online programs that has shown its usefulness in the training of the most studied SC domains. Compared with other available programs (28, 72), this program allows online self-training and follow-up by therapists, thus filling the lack of similar intervention instruments in our community.

Our study has a number of limitations, including the fact that most participants in the sample underwent drug treatment; therefore, we do not know whether the relationships found in this study can be replicated in other populations, including individuals who refuse to undergo treatment. Most of the participants in our sample had a diagnosis of chronic stable schizophrenia; therefore, we ignore the performance and feasibility of ET in first episodes or in individuals at high risk for psychosis. Moreover, the majority of the study participants were men, and therefore, the generalizability of the results must be regarded with caution. However, it is a well-known fact that schizophrenia is more severe in men than in women, and therefore, day hospitals and day centers are more frequented by men than women (73).

Regarding participation remarkably, attendance in our sample was perfect. Although this fact could be surprising, it is worth noting that research studies in schizophrenia in our community are scarce, and therefore, it is easier to raise the interest of patients as well as therapists and evaluators, especially if the active treatment is a computerized online program with an attractive interface, cognitively not demanding and allowing self-training, factors that should be taken into account to explain the adherence of patients during the study.

Regarding our results on social functioning, measured with the SFS-SR, the lack of significance of our findings should be considered in the light of the following facts: given that chronic patients have insight and metacognitive deficits, using a self-evaluated scale to measure social functioning was not the best idea. Moreover, it seems to us that social functioning has to be the goal but probably a standard too high for computerized interventions. This is a common place in other clinical domains, for instance in Alzheimer's, where generalizability of computerized interventions to daily living is currently absent (74). Our aim is to create an online tool for helping patients to practice ER and ToM interactions but by no means to substitute group therapy or social skills training. In our opinion, computerized tools give the patients the opportunity to drill and practice skills hardly rehearsable outside the virtual realm, but at least in chronic cases, these skills should be trained *in vivo* in protected environments before aspiring to show generalization in the real world.

Finally, the study was conducted vs. TAU and not vs. another active condition. This is obviously not the best design, but our inspiration was based in recent studies in SC rehabilitation both in group therapy and with computerized tools (32, 35, 75–79). However, it must be taken into account that there is a scarcity of data regarding efficacy of computerized programs for SC and that comparing at this point a computerized tool with group strategies seems at least to us unfair.

In terms of the program's future, version 2.0 is now available, including version 1.0 games and ER tasks devised to improve processing speed, mimicry abilities, and prosodic recognition. Regarding ToM, a short film with real actors and a 2.5 h gameplay graphic adventure with puzzles on ToM and moral dilemmas have been included, and their aim is to offer a gradual and longer training maintaining the attention of patients and their will to improve. The environment has been created with game mechanics, and it has metacognitive hyperlinks designed for self-training.

ETHICS STATEMENT

The study was approved by the Clinical Research Ethics Committee of Galicia (EC) and met all applicable ethical and legal standards (registration code 2014/459) and it has been registered in BioMed Center an international RCT database (ISRCTN83459317).

AUTHOR CONTRIBUTIONS

AGC (principal investigator), YMS, and MVC are the creators of e-Motional Training. YMS, MVC, and AGC designed the study, selected participants, applied the intervention, extracted data, and supervised the study. FDL participated in patient selection and in obtaining and extracting data, and MRA and RM reviewed the manuscript. Moreover, RM contributed to perform statistical analysis and writing of the final version. The manuscript was authored by YMS, MVC, and AGC. All the authors have had full access to data in the study, have personally reviewed the manuscript, and gave final approval of the version attached.

ACKNOWLEDGMENTS

We would like to thank the following patient's associations: APEM, DOA, Virxe da Cerca and MOREA for their collaboration. María Teresa Alves (MSci) for her statistical support and Dr. Díaz del Valle, Dr. Serrano, Dr. Paramo, and Dr. Olivares for their help. The project was 80% funded by the Galician Department of Health with European Regional Development Funds through the PRIS program (Program for the precommercial development of Research results of the public health System of Galicia). It has also received the Siota Grant VIII Edition of the College of Psychologists of Galicia, the 2013 Biannual Grant of the Medical- Surgical Academy of Ourense, the II Spanish National award Innova-eVia 2015, and the National Award for the Best Videogame of Health at e-Health Spanish Congress in Madrid 2016. Finally the authors would like to express their gratitute to the referees for their comments that help to improve the manuscript.

REFERENCES

1. Green MF, Penn DL, Bentall R, Carpenter WT, Gaebel W, Gur RC, et al. Social cognition in schizophrenia: an NIMH workshop on definitions, assessment, and research opportunities. *Schizophr Bull* (2008) 34(6):1211–20. doi:10.1093/schbul/sbm145
2. Pinkham AE, Harvey PD, Penn DL. Social cognition psychometric evaluation: results of the Final Validation Study. *Schizophr Bull* (2017). doi:10.1093/schbul/sbx117
3. Green MF, Olivier B, Crawley JN, Penn DL, Silverstein S. Social cognition in schizophrenia: recommendations from the measurement and treatment research to improve cognition in schizophrenia new approaches conference. *Schizophr Bull* (2005) 31(4):882–7. doi:10.1093/schbul/sbi049
4. Lysaker PH, Gumley A, Luedtke B, Buck KD, Ringer JM, Olesek K, et al. Social cognition and metacognition in schizophrenia: evidence of their independence and linkage with outcomes. *Acta Psychiatr Scand* (2013) 127(3):239247. doi:10.1111/acps.12012
5. Pinkham AE, Penn DL, Perkins DO, Lieberman J. Implications for the neural basis of social cognition for the study of schizophrenia. *Am J Psychiatry* (2003) 160(5):815–24. doi:10.1176/appi.ajp.160.5.815
6. Hooker C, Park S. Emotion processing and its relationship to social functioning in schizophrenia patients. *Psychiatry Res* (2002) 112(1):41–50. doi:10.1016/S01651781(02)00177-4
7. Maat A, van Montfort SJ, de Nijs J, Derks EM, Kahn RS, Linszen DH, et al. Emotion processing in schizophrenia is state and trait dependent. *Schizophr Res* (2015) 161(2–3):392–8. doi:10.1016/j.schres.2014.11.027
8. Edwards J, Pattison PE, Jackson HJ, Wales RJ. Facial affect and affective prosody recognition in first-episode schizophrenia. *Schizophr Res* (2001) 48(2–3):235–53. doi:10.1016/S0920-9964(00)00099-2
9. Evangeli M, Broks P. Face processing in schizophrenia: parallels with the effects of amygdala damage. *Cogn Neuropsychiatry* (2000) 5(2):81–104. doi:10.1080/135468000395754
10. Kohler CG, Bilker W, Hagendoorn M, Gur RE, Gur RC. Emotion recognition deficit in schizophrenia: association with symptomatology and cognition. *Biol Psychiatry* (2000) 48:127–36. doi:10.1016/S0006-3223(00)00847-7
11. Erol A, Putgul G, Kosger F, Ersoy B. Facial emotion recognition in schizophrenia: the impact of gender. *Psychiatry Investig* (2013) 10(1):69–74. doi:10.4306/pi.2013.10.1.69
12. Amminger GP, Schäfer MR, Klier CM, Schlögelhofer M, Mossaheb N, Thompson A, et al. Facial and vocal affect perception in people at ultra-high risk of psychosis, first-episode schizophrenia and healthy controls. *Early Interv Psychiatry* (2012) 6(4):450–4. doi:10.1111/j.1751-7893.2012.00362.x
13. Vohs JL, Lysaker PH, Francis MM, Hamm J, Buck KD, Olesek K, et al. Metacognition, social cognition, and symptoms in patients with first episode and prolonged psychoses. *Schizophr Res* (2014) 153(1–3):54–9. doi:10.1016/j.schres.2014.01.012

14. Penn DL, Spaulding W, Reed D, Sullivan M. The relationship of social cognition to ward behavior in chronic schizophrenia. *Schizophr Res* (1996) 20(3):327–35. doi:10.1016/0920-9964(96)00010-2
15. Ihnen G, Penn DL, Corrigan PW, Martin J. Social perception and social skill in schizophrenia. *Psychiatry Res* (1998) 80:275–86. doi:10.1016/S0165-1781(98)00079-1
16. Frith C, Corcoran R. Exploring "theory of mind" in people with schizophrenia. *Psychol Med* (1996) 26(3):521–30. doi:10.1017/S0033291700035601
17. Horan WP, Green MF. Treatment social cognition in schizophrenia: current status and future directions. *Schizophr Res* (2017). doi:10.1016/j.schres.2017.07.013
18. Buck BE, Healey KM, Gagen EC, Roberts DL, Penn DL. Social cognition in schizophrenia: factor structure, clinical and functional correlates. *J Ment Health* (2016) 8237:1–8. doi:10.3109/09638237.2015.1124397
19. Flood AM, Julian Hare D, Wallis P. An investigation into social information processing in young people with Asperger syndrome. *Autism* (2011) 15(5):601–24. doi:10.1177/1362361310387803
20. Fett AKJ, Viechtbauer W, Dominguez MD, Penn DL, van Os J, Krabbendam L. The relationship between neurocognition and social cognition with functional outcomes in schizophrenia: a meta-analysis. *Neurosci Biobehav Rev* (2011) 35(3):573–88. doi:10.1016/j.neubiorev.2010.07.001
21. Couture SM, Penn DL, Roberts DL. The functional significance of social cognition in schizophrenia: a review. *Schizophr Bull* (2006) 32(Suppl 1):S44–63. doi:10.1093/schbul/sbl029
22. Schmidt SJ, Mueller DR, Roder V. Social cognition as a mediator variable between neurocognition and functional outcome in schizophrenia: empirical review and new results by structural equation modeling. *Schizophr Bull* (2011) 37(Suppl 2):S41–54. doi:10.1093/schbul/sbr079
23. Couture S, Granholm E, Fish S. A path model investigation of neurocognition, theory of mind, social competence, negative symptoms and real-world functioning in schizophrenia. *Schizophr Res* (2011) 125(2–3):152–60. doi:10.1016/j.schres.2010.09.020.A
24. Addington J, Saeedi H, Addington D. Influence of social perception and social knowledge on cognitive and social functioning in early psychosis. *Br J Psychiatry* (2006) 189:373–8. doi:10.1192/bjp.bp.105.021022
25. Vauth R, Rüsch N, Wirtz M, Corrigan PW. Does social cognition influence the relation between neurocognitive deficits and vocational functioning in schizophrenia? *Psychiatry Res* (2004) 128(2):155–65. doi:10.1016/j.psychres.2004.05.018
26. Berry K, Bucci S, Kinderman P, Emsley R, Corcoran R. An investigation of attributional style, theory of mind and executive functioning in acute paranoie and remission. *Psychiatry Res* (2015) 226(1):84–90. doi:10.1016/j.psychres.2014.12.009
27. Fretland RA, Andersson S, Sundet K, Andreassen OA, Melle I, Vaskinn A. Theory of mind in schizophrenia: error types and associations with symptoms. *Schizophr Res* (2015) 162(3):42–6. doi:10.1016/j.schres.2015.01.024
28. Lahera G, Benito A, Montes JM, Fernández-Liria A, Olbert CM, Penn DL. Social cognition and interaction training (SCIT) for outpatients with bipolar disorder. *J Affect Disord* (2013) 146(1):132–6. doi:10.1016/j.jad.2012.06.032
29. Sparks A, McDonald S, Lino B, O'Donnell M, Green MJ. Social cognition, empathy and functional outcome in schizophrenia. *Schizophr Res* (2010) 122(1–3):172–8. doi:10.1016/j.schres.2010.06.011
30. Wölwer W, Frommann N. Social-cognitive remediation in schizophrenia: generalization of effects of the training of affect recognition (TAR). *Schizophr Bull* (2011) 37(Suppl 2):63–70. doi:10.1093/schbul/sbr071
31. Hempel RJ, Dekker JA, van Beveren NJ, Tulen JH, Hengeveld MW. The effect of antipsychotic medication on facial affect recognition in schizophrenia: a review. *Psychiatry Res* (2010) 178(1):1–9. doi:10.1016/j.psychres.2008.07.025
32. Roberts DL, Combs DR, Willoughby M, Mintz J, Gibson C, Rupp B, et al. A randomized, controlled trial of Social Cognition and Interaction Training (SCIT) for outpatients with schizophrenia spectrum disorders. *Br J Clin Psychol* (2014) 53(3):281–98. doi:10.1111/bjc.12044
33. Fernandez-Gonzalo S, Turon M, Jodar M, Pousa E, Hernandez Rambla C, Palao D. A new computerized cognitive and social cognition training specifically designed for patients with schizophrenia/schizoaffective disorder in early stages of illness: a pilot study. *Psychiatry Res* (2015) 228(3):501–9. doi:10.1016/j.psychres.2015.06.007
34. Lindenmayer JP, McGurk SR, Khan A, Kaushik S, Thanju A, Hoffman L, et al. Improving social cognition in schizophrenia: a pilot intervention combining computerized social cognition training with cognitive remediation. *Schizophr Bull* (2013) 39(3):507–17. doi:10.1093/schbul/sbs120
35. Granholm E, McQuaid JR, McClure FS, Link PC, Perivoliotis D, Gottlieb JD, et al. Randomized controlled trial of cognitive behavioral social skills training for older people with schizophrenia: 12-month follow-up. *J Clin Psychiatry* (2007) 68(5):730–7. doi:10.4088/JCP.v68n0510
36. Park KM, Ku J, Choi SH, Jang HJ, Park JY, Kim SI, et al. A virtual reality application in role plays of social skills training for schizophrenia: a randomized, controlled trial. *Psychiatry Res* (2011) 189(2):166–72. doi:10.1016/j.psychres.2011.04.003
37. Rus-Calafell M, Gutiérrez-Maldonado J, Ribas-Sabaté J. A virtual reality-integrated program for improving social skills in patients with schizophrenia: a pilot study. *J Behav Ther Exp Psychiatry* (2014) 45(1):81–9. doi:10.1016/j.jbtep.2013.09.002
38. Seo JM, Ahn S, Byun EK, Kim CK. Social skills training as nursing intervention to improve the social skills and self-esteem of inpatients with chronic schizophrenia. *Arch Psychiatr Nurs* (2007) 21(6):317–26. doi:10.1016/j.apnu.2006.09.005
39. Drusch K, Stroth S, Kamp D, Frommann N, Wölwer W. Effects of Training of Affect Recognition on the recognition and visual exploration of emotional faces in schizophrenia. *Schizophr Res* (2014) 159(2–3):485–90. doi:10.1016/j.schres.2014.09.003
40. Russell TA, Chu E, Phillips ML. A pilot study to investigate the effectiveness of emotion recognition remediation in schizophrenia using the micro-expression training tool. *Br J Clin Psychol* (2006) 45(Pt 4):579–83. doi:10.1348/014466505X90866
41. Marsh P, Langdon R, McGuire J, Harris A, Polito V, Coltheart M. An open clinical trial assessing a novel training program for social cognitive impairment in schizophrenia. *Australas Psychiatry* (2013) 21(2):122–6. doi:10.1177/1039856213475683
42. Roberts DL, Kleinlein P, Stevens B. An alternative to generating alternative interpretations in social cognitive therapy for psychosis. *Behav Cogn Psychother* (2012) 40(4):491–5. doi:10.1017/S1352465812000082
43. Mazza M, Lucci G, Pacitti F, Pino MC, Mariano M, Casacchia M, et al. Could schizophrenic subjects improve their social cognition abilities only with observation and imitation of social situations? *Neuropsychol Rehabil* (2010) 20(5):675–703. doi:10.1080/09602011.2010.486284
44. Bechi M, Spangaro M, Bosia M, Zanoletti A, Fresi F, Buonocore M, et al. Theory of Mind intervention for outpatients with schizophrenia. *Neuropsychol Rehabil* (2013) 23(3):383–400. doi:10.1080/09602011.2012.762751
45. Tan BL, Lee SA, Lee J. Social cognitive interventions for people with schizophrenia: a systematic review. *Asian J Psychiatr* (2016). doi:10.1016/j.ajp.2016.06.013
46. Kurtz MM, Richardson CL. Social cognitive training for schizophrenia: a meta analytic investigation of controlled research. *Schizophr Bull* (2012) 38(5):1092–104. doi:10.1093/schbul/sbr036
47. Nahum M, Fisher M, Loewy R, Poelke G, Ventura J, Nuechterlein KH, et al. A novel, online social cognitive training program for young adults with schizophrenia: a pilot study. *Schizophr Res Cogn* (2014) 1(1):e11–9. doi:10.1016/j.scog.2014.01.003
48. Brenner HD, Hodel B, Kube G, Roder V. [Cognitive therapy of schizophrenic patients:problem analysis and empirical results]. *Nervenarzt* (1987) 58(2):72–83.
49. Ochsner KN. The social-emotional processing stream: five core constructs and their translational potential for schizophrenia and beyond. *Biol Psychiatry* (2008) 64(1):48–61. doi:10.1016/j.biopsych.2008.04.024
50. Zubin J, Spring B. Vulnerability – a new view of schizophrenia. *J Abnorm Psychol* (1977) 86(2):103–26. doi:10.1037/0021-843X.86.2.103
51. Kay SR, Fiszbein A, Qpjer LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* (1987) 13(2):261–76.
52. Cordero A, Calonge I. *K-BIT, Test Breve de Inteligencia de Kaufman Adaptación española*. 2nd ed. Madrid: TEA Ediciones (1996).
53. Young A, Perrett D, Calde A, Sprengelmeyer R, Ekman P. *Facial Expressions of Emotion: Stimuli and Tests (FEEST)*. Bury St Edmunds: Thames Valley Test Company (2002).

54. Gil D, Fernández-Modamio M, Bengochea R, Arrieta M. Adaptación al español de la prueba de teoría de la mente Hinting Task. *Rev Psiquiatr Salud Ment* (2012) 5(2):79–88. doi:10.1016/j.rpsm.2011.11.004
55. Janssen I, Krabbendam L, Jolles J, van Os J. Alterations in theory of mind in patients with schizophrenia and non-psychotic relatives. *Acta Psychiatr Scand* (2003) 108(2):110117. doi:10.1034/j.1600-0447.2003.00092.x
56. Baron-Cohen S, Jolliffe T, Mortimore C, Robertson M. Another advanced test of theory of mind: evidence from very high functioning adults with autism or asperger syndrome. *J Child Psychol Psychiatry* (1997) 38(7):813–22. doi:10.1111/j.1469-7610.1997.tb01599.x
57. Stone V. *Faux Pas Recognition Test (Adult Version) Created by Valerie Stone & Simon Baron Cohen Correct Citations for Use of This Test*. (2002). p. 407–18. Available from: http://docs.autismresearchcentre.com/tests/FauxPas_Adult.pdf
58. Pousa E. *Measurement of Theory of Mind in Healthy Adolescents: Translation and Cultural Adaptation of F. Happé's Theory of Mind Stories*. Barcelona: Tesis Doctoral, Universidad Autónoma de Barcelona (1999).
59. Lahera G, Boada L, Pousa E, Mirapeix I, Morón-Nozaleda G, Marinas L, et al. Movie for the Assessment of Social Cognition (MASC): Spanish validation. *J Autism Dev Disord* (2014) 44(8):1886–96. doi:10.1007/s10803-014-2061-6
60. Combs DR, Penn DL, Wicher M, Waldheter E. The Ambiguous Intentions Hostility Questionnaire (AIHQ): a new measure for evaluating hostile social-cognitive biases in paranoia. *Cogn Neuropsychiatry* (2007) 12(2): 128–43. doi:10.1080/13546800600787854
61. Extremera N, Fernández-Berrocal P. *Adaptación Española Del Test de Inteligencia Emocional de Mayer-Salovey-Caruso (MSCEIT): Manual Y Cuadernillo*. Madrid: TEA (2009).
62. Torres A, Olivares J. Validación en castellano de la Social Functioning Scales (Escala de Funcionamiento Social). *Actas Esp Psiquiatr* (2005) 33:216–20.
63. Vázquez-Campo M, Maroño Y, Lahera G, Mateos R, García-Caballero A. e-motional training®: pilot study on a novel online training program on social cognition for patients with schizophrenia. *Schizophr Res Cogn* (2016) 4:10–7. doi:10.1016/j.scog.2015.11.007
64. Roberts DL, Penn DL, Labate D, Margolis SA, Sterne A. Transportability and feasibility of Social Cognition and Interaction Training (SCIT) in community settings. *Behav Cogn Psychother* (2010) 38(1):35–47. doi:10.1017/S1352465809990464
65. Dodich A, Cerami C, Canessa N, Crespi C, Marcone A, Arpone M, et al. Emotion recognition from facial expressions: a normative study of the Ekman 60-Faces Test in the Italian population. *Neurol Sci* (2014) 35(7):10151021. doi:10.1007/s10072-014-1631-x
66. Marsh PJ, Luckett G, Russell T, Coltheart M, Green MJ. Effects of facial emotion recognition remediation on visual scanning of novel face stimuli. *Schizophr Res* (2012) 141(2–3):234–40. doi:10.1016/j.schres.2012.08.006
67. Koelkebeck K, Kohl W, Luettgenau J, Triantafillou S, Ohrmann P, Satoh S, et al. Benefits of using culturally unfamiliar stimuli in ambiguous emotion identification: a cross-cultural study. *Psychiatry Res* (2015) 228:39–45. doi:10.1016/j.psychres.2015.04.005
68. Gohar S, Hamdi E, El Ray L, Horan W, Green M. Adapting and evaluating a social cognitive remediation program for schizophrenia in Arabic. *Schizophr Res* (2013) 148(13):12–7. doi:10.1016/j.schres.2013.05.008
69. Peyroux E, Franck N. Improving social cognition in people with schizophrenia with RC2S: two single-case studies. *Front Psychiatry* (2016) 7:66. doi:10.3389/fpsyt.2016.00066
70. Rocha N, Queirós C. Metacognitive and social cognition training (MSCT) in schizophrenia: a preliminary efficacy study. *Schizophr Res* (2013) 150(1):64–8. doi:10.1016/j.schres.2013.07.057
71. Maul A. Examining the structure of emotional intelligence at the item level: new perspectives, new conclusions. *Cogn Emot* (2012) 26(3):503–20. doi:10.1080/02699931.2011.588690
72. Kleinlein P. Social Cognition and Interaction Training (SCIT) for individuals with schizophrenia spectrum disorders in outpatient treatment settings. (2010). Available from: http://digitalcommons.unl.edu/psychdiss/18/
73. Aleman A, Kahn RS, Selten JP. Sex differences in the risk of schizophrenia: evidence from metaanalysis. *Arch Gen Psychiatry* (2003) 60(6):565–71. doi:10.1001/archpsyc.60.6.565
74. Hill NT, Mowszowski L, Naismith SL, Chadwick VL, Valenzuela M, Lampit A. Computerized cognitive training in older adults with mild cognitive impairment or dementia: a systematic review and meta-analysis. *Am J Psychiatry* (2017) 174(4):329–40. doi:10.1176/appi.ajp.2016.16030360
75. Mueller DR, Schmidt SJ, Roder V. One-year randomized controlled trial and follow up of integrated neurocognitive therapy for schizophrenia outpatients. *Schizophr Bull* (2015) 41(3):604–16. doi:10.1093/schbul/sbu223
76. Sachs G, Winklbaur B, Jagsch R, Lasser I, Kryspin-Exner I, Frommann N, et al. Training of affect recognition (TAR) in schizophrenia – impact on functional outcome. *Schizophr Res* (2012) 138(2–3):262–7. doi:10.1016/j.schres.2012.03.005
77. Wang Y, Roberts DL, Xu B, Cao R, Yan M, Jiang Q. Social cognition and interaction training for patients with stable schizophrenia in Chinese community settings. *Psychiatry Res* (2013) 210(3):751–5. doi:10.1016/j.psychres.2013.08.038
78. Combs DR, Elerson K, Penn DL, Tiegreen JA, Nelson A, Ledet SN, et al. Stability and generalization of Social Cognition and Interaction Training (SCIT) for schizophrenia: six-month follow-up results. *Schizophr Res* (2009) 112(1–3):196–7. doi:10.1016/j.schres.2009.04.010
79. Roberts DL, Penn DL. Social cognition and interaction training (SCIT) for outpatients with schizophrenia: a preliminary study. *Psychiatry Res* (2009) 166(2–3):141–7. doi:10.1016/j.psychres.2008.02.007

Conflict of Interest Statement: AC, YS, and MC are the intellectual authors of *e-Motional Training*® 1.0, a program funded by the Galician Department through the PRIS program, whose objective is to generate a spin-off.

Copyright © 2018 Maroño Souto, Vázquez Campo, Díaz Llenderrozas, Rodríguez Álvarez, Mateos and García Caballero. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

REVIEW
published: 30 October 2018
doi: 10.3389/fpsyt.2018.00537

Multidimensional Connectomics and Treatment-Resistant Schizophrenia: Linking Phenotypic Circuits to Targeted Therapeutics

Mary-Anne B. MacKay, John W. Paylor, James T. F. Wong, Ian R. Winship, Glen B. Baker and Serdar M. Dursun*

Neurochemical Research Unit and Bebensee Schizophrenia Research Unit, Department of Psychiatry, University of Alberta, Edmonton, AB, Canada

OPEN ACCESS

Edited by:
Felice Iasevoli, Università degli Studi di Napoli Federico II, Italy

Reviewed by:
Lucy Denise Vanes, University College London, United Kingdom
Roberto Cavallaro, Università Vita-Salute San Raffaele, Italy
Guusje Collin, Rudolf Magnus Institute of Neuroscience, Netherlands

***Correspondence:**
Mary-Anne B. MacKay
marnie.mackay@ualberta.ca

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *17 July 2018*
Accepted: *10 October 2018*
Published: *30 October 2018*

Citation:
MacKay M-AB, Paylor JW, Wong JTF, Winship IR, Baker GB and Dursun SM (2018) Multidimensional Connectomics and Treatment-Resistant Schizophrenia: Linking Phenotypic Circuits to Targeted Therapeutics. Front. Psychiatry 9:537. doi: 10.3389/fpsyt.2018.00537

Schizophrenia is a very complex syndrome that involves widespread brain multi-dysconnectivity. Neural circuits within specific brain regions and their links to corresponding regions are abnormal in the illness. Theoretical models of dysconnectivity and the investigation of connectomics and brain network organization have been examined in schizophrenia since the early nineteenth century. In more recent years, advancements have been achieved with the development of neuroimaging tools that have provided further clues to the structural and functional organization of the brain and global neural networks in the illness. Neural circuitry that extends across prefrontal, temporal and parietal areas of the cortex as well as limbic and other subcortical brain regions is disrupted in schizophrenia. As a result, many patients have a poor response to antipsychotic treatment and treatment failure is common. Treatment resistance that is specific to positive, negative, and cognitive domains of the illness may be related to distinct circuit phenotypes unique to treatment-refractory disease. Currently, there are no customized neural circuit-specific and targeted therapies that address this neural dysconnectivity. Investigation of targeted therapeutics that addresses particular areas of substantial regional dysconnectivity is an intriguing approach to precision medicine in schizophrenia. This review examines current findings of system and circuit-level brain dysconnectivity in treatment-resistant schizophrenia based on neuroimaging studies. Within a connectome context, on-off circuit connectivity synonymous with excitatory and inhibitory neuronal pathways is discussed. Mechanistic cellular, neurochemical and molecular studies are included with specific emphasis given to cell pathology and synaptic communication in glutamatergic and GABAergic systems. In this review we attempt to deconstruct how augmenting treatments may be applied within a circuit context to improve circuit integration and treatment response. Clinical studies that have used a variety of glutamate receptor and GABA interneuron modulators, nitric oxide-based therapies and a variety of other strategies as augmenting treatments with

antipsychotic drugs are included. This review supports the idea that the methodical mapping of system-level networks to both on (excitatory) and off (inhibitory) cellular circuits specific to treatment-resistant disease may be a logical and productive approach in directing future research toward the advancement of targeted pharmacotherapeutics in schizophrenia.

Keywords: schizophrenia, treatment-resistant, connectomics, dysconnectivity, gamma band oscillations, NMDA receptors, GABA interneurons

INTRODUCTION

Treatment-resistant schizophrenia (TRS) remains one of the greatest therapeutic challenges in psychiatry. Schizophrenia is a complex neurodevelopmental syndrome; with disease processes occurring *in utero* that may disrupt the formation of critical neural circuits and result in widespread brain dysconnectivity. Hints of altered neural circuitry, for example delays in gross and fine motor skill development, often evolve during childhood and may precede the first subtle signs of psychosis during late adolescence in those who will develop the illness (1–4). Adolescents with disrupted neural circuit development and circuit dysconnectivity related to the progression of the disease often begin to exhibit sub-threshold psychotic symptoms during developmental periods associated with increasing gray matter (GM) volume and refinement of cortical circuits including synaptic pruning, reinforcement, and neuronal synchronization (5–8). The gradual alterations in brain connectivity and subsequent symptoms can persist for years before psychosis emerges and diagnosis and antipsychotic medications are initiated. In most cases, individuals with schizophrenia progress with an illness that is characterized by periods of exacerbation and remission of psychosis. Recovery is dependent on compliance with and response to optimized antipsychotic medication, the development of a strong therapeutic alliance to treatment team members, and intensive social and vocational support (9). Even with the best antipsychotic treatments that are available today and access to full functional supports, a sub-population of patients with schizophrenia will never attain an optimal response to treatment and remain very ill. These are the patients who have treatment-refractory illness or in the case of non-response to clozapine, ultra-resistant disease (10, 11).

Identifying treatments that will benefit patients with TRS remains a significant challenge. Our understanding of personalized treatment response and resistance to medication is limited by an inability to accurately pinpoint the individual genetic, cellular and neural circuit drivers of psychoses. Investigations of neuronal ensembles and cortical networks at the micro-scale level are not possible using the clinical diagnostic and macro-scale imaging tools that are currently available. Moreover, inconsistent clinical definitions of positive, negative or cognitive symptom-specific differences in TRS lead to ambiguous treatment guideline recommendations and a wide variation in clinical approaches to treat TRS in practice. Different phenotypes of psychoses may respond to different targeted treatments that are cellular or neural circuit-specific, but at present we do not have the ability to identify the appropriate targeted therapies for different TRS phenotypes.

The Treatment Response and Resistance in Psychosis (TRRIP) working group recently addressed these challenges (12). Members are researchers and clinicians who have expertise in TRS and attended specific TRRIP working group meetings at international schizophrenia and neuropsychopharmacology research conferences to establish criteria to standardize the definition of treatment resistance in schizophrenia. In addition to capturing a core definition of treatment resistance that can be included and shared across all clinical treatment guidelines worldwide, recommendations were also made on the importance of identification of all clinical sub-specifiers or symptom phenotypes common to TRS (12). The standardization of clinical criteria of TRS has been an important advancement and will benefit future TRS research and clinical translation.

Treatment resistance has been most characterized in schizophrenia by how responsive the positive symptom domain is to antipsychotic medications. It is estimated that 70–80% of patients with schizophrenia have a phenotype of psychosis that is responsive to dopamine-blocking treatment (13). However, in over 100 years of treatment history and despite the improvements made to the functional selectivity and potency of antipsychotic medications, 60% of patients continue to fail to achieve symptom improvement after several weeks on drug therapy (14).

Many treatment-refractory patients present with a psychosis that is positive symptom domain responsive, but have symptoms that are non-responsive within the negative or cognitive symptom subdomains and associated circuits. It is now recommended that patients with symptom profiles that do not respond to antipsychotic medication and are considered treatment resistant be identified as: TRS-positive symptom domain-, TRS-negative symptom domain-, and TRS-cognitive symptom domain-specific. For those patients with combined treatment resistance in more than one domain (multidimensional resistance), identifying all of those specific symptom domains will provide further clarification (12).

Traditionally, for those patients who are unable to obtain adequate positive symptom control or sustain a response with at least 2 dopamine receptor-2 (D_2)-blocking agents at therapeutic doses for at least 6 weeks, clozapine is the recommended drug of choice. An estimated 30–60% of these patients will respond to clozapine and have what can be described as a clozapine-responsive psychosis (10, 15, 16). Patients who do not have

an optimal response to clozapine and continue to experience prominent positive symptoms have clozapine-resistant psychosis or an ultra-resistant psychotic disease (11). Currently, there are no therapies that address this most severe form of neural-dysconnectivity in schizophrenia.

In this review, we examine TRS from a circuit-based perspective. We start by highlighting the historical development of connectome science in schizophrenia, identifying those early pioneers in psychiatry who originally recognized the disease as an illness of widespread disconnectivity and their valuable contribution to the evolution of network science today. We then examine neuroimaging studies that support both systemic and circuit-level brain dysconnectivity specific to treatment resistance and attempt to explain underlying circuit biology and brain topology that may be unique to this most severe form of the illness. Within a connectome context, attempts to map on-off circuit connectivity synonymous with excitatory and inhibitory neuronal pathways are discussed. Functional correlates of dysconnectivity in schizophrenia are also considered with a focus on cortical network oscillations, giving particular emphasis to the role of gamma band oscillations (GBOs) and their ability to integrate information across large populations of neurons in the illness. Mechanistic models describing underlying neural circuitry and the complex relationship involved in the synchronized firing between excitatory pyramidal cells and inhibitory gamma-aminobutyric acid (GABA)-ergic interneurons are also reviewed to help visualize and understand the inter-relationship between neuronal ensembles within the brain and the complex mechanisms behind their dysfunctional communication in schizophrenia. Finally, we deconstruct how augmenting pharmacological treatments, such as glutamate *N*-methyl-D-aspartate (NMDA) receptor and GABA interneuron modulators as well as nitric oxide (NO)-based treatments may be applied within a circuit context to improve circuit integration and treatment response in TRS. Updates on neurosurgical and neuromodulation targets under investigation in TRS are also included and provide an overview of beneficial circuit-based targets that may improve treatment resistant symptoms in those patients that remain refractory to pharmacological approaches.

This review supports the idea that the mapping of cellular and system-level networks to both on (excitatory) and off (inhibitory) circuit phenotypes specific to treatment-resistant disease may be a productive strategy in expanding future research toward customized neural circuit-specific pharmacotherapeutics and directed neuromodulation treatments in schizophrenia. Targeted therapeutics that can improve particular areas of regional functional dysconnectivity that are found to be substantially affected in TRS is an intriguing approach to precision medicine in schizophrenia.

HISTORY OF CONNECTOMICS IN SCHIZOPHRENIA-THE EARLY CONNECTIONISTS

Theoretical models of disconnectivity and the investigation of connectomics and brain network organization have been examined in schizophrenia since the early nineteenth century. Historically, there have been a number of influential figures who have made major contributions to the development of modern day network-based science known as connectomics. One of the very first connectionist pioneers in psychiatry was Wilhelm Griesinger (1817–1868), a German neurologist and psychiatrist who initially proposed that mental illnesses are brain disorders with pathological and neuroanatomical origins similar to neurological disorders (17). From his teachings, his student Theodor Hermann Meynert (1833–1892), a German-Austrian neuropathologist, anatomist and psychiatrist, made further contributions to this biological model of mental illness (18). His work was based primarily on neuroanatomical and histological studies where he worked to characterize various afferent and efferent white matter (WM) fiber tracts of the cerebral cortex. Meynert believed that association fibers connecting regional areas of the brain are the most disrupted in psychiatric diseases, which has been consistently demonstrated by several structural and functional magnetic resonance imaging (MRI) studies of schizophrenia in recent times (18–21).

Meynert's student Carl Wernicke (1848–1905) further developed the disconnectivity theory of schizophrenia. Although he was best known for his theories regarding the neural circuits involved in higher cognitive functions and the neuropathology of aphasia, he also studied the neuroanatomical and functional aspects of schizophrenia. In his textbook *Grundriss der Psychiatrie* (Outlines of Psychiatry 1900) which was written based on detailed reviews of his clinical cases, he outlined his hypothesis that there is a deficiency in association fiber connectivity in schizophrenia that contributes to an over-activation of cortical sensory regions that can then lead to the development of psychosis (22).

One of the most well-known clinicians in the history of psychiatry and recognized as the founder of modern psychiatry was Emil Kraepelin (1856–1926), a German psychiatrist who conceptualized schizophrenia as a disorder with both neurodevelopmental and biological origins. Kraepelin was the first to develop a classification system of psychiatric disorders and divided endogenous psychoses into two distinct forms based on disease course and outcome. He described the psychosis involved in schizophrenia as a dementia praecox, a term that combined the cognitive symptoms (dementia) of the illness with an early development of the disorder (praecox) vs. the episodic nature of manic depressive (affective) psychosis (23).

It was the Swiss psychiatrist Eugen Bleuler (1857–1939) who then coined the term schizophrenia (from the Greek verb *schizein* meaning split and *phren* meaning soul, spirit or mind) to highlight the fragmented thinking or thought disorder that is common to the functional disconnectivity of the illness. Bleuler replaced the term dementia praecox to clearly distinguish schizophrenia from a degenerative illness with a poor outcome. He recognized that progressive cognitive deterioration (characteristic of dementia) was not common in schizophrenia and the onset of symptoms does not always occur early in life (24). For a detailed overview see Collin et al. (19).

MODERN-DAY CONNECTIONISTS

With the advancement of neuroimaging techniques, such as positron emission tomography (PET) and MRI that are able to detail both anatomical and functional connectivity, the disconnection hypothesis of schizophrenia has been refined further. The modern-day disconnectivity hypothesis of schizophrenia initially emphasized the link between the signs and symptoms of schizophrenia and the dysfunctional integration between different cortical areas of the brain, directly related to the underlying abnormalities in neurons and synaptic functioning (25). Abnormal modulation of NMDA receptor function and impaired control of synaptic plasticity is thought to be the underlying key to dysfunction and directly contributes to an extended pattern of "dysconnection" of the structural and functional integration of the brain (26–30). Today, network scientists integrate the mathematical analysis of graph theory as a framework for studying and tracing these macro-scale brain networks through non-invasive neuroimaging and MRI methods (31–33). Through these methods they are able to create a "connectome," the neuronal map of the brain's anatomical and functional connectivity architecture, and elucidate the complex organization of the neuronal elements that underlies brain function (31–34).

THE SCIENCE OF CONNECTOMICS

The scientific study of connectomics involves mapping out the detailed connectivity of brain regions to characterize the architectural networks of the human brain. Connectomics is therefore a powerful tool to visualize the structural and functional dysconnections associated with schizophrenia. The human connectome provides a detailed map of brain-wide circuit connectivity and allows inference into how brain function may be affected by disruption of the structural organizational network (31, 34). At the micro-scale, the physical wiring of single neurons and their synaptic connections to other neurons through dendritic and axonal connections comprise local network circuits. At the meso-scale (local populations of 80–100 neurons that span all cortical layers), connectivity is at the level of functionally specialized subnetworks within single cortical columns that are selectively connected within and between neighboring cortical columns and constitute a major functional element for cortical information processing. At the macro-scale, inter-regional connectivity of cerebral lobes via WM interhemispheric tracts is responsible for the integration and relay of information between various parts of the brain (34).

Connectomics heavily utilizes graph theory, a specialized discipline of mathematics concerned with the study of graphs or models that represent relations between objects. Large collections of algorithms are used to calculate topological characteristics of both structural and functional brain imaging connectivity data that can be represented in the form of a graph. The graph consists of nodes that represent single neurons or brain regions that are defined by connection endpoints of two line segments that are then linked by edges that illustrate their direct connection to each other via axonal projections, WM pathways or functional coupling between inter-regional brain areas (31). The closeness of neuronal and brain region nodes represents a higher probability of being connected, as long axonal projections are functionally expensive in terms of wiring costs. The tendency of nodes to cluster and form shorter communication paths allows for more efficient integration between spatially disconnected node pairs. The degree or number of edges each node possesses and how close they are to each other (centrality) represents the interconnectivity of a node to other nodes within the entire brain network. Nodes that have a high degree of edges and possess high centrality are known as hubs (35–37). In turn, brain hubs that are "rich" in connectivity and more densely interconnected to each other in comparison to what their high degree alone would predict form a central "rich club organization" essential for the integration of global information and brain communication (37) as illustrated in **Figure 1**. Disruption to central "rich club" hubs of the human connectome has been associated with several brain disorders (38). Notably, hub lesions that are highly concentrated within cortical hubs of the frontal and temporal lobes are found to be specifically affected in schizophrenia (38).

THE CONNECTOMICS OF SCHIZOPHRENIA

Neuroimaging studies show impaired structural and functional connectivity in individuals diagnosed with schizophrenia (39–41). The dysconnection between different brain regions of GM and the WM circuits that connect them are consistent with reduced functional connectivity revealed in both resting state and task-based functional (fMRI) studies (32, 41). Recent advances in the use of MRI and in particular diffusion-weighted imaging (DTI) have brought insight into the extent of structural WM dysconnectivity and alterations in the macro-scale neuronal wiring in schizophrenia. Most studies have investigated fractional anisotropy (FA), a neuroimaging marker that indexes the constraint of the direction of water diffusion in WM and can be a measure of an abnormality in the integrity of myelin microstructure or axonal integrity or differences in the orientation of how axonal fibers are organized. White matter in the frontal and temporal lobes have been the most frequently reported with reduced FA integrity in DTI studies of those with schizophrenia (39–41). Meta-analyses of voxel-based DTI studies in schizophrenia have found significant decreases in two main brain regions, the left frontal deep WM and left temporal deep WM (42), with overlapping GM and WM structural abnormalities (43). A more recent meta-analysis that included 29 independent international studies found global WM microstructural disruptions throughout the entire brain (44).

Consistent with these findings, an additional imaging study found significant decreases in WM FA to not only involve the fronto-temporal regions, but also to be widespread throughout each lobe of the brain, including the cerebellum. Major fiber bundles that connect the cortical lobes including the corpus callosum, cingulum and thalamic radiations exhibited the most severe pathology. More than 50% of the cortico-cortical and

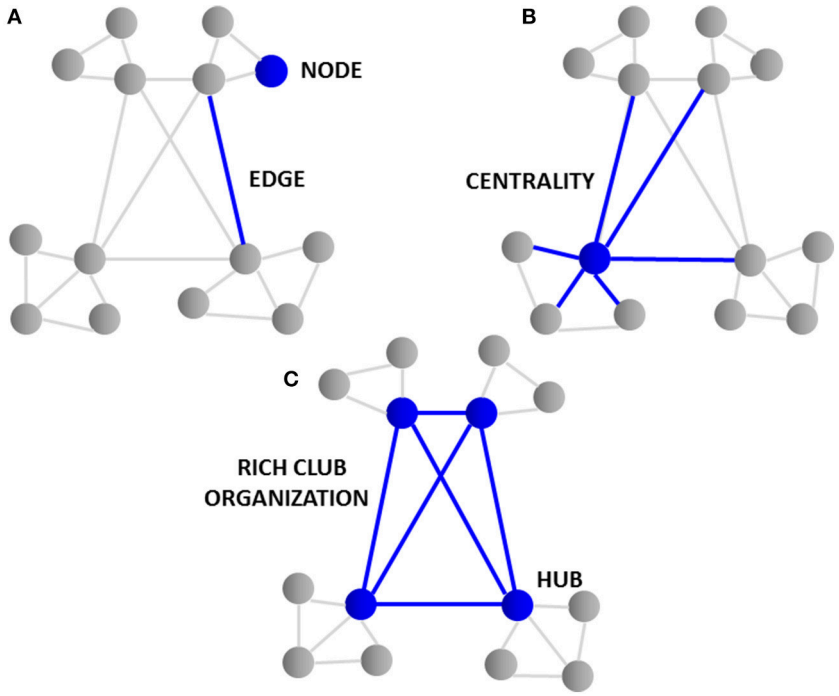


FIGURE 1 | Topological graph features of the connectome. **(A)** The graph consists of "nodes" that represent single neurons or brain regions and are linked by "edges" illustrating their connection to each other via axonal projections. **(B)** The "degree" or number of edges and how close they are to each other "centrality" represents the interconnectivity of nodes. **(C)** Nodes having a high degree of edges and high centrality are knowns as "hubs." Brain hubs "rich" in connectivity to each other and found centrally form the "rich club organization." The rich club hubs found in cortical and frontal lobe regions of the brain are affected in schizophrenia.

cortico-subcortical WM fibers that provide the connections between those hub regions that contribute to the "rich club" in the brain were affected (45) and network hubs located in association cortex particularly affected (20, 21). These significant structural disturbances may be responsible for the widespread disruption of cortical information processing and integration of information across multiple regions of the brain in schizophrenia.

Functional MRI studies have also suggested abnormalities in the connectivity of brain networks in schizophrenia and relate to the structural disturbances that interconnect them. While reduced functional connectivity is a replicated finding among many studies (32, 41, 46, 47), there have also been reports of increase in functional connectivity in the illness (48, 49). The discrepancy may simply be related to non-uniform changes in brain connectivity, such as hyper-synchrony of neuronal ensembles vs. dysregulated networks, fMRI preprocessing errors, or abnormalities in neuronal wiring and oscillatory firing and compensatory hyper-connectivity of important hubs within the association cortex as a consequence of the illness (50).

THE CONNECTOMICS OF TREATMENT RESISTANCE

Widespread dysfunction throughout the entire neural network that involves both cortical and subcortical regions is pronounced in TRS and may have an underlying circuit biology that is unique to this most severe form of the illness. Anatomical regions and neural circuits that have been examined comparing those individuals with treatment resistant vs. treatment responsive disease have uncovered more severe pathological findings in all cortical tissues that have been measured. A number of imaging studies using a variety of structural and fMRI methods have examined TRS to elucidate the difference between the phenotypic subtypes of responsive and non-responsive illness. For detailed reviews see Mouchlianitis et al. (51) and Nakajima et al. (52).

The loss of neuronal elements that underlie the symptoms of both TRS and ultra-resistant schizophrenia (clozapine-resistant psychosis) may be more substantial than what is found in those patient phenotypes who have responded to antipsychotic treatment (51, 52). Volumetric, DTI and fMRI studies that have examined intra-regional brain morphology (53–56) inter-regional WM circuit integrity (43, 57–59), and functional connectivity (60–63) specific to TRS have consistently identified a disruption to frontal and temporal lobe regions and the major fiber bundles that connect them.

Studies that have specifically compared patients with treatment responsive schizophrenia vs. TRS have reported greater global volumetric reductions of GM in treatment resistant and ultra-resistant patients. There have been consistent reports of reduced GM volumes predominantly within the dorsolateral prefrontal cortex (DLPFC) (53–56), as well as posterior cortical regions, such as the temporal cortex (53–56), parietal cortex (53, 56) and also within the occipital cortex

(53, 55, 56) in TRS. Abnormalities in all regions of the corpus callosum as well as commissural and association long axonal fiber pathways connecting prefrontal, temporal, parietal and occipital regions have also been found, with reduced axonal integrity and more severe structural damage in both chronic illness and treatment-resistant populations (43, 57–59, 64). This evidence seems to suggest that on the spectrum of cellular and circuit disruption characteristic of schizophrenia in general, TRS may involve a more severe type of multi-dysconnectivity of brain networks that spans across almost every region of the brain.

The reduction in cortical GM and WM volumes and distinct WM tract disturbances in TRS may be a consequence of disrupted macro-scale neural architecture and network dysconnectivity that originate within distinct micro-scale neuronal ensembles. Morphometric studies that have been investigated in schizophrenia suggest that cortical volume loss is not related to the reduction of the number of neurons in the cortex, but to architectural neuronal disorganization, reduction in neuronal size, and diminished neuropil (axons, dendrites, and synaptic terminals) (65, 66). The etiology behind the loss of dendritic spines and dendritic length of cortical pyramidal neurons is not entirely clear but may originate from hypofunctioning NMDA glutamate receptors on pyramidal cells and interneurons (67–69). From a circuit perspective, hypofunction of NMDA receptors on GABAergic inhibitory interneurons disinhibits associated pyramidal neurons in the circuit and causes a potentially pathological glutamatergic excitatory effect (70, 71).

Hyperglutamatergia may be a distinct feature of TRS and be differentiated from treatment-responsive disease since greater abnormalities in glutamate function have been found in those patients with TRS while maintaining a relatively normal and intact dopamine function. Neuroimaging measures using fluorine-18-L-dihydroxyphenylalanine (^{18}F-DOPA) as a PET radiotracer found a higher level of striatal dopamine synthesis capacity in patients with schizophrenia who responded to treatment vs. those patients with TRS who had equivalent striatal dopamine levels found in healthy controls (72). The same group later utilized proton magnetic resonance spectroscopy (^{1}H-MRS) imaging in TRS to examine glutamate changes that may be specific to antipsychotic treatment-resistance (73). This was the first group to report high glutamate and glutamine levels in the anterior cingulate cortex (ACC) in TRS as compared to those with schizophrenia in remission, and another group has since replicated this finding (74).

Increased concentrations of glutamate found in the ACC that are specific to TRS are consistent with both the glutamate hyperfunction and the NMDA receptor hypofunction hypotheses of schizophrenia. Normally, glutamate is responsible for regulating inhibitory tone in the brain by binding to NMDA receptors on GABAergic interneurons. The structural mechanism that may cause NMDA receptor hypofunction in TRS can lead to disinhibition of pyramidal neurons and excitatory pathways by the understimulation of inhibitory GABA interneurons (75). The downstream effect can then cause an increase in glutamate release from presynaptic pyramidal neurons and binding to α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate receptors and may be a compensatory effect of the NMDA blockade (75–78). The hyperglutamatergic state can initiate calcium influx and cellular toxicity which, over time, can be detrimental to neuronal networks (79). In treatment-resistant disease, excitatory inputs from pyramidal neurons within the ACC circuit could also be disinhibited, leading to increased glutamate efflux and generating symptoms that fail to respond to D_2-blocking medications. Glutamate-mediated excitotoxicity may be responsible for the widespread brain abnormalities and severity of symptoms that are found in TRS.

Disturbances in the functional activity of neural circuits have consistently been reported in TRS. Functional MRI studies that have examined changes in neurophysiological measures also may indicate disordered firing and pathological oscillatory activity that may be more pronounced in TRS (63). Persistent auditory hallucinations are a core feature of psychosis. Poor control of this symptom within the positive symptom domain that persists despite adequate trials of antipsychotic medications is often the clearest and most common indicator of severe treatment resistance. Patients with specific TRS-positive symptom domain phenotypes and experiencing auditory verbal hallucinations (AVH) have been investigated in fMRI studies (60–63).

Functional MRI using magnetically labeled blood water protons as an endogenous tracer (arterial spin labeling) to measure tissue perfusion found increased cerebral blood flow in the left superior temporal gyrus, right supramarginal gyrus, and temporal polar cortex in patients with treatment-resistant AVH (63). Functional resting-state MRI studies that investigated connectivity alterations in the default network in patients with chronic non-responsive AVH and treated patients without AVH found that treatment-resistant patients had increased functional connectivity between the dorsomedial prefrontal cortex and other frontotemporal regions, but reduced connectivity between the ventromedial prefrontal cortex and areas of the cingulate cortex (60). Reduced functional connectivity between the left temporo-parietal junction (TPJ) and right Broca's area and ACC and temporo-cingulate pathways have also been implicated in patients with persistent AVH (61, 62). All functional alterations found were greater in those patients with persistent treatment-resistant symptoms, indicating there may be fundamental differences within these brain network properties that are also specific to TRS.

Network-based statistics can be applied to fMRI data to investigate brain networks and to better delineate the differences in the connectome unique to TRS. Although there have been a number of network-based studies in schizophrenia (31, 32, 45–47), Ganella et al. were the first to measure the connectivity and global and local efficiency of whole-brain functional networks from resting state fMRI data in individuals with TRS compared to healthy controls (80). Whole-brain connectivity analysis in this study showed reductions in functional connectivity between all of the brain lobes, with the majority of reduced connections between fronto-temporal, fronto-occipital, temporo-occipital and temporo-temporal subregions. The majority of reduced functional connections in TRS were found in the temporal lobe

(between Heschl's gyrus and the frontal lobe), the occipital lobe (between the cuneus and the frontal lobe), and the frontal lobe (between the paracentral lobule and the occipital lobe). Treatment-resistant individuals showed reduced functional connectivity in the temporal lobes as regions most implicated. Decreased connectivity between frontal and temporal brain hubs regions is a particularly vulnerable circuit consistently reported in several studies in schizophrenia and is also characteristic of the circuit pathophysiology of TRS (80).

In terms of network-based analysis, global network efficiency was significantly reduced in the TRS group compared to controls with significant increases in local efficiency. Reduced global network efficiency indicates that the reduction of functional connectivity and integration between different brain hubs in TRS as a result of the disease process may create surrogate or back-up connections locally (increase in local efficiency) as a homeostatic mechanism and an attempt to compensate for the reduction in longer-range connectivity and restore integration (46, 80).

THE SYNCHRONIZATION OF CORTICAL CIRCUITS

One possible functional correlate of the aberrant connectivity observed in TRS is disturbances in cortical network oscillations. Oscillations in network activity include the theta (∼4–8 Hz), alpha (∼8–13 Hz), and gamma (∼30–80 Hz) bands. These oscillations are measurable by electroencephalography (EEG) and magnetoencephalography (MEG) and are thought to be reflective of cortical information processing and integration (79, 81) Importantly, they reflect the synchronous activity of large populations of neurons that integrate information across multiple brain regions. With regard to schizophrenia, specific interest has been paid to the gamma band oscillation (GBO) (82–85). The GBO plays an important role in a variety of cognitive tasks including sensory processing, working memory, attention, and cognitive control–all of which are disturbed in the illness (86–91). More generally, it is thought to be critical to the process of feature binding, in which sensory information of a variety of modalities is integrated coherently into a unified representation (92). Fittingly, it has been suggested that the underlying dysfunction in schizophrenia is the inability to integrate the activity of distributed neuronal networks. These disturbances in the GBO could underlie the dysfunctional communication observed between disparate brain regions in the illness.

The GBO has been shown to be disrupted in schizophrenia patients during the performance of a wide variety of tasks, including simpler perceptual tasks and more complex and cognitively demanding tasks (93–96). In patients diagnosed with schizophrenia, EEG studies have shown that the GBO is impaired in working memory tasks at frontal and posterior sites, as well as in the frontal cortex during cognitive control tasks (97–100). Performance of these tasks is typically associated with increase in GBO activity in healthy subjects. However, in subjects with schizophrenia this demand-related modulation of the GBO is absent or diminished. The deficit in task-related modulation of the GBO is also present in first-episode patients, suggesting that this is driven by the underlying disease process rather than illness chronicity or long-term use of antipsychotic medications (99). Several of these studies have also shown that deficits in cognitive control in patients with schizophrenia are correlated with their deficits in GBO activity (91, 98). Convergent evidence from fMRI studies has also shown a lack of task-demand related modulation of activity in the PFC in schizophrenia patients (101). These findings suggest that for cognitive tasks, particularly those that may depend on integration of information, the GBO is a reflection of disturbed functional connectivity between communicating brain regions.

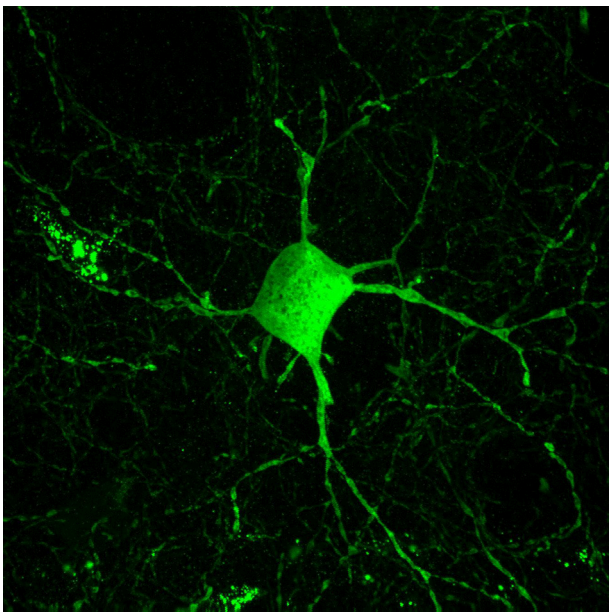


FIGURE 2 | Parvalbumin interneurons contribute to the inhibitory dysfunction in schizophrenia. Parvalbumin interneurons are fast-spiking inhibitory interneurons characterized by the calcium binding protein parvalbumin. These interneurons are innervated by excitatory glutamatergic cells and in turn their projections target the cell soma of excitatory pyramidal cells. This excitatory-inhibitory interplay is thought to give rise to the GBO, which is reflective of parvalbumin interneurons role in synchronizing large populations of excitatory cells. The GBO is disturbed in schizophrenia, and dysfunction within parvalbumin interneurons is thought to be central to these abnormalities.

Multiple models have been generated to describe the underlying neural circuitry that gives rise to the GBO. Two prominent ones include the Interneuron Network Gamma (ING) model and the Pyramidal Interneuron Network Gamma (PING) model (102). In the ING, pyramidal cells are synchronized by the activity of interneurons, but pyramidal cells themselves are not directly involved in the generation of the GBO. In PING, oscillations are generated via the recurrent synaptic connectivity between the excitatory activity of pyramidal cells and feedback inhibition of interneurons. While this process is still not fully understood, experimental observations favor the PING model of GBO generation. In this case, synaptic inhibition via GABAergic interneurons defines the timing and firing rate of pyramidal

neurons, creating precise windows within which large groups of excitatory cells can fire synchronously (103–105). In turn, excitatory cells also provide input onto GABAergic interneurons, creating a loop for entrainment of cortical networks across brain regions. Support for the PING model comes from findings that interneuron activity follows pyramidal cell activity by a short delay, consistent with pyramidal cell excitatory drive as the main stimulus for interneuron excitation in the model (106, 107). Within excitatory cells, α1-containing $GABA_A$ receptors post-synaptic to a subset of inhibitory interneuron processes produce currents with decay periods fitting for the production of gamma oscillations (84). Lastly, it has been shown that with genetic knockout of AMPA glutamate receptors within specific populations of inhibitory interneurons, synaptic excitation of these inhibitory interneurons is diminished and the power of the gamma oscillation severely reduced (108). These findings support the theory that the GBO arises from a complicated interplay between excitatory pyramidal cells and inhibitory interneurons.

Consistent with the PING model, there is ample evidence to suggest that both excitatory glutamatergic and inhibitory GABAergic activity are disturbed in schizophrenia (84, 85). Deficits in excitatory glutamatergic signaling have been identified as a possible core feature behind the pathophysiology of schizophrenia that gave rise to the NMDA receptor hypofunction hypothesis (71). This hypothesis arose from the observation that NMDA receptor antagonists (e.g., ketamine, PCP) can reproduce some of the symptoms of schizophrenia. Subsequent studies have identified widespread dysfunction of NMDA receptors in schizophrenia. Interestingly, given that the GBO is thought to be generated by the activity of inhibitory interneurons, much of the observed dysfunction in NMDA receptors has been specific to inhibitory interneurons themselves. For example, post-mortem analysis of the PFC of schizophrenia patients has shown a 50% reduction in the expression of the NR2A subunit within inhibitory interneurons that express parvalbumin, a calcium binding protein (109). Moreover, chronic NMDA receptor antagonist administration in rodent models reduces the expression of the parvalbumin protein and GAD67 (the primary GABA-synthesizing enzyme glutamic acid decarboxylase) in parvalbumin-positive(+) inhibitory interneurons (110, 111). Acute administration of NMDA receptor antagonists has also been shown to decrease the activity of interneurons with a corresponding increase in the activity of pyramidal cells (70). Thus, NMDA receptor antagonism may reduce the function of inhibitory interneurons which subsequently disinhibits the activity of pyramidal cells. Within the context of schizophrenia, NMDA receptor hypofunction may result in the diminished excitation of inhibitory interneurons within cortical networks.

Inhibitory interneurons are particularly sensitive to NMDA receptor antagonists (70, 112, 113). In combination with findings of altered expression of NMDA receptors within these interneurons, it is well-supported that inhibitory interneurons, particularly those expressing the calcium-binding protein parvalbumin, are a locus for dysfunction in schizophrenia (shown in **Figure 2**) (84, 114, 115). A number of studies have shown that parvalbumin+ cells are critical to the generation and maintenance of the GBO (106, 113, 116, 117). These interneurons have extremely fast-spiking properties and their rapid synaptic activation is consistent with the frequency required for entrainment of the GBO (118). Parvalbumin+ cells also show the strongest coupling to the gamma oscillation cycle relative to other interneuron types (e.g., calbindin, calrentin) (119, 120). Parvalbumin+ interneurons are typically fast-spiking and provide perisomatic inhibition onto excitatory pyramidal cells. Parvalbumin+ interneurons can present morphologically as either basket (project to the soma and proximal dendrites of neurons) or chandelier cells (project to the initial axon segment of neurons) as illustrated in **Figure 3**. While both parvalbumin+ basket and chandelier cells are active during GBO, parvalbumin+ basket cell activity is more strongly coupled with the GBO (121). Studies have also shown that GBO power is markedly reduced by opioid receptor activation, which dampens the activity of synaptic inputs from parvalbumin basket cells onto pyramidal neurons but does not affect chandelier neurons (122). These findings emphasize the critical importance of parvalbumin+ basket cells specifically to the generation of the GBO and their dysfunction in schizophrenia. In support of this, it has been shown that reductions in the firing rate of parvalbumin+ interneurons via optogenetics can reduce the power of GBO (114). Conversely, non-rhythmic stimulation provided to parvalbumin+ interneurons can increase the power of the GBO.

Parvalbumin+ cells have been extensively studied in schizophrenia and evidence of their dysfunction extends well beyond their contribution to the GBO (85, 115, 123). Parvalbumin+ cells have a reduction in mRNA and protein levels of parvalbumin itself despite unaltered neuronal density in patients with schizophrenia observed post-mortem (124–126). Parvalbumin+ cells also have reduced protein and mRNA levels of GAD67 and up to 50% of parvalbumin+ cells are wholly devoid of GAD67 (124). This loss of GAD67 represents a significant decrease in the strength of inhibitory inputs on the pyramidal cells they target (115). Moreover, this deficit has been observed in parvalbumin+ cells across multiple cortical regions including the DLPFC and ACC (127–129). Two hypotheses have been generated to account for the convergent evidence of dysfunction localized to parvalbumin+ basket cells (84). One hypothesis emphasizes the inhibitory contribution to this network interplay and the other excitatory activity. First, lower GAD67 levels in parvalbumin+ basket cells could result in a disinhibition of pyramidal cells. Alternatively, the loss of GAD67 in parvalbumin+ basket cells could be a development disruption due to lack of excitatory input onto these cells to drive their activity. Consistent findings of dendritic spine loss on pyramidal cells in areas like the DLPFC and dysfunction within glutamatergic channels (e.g., NMDA, AMPA) could contribute to this loss of excitatory input onto parvalbumin+ basket cells in schizophrenia (68). These findings support the central importance of parvalbumin+ inhibitory interneurons in schizophrenia pathophysiology but whether this is a primary pathology or homeostatic mechanism in response to diminished pyramidal cell input is still unclear.

Despite an improved understanding of the underlying pathophysiology of schizophrenia, particularly with regard to

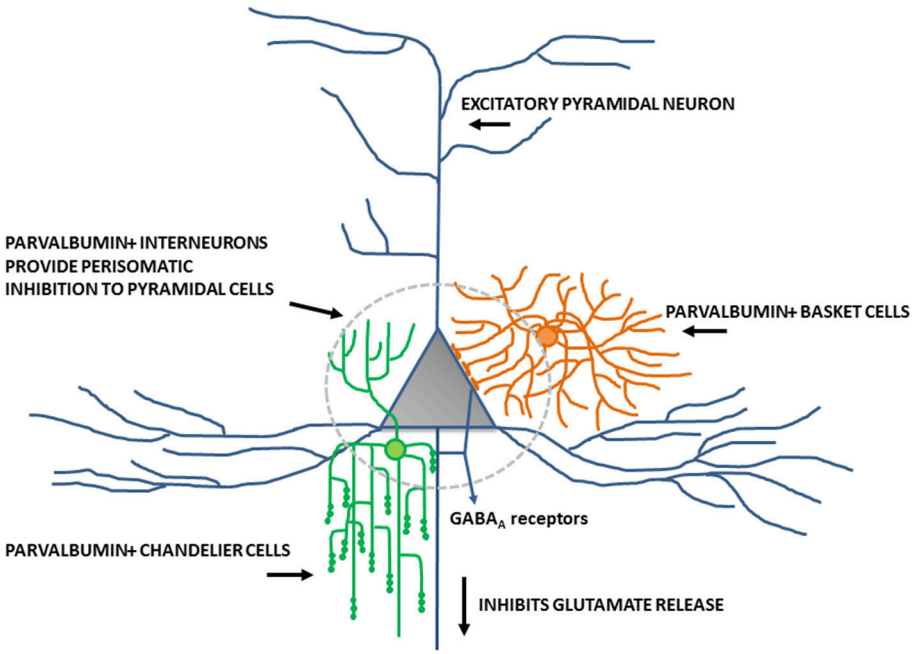


FIGURE 3 | Schematic diagram of perisomatic inhibition of cortical pyramidal cells by parvalbumin+ basket cells and chandelier interneurons. Adapted from Lewis et al. (115).

cellular mechanisms contributing to the GBO, a multitude of questions remain. Of utmost importance to the current review is the validity of these findings, many of which have been garnered from animal models of schizophrenia, to TRS. Current cellular and animal models have significant limitations in modeling the illness and few, if any, attempts have been made to replicate the treatment-resistant presentation of the disorder. Secondly, further investigation is required to understand the complex interplay between excitatory glutamatergic cells and inhibitory interneurons in the dysfunctional circuitry of schizophrenia. Specifically, a better understanding of the cellular properties that give rise to the GBO are necessary to better understand approaches for treatment. And lastly, novel treatments and therapeutics need to be designed to target the pathophysiological functioning of GBO circuitry. These approaches may include pharmacological stimulation of the neural circuitry or might be targeted through novel non-pharmacological approaches, such as rTMS which can directly stimulate the GBO.

SYMPTOM DOMAIN CIRCUITS

Patients who have a phenotype of psychosis that is responsive to dopamine-blocking medication may have dysregulated striatal hyperdopaminergia related to circuit abnormalities within the fronto-striatal complex of the mesolimbic dopaminergic pathway. Glutamatergic projections from the PFC to the ventral tegmental area (VTA) normally regulate dopamine release in the nucleus accumbens. Within this circuit phenotype, hypofunctioning NMDA glutamate receptors on cortical parvalbumin+ GABAergic interneurons will cause an excessive release of glutamate within the VTA. Hyperglutamatergia then leads to overstimulation (on circuit phenotype) of dopaminergic neurons within the mesolimbic dopamine pathway and excessive release of dopamine within limbic structures, such as the nucleus accumbens, amygdala and hippocampus (130, 131). Hyperdopaminergia within the fronto-striatal circuit underlies the beneficial positive symptom domain response that treatment-responsive patients achieve with D_2-blocking medications.

Negative and cognitive symptom domain circuitry involves cortical brainstem glutamate projections that communicate within the mesocortical dopamine circuit. Glutamatergic projections from the cortex onto hypofunctioning NMDA glutamate receptors located on cortical parvalbumin+ interneurons leads to the excessive release of glutamate in the VTA. The excessive stimulation of pyramidal VTA neurons then leads to the inhibition (off circuit phenotype) of mesocortical dopamine neurons and insufficient dopamine release in the PFC and subsequent negative and cognitive symptoms in schizophrenia (130, 131).

In those patients who fail to respond to antipsychotic medication, it has been demonstrated that although D_2 receptor occupancy is identical to treatment-responsive patients, the lack of efficacy from D_2-blocking medication may indicate that hyperdopaminergia may not be related to the symptoms associated with non-response to medications (132). Higher levels of striatal dopamine synthesis capacity have been found in patients with schizophrenia who responded to treatment vs. those patients with TRS who have much lower striatal

dopamine levels comparable to healthy controls (72). Also, fronto-striatal dysconnectivity is more pervasive and widely distributed anatomically in TRS as compared to treatment-responsive individuals which may also explain the limited efficacy of dopamine-blocking medication targeting D_2 receptors within the fronto-striatal circuit in TRS (133).

The neurobiology unique to treatment resistance may involve more glutamatergic related abnormalities than disruptions involving dopamine. Clozapine has a unique and complex pharmacological profile (having a higher affinity to D_4 receptors than to D_2 receptors) and a higher binding affinity to many other non-dopaminergic receptors. Clozapine is able to normalize glutamate neurotransmission by increasing NMDA receptor activity in the cortex by a number of different mechanisms. It has been demonstrated that antagonism of D_4 receptors can regulate glutamatergic transmission by upregulating AMPA receptors and providing homeostatic stabilization of the excitation of PFC pyramidal neurons by indirect enhancement of NMDA activity (134). Clozapine has also been shown to reduce the reuptake of glutamate in the cortex by decreasing the expression of glutamate transporters located on both glial and neuronal cells in cortical and subcortical areas (135). Clozapine has the ability to antagonize glycine transporter-1 (GlyT1) sites for reuptake of glycine by glial cells (136), and can increase glial D-serine release and enhance the release of glutamate via activation of NMDA receptors (137) which may help to regulate some of the downstream glutamate abnormalities that have been found in TRS (73, 74).

It is difficult to map the underlying circuit pathology in ultra-resistant schizophrenia due to the heterogeneity of the illness and limited studies that have explicitly examined this population. Due to multidimensional symptom domains resistant to clozapine, ultra-resistant schizophrenia can be described as the most severe phenotype of the illness that is mediated by multiple mechanisms far beyond dysregulated striatal hyperdopaminergia and glutamate NMDA receptor hypofunction.

CIRCUIT-BASED PHARMACOLOGICAL TREATMENTS

Currently, there are no customized neural circuit-specific and targeted therapies that can address the neural-dysconnectivity in schizophrenia. Despite the lack of precision and ubiquitous targets of pharmacological methods, the use of adjunctive agents to antipsychotic medications may be conceptualized within a circuit context to help improve neuronal network integration and treatment response in TRS. In many cases, augmentation strategies are needed to improve the residual psychopathology symptom domains that have been non-responsive to antipsychotic drugs (including clozapine). Usually in those patients who have not responded to clozapine, a variety of other antipsychotic medications, antidepressants, anticonvulsants, benzodiazepines or a variety of glutamate augmenting agents have been attempted. Clinical studies have used a variety of agents that can enhance glutamate NMDA receptors (on connectomic) function in an attempt to improve downstream GABAergic (off connectomic) inhibitory effects. GABA interneuron modulators have also been recently investigated as an attempt to inhibit pyramidal cell firing, as well as NO-based therapies to improve intracellular NMDA receptor signaling and other direct circuit-targeted neurosurgical and neuromodulation strategies for their therapeutic benefit in treatment resistant disease.

GLUTAMATERGIC AGENTS

Many drugs that target and co-activate glutamatergic pathways have been of interest as a non-dopaminergic approach to improve antipsychotic treatment in schizophrenia. Strategies to improve glutamate NMDA receptor hypoactivity on GABAergic interneurons have targeted extracellular binding sites on the receptor. The glycine modulatory site has been investigated as a target to improve NMDA receptor hypofunction in schizophrenia and several agonists or partial agonists of this binding site on the NMDA receptor have been studied in clinical trials (138).

The amino acid glycine is a co-agonist of the NMDA receptor and it is required along with glutamate to activate the NMDA ion channel (139, 140). The binding site for glycine (located on the NR1 subunit) of the NMDA receptor was first discovered by Johnson and Ascher (1987) by preclinical electrophysiology studies using the outside-out patch clamp method. The NMDA receptor response was then observed to be potentiated by glycine. The distinct binding site (glycine B receptor) was separate from the strychnine-sensitive glycine inhibitory receptor as NMDA receptor potentiation by glycine was not blocked by strychnine (139). In clinical studies, reduced plasma concentrations of glycine have been found in patients with schizophrenia and have been correlated with a greater number of negative symptoms (141, 142), supporting the use of glycine as a strategy to improve NMDA receptor functioning in those patients identified as having treatment resistance specific to the negative symptom domain (138).

Glycine was first used as an augmenting treatment in schizophrenia close to 30 years ago in a few small open-label clinical trials used at doses between 5 and 25 g per day (138, 143–145). In subsequent controlled trials, 60 g of glycine augmented with first-generation or second-generation antipsychotic medication was reported to improve not only the negative symptoms (146–150), but also cognitive symptoms (147, 148, 150) and the depressive symptoms of the illness (148). Glycine is not able to cross the blood-brain barrier easily as it has no specific amino acid transporter, so higher doses must be used that impacts patients' tolerability to glycine. The benefits reported of using glycine as an augmenting treatment to antipsychotic medications to improve the cognitive and negative symptoms domains of the illness has since been disputed. In a subsequent review, glycine was found to have moderate effect in reducing negative symptoms and it was uncertain whether it had any benefit at improving cognitive symptoms (151). The multicentre Cognitive and Negative Symptoms in Schizophrenia

Trial (CONSIST), found no significant differences between glycine and placebo at improving the negative or cognitive symptom domains of the illness (152). Overall, glycine may be beneficial for those patients that have treatment resistance specific to the negative and cognitive symptom domains; (153) however it has not been a beneficial augmenting strategy in patients with TRS on clozapine (154).

An alternative approach to increasing endogenous brain glycine concentrations has been to block its reuptake and thus improve glutamatergic tone. The amino acid sarcosine, a GlyT1 inhibitor, has also been demonstrated to improve the negative, cognitive and depressive symptom domains of schizophrenia (155, 156). Unfortunately, significant side-effects have since been reported including ataxia, hypoactivity and respiratory depression with the use of sarcosine, perhaps in relation to mechanisms involved in the overstimulation of the strychnine-sensitive glycine inhibitory glycine receptor (157, 158). When used as an augmenting strategy in patients with TRS, sarcosine was also not effective (159). This may be related to clozapine's glutamatergic effects and known GlyT1 antagonist properties (136, 138). Bitopertin, a non-sarcosine-based selective GlyT1 inhibiting drug, has also been investigated as an adjunct to antipsychotics (at doses of 10 and 30 mg per day) to mainly target the negative symptom domain of the illness (160). In subsequent phase III trials (SearchLyte trial programme), bitopertin was unsuccessful at improving the primary outcome measure of Positive and Negative Syndrome Scale (PANSS) (161) negative symptom scores over placebo which led the manufacturer Hoffmann-La Roche to discontinue the programme prematurely (138).

D-serine, an allosteric modulator at the glycine co-agonist binding site, has also been investigated as an augmenting strategy primarily for improving the deficit symptoms of schizophrenia. D-serine may be more effective than glycine as it has a greater affinity for the glycine/serine binding site and also has an increased ability to cross the blood-brain barrier (162–164). Serum concentrations of D-serine have also been found to be reduced in schizophrenia (165). D-serine selectively binds to synaptic NMDA receptors and may strengthen circuit connectivity and have more of a neuroprotective effect as compared to glycine, which binds to both synaptic and extrasynaptic NMDA receptors (138, 166). The therapeutic effects of D-serine to improve refractory negative symptoms in schizophrenia have been demonstrated when added to antipsychotic therapy in patients with acute (156), chronic (167), and treatment-resistant illness (168). D-serine is well-tolerated and has been reported to be safe and effective used at dosages up to 120 mg/kg per day (169). D-cycloserine, a drug that was initially used to treat tuberculosis and an anolog of D-serine, is also active at the glycine site and has been reported to benefit the negative symptom domain of schizophrenia (170–172). Unfortunately, in patients with TRS, glycine, D-serine, and D-cycloserine have all been reported to be less effective at improving the negative and cognitive symptom domains in those patients receiving clozapine therapy (138, 152, 154, 172, 173).

Drugs that can downregulate presynaptic disinhibited glutamate release on secondary downstream glutamate neurons have also been explored in patients with TRS and may also work to modulate circuit connectivity. Lamotrigine, an anticonvulsant drug that suppresses presynaptic glutamate release by the blockade of voltage-sensitive sodium channels has been shown to improve clinical response when used as an adjunct to clozapine treatment in ultra-resistant schizophrenia (138, 174–178). The beneficial effects may be associated with clozapine's low affinity to the D2-receptor and involvement with the glutamate system (in comparison to other antipsychotic drugs) which may be further enhanced by lamotrigine (138, 175). More recent clinical trials have studied the efficacy between the metabotropic glutamate 2/3 (mGlu2/3) receptor agonist pomaglumetad methionil (also known as LY2140023) and atypical antipsychotics (138, 179, 180). In a phase II study, it was found to be less effective than the comparator atypical antipsychotic (180) and Eli Lilly subsequently stopped a phase III trial investigating the compound as it failed to meet its primary endpoint.

NITRIC OXIDE-BASED TREATMENTS

An alternative and novel approach that may improve glutamate NMDA receptor signaling and circuit connectivity in schizophrenia is to target the glutamate-NO-cyclic guanosine monophosphate (cGMP) signaling cascade. Nitric oxide is produced in the brain by a complex interaction with a functional glutamate NMDA receptor and there have been a number of clinical studies suggesting that signaling within the glutamate-NO-cGMP pathway may be disrupted in the illness (138, 181–187). As a gaseous signaling molecule, NO is classified as a neuromodulator or second messenger due to its ability to generate the production of cGMP. Nitric oxide-mediated signal transduction is an important driver for a variety of cellular processes throughout the body, including those critical for the establishment and maintenance of functional neuronal circuits and synaptogenesis (138, 188). In the cerebral cortex, neurons that produce NO are among the earliest differentiating cells that develop (138, 189). The presence of NO-producing neurons during critical developmental growth periods suggests that NO may be required for the formation and subsequent migration of neurons in the brain, and interruption of NO synthesis could lead to impairment in neuronal connectivity as is observed in schizophrenia.

Studies examining the effects of the NO donor drug sodium nitroprusside (SNP) in PCP-treated rats has contributed insight into the role of NO in psychosis (138, 190, 191). The results then stimulated the investigation of the therapeutic effects of SNP in schizophrenia (192, 193). Sodium nitroprusside is a nitrovasodilator drug traditionally used for hypertensive crisis (194). When SNP is administered, it reacts with oxyhemoglobin molecules that are within erythrocytes to form methemoglobin which causes the molecule to become unstable and immediately release NO (138, 194).

The first investigational clinical trial of NO in schizophrenia was conducted at the University Teaching Hospital in Ribeirao

Preto, Sao Paulo, Brazil. In this clinical trial, an intravenous infusion of SNP in patients who were already on antipsychotics produced rapid improvement of symptoms (within 4 h of a single infusion) as compared to those patients who received a placebo infusion (138, 192). Symptom improvement continued for 4 weeks following the infusion (although antipsychotic medication adjustments were permitted 7 days following the infusion). The lasting benefits are thought to be related to cGMP's ability to stimulate early gene products and subsequent modulatory effects on the NMDA receptor itself. Sodium nitroprusside has been beneficial in both early stage schizophrenia and in a few case reports of ultra-resistant schizophrenia and did improve a wide spectrum of symptom domains, including the positive, negative, and anxiety symptoms of the illness (138, 192, 193). The results were not replicated in a subsequent trial testing SNP in a population of long-term chronically ill patients (195), which may suggest that SNP-based therapies may be most effective when used within the earlier stages of the illness in those patients experiencing acute symptoms.

In relation to these findings, Dr. Paul Morrison (King's College London) is currently testing the NO-based compound glyceryl trinitrate (GTN) for its ability to improve the cognitive symptom domain of patients experiencing acute psychosis and who are requiring hospitalization (Clinicaltrials.gov Identifier: NCT02906553). Glyceryl trinitrate is another nitrovasodilator drug that has been used to treat angina and other cardiac conditions including myocardial infarction and congestive heart failure. The biotransformation of GTN involves both enzymatic and nonenzymatic pathways that are linked to the pharmacokinetic and pharmacodynamics properties of the drug (138, 196). The metabolic conversion of GTN to NO may also improve downstream glutamate signaling. This clinical trial aims to assess the role of the NO system in cognition and will initiate a sublingual GTN spray 0.4 mg dose, once per day for 3 days or matching placebo formulation spray not containing GTN before the patients are initiated on antipsychotic medication. Glyceryl trinitrate in sublingual spray formulation is a much more convenient and less invasive approach to drug delivery than intravenous infusion of SNP in patients with schizophrenia and may be a promising approach to further improve treatment-resistant cognitive symptoms in the illness.

$GABA_{ergic}$ INTERNEURON MODULATORS

Pharmacological strategies that target GABAergic interneurons that may correct dysfunctional inhibitory feedback within corticolimbic circuits are also being investigated. Specifically, parvalbumin+ cells are now also being explored as a novel approach to repairing DLPFC neural circuitry and improving the cognitive symptom domain in schizophrenia (Clinicaltrials.gov Identifier: NCT03164876). Parvalbumin+ cells innervate multiple pyramidal cells and contain lower mRNA for parvalbumin and GAD67 in those with schizophrenia (124) and reduced expression of the potassium channel *KCNS3* gene which encodes the Kv9.3 potassium channel α subunit and is essential for control over its fast-spiking abilities (197). Inhibitory parvalbumin+ interneurons contribute to the cognitive deficits in schizophrenia (115) and in unmedicated patients with the illness. Kv3.1 channels located on parvalbumin+ cells are reduced by disease and then normalized with the use of antipsychotic drugs (198). Dr. Charles Large (Autifony Therapeutics) has recently completed a phase I study of AUT00206, a Kv3.1 channel modulator in healthy volunteers (Clinicaltrials.gov Identifer: NCT02589262) and in collaboration with Dr. Oliver Howes (King's College London), his team are currently recruiting for a continued phase I study to explore its safety, tolerability, pharmacokinetics and treatment effects on relevant biomarkers in patients with schizophrenia (Clinicaltrials.gov Identifier: NCT03164876).

CIRCUIT-BASED NEUROSURGERY

Surgical modalities that can precisely target particular regions of focal and well-localized dysconnectivity in the brain are currently being tested as a more circuit-specific approach to precision medicine in schizophrenia. Deep brain stimulation (DBS) has been a well-established targeted therapeutic approach that has been used to improve the treatment-resistant symptoms of Parkinson's disease, obsessive-compulsive disorder and treatment refractory depression (199–202).

Neurosurgical DBS strategies are also now being considered to be used in ultra-resistant schizophrenia to target those relevant brain hubs that may improve the interconnectivity of relevant neuronal circuits. The implantation of electrodes into accessible anatomical nodes can be targeted to normalize or reset abnormal patterns of cortical network GBO activity that disrupt neural circuits. The stimulation settings of the electrodes can be titrated to tune the neurons to specific frequencies and recalibrate neuronal asynchrony. There is current interest in targeting several important network hubs using DBS in ultra-resistant schizophrenia involved in basal ganglia-thalamocortical and DLPFC brain circuits. Hubs identified include the hippocampus, ventral and associated striatum, medial and DLPFC, substantia nigra, nucleus accumbens and the mediodorsal nucleus of the thalamus (203–205). These hubs have been chosen primarily based on known pathological findings in schizophrenia and/or their interconnectedness to other brain hubs that are circuit-specific and related to the excessive and mistimed dopamine release in the striatum. Hippocampal dysfunction that drives downstream dopamine release in the striatum contributing to persistent positive symptoms is one of the clinical hallmarks for treatment-resistant disease (206).

Currently there are two phase I DBS trials investigating this approach in ultra-resistant schizophrenia that are recruiting patients. The first trial at Hospital Santa Creu i Sant Pau in Barcelona (Clinicaltrials.gov Identifier: NCT02377505) is targeting electrode placement in either the nucleus accumbens or the subgenual ACC. The participants will be randomized to receive stimulation to either of these neuroanatomical sites with the stimulation remaining on until a full 6 months of stabilization

is achieved. Those patients who are responsive will then be crossed-over to stimulation-on or stimulation-off groups for 3 months.

The principal investigator, Dr. Iluminada Corripio has recently reported positive findings in the first subject who participated in this clinical trial. The patient had a long history of ultra-resistant schizophrenia-positive symptom domain refractory symptoms including manifestations of persecutory, control and delusions of reference. Her referential delusions had become so pronounced that she was unable to leave her home. The patient had a long treatment history typical of ultra-resistant schizophrenia including many trials with a number of different antipsychotic medications, including the use of clozapine (600 mg/day) with little benefit. The patient underwent bilateral electrode implantation in the nucleus accumbens and left-sided unilateral stimulation. Improvement was achieved in both positive and negative symptoms measured 4 weeks post-implantation and after 11 months of open treatment, the patient experienced over a 60% reduction in positive symptoms as measured by the positive symptoms subscale of the PANSS as well as a 33% reduction in negative symptoms, 50% reduction in the PANSS disorganization factor, 33% reduction in PANSS excited factor and 16.7% increase in the depressed factor. The patient continues to do well and is now able to leave her home and has made significant improvements to her overall functioning. For this patient with ultra-resistant schizophrenia, this DBS treatment option was of substantial benefit to otherwise untreatable refractory symptoms (207).

The second DBS trial in ultra-resistant schizophrenia is out of Johns Hopkins University where the study team led by Dr. William Anderson will be recruiting three ultra-refractory patients and will be targeting the local inhibition of the substantia nigra pars reticulata (SNr), a major outflow nucleus of the basal ganglia with the intention of disinhibition and driving the activity of the mediodorsal nucleus of the thalamus (Clinicaltrials.gov Identifier: NCT02361554). The structure and hypofunction of the mediodorsal nucleus of the thalamus has been investigated in several imaging and post-mortem studies in schizophrenia (208). All of the DBS studies in ultra-resistant schizophrenia are only recruiting those patients who have exhausted all other therapeutic alternatives and continue to have severe and disabling clinical symptoms and poor functioning.

CIRCUIT-BASED NEUROMODULATION

The use of external neuromodulation devices, a less invasive circuit-based treatment approach than DBS has also become an alternative treatment option for refractory schizophrenia. Repetitive transcranial magnetic stimulation (rTMS) has been the method most investigated. In rTMS time-varying currents are generated in an induction coil and are held over the scalp and applied to stimulate and improve the functioning and synchrony of the GBO networks and GABA inhibitory mechanisms within the brain circuits beneath it. There have been several randomized studies conducted to show that stimulation targeted over the left TPJ, a critical hub involved in the pathophysiology of AVH, can reduce these symptoms (209–215).

Transcranial direct current stimulation (tDCS) is an alternative non-invasive form of neuromodulation that has been used to target specific circuits of the brain to improve treatment-refractory symptom domains of schizophrenia. It is a smaller, lightweight, portable and less expensive option than TMS and could be easily used at home to reduce the burden of having to receive daily treatments within a clinical setting (216). In this approach, two sponge electrodes are positioned on the scalp to facilitate a low-intensity electrical current (1–2 mA) that is passed between them. The transcranial current that is generated is continuous and flows in a direct current from an anode (current that enters the body) to induce prolonged depolarization to a cathode (a current that exits the body) to induce hyperpolarization under the cathode (217–220). It is thought that the mechanisms involved in the longer-lasting effects of tDCS are protein synthesis-dependent and in the modification of intracellular cascades beyond the membrane potential to influence cellular features associated with NMDA receptor functioning (216, 217). tDCS is increasingly being investigated by more independent schizophrenia researchers and primarily for improvement of positive (AVH) and negative symptom domain refractory symptoms.

Based on observations of the dysconnectivity of fronto-temporal circuits from functional neuroimaging studies of patients experiencing AVH (60–62), clinical studies have used tDCS to improve the dysconnectivity of these circuits to decrease AVH in patients with schizophrenia. In these studies, the anode electrode is applied over the left DLPFC (abnormally hypoactive) with the cathode electrode applied over the TPJ (abnormally hyperactive) to modulate the circuit and alleviate the severity of the AVH in schizophrenia (218, 221, 222). Results have been mixed in the ability of tDCS to reduce severity and frequency of AVH. For reviews see Li et al. (223), Ponde et al. (224), and Agarwal et al. (225). Studies that have reported a stronger and longer lasting response have had a higher number of treatment sessions and/or shorter time interval between sessions within their design (221, 226).

Open-label and randomized clinical trials that have examined the effects of tDCS to target negative symptoms of schizophrenia have placed the anode over the left DLPFC and the cathode over the right DLPFC or the right supraorbital region or placed it extra-cephalically (221, 227–229). A meta-analysis concluded that tDCS treatment is beneficial for improving negative symptom domain indications (211). There has been direct support for the safety of tDCS in human clinical trials with the most often reported side-effect of mild skin erythema, itching, tingling and burning under the electrode placement as well as temporary headache and dizziness which resolves after stimulation (218, 220).

CONCLUDING REMARKS

Treatment resistance in schizophrenia continues to be a therapeutic challenge in psychiatry. Within the spectrum of the disease, neural circuits within specific brain regions

and their structural and functional links to corresponding regions seem to be further disrupted in TRS. In this review, we have examined TRS from a circuit-based perspective. We highlighted attempts by leading schizophrenia clinicians and researchers to standardize the definition of treatment resistance in schizophrenia and have identified and incorporated recommended terminology with regards to the clinical sub-specifiers or symptom phenotypes that are common to TRS. We discussed the developments of network-based science from the early pioneers who recognized psychiatric illness and schizophrenia as a disease of neuronal and functional disconnectivity. With the development of neuroimaging methods, modern-day connectionists have built upon these theories and have continued to develop and advance network connectomic science today.

Our review of schizophrenia and TRS within a connectome context suggests that the structural and functional alterations may be greater in those patients with persistent treatment-resistant symptoms, indicating that there may be fundamental differences within brain network properties that contribute to the inability to integrate the activity and function of distributed neuronal networks that are specific to TRS. Cortical network oscillations and GBO in particular have been reviewed to understand their role in the integration of neuronal information across large neuronal ensembles in the illness. The complex relationship involved in the synchronized firing between excitatory pyramidal cells and inhibitory GABAergic interneurons were also reviewed, including findings specific to dysfunctional inhibitory networks in schizophrenia and parvalbumin interneuron dysfunction and what role these cells may play in dysfunctional pyramidal cell inhibition in schizophrenia.

We conclude the review with an overview of several augmenting pharmacological treatments, such as glutamate NMDA receptor and GABA interneuron modulators as well as NO-based treatments and how they may be viewed within a circuit context. Neurosurgical and neuromodulatory approaches were also discussed to highlight a number of beneficial circuit-based targets that may improve circuit integration and treatment response in TRS and improve treatment refractory symptoms in patients who have demonstrated poor response to alternative treatment approaches. The precise mapping of cellular and system-level networks to both on (excitatory) and off (inhibitory) circuit phenotypes specific to treatment-resistant disease remains challenging. Understanding the complexity of the cellular properties that are involved in dysfunctional brain networks in TRS will be critical toward future research in neural circuit-specific pharmacotherapeutics and directed neuromodulation treatments in schizophrenia. The ongoing interest and innovation that has been dedicated toward the understanding of the neural circuitry of schizophrenia and targeted treatment of TRS will hopefully improve personalized outcomes of those suffering from this debilitating disease.

AUTHOR CONTRIBUTIONS

M-AM, JP, and JW conducted the literature review. M-AM and JP wrote the first draft of the review. JW, IW, GB, and SD all contributed to and approved the final manuscript.

ACKNOWLEDGMENTS

The authors are grateful to the Canadian Institutes of Health Research (CIHR), the Canadian Foundation for Innovation (CFI) and the University of Alberta for funding.

REFERENCES

1. Fish B, Marcus J, Hans SL, Auerbach JG, Perdue S. Infants at risk for schizophrenia: sequelae of a genetic neurointegrative defect. A review and replication analysis of pandysmaturation in the Jerusalem Infant Development Study. *Arch Gen Psychiatry* (1992) 49: 221–35.
2. Jones P, Rodgers B, Murray R, Marmot M. Child development risk factors for adult schizophrenia in the British 1946 birth cohort. *Lancet* (1994) 344:1398–402.
3. Cannon M, Caspi A, Moffitt TE, Harrington H, Taylor A, Murray RM, et al. Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder: results from a longitudinal birth cohort. *Arch Gen Psychiatry* (2002) 59:449–56. doi: 10.1001/archpsyc.59.5.449
4. Walker EF, Savoie T, Davis D. Neuromotor precursors of schizophrenia. *Schizophr Bull.* (1994) 20:441–51.
5. Huttenlocher PR. Synaptic density in human frontal cortex–developmental changes and effects of aging. *Brain Res*. (1979) 163:195–205.
6. Feinberg I. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *J Psychiatr Res*. (1982) 17: 319–34.
7. Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science* (1986) 232:232–5.
8. McGlashan TH. Early detection and intervention in schizophrenia: research. *Schizophr Bull*. (1996) 22:327–45.
9. Lieberman JA, Perkins D, Belger A, Chakos M, Jarskog F, Boteva K, et al. The early stages of schizophrenia: speculations on pathogenesis, pathophysiology, and therapeutic approaches. *Biol Psychiatry* (2001) 50:884–97. doi: 10.1016/S0006-3223(01)01303-8
10. Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch Gen Psychiatry* (1988) 45:789–96.
11. Mouaffak F, Tranulis C, Gourevitch R, Poirier MF, Douki S, Olie JP, et al. Augmentation strategies of clozapine with antipsychotics in the treatment of ultraresistant schizophrenia. *Clin Neuropharmacol*. (2006) 29:28–33.
12. Howes OD, McCutcheon R, Agid O, de Bartolomeis A, van Beveren NJ, Birnbaum ML, et al. Treatment-resistant schizophrenia: Treatment Response and Resistance in Psychosis (TRRIP) Working Group consensus guidelines on diagnosis and terminology. *Am J Psychiatry* (2017) 174:216–29. doi: 10.1176/appi.ajp.2016.16050503
13. Agid O, Arenovich T, Sajeev G, Zipursky RB, Kapur S, Foussias G, et al. An algorithm-based approach to first-episode schizophrenia: response rates over 3 prospective antipsychotic trials with a retrospective data analysis. *J Clin Psychiatry* (2011) 72:1439–44. doi: 10.4088/JCP.09m05785yel
14. Hegarty JD, Baldessarini RJ, Tohen M, Waternaux C, Oepen G. One hundred years of schizophrenia: a meta-analysis of the outcome literature. *Am J Psychiatry* (1994) 151:1409–16.

15. Lieberman JA, Safferman AZ, Pollack S, Szymanski S, Johns C, Howard A, et al. Clinical effects of clozapine in chronic schizophrenia: response to treatment and predictors of outcome. *Am J Psychiatry* (1994)151:1744–52.
16. Meltzer HY. Treatment-resistant schizophrenia–the role of clozapine. *Curr Med Res Opin*. (1997) 14:1–20. doi: 10.1185/030079997091 13338
17. Marx OM. Wilhelm Griesinger and the history of psychiatry: a reassessment. *Bull Hist Med*. (1972) 46:519–44.
18. Seitelberger F. Theodor Meynert (1833–1892), pioneer and visionary of brain research. *J Hist Neurosci*. (1997) 6:264–74. doi: 10.1080/096470497095 25713
19. Collin G, Turk E, van den Heuvel MP. Connectomics in Schizophrenia: from early pioneers to recent brain network findings. *Biol Psychiatry Cogn Neurosci Neuroimaging* (2016) 1:199–208. doi: 10.1016/j.bpsc.2016. 01.002
20. van den Heuvel MP, Mandl RC, Stam CJ, Kahn RS, Hulshoff Pol HE. Aberrant frontal and temporal complex network structure in schizophrenia: a graph theoretical analysis. *J Neurosci*. (2010) 30:15915–26. doi: 10.1523/JNEUROSCI.2874-10.2010
21. van den Heuvel MP, Sporns O, Collin G, Scheewe T, Mandl RC, Cahn W, et al. Abnormal rich club organization and functional brain dynamics in schizophrenia. *JAMA Psychiatry* (2013) 70:783–92. doi: 10.1001/jamapsychiatry.2013.1328
22. Wernicke C. *Grundriss der Psychiatrie*: Leipzig: Thieme (1906).
23. Kraepelin E. *Dementia Praecox and Paraphrenia*. Edinburgh: Livingstone (1919).
24. Bleuler E. *Dementia Praecox or the Group of Schizophrenias*. New York, NY: New York International University Press (1911).
25. Friston KJ, Frith CD. Schizophrenia: a disconnection syndrome? *Clin Neurosci*. (1995) 3:89–97.
26. Weinberger DR. A connectionist approach to the prefrontal cortex. *J Neuropsychiatry Clin Neurosci*. (1993) 5:241–53. doi: 10.1176/jnp.5.3.241
27. Andreasen NC, Paradiso S, O'Leary DS. "Cognitive dysmetria" as an integrative theory of schizophrenia: a dysfunction in cortical-subcortical-cerebellar circuitry? *Schizophr Bull*. (1998) 24:203–18.
28. Bullmore ET, Frangou S, Murray RM. The dysplastic net hypothesis: an integration of developmental and dysconnectivity theories of schizophrenia. *Schizophr Res*. (1997) 28:143–56.
29. Stephan KE, Baldeweg T, Friston KJ. Synaptic plasticity and dysconnection in schizophrenia. *Biol Psychiatry* (2006) 59:929–39. doi: 10.1016/j.biopsych.2005.10.005
30. Stephan KE, Friston KJ, Frith CD. Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. *Schizophr Bull*. (2009) 35:509–27. doi: 10.1093/schbul/sbn176
31. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci*. (2009) 10:186–98. doi: 10.1038/nrn2575
32. Fornito A, Zalesky A, Pantelis C, Bullmore ET. Schizophrenia, neuroimaging and connectomics. *Neuroimage* (2012) 62:2296–314. doi: 10.1016/j.neuroimage.2011.12.090
33. Hagmann P, Cammoun L, Gigandet X, Gerhard S, Grant PE, Wedeen V, et al. MR connectomics: principles and challenges. *J Neurosci Methods* (2010) 194:34–45. doi: 10.1016/j.jneumeth.2010.01.014
34. Sporns O, Tononi G, Kotter R. The human connectome: a structural description of the human brain. *PLoS Comput Biol*. (2005) 1:e42. doi: 10.1371/journal.pcbi.0010042
35. Sporns O, Honey CJ, Kotter R. Identification and classification of hubs in brain networks. *PLoS ONE* (2007) 2:e1049. doi: 10.1371/journal.pone.0001049
36. Bullmore E, Sporns O. The economy of brain network organization. *Nat Rev Neurosci*. (2012) 13:336–49. doi: 10.1038/nrn3214
37. van den Heuvel MP, Sporns O. Rich-club organization of the human connectome. *J Neurosci*. (2011) 31:15775–86. doi: 10.1523/JNEUROSCI.3539-11.2011
38. Crossley NA, Mechelli A, Scott J, Carletti F, Fox PT, McGuire P, et al. The hubs of the human connectome are generally implicated in the anatomy of brain disorders. *Brain* (2014) 137:2382–95. doi: 10.1093/brain/awu132
39. Kubicki M, McCarley R, Westin CF, Park HJ, Maier S, Kikinis R, et al. A review of diffusion tensor imaging studies in schizophrenia. *J Psychiatr Res*. (2007) 41:15–30. doi: 10.1016/j.jpsychires.2005.05.005
40. Kubicki M, Shenton ME. Diffusion tensor imaging findings and their implications in schizophrenia. *Curr Opin Psychiatry* (2014) 27:179–84. doi: 10.1097/YCO.0000000000000053
41. Pettersson-Yeo W, Allen P, Benetti S, McGuire P, Mechelli A. Dysconnectivity in schizophrenia: where are we now? *Neurosci Biobehav Rev*. (2011) 35:1110–24. doi: 10.1016/j.neubiorev.2010.11.004
42. Ellison-Wright I, Bullmore E. Meta-analysis of diffusion tensor imaging studies in schizophrenia. *Schizophr Res*. (2009) 108:3–10. doi: 10.1016/j.schres.2008.11.021
43. Bora E, Fornito A, Radua J, Walterfang M, Seal M, Wood SJ, et al. Neuroanatomical abnormalities in schizophrenia: a multimodal voxelwise meta-analysis and meta-regression analysis. *Schizophr Res*. (2011) 127:46–57. doi: 10.1016/j.schres.2010.12.020
44. Kelly S, Jahanshad N, Zalesky A, Kochunov P, Agartz I, Alloza C, et al. Widespread white matter microstructural differences in schizophrenia across 4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. *Mol Psychiatry* (2018) 23:1261–9. doi: 10.1038/mp.2017.170
45. Klauser P, Baker ST, Cropley VL, Bousman C, Fornito A, Cocchi L, et al. White matter disruptions in schizophrenia are spatially widespread and topologically converge on brain network hubs. *Schizophr Bull*. (2017) 43:425–35. doi: 10.1093/schbul/sbw100
46. Lynall ME, Bassett DS, Kerwin R, McKenna PJ, Kitzbichler M, Muller U, et al. Functional connectivity and brain networks in schizophrenia. *J Neurosci*. (2010) 30:9477–87. doi: 10.1523/JNEUROSCI.0333-10.2010
47. Zalesky A, Fornito A, Seal ML, Cocchi L, Westin CF, Bullmore ET, et al. Disrupted axonal fiber connectivity in schizophrenia. *Biol Psychiatry* (2011) 69:80–9. doi: 10.1016/j.biopsych.2010.08.022
48. Whitfield-Gabrieli S, Thermenos HW, Milanovic S, Tsuang MT, Faraone SV, McCarley RW, et al. Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. *Proc Natl Acad Sci USA*. (2009) 106:1279–84. doi: 10.1073/pnas.0809141106
49. Hoffman RE, Fernandez T, Pittman B, Hampson M. Elevated functional connectivity along a corticostriatal loop and the mechanism of auditory/verbal hallucinations in patients with schizophrenia. *Biol Psychiatry* (2011) 69:407–14. doi: 10.1016/j.biopsych.2010.09.050
50. Fornito A, Bullmore ET. Reconciling abnormalities of brain network structure and function in schizophrenia. *Curr Opin Neurobiol*. (2015) 30:44–50. doi: 10.1016/j.conb.2014.08.006
51. Mouchlianitis E, McCutcheon R, Howes OD. Brain-imaging studies of treatment-resistant schizophrenia: a systematic review. *Lancet Psychiatry* (2016) 3:451–63. doi: 10.1016/S2215-0366(15)00540-4
52. Nakajima S, Takeuchi H, Plitman E, Fervaha G, Gerretsen P, Caravaggio F, et al. Neuroimaging findings in treatment-resistant schizophrenia: a systematic review: lack of neuroimaging correlates of treatment-resistant schizophrenia. *Schizophr Res*. (2015) 164:164–75. doi: 10.1016/j.schres.2015.01.043
53. Anderson VM, Goldstein ME, Kydd RR, Russell BR. Extensive gray matter volume reduction in treatment-resistant schizophrenia. *Int J Neuropsychopharmacol*. (2015) 18:pyv016. doi: 10.1093/ijnp/pyv016
54. Kubera KM, Sambataro F, Vasic N, Wolf ND, Frasch K, Hirjak D, et al. Source-based morphometry of gray matter volume in patients with schizophrenia who have persistent auditory verbal hallucinations. *Prog Neuropsychopharmacol Biol Psychiatry* (2014) 50:102–9. doi: 10.1016/j.pnpbp.2013.11.015
55. Molina V, Reig S, Sanz J, Palomo T, Benito C, Sarramea F, et al. Differential clinical, structural and P300 parameters in schizophrenia patients resistant to conventional neuroleptics. *Prog Neuropsychopharmacol Biol Psychiatry* (2008) 32:257–66. doi: 10.1016/j.pnpbp.2007.08.017
56. Zugman A, Gadelha A, Assuncao I, Sato J, Ota VK, Rocha DL, et al. Reduced dorso-lateral prefrontal cortex in treatment resistant schizophrenia. *Schizophr Res*. (2013) 148:81–6. doi: 10.1016/j.schres.2013.05.002
57. Friedman JI, Tang C, Carpenter D, Buchsbaum M, Schmeidler J, Flanagan L, et al. Diffusion tensor imaging findings in first-episode

and chronic schizophrenia patients. *Am J Psychiatry* (2008) 165:1024–32. doi: 10.1176/appi.ajp.2008.07101640
58. Kong X, Ouyang X, Tao H, Liu H, Li L, Zhao J, et al. Complementary diffusion tensor imaging study of the corpus callosum in patients with first-episode and chronic schizophrenia. *J Psychiatry Neurosci*. (2011) 36:120–5. doi: 10.1503/jpn.100041
59. Holleran L, Ahmed M, Anderson-Schmidt H, McFarland J, Emsell L, Leemans A, et al. Altered interhemispheric and temporal lobe white matter microstructural organization in severe chronic schizophrenia. *Neuropsychopharmacology* (2014) 39:944–54. doi: 10.1038/npp.2013.294
60. Alonso-Solis A, Vives-Gilabert Y, Grasa E, Portella MJ, Rabella M, Sauras RB, et al. Resting-state functional connectivity alterations in the default network of schizophrenia patients with persistent auditory verbal hallucinations. *Schizophr Res*. (2015) 161:261–8. doi: 10.1016/j.schres.2014.10.047
61. Vercammen A, Knegtering H, den Boer JA, Liemburg EJ, Aleman A. Auditory hallucinations in schizophrenia are associated with reduced functional connectivity of the temporo-parietal area. *Biol Psychiatry* (2010) 67:912–8. doi: 10.1016/j.biopsych.2009.11.017
62. Wolf ND, Sambataro F, Vasic N, Frasch K, Schmid M, Schonfeldt-Lecuona C, et al. Dysconnectivity of multiple resting-state networks in patients with schizophrenia who have persistent auditory verbal hallucinations. *J Psychiatry Neurosci*. (2011) 36:366–74. doi: 10.1503/jpn.110008
63. Wolf ND, Gron G, Sambataro F, Vasic N, Frasch K, Schmid M, et al. Magnetic resonance perfusion imaging of auditory verbal hallucinations in patients with schizophrenia. *Schizophr Res*. (2012) 134:285–7. doi: 10.1016/j.schres.2011.11.018
64. Mitelman SA, Brickman AM, Shihabuddin L, Newmark RE, Hazlett EA, Haznedar MM, et al. A comprehensive assessment of gray and white matter volumes and their relationship to outcome and severity in schizophrenia. *Neuroimage* (2007) 37:449–62. doi: 10.1016/j.neuroimage.2007.04.070
65. Selemon LD, Rajkowska G, Goldman-Rakic PS. Abnormally high neuronal density in the schizophrenic cortex. A morphometric analysis of prefrontal area 9 and occipital area 17. *Arch Gen Psychiatry* (1995) 52:805–20.
66. Selemon LD, Goldman-Rakic PS. The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biol Psychiatry* (1999) 45:17–25.
67. Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, et al. Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J Neurol Neurosurg Psychiatry* (1998) 65:446–53.
68. Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry* (2000) 57:65–73.
69. Black JE, Kodish IM, Grossman AW, Klintsova AY, Orlovskaya D, Vostrikov V, et al. Pathology of layer V pyramidal neurons in the prefrontal cortex of patients with schizophrenia. *Am J Psychiatry* (2004) 161:742–4. doi: 10.1176/appi.ajp.161.4.742
70. Homayoun H, Moghaddam B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J Neurosci*. (2007) 27:11496–500. doi: 10.1523/JNEUROSCI.2213-07.2007
71. Olney JW, Newcomer JW, Farber NB. NMDA receptor hypofunction model of schizophrenia. *J Psychiatr Res*. (1999) 33:523–33.
72. Demjaha A, Murray RM, McGuire PK, Kapur S, Howes OD. Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. *Am J Psychiatry* (2012) 169:1203–10. doi: 10.1176/appi.ajp.2012.12010144
73. Demjaha A, Egerton A, Murray RM, Kapur S, Howes OD, Stone JM, et al. Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function. *Biol Psychiatry* (2014) 75:e11–3. doi: 10.1016/j.biopsych.2013.06.011
74. Mouchlianitis E, Bloomfield MA, Law V, Beck K, Selvaraj S, Rasquinha N, et al. Treatment-resistant schizophrenia patients show elevated anterior cingulate cortex glutamate compared to treatment-responsive. *Schizophr Bull*. (2016) 42:744–52. doi: 10.1093/schbul/sbv151
75. Thornberg SA, Saklad SR. A review of NMDA receptors and the phencyclidine model of schizophrenia. *Pharmacotherapy* (1996) 16:82–93.
76. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry* (1995) 52:998–1007.
77. Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* (2012) 37:4–15. doi: 10.1038/npp.2011.181
78. Laruelle M. Schizophrenia: from dopaminergic to glutamatergic interventions. *Curr Opin Pharmacol*. (2014) 14:97–102. doi: 10.1016/j.coph.2014.01.001
79. Fries P. Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annu Rev Neurosci*. (2009) 32:209–24. doi: 10.1146/annurev.neuro.051508.135603
80. Ganella EP, Bartholomeusz CF, Seguin C, Whittle S, Bousman C, Phassouliotis C, et al. Functional brain networks in treatment-resistant schizophrenia. *Schizophr Res*. (2017) 184:73–81. doi: 10.1016/j.schres.2016.12.008
81. Wang XJ. Neurophysiological and computational principles of cortical rhythms in cognition. *Physiol Rev*. (2010) 90:1195–268. doi: 10.1152/physrev.00035.2008
82. Uhlhaas PJ, Singer W. Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci*. (2010) 11:100–13. doi: 10.1038/nrn2774
83. Uhlhaas PJ, Haenschel C, Nikolic D, Singer W. The role of oscillations and synchrony in cortical networks and their putative relevance for the pathophysiology of schizophrenia. *Schizophr Bull*. (2008) 34:927–43. doi: 10.1093/schbul/sbn062
84. Gonzalez-Burgos G, Cho RY, Lewis DA. Alterations in cortical network oscillations and parvalbumin neurons in schizophrenia. *Biol Psychiatry* (2015) 77:1031–40. doi: 10.1016/j.biopsych.2015.03.010
85. McNally JM, McCarley RW, Brown RE. Impaired GABAergic neurotransmission in schizophrenia underlies impairments in cortical gamma band oscillations. *Curr Psychiatry Rep*. (2013) 15:346, 012-0346. doi: 10.1007/s11920-012-0346-z
86. Bosman CA, Lansink CS, Pennartz CM. Functions of gamma-band synchronization in cognition: from single circuits to functional diversity across cortical and subcortical systems. *Eur J Neurosci*. (2014) 39:1982–99. doi: 10.1111/ejn.12606
87. Cheng CH, Chan PY, Niddam DM, Tsai SY, Hsu SC, Liu CY. Sensory gating, inhibition control and gamma oscillations in the human somatosensory cortex. *Sci Rep*. (2016) 6:20437. doi: 10.1038/srep20437
88. Leicht G, Karch S, Karamatskos E, Giegling I, Moller HJ, Hegerl U, et al. Alterations of the early auditory evoked gamma-band response in first-degree relatives of patients with schizophrenia: hints to a new intermediate phenotype. *J Psychiatr Res*. (2011) 45:699–705. doi: 10.1016/j.jpsychires.2010.10.00
89. Tallon-Baudry C, Bertrand O, Peronnet F, Pernier J. Induced gamma-band activity during the delay of a visual short-term memory task in humans. *J Neurosci*. (1998) 18:4244–54.
90. Womelsdorf T, Fries P, Mitra PP, Desimone R. Gamma-band synchronization in visual cortex predicts speed of change detection. *Nature* (2006) 439:733–6. doi: 10.1038/nature04258
91. Lesh TA, Niendam TA, Minzenberg MJ, Carter CS. Cognitive control deficits in schizophrenia: mechanisms and meaning. *Neuropsychopharmacology* (2011) 36:316–38. doi: 10.1038/npp.2010.156
92. Fries P, Roelfsema PR, Engel AK, Konig P, Singer W. Synchronization of oscillatory responses in visual cortex correlates with perception in interocular rivalry. *Proc Natl Acad Sci USA*. (1997) 94: 12699–704.
93. Kwon JS, O'Donnell BF, Wallenstein GV, Greene RW, Hirayasu Y, Nestor PG, et al. Gamma frequency-range abnormalities to auditory stimulation in schizophrenia. *Arch Gen Psychiatry* (1999) 56:1001–5.
94. Brenner CA, Kieffaber PD, Clementz BA, Johannesen JK, Shekhar A, O'Donnell BF, et al. Event-related potential abnormalities in schizophrenia: a failure to "gate in" salient information? *Schizophr Res*. (2009) 113:332–8. doi: 10.1016/j.schres.2009.06.012
95. Basar-Eroglu C, Brand A, Hildebrandt H, Karolina Kedzior K, Mathes B, Schmiedt C. Working memory related gamma oscillations in schizophrenia patients. *Int J Psychophysiol*. (2007) 64:39–45. doi: 10.1016/j.ijpsycho.2006.07.007
96. Spencer KM, Nestor PG, Perlmutter R, Niznikiewicz MA, Klump MC, Frumin M, et al. Neural synchrony indexes disordered perception and cognition in schizophrenia. *Proc Natl Acad Sci USA*. (2004) 101:17288–93. doi: 10.1073/pnas.0406074101
97. Haenschel C, Bittner RA, Waltz J, Haertling F, Wibral M, Singer W, et al. Cortical oscillatory activity is critical for working memory as revealed

by deficits in early-onset schizophrenia. *J Neurosci*. (2009) 29:9481–9. doi: 10.1523/JNEUROSCI.1428-09.2009
98. Cho RY, Konecky RO, Carter CS. Impairments in frontal cortical gamma synchrony and cognitive control in schizophrenia. *Proc Natl Acad Sci USA*. (2006) 103:19878–83. doi: 10.1073/pnas.0609440103
99. Minzenberg MJ, Firl AJ, Yoon JH, Gomes GC, Reinking C, Carter CS. Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia. *Neuropsychopharmacology* (2010) 35:2590–9. doi: 10.1038/npp.2010.150
100. Chen CM, Stanford AD, Mao X, Abi-Dargham A, Shungu DC, Lisanby SH, et al. GABA level, gamma oscillation, and working memory performance in schizophrenia. *Neuroimage Clin*. (2014) 4:531–9. doi: 10.1016/j.nicl.2014.03.007
101. Manoach DS. Prefrontal cortex dysfunction during working memory performance in schizophrenia: reconciling discrepant findings. *Schizophr Res*. (2003) 60:285–98. doi: 10.1016/S0920-9964(02)00294-3
102. Whittington MA, Traub RD, Kopell N, Ermentrout B, Buhl EH. Inhibition-based rhythms: experimental and mathematical observations on network dynamics. *Int J Psychophysiol*. (2000) 38:315–36.
103. Whittington MA, Faulkner HJ, Doheny HC, Traub RD. Neuronal fast oscillations as a target site for psychoactive drugs. *Pharmacol Ther*. (2000) 86:171–90. doi: 10.1016/S0163-7258(00)00038-3
104. Fries P, Nikolic D, Singer W. The gamma cycle. *Trends Neurosci*. (2007) 30:309–16. doi: 10.1016/j.tins.2007.05.005
105. Traub RD, Bibbig A, LeBeau FE, Buhl EH, Whittington MA. Cellular mechanisms of neuronal population oscillations in the hippocampus *in vitro*. *Annu Rev Neurosci*. (2004) 27:247–78. doi: 10.1146/annurev.neuro.27.070203.144303
106. Korotkova T, Fuchs EC, Ponomarenko A, von Engelhardt J, Monyer H. NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. *Neuron* (2010) 68:557–69. doi: 10.1016/j.neuron.2010.09.017
107. Senior TJ, Huxter JR, Allen K, O'Neill J, Csicsvari J. Gamma oscillatory firing reveals distinct populations of pyramidal cells in the CA1 region of the hippocampus. *J Neurosci*. (2008) 28:2274–86. doi: 10.1523/JNEUROSCI.4669-07.2008
108. Wulff P, Ponomarenko AA, Bartos M, Korotkova TM, Fuchs EC, Bahner F, et al. Hippocampal theta rhythm and its coupling with gamma oscillations require fast inhibition onto parvalbumin-positive interneurons. *Proc Natl Acad Sci USA*. (2009) 106:3561–6. doi: 10.1073/pnas.0813176106
109. Bitanihirwe BK, Lim MP, Kelley JF, Kaneko T, Woo TU. Glutamatergic deficits and parvalbumin-containing inhibitory neurons in the prefrontal cortex in schizophrenia. *BMC Psychiatry* (2009) 9:71, 244X-9-71. doi: 10.1186/1471-244X-9-71
110. Behrens MM, Ali SS, Dao DN, Lucero J, Shekhtman G, Quick KL, et al. Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. *Science* (2007) 318:1645–7. doi: 10.1126/science.1148045
111. Keilhoff G, Becker A, Grecksch G, Wolf G, Bernstein HG. Repeated application of ketamine to rats induces changes in the hippocampal expression of parvalbumin, neuronal nitric oxide synthase and cFOS similar to those found in human schizophrenia. *Neuroscience* (2004) 126:591–8. doi: 10.1016/j.neuroscience.2004.03.039
112. Xi D, Zhang W, Wang HX, Stradtman GG, Gao WJ. Dizocilpine (MK-801) induces distinct changes of N-methyl-D-aspartic acid receptor subunits in parvalbumin-containing interneurons in young adult rat prefrontal cortex. *Int J Neuropsychopharmacol*. (2009) 12:1395–408. doi: 10.1017/S146114570900042X
113. Carlen M, Meletis K, Siegle JH, Cardin JA, Futai K, Vierling-Claassen D, et al. A critical role for NMDA receptors in parvalbumin interneurons for gamma rhythm induction and behavior. *Mol Psychiatry* (2012) 17:537–48. doi: 10.1038/mp.2011.31
114. Sohal VS, Zhang F, Yizhar O, Deisseroth K. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* (2009) 459:698–702. doi: 10.1038/nature07991
115. Lewis DA, Curley AA, Glausier JR, Volk DW. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. *Trends Neurosci*. (2012) 35:57–67. doi: 10.1016/j.tins.2011.10.004
116. Belforte JE, Zsiros V, Sklar ER, Jiang Z, Yu G, Li Y, et al. Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat Neurosci*. (2010) 13:76–83. doi: 10.1038/nn.2447
117. Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* (2009) 459:663–7. doi: 10.1038/nature08002
118. Hu H, Martina M, Jonas P. Dendritic mechanisms underlying rapid synaptic activation of fast-spiking hippocampal interneurons. *Science* (2010) 327:52–8. doi: 10.1126/science.1177876
119. Klausberger T, Somogyi P. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* (2008) 321:53–7. doi: 10.1126/science.1149381
120. Tukker JJ, Fuentealba P, Hartwich K, Somogyi P, Klausberger T. Cell type-specific tuning of hippocampal interneuron firing during gamma oscillations *in vivo*. *J Neurosci*. (2007) 27:8184–9. doi: 10.1523/JNEUROSCI.1685-07.2007
121. Massi L, Lagler M, Hartwich K, Borhegyi Z, Somogyi P, Klausberger T. Temporal dynamics of parvalbumin-expressing axo-axonic and basket cells in the rat medial prefrontal cortex *in vivo*. *J Neurosci*. (2012) 32:16496–502. doi: 10.1523/JNEUROSCI.3475-12.2012
122. Gulyas AI, Szabo GG, Ulbert I, Holderith N, Monyer H, Erdelyi F, et al. Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. *J Neurosci*. (2010) 30:15134–45. doi: 10.1523/JNEUROSCI.4104-10.2010
123. Ferguson BR, Gao WJ. PV Interneurons: Critical regulators of E/I Balance for prefrontal cortex-dependent behavior and psychiatric disorders. *Front Neural Circuits* (2018) 12:37. doi: 10.3389/fncir.2018.00037
124. Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, et al. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci*. (2003) 23:6315–26. doi: 10.1523/JNEUROSCI.23-15-06315.2003
125. Fung SJ, Webster MJ, Sivagnanasundaram S, Duncan C, Elashoff M, Weickert CS. Expression of interneuron markers in the dorsolateral prefrontal cortex of the developing human and in schizophrenia. *Am J Psychiatry* (2010) 167:1479–88. doi: 10.1176/appi.ajp.2010.09060784
126. Woo TU, Miller JL, Lewis DA. Schizophrenia and the parvalbumin-containing class of cortical local circuit neurons. *Am J Psychiatry* (1997) 154:1013–5. doi: 10.1176/ajp.154.7.1013
127. Woo TU, Walsh JP, Benes FM. Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express the N-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. *Arch Gen Psychiatry* (2004) 61:649–57. doi: 10.1001/archpsyc.61.7.649
128. Frankle WG, Cho RY, Prasad KM, Mason NS, Paris J, Himes ML, et al. *In vivo* measurement of GABA transmission in healthy subjects and schizophrenia patients. *Am J Psychiatry* (2015) 172:1148–59. doi: 10.1176/appi.ajp.2015.14081031
129. Curley AA, Arion D, Volk DW, Asafu-Adjei JK, Sampson AR, Fish KN, et al. Cortical deficits of glutamic acid decarboxylase 67 expression in schizophrenia: clinical, protein, and cell type-specific features. *Am J Psychiatry* (2011) 168:921–9. doi: 10.1176/appi.ajp.2011.11010052
130. Fallon J, Opole I, Potkin S. The neuroanatomy of schizophrenia: circuitry and neurotransmitter systems. *Clin Neurosci Res*. (2003) 3:77–107. doi: 10.1016/S1566-2772(03)00022-7
131. Stahl SM. Symptoms and circuits, part 3: schizophrenia. *J Clin Psychiatry* (2004) 65:8–9.
132. Wolkin A, Barouche F, Wolf AP, Rotrosen J, Fowler JS, Shiue CY, et al. Dopamine blockade and clinical response: evidence for two biological subgroups of schizophrenia. *Am J Psychiatry* (1989) 146:905–8. doi: 10.1176/ajp.146.7.905
133. White TP, Wigton R, Joyce DW, Collier T, Fornito A, Shergill SS. Dysfunctional striatal systems in treatment-resistant schizophrenia. *Neuropsychopharmacology* (2016) 41:1274–85. doi: 10.1038/npp.2015.277

134. Yuen EY, Zhong P, Yan Z. Homeostatic regulation of glutamatergic transmission by dopamine D4 receptors. *Proc Natl Acad Sci USA*. (2010) 107:22308–13. doi: 10.1073/pnas.1010025108
135. Schmitt A, Zink M, Petroianu G, May B, Braus DF, Henn FA. Decreased gene expression of glial and neuronal glutamate transporters after chronic antipsychotic treatment in rat brain. *Neurosci Lett*. (2003) 347:81–4. doi: 10.1016/S0304-3940(03)00653-0
136. Williams JB, Mallorga PJ, Conn PJ, Pettibone DJ, Sur C. Effects of typical and atypical antipsychotics on human glycine transporters. *Schizophr Res*. (2004) 71:103–12. doi: 10.1016/j.schres.2004.01.013
137. Tanahashi S, Yamamura S, Nakagawa M, Motomura E, Okada M. Clozapine, but not haloperidol, enhances glial D-serine and L-glutamate release in rat frontal cortex and primary cultured astrocytes. *Br J Pharmacol.* (2012) 165:1543–55. doi: 10.1111/j.1476-5381.2011.01638.x
138. MacKay MB. The use of adjuvant L-arginine in schizophrenia: a behavioural and neurochemical analysis. Dissertation, Edmonton: University of Alberta (2016).
139. Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* (1987) 325:529–31. doi: 10.1038/325529a0
140. Kleckner NW, Dingledine R. Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. *Science* (1988) 241:835–7.
141. Sumiyoshi T, Anil AE, Jin D, Jayathilake K, Lee M, Meltzer HY. Plasma glycine and serine levels in schizophrenia compared to normal controls and major depression: relation to negative symptoms. *Int J Neuropsychopharmacol*. (2004) 7:1–8. doi: 10.1017/S1461145703003900
142. Neeman G, Blanaru M, Bloch B, Kremer I, Ermilov M, Javitt DC, et al. Relation of plasma glycine, serine, and homocysteine levels to schizophrenia symptoms and medication type. *Am J Psychiatry* (2005)162:1738–40. doi: 10.1176/appi.ajp.162.9.1738
143. Waziri R. Glycine therapy of schizophrenia. *Biol Psychiatry* (1988) 23:210–1. doi: 10.1016/0006-3223(88)90093-5
144. Rosse RB, Theut SK, Banay-Schwartz M, Leighton M, Scarcella E, Cohen CG, et al. Glycine adjuvant therapy to conventional neuroleptic treatment in schizophrenia: an open-label, pilot study. *Clin Neuropharmacol*. (1989) 12:416–24.
145. Costa J, Khaled E, Sramek J, Bunney W Jr, Potkin SG. An open trial of glycine as an adjunct to neuroleptics in chronic treatment-refractory schizophrenics. *J Clin Psychopharmacol*. (1990) 10:71–2.
146. Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U, Lindenmayer JP. Amelioration of negative symptoms in schizophrenia by glycine. *Am J Psychiatry* (1994) 151:1234–6.
147. Javitt DC, Silipo G, Cienfuegos A, Shelley AM, Bark N, Park M, et al. Adjunctive high-dose glycine in the treatment of schizophrenia. *Int J Neuropsychopharmacol*. (2001) 4:385–91. doi: 10.1017/S1461145701002590
148. Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Horowitz A, Kelly D. Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia. *Br J Psychiatry* (1996) 169:610–7.
149. Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. *Arch Gen Psychiatry* (1999) 56:29–36.
150. Heresco-Levy U, Ermilov M, Lichtenberg P, Bar G, Javitt DC. High-dose glycine added to olanzapine and risperidone for the treatment of schizophrenia. *Biol Psychiatry* (2004) 55:165–71. doi: 10.1016/S0006-3223(03)00707-8
151. Tuominen HJ, Tiihonen J, Wahlbeck K. Glutamatergic drugs for schizophrenia. *Cochrane Database Syst Rev*. (2006) 2:CD003730. doi: 10.1002/14651858.CD003730.pub2
152. Buchanan RW, Javitt DC, Marder SR, Schooler NR, Gold JM, McMahon RP, et al. The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): the efficacy of glutamatergic agents for negative symptoms and cognitive impairments. *Am J Psychiatry* (2007) 164:1593–602. doi: 10.1176/appi.ajp.2007.06081358
153. Tsai GE, Lin PY. Strategies to enhance N-methyl-D-aspartate receptor-mediated neurotransmission in schizophrenia, a critical review and meta-analysis. *Curr Pharm Des*. (2010) 16:522–37. doi: 10.2174/138161210790361452
154. Diaz P, Bhaskara S, Dursun SM, Deakin B. Double-blind, placebo-controlled, crossover trial of clozapine plus glycine in refractory schizophrenia negative results. *J Clin Psychopharmacol*. (2005) 25:277–8.
155. Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry (2004) 55:452–6. doi: 10.1016/j.biopsych.2003.09.012
156. Lane HY, Chang YC, Liu YC, Chiu CC, Tsai GE. Sarcosine or D-serine add-on treatment for acute exacerbation of schizophrenia: a randomized, double-blind, placebo-controlled study. *Arch Gen Psychiatry* (2005) 62:1196–204. doi: 10.1001/archpsyc.62.11.1196
157. Chue P. Glycine reuptake inhibition as a new therapeutic approach in schizophrenia: focus on the glycine transporter 1 (GlyT1). *Curr Pharm Des*. (2013) 19:1311–20. doi: 10.2174/138161213804805766
158. Perry KW, Falcone JF, Fell MJ, Ryder JW, Yu H, Love PL, et al. Neurochemical and behavioral profiling of the selective GlyT1 inhibitors ALX5407 and LY2365109 indicate a preferential action in caudal vs. cortical brain areas. *Neuropharmacology* (2008) 55:743–54. doi: 10.1016/j.neuropharm.2008.06.016
159. Lane HY, Huang CL, Wu PL, Liu YC, Chang YC, Lin PY, et al. Glycine transporter I inhibitor, N-methylglycine (sarcosine), added to clozapine for the treatment of schizophrenia. *Biol Psychiatry* (2006) 60:645–9. doi: 10.1016/j.biopsych.2006.04.005
160. Umbricht D, Alberati D, Martin-Facklam M, Borroni E, Youssef EA, Ostland M, et al. Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: a randomized, double-blind, proof-of-concept study. *JAMA Psychiatry* (2014) 71:637–46. doi: 10.1001/jamapsychiatry.2014.163
161. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull*. (1987) 13:261–76.
162. Nunes EA, MacKenzie EM, Rossolatos D, Perez-Parada J, Baker GB, Dursun SM. D-serine and schizophrenia: an update. *Expert Rev Neurother*. (2012) 12:801–12. doi: 10.1586/ern.12.65
163. Matsui T, Sekiguchi M, Hashimoto A, Tomita U, Nishikawa T, Wada K. Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. *J Neurochem*. (1995) 65:454–8.
164. Bauer D, Hamacher K, Broer S, Pauleit D, Palm C, Zilles K, et al. Preferred stereoselective brain uptake of d-serine–a modulator of glutamatergic neurotransmission. *Nucl Med Biol*. (2005) 32:793–7. doi: 10.1016/j.nucmedbio.2005.07.004
165. Hashimoto K, Fukushima T, Shimizu E, Komatsu N, Watanabe H, Shinoda N, et al. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. *Arch Gen Psychiatry* (2003) 60:572–6. doi: 10.1001/archpsyc.60.6.572
166. Vizi ES, Kisfali M, Lorincz T. Role of nonsynaptic GluN2B-containing NMDA receptors in excitotoxicity: evidence that fluoxetine selectively inhibits these receptors and may have neuroprotective effects. *Brain Res Bull*. (2013) 93:32–8. doi: 10.1016/j.brainresbull.2012.10.005
167. Lane HY, Lin CH, Huang YJ, Liao CH, Chang YC, Tsai GE. A randomized, double-blind, placebo-controlled comparison study of sarcosine (N-methylglycine) and D-serine add-on treatment for schizophrenia. *Int J Neuropsychopharmacol*. (2010) 13:451–60. doi: 10.1017/S1461145709990939
168. Tsai G, Yang P, Chung LC, Lange N, Coyle JT. D-serine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* (1998) 44:1081–9.
169. Kantrowitz JT, Malhotra AK, Cornblatt B, Silipo G, Balla A, Suckow RF, et al. High dose D-serine in the treatment of schizophrenia. *Schizophr Res*. (2010) 121:125–30. doi: 10.1016/j.schres.2010.05.012
170. Heresco-Levy U, Javitt DC, Ermilov M, Silipo G, Shimoni J. Double-blind, placebo-controlled, crossover trial of D-cycloserine adjuvant therapy for treatment-resistant schizophrenia. *Int J Neuropsychopharmacol*. (1998) 1:131–5. doi: 10.1017/S1461145798001242
171. Heresco-Levy U, Ermilov M, Shimoni J, Shapira B, Silipo G, Javitt DC. Placebo-controlled trial of D-cycloserine added to conventional neuroleptics,

olanzapine, or risperidone in schizophrenia. *Am J Psychiatry* (2002) 159:480–2. doi: 10.1176/appi.ajp.159.3.480
172. Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, et al. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. *Arch Gen Psychiatry* (1999) 56:21–7.
173. Tsai GE, Yang P, Chung LC, Tsai IC, Tsai CW, Coyle JT. D-serine added to clozapine for the treatment of schizophrenia. *Am J Psychiatry* (1999) 156:1822–5. doi: 10.1176/ajp.156.11.1822
174. Dursun SM, McIntosh D, Milliken H. Clozapine plus lamotrigine in treatment-resistant schizophrenia. *Arch Gen Psychiatry* (1999) 56:950.
175. Dursun SM, Deakin JF. Augmenting antipsychotic treatment with lamotrigine or topiramate in patients with treatment-resistant schizophrenia: a naturalistic case-series outcome study. *J Psychopharmacol.* (2001) 15:297–301. doi: 10.1177/026988110101500409
176. Tiihonen J, Hallikainen T, Ryynanen OP, Repo-Tiihonen E, Kotilainen I, Eronen M, et al. Lamotrigine in treatment-resistant schizophrenia: a randomized placebo-controlled crossover trial. *Biol Psychiatry* (2003) 54:1241–8. doi: 10.1016/S0006-3223(03)00524-9
177. Kremer I, Vass A, Gorelik I, Bar G, Blanaru M, Javitt DC, et al. Placebo-controlled trial of lamotrigine added to conventional and atypical antipsychotics in schizophrenia. *Biol Psychiatry* (2004) 56:441–6. doi: 10.1016/j.biopsych.2004.06.029
178. Zoccali R, Muscatello MR, Bruno A, Cambria R, Mico U, Spina E, et al. The effect of lamotrigine augmentation of clozapine in a sample of treatment-resistant schizophrenic patients: a double-blind, placebo-controlled study. *Schizophr Res*. (2007) 93:109–16. doi: 10.1016/j.schres.2007.02.009
179. Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized phase 2 clinical trial. *Nat Med*. (2007) 13:1102–7. doi: 10.1038/nm1632
180. Kinon BJ, Zhang L, Millen BA, Osuntokun OO, Williams JE, Kollack-Walker S, et al. A multicenter, inpatient, phase 2, double-blind, placebo-controlled dose-ranging study of LY2140023 monohydrate in patients with DSM-IV schizophrenia. *J Clin Psychopharmacol*. (2011) 31:349–55. doi: 10.1097/JCP.0b013e318218dcd5
181. Nakano Y, Yoshimura R, Nakano H, Ikenouchi-Sugita A, Hori H, Umene-Nakano W, et al. Association between plasma nitric oxide metabolites levels and negative symptoms of schizophrenia: a pilot study. *Hum Psychopharmacol*. (2010) 25:139–44. doi: 10.1002/hup.1102
182. Lee BH, Kim YK. Reduced plasma nitric oxide metabolites before and after antipsychotic treatment in patients with schizophrenia compared to controls. *Schizophr Res*. (2008) 104:36–43. doi: 10.1016/j.schres.2008.06.005
183. Suzuki E, Nakaki T, Nakamura M, Miyaoka H. Plasma nitrate levels in deficit versus non-deficit forms of schizophrenia. *J Psychiatry Neurosci*. (2003) 28:288–92.
184. Ramirez J, Garnica R, Boll MC, Montes S, Rios C. Low concentration of nitrite and nitrate in the cerebrospinal fluid from schizophrenic patients: a pilot study. *Schizophr Res*. (2004) 68:357–61. doi: 10.1016/S0920-9964(03)00070-7
185. Maia-de-Oliveira JP, Trzesniak C, Oliveira IR, Kempton MJ, Rezende TM, Iego S, et al. Nitric oxide plasma/serum levels in patients with schizophrenia: a systematic review and meta-analysis. *Rev Bras Psiquiatr*. (2012) 34(Suppl. 2):S149–55. doi: 10.1016/j.rbp.2012.07.001
186. Lauer M, Johannes S, Fritzen S, Senitz D, Riederer P, Reif A. Morphological abnormalities in nitric-oxide-synthase-positive striatal interneurons of schizophrenic patients. *Neuropsychobiology* (2005) 52:111–7. doi: 10.1159/000087555
187. Xing G, Chavko M, Zhang LX, Yang S, Post RM. Decreased calcium-dependent constitutive nitric oxide synthase (cNOS) activity in prefrontal cortex in schizophrenia and depression. *Schizophr Res*. (2002) 58:21–30. doi: 10.1016/S0920-9964(01)00388-7
188. Garthwaite J, Boulton CL. Nitric oxide signaling in the central nervous system. *Annu Rev Physiol.* (1995) 57:683–706. doi: 10.1146/annurev.ph.57.030195.003343
189. Judas M, Sestan N, Kostovic I. Nitrinergic neurons in the developing and adult human telencephalon: transient and permanent patterns of expression in comparison to other mammals. *Microsc Res Tech*. (1999) 45:401–19. doi: 10.1002/(SICI)1097-0029(19990615)45:6<401::AID-JEMT7>3.0.CO;2-Q
190. Bujas-Bobanovic M, Bird DC, Robertson HA, Dursun SM. Blockade of phencyclidine-induced effects by a nitric oxide donor. *Br J Pharmacol*. (2000) 130:1005–12. doi: 10.1038/sj.bjp.0703406
191. Bird DC, Bujas-Bobanovic M, Robertson HA, Dursun SM. Lack of phencyclidine-induced effects in mice with reduced neuronal nitric oxide synthase. *Psychopharmacology (Berl)*. (2001) 155:299–309. doi: 10.1007/S002130100705
192. Hallak JE, Maia-de-Oliveira JP, Abrao J, Evora PR, Zuardi AW, Crippa JA, et al. Rapid improvement of acute schizophrenia symptoms after intravenous sodium nitroprusside: a randomized, double-blind, placebo-controlled trial. *JAMA Psychiatry* (2013) 70:668–76. doi: 10.1001/jamapsychiatry.2013.1292
193. Maia-de-Oliveira JP, Belmonte-de-Abreu P, Bressan RA, Cachoeira C, Baker GB, Dursun SM, et al. Sodium nitroprusside treatment of clozapine-refractory schizophrenia. *J Clin Psychopharmacol*. (2014) 34:761–3. doi: 10.1097/JCP.0000000000000217
194. Friederich JA, Butterworth JF IV. Sodium nitroprusside: twenty years and counting. *Anesth Analg*. (1995) 81:152–62.
195. Stone JM, Morrison PD, Koychev I, Gao F, Reilly TJ, Kolanko M, et al. The effect of sodium nitroprusside on psychotic symptoms and spatial working memory in patients with schizophrenia: a randomized, double-blind, placebo-controlled trial. *Psychol Med*. (2016) 46:3443–50. doi: 10.1017/S0033291716002245
196. Hashimoto S, Kobayashi A. Clinical pharmacokinetics and pharmacodynamics of glyceryl trinitrate and its metabolites. *Clin Pharmacokinet*. (2003) 42:205–21. doi: 10.2165/00003088-200342030-00001
197. Georgiev D, Yoshihara T, Kawabata R, Matsubara T, Tsubomoto M, Minabe Y, et al. Cortical gene expression after a conditional knockout of 67 kDa glutamic acid decarboxylase in parvalbumin neurons. *Schizophr Bull*. (2016) 42:992–1002. doi: 10.1093/schbul/sbw022
198. Yanagi M, Joho RH, Southcott SA, Shukla AA, Ghose S, Tamminga CA. Kv3.1-containing K(+) channels are reduced in untreated schizophrenia and normalized with antipsychotic drugs. *Mol Psychiatry* (2014) 19:573–9. doi: 10.1038/mp.2013.49
199. de Hemptinne C, Swann NC, Ostrem JL, Ryapolova-Webb ES, San Luciano M, Galifianakis NB, et al. Therapeutic deep brain stimulation reduces cortical phase-amplitude coupling in Parkinson's disease. *Nat Neurosci*. (2015) 18:779–86. doi: 10.1038/nn.3997
200. Volkmann J, Wolters A, Kupsch A, Muller J, Kuhn AA, Schneider GH, et al. Pallidal deep brain stimulation in patients with primary generalised or segmental dystonia: 5-year follow-up of a randomised trial. *Lancet Neurol*. (2012) 11:1029–38. doi: 10.1016/S1474-4422(12)70257-0
201. Greenberg BD, Rauch SL, Haber SN. Invasive circuitry-based neurotherapeutics: stereotactic ablation and deep brain stimulation for OCD. *Neuropsychopharmacology* (2010) 35:317–36. doi: 10.1038/npp.2009.128
202. Anderson RJ, Frye MA, Abulseoud OA, Lee KH, McGillivray JA, Berk M, et al. Deep brain stimulation for treatment-resistant depression: efficacy, safety and mechanisms of action. *Neurosci Biobehav Rev*. (2012) 36:1920–33. doi: 10.1016/j.neubiorev.2012.06.001
203. Gault JM, Davis R, Cascella NG, Saks ER, Corripio-Collado I, Anderson WS, et al. Approaches to neuromodulation for schizophrenia. *J Neurol Neurosurg Psychiatry* (2018) 89:777–87. doi: 10.1136/jnnp-2017-316946
204. Mikell CB, Sinha S, Sheth SA. Neurosurgery for schizophrenia: an update on pathophysiology and a novel therapeutic target. *J Neurosurg.* (2016) 124:917–28. doi: 10.3171/2015.4.JNS15120
205. Salgado-Lopez L, Pomarol-Clotet E, Roldan A, Rodriguez R, Molet J, Sarro S, et al. Letter to the Editor: deep brain stimulation for schizophrenia. *J Neurosurg.* (2016) 125:229–30. doi: 10.3171/2015.12.JNS152874
206. Silbersweig DA, Stern E, Frith C, Cahill C, Holmes A, Grootoonk S, et al. A functional neuroanatomy of hallucinations in schizophrenia. *Nature* (1995) 378:176–9. doi: 10.1038/378176a0
207. Corripio I, Sarro S, McKenna PJ, Molet J, Alvarez E, Pomarol-Clotet E, et al. Clinical improvement in a treatment-resistant patient with schizophrenia treated with deep brain stimulation. *Biol Psychiatry* (2016) 80:e69–70. doi: 10.1016/j.biopsych.2016.03.1049

208. Byne W, Hazlett EA, Buchsbaum MS, Kemether E. The thalamus and schizophrenia: current status of research. *Acta Neuropathol*. (2009) 117:347–68. doi: 10.1007/s00401-008-0404-0
209. Hoffman RE, Boutros NN, Berman RM, Roessler E, Belger A, Krystal JH, et al. Transcranial magnetic stimulation of left temporoparietal cortex in three patients reporting hallucinated "voices". *Biol Psychiatry* (1999) 46:130–2.
210. Aleman A, Sommer IE, Kahn RS. Efficacy of slow repetitive transcranial magnetic stimulation in the treatment of resistant auditory hallucinations in schizophrenia: a meta-analysis. *J Clin Psychiatry* (2007) 68:416–21.
211. Aleman A, Enriquez-Geppert S, Knegtering H, Dlabac-de Lange JJ. Moderate effects of noninvasive brain stimulation of the frontal cortex for improving negative symptoms in schizophrenia: meta-analysis of controlled trials. *Neurosci Biobehav Rev*. (2018) 89:111–8. doi: 10.1016/j.neubiorev.2018.02.009
212. Freitas C, Fregni F, Pascual-Leone A. Meta-analysis of the effects of repetitive transcranial magnetic stimulation (rTMS) on negative and positive symptoms in schizophrenia. *Schizophr Res*. (2009) 108:11–24. doi: 10.1016/j.schres.2008.11.027
213. Slotema CW, Aleman A, Daskalakis ZJ, Sommer IE. Meta-analysis of repetitive transcranial magnetic stimulation in the treatment of auditory verbal hallucinations: update and effects after one month. *Schizophr Res*. (2012) 142:40–5. doi: 10.1016/j.schres.2012.08.025
214. Slotema CW, Blom JD, Sommer IE. Treatment strategies for auditory verbal hallucinations. *Tijdschr Psychiatr*. (2014) 56:247–56. doi: 10.1017/S0033291712000165
215. Tranulis C, Sepehry AA, Galinowski A, Stip E. Should we treat auditory hallucinations with repetitive transcranial magnetic stimulation? A metaanalysis. *Can J Psychiatry* (2008) 53:577–86. doi: 10.1177/070674370805300904
216. Priori A, Hallett M, Rothwell JC. Repetitive transcranial magnetic stimulation or transcranial direct current stimulation? *Brain Stimul*. (2009) 2:241–5. doi: 10.1016/j.brs.2009.02.004
217. Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: state of the art 2008. *Brain Stimul*. (2008) 1:206–23. doi: 10.1016/j.brs.2008.06.004
218. Brunoni AR, Nitsche MA, Bolognini N, Bikson M, Wagner T, Merabet L, et al. Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. *Brain Stimul*. (2012) 5:175–95. doi: 10.1016/j.brs.2011.03.002
219. Woods AJ, Antal A, Bikson M, Boggio PS, Brunoni AR, Celnik P, et al. A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin Neurophysiol*. (2016) 127:1031–48. doi: 10.1016/j.clinph.2015.11.012
220. Bikson M, Grossman P, Thomas C, Zannou AL, Jiang J, Adnan T, et al. Safety of transcranial direct current stimulation: evidence based update 2016. *Brain Stimul*. (2016) 9:641–61. doi: 10.1016/j.brs.2016.06.004
221. Brunelin J, Mondino M, Gassab L, Haesebaert F, Gaha L, Suaud-Chagny MF, et al. Examining transcranial direct-current stimulation (tDCS) as a treatment for hallucinations in schizophrenia. *Am J Psychiatry* (2012) 169:719–24. doi: 10.1176/appi.ajp.2012.11071091
222. Mondino M, Jardri R, Suaud-Chagny MF, Saoud M, Poulet E, Brunelin J. Effects of fronto-temporal transcranial direct current stimulation on auditory verbal hallucinations and resting-state functional connectivity of the left temporo-parietal junction in patients with schizophrenia. *Schizophr Bull*. (2016) 42:318–26. doi: 10.1093/schbul/sbv114
223. Li H, Wang Y, Jiang J, Li W, Li C. Effects of transcranial direct current stimulation (tDCS) for auditory hallucinations: a systematic review. *Shanghai Arch Psychiatry* (2016) 28:301–8. doi: 10.11919/j.issn.1002-0829.216121
224. Ponde PH, de Sena EP, Camprodon JA, de Araujo AN, Neto MF, DiBiasi M, et al. Use of transcranial direct current stimulation for the treatment of auditory hallucinations of schizophrenia–a systematic review. *Neuropsychiatr Dis Treat*. (2017) 13:347–55. doi: 10.2147/NDT.S122016
225. Agarwal SM, Shivakumar V, Bose A, Subramaniam A, Nawani H, Chhabra H, et al. Transcranial direct current stimulation in schizophrenia. *Clin Psychopharmacol Neurosci*. (2013) 11:118–25. doi: 10.9758/cpn.2013.11.3.118
226. Mondino M, Brunelin J, Palm U, Brunoni AR, Poulet E, Fecteau S. Transcranial direct current stimulation for the treatment of refractory symptoms of schizophrenia. Current evidence and future directions. *Curr Pharm Des*. (2015) 21:3373–83.
227. Gomes JS, Shiozawa P, Dias AM, Valverde Ducos D, Akiba H, Trevizol AP, et al. Left dorsolateral prefrontal cortex anodal tDCS effects on negative symptoms in schizophrenia. *Brain Stimul*. (2015) 8:989–91. doi: 10.1016/j.brs.2015.07.033
228. Kurimori M, Shiozawa P, Bikson M, Aboseria M, Cordeiro Q. Targeting negative symptoms in schizophrenia: results from a proof-of-concept trial assessing prefrontal anodic tDCS protocol. *Schizophr Res*. (2015) 166:362–3. doi: 10.1016/j.schres.2015.05.029
229. Palm U, Keeser D, Hasan A, Kupka MJ, Blautzik J, Sarubin N, et al. Prefrontal transcranial direct current stimulation for treatment of schizophrenia with predominant negative symptoms: a double-blind, sham-controlled proof-of-concept study. *Schizophr Bull*. (2016) 42:1253–61. doi: 10.1093/schbul/sbw041

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 MacKay, Paylor, Wong, Winship, Baker and Dursun. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

BRIEF RESEARCH REPORT
published: 31 October 2018
doi: 10.3389/fpsyt.2018.00553

Disease Severity in Treatment Resistant Schizophrenia Patients Is Mainly Affected by Negative Symptoms, Which Mediate the Effects of Cognitive Dysfunctions and Neurological Soft Signs

Felice Iasevoli, Camilla Avagliano, Benedetta Altavilla, Annarita Barone, Luigi D'Ambrosio, Marta Matrone, Danilo Notar Francesco, Eugenio Razzino and Andrea de Bartolomeis*

Section of Psychiatry - Unit on Treatment Resistant Psychosis, Laboratory of Molecular and Translational Psychiatry, Department of Neuroscience, University School of Medicine Federico II, Naples, Italy

OPEN ACCESS

Edited by:
Błazej Misiak, Wroclaw Medical University, Poland

Reviewed by:
Xijia Xu, Nanjing Brain Hospital Affiliated to Nanjing Medical University, China
Daniel Berge, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Spain

***Correspondence:**
Felice Iasevoli
felice.iasevoli@unina.it

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *17 September 2018*
Accepted: *15 October 2018*
Published: *31 October 2018*

Citation:
Iasevoli F, Avagliano C, Altavilla B, Barone A, D'Ambrosio L, Matrone M, Notar Francesco D, Razzino E and de Bartolomeis A (2018) Disease Severity in Treatment Resistant Schizophrenia Patients Is Mainly Affected by Negative Symptoms, Which Mediate the Effects of Cognitive Dysfunctions and Neurological Soft Signs. Front. Psychiatry 9:553. doi: 10.3389/fpsyt.2018.00553

This *post-hoc* study was aimed at assessing whether disease severity was higher in a sample of Treatment Resistant Schizophrenia patients (TRS) compared to schizophrenia patients responsive to antipsychotics (non-TRS). Determinants of disease severity were also investigated in these groups. Eligible patients were screened by standardized diagnostic algorithm to categorize them as TRS or non-TRS. All patients underwent the following assessments: CGI-S; PANSS; DAI; NES; a battery of cognitive tests. Socio-demographic and clinical variables were also recorded. TRS patients exhibited significantly higher disease severity and psychotic symptoms, either as PANSS total score or subscales' scores. A preliminary correlation analysis ruled out clinical and cognitive variables not associated with disease severity in the two groups. Hierarchical linear regression showed that negative symptoms were the clinical variable explaining the highest part of variation in disease severity in TRS, while in non-TRS patients PANSS-General Psychopathology was the variable explaining the highest variation. Mediation analysis showed that negative symptoms mediate the effects of verbal fluency dysfunctions and high-level neurological soft signs (NSS) on TRS' disease severity. These results show that determinants of disease severity sharply differ in TRS and non-TRS patients, and let hypothesize that TRS may stem from cognitive disfunctions and putatively neurodevelopmental aberrations.

Keywords: psychosis, refractory, clozapine, antipsychotics, positive symptoms, response

INTRODUCTION

Treatment Resistant Schizophrenia (TRS) is a major challenge in clinical management and therapy of schizophrenia (1), which *per se* is among the most relevant causes of morbidity worldwide (2). TRS is defined as the lack of response to a number of antipsychotic agents, which causes the patients to be actively symptomatic and to not gain symptom remission and functional recovery (3).

Accordingly, TRS has been associated to more severe social disability (4), whose determinants appear to strongly diverge from that in responder schizophrenia patients (i.e., non-TRS) (5, 6). Also, TRS may represent a categorically distinct subtype of schizophrenia (7), as also suggested by clinical data showing higher severity of neurological soft signs (NSS) in these patients (8), a marker of aberrant brain development (9).

In this study, we evaluated whether disease severity differed in TRS vs. non-TRS patients. As a subsequent step, we tried to delineate the clinical factors influencing disease severity in these two groups.

METHODS

This *post-hoc* analysis used data from a previous cross-sectional naturalistic study (6). Patients' recruitment continued after the above-mentioned report, and therefore the present study includes data from an expanded sample compared to that earlier one.

Patients were referred to our academic Outpatient Unit on Treatment Resistant Psychosis, University "Federico II" of Naples, by community psychiatrists for evaluation of putative TRS, as they suffered from psychotic symptoms apparently non-responding to antipsychotic agents. All consecutive patients meeting criteria for eligibility were recruited.

Inclusion criteria were: (i) age within the 18–65-year range; (ii) diagnosis of schizophrenia; (iii) being treated with antipsychotics; (iv) stabilized symptoms, including persistent psychotic symptoms with no evidence of actual or recent (i.e., in the last 3 months prior assessments) worsening. Exclusion criteria were: (i) intellectual disability (according to DSM-5 diagnostic criteria); (ii) severe medical diseases; (iii) non-schizophrenia psychotic disorders; (iv) psychotic symptoms due to another medical condition or to substances/medications.

All patients signed a written informed consent form, approved by the local Ethical Committee. All procedures carried out herein complied with the principles laid down by the Declaration of Helsinki, revised Hong Kong 1989.

A preliminary screening procedure was carried out for identifying non-schizophrenia psychotic disorders, pseudo-TRS, non-TRS, and TRS patients. This procedure has been described elsewhere (6). For all patients, the following set of clinical-demographic data were recorded: age; gender; education years; age at disease onset (AaO); duration of illness (DoI); age at first psychiatric evaluation; history of substance, alcohol, or drug abuse; everyday living functional milestones (4). The following rating scales were administered by two experienced raters: the Clinical Global Impression-Severity (CGI-S); the Positive and Negative Syndrome Scale (PANSS); the Neurological Evaluation Scale (NES) (10); the Drug Attitude Inventory (11).

Patients were assessed for the following cognitive domains' performances: Sustained and Selective Attention by the Continuous Performance Task (CPT); Verbal Memory by the List Learning task; Visuospatial Memory (VSM) by the Brief Visuospatial Memory test-Revisited; Working Memory by the Digit Sequencing task; Verbal Fluency by the Category Instances task and the Controlled Oral Word Association test; Problem Solving by the Tower of London task; Speed of Information Processing by the Symbol Coding task. Raw data from each task were adjusted in corrected scores, according to values in the Italian normative population (12–14). High corrected scores corresponded to better preservation of cognitive status.

All statistical procedures were carried out by using the SPSS 24.0®. Descriptive statistics were used to report clinical and socio-demographic data. Independent-sample Student's *T*-test was used to compare quantitative data among diagnostic groups. In all tests, significance was set at $p < 0.05$ (two-tailed). Analysis of correlation was performed by Pearons's or Spearman's test, for continuous and categorical variables respectively. Multivariate linear regression analysis was used to perform both hierarchical linear regression (HLR) and mediation analyses.

RESULTS

Group Comparison

A total of 73 schizophrenia patients enrolled in the study were subdivided in TRS ($n = 41$) and non-TRS ($n = 32$) ones. Age [$t_{(1,71)} = 1.66$; $p > 0.05$], gender ($\chi = 1.64$; $p > 0.05$), and education age [$t_{(1,71)} = 1.45$; $p > 0.05$] were not significantly different between groups. Disease severity and psychotic symptoms were significantly more severe in TRS patients compared to non-TRS [Student's *t*-test; CGI-S: $t_{(1,71)} = 3.48$; $p = 0.001$; PANSS Positive Score: $t_{(1,71)} = 1.92$; $p = 0.059$; PANSS Negative Score: $t_{(1,71)} = 3.99$; $p < 0.0005$; PANSS General Psychopathology (GP) Score: $t_{(1,71)} = 3.21$; $p = 0.002$; PANSS Total Score: $t_{(1,71)} = 3.79$; $p < 0.0005$] (**Figure 1**).

Correlation Analysis

In TRS patients, Pearson's test revealed significant positive correlations between disease severity and psychotic symptoms (PANSS Positive: $r = 0.51$, $p = 0.001$; PANSS Negative: $r = 0.59$, $p < 0.0005$; PANSS-GP: $r = 0.58$, $p < 0.0005$) or NSS (NES score: $r = 0.44$, $p = 0.005$), and inverse significant correlations between disease severity and verbal fluency performances ($r = -0.35$, $p = 0.03$) or VSM score ($r = -0.33$, $p = 0.03$).

In non-TRS patients, disease severity showed significant negative correlations with age ($r = -0.38$, $p = 0.03$) and duration of disease ($r = -0.36$, $p = 0.04$) and significant positive correlations with psychopathology (PANSS Positive: $r = 0.50$, $p = 0.004$; PANSS Negative: $r = 0.41$, $p = 0.02$; PANSS-GP: $r = 0.56$, $p = 0.001$), but not with NSS. Lifetime work occupation ($\rho = -0.37$, $p = 0.03$), residential status ($\rho = -0.40$; $p = 0.02$), and history of drug abuse ($\rho = 0.42$; $p = 0.02$) were also significantly correlated with disease severity at the Spearman's ρ test in these patients.

Hierarchical Linear Regression

We used a hierarchical linear regression (HLR) approach to evaluate which variables explained the most part of variation in CGI-S score. PANSS Negative score was the variable that explained the most variance in CGI-S (Model 1: $F = 21.22$; $p < 0.0005$; $R^2 = 0.36$; standardized $\beta = 0.599$). PANSS Positive score was the only other variable whose addition in the model led to

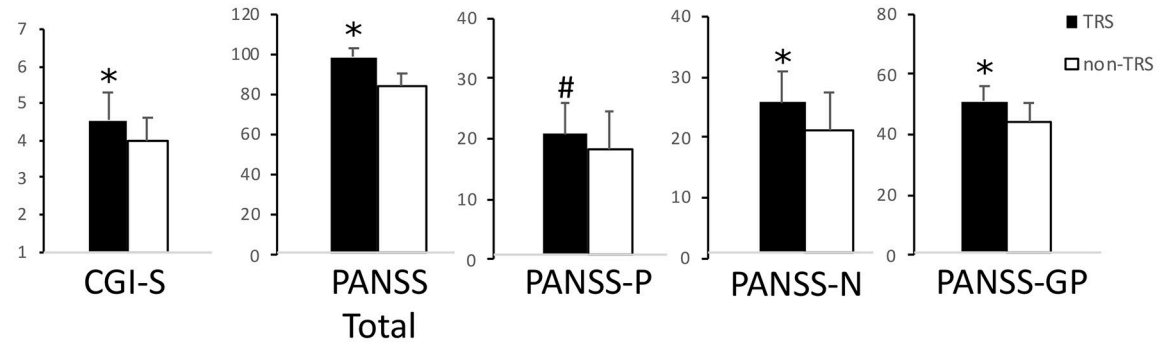


FIGURE 1 | Disease severity and psychotic symptoms. In this picture are reported TRS and non-TRS groups' mean scores + standard deviations on the (from left to right): Clinical Global Impression-Severity (CGI-S) scale; Positive and Negative Syndrome Scale (PANSS) Total score; PANSS Positive Symptoms' Subscale (PANSS-P); PANSS Negative Symptoms' Subscale (PANSS-N); PANSS General Psychopathology Subscale (PANSS-GP). Note the different scales on multiple graphics. *$p < 0.05$ at the Student's *t*-test. #Trend toward significance ($p = 0.06$).

a statistically significant increase in R^2 (Model 2: $F = 17.87$; $p < 0.0005$; $R^2 = 0.49$; standardized β PANSS Negative = 0.492; standardized β PANSS Positive = 0.380).

In non-TRS patients, the HLR approach showed that inclusion of PANSS-GP score explained substantial variation in CGI-S (Model 1: 13.64; $p = 0.001$; $R^2 = 0.313$; standardized β = 0.559) and no other variable added significant variation to the equation.

Mediation Analysis

In order to make the relationships among these variables clearer, we performed a series of mediation analysis based on the Baron and Kenny four-step model (15). We started from the hypothesis that the variables responsible for the highest variance in HLR may mediate the relations with disease severity of the variables found associated to CGI-S in the correlation analysis.

According to correlation analysis, all variables included in the regression analysis were significant predictors of the outcome variable CGI-S (Step 1).

In TRS patients, the putative mediator variables were PANSS Negative score or PANSS Positive score. Verbal Fluency, NSS, and PANSS Positive score were significant predictors of the outcome variable PANSS Negative score (Step 2), while VSM score and PANSS-GP were not (Step 2 not met; analysis stopped). PANSS Negative score was significantly predictive of the outcome variable CGI-S when controlled for either Verbal Fluency, NSS, or PANSS Positive (Step 3). Verbal Fluency and NSS were no more significantly predictive of CGI-S score when controlled for PANSS Negative (Step 4), indicating that their relations with CGI-S may be partially mediated by negative symptoms. On the contrary, PANSS Positive was still significantly predictive of CGI-S when controlled for PANSS Negative, indicating that negative symptoms did not mediate the relation between positive symptoms and disease severity. VSM score, however, was significantly predictive of the outcome variable PANSS Positive (Step 2). PANSS Positive was predictive of CGI-S score after controlling for VSM score (Step 3), while VSM score was no more significantly predictive of CGI-S score after controlling for PANSS Positive (Step 4), thereby indicating that the relation between VSM and CGI-S was partially mediated by positive symptoms. Alternative models, using different combinations of causal and moderator variables, were investigated, however none of these yielded significant results (data not shown). The results of this analysis are illustrated in **Figure 2A**.

In non-TRS patients, mediation analysis showed that the most important mediator variable was PANSS-GP, which agreed with results of the HLR. Among the variables correlated with CGI-S, PANSS Positive, PANSS Negative, and age were significantly predictive of the outcome variable PANSS-GP (Step 2). PANSS Positive, PANSS Negative, and age were no more significantly predictive of CGI-S score when controlled for PANSS-GP (Step 3). PANSS-GP was still significantly predictive of CGI-S score after controlling for PANSS Positive, PANSS Negative, or age (Step 4). Alternative models were also investigated. The only other significant mediation model was found for PANSS Positive as a mediation variable for age and duration of illness effects on CGI-S. The results of this analysis are illustrated in **Figure 2B**.

DISCUSSION

The present work was aimed at dissecting some of the distinctive clinical features that affect disease severity in schizophrenia patients responsive to antipsychotic medications compared to TRS ones. We observed directional relationships among the variables accounted herein and disease severity, that were sharply divergent for TRS and non-TRS. Indeed, TRS has been considered a unique neurobiological clinical entity (16–18), with its proper pathophysiology, clinical presentation, and disease course (5, 7). The differences in clinical determinants of disease severity found in the present study comply with this view.

Notably, in TRS patients the most relevant clinical variable in determining disease severity was found to be the extent of negative symptoms. The impact of negative symptoms on disease severity does not appear attributable to their higher severity in TRS, since global psychotic symptoms as well as each psychotic symptom domain have been found more severe in TRS compared to non-TRS patients herein. Indeed, the

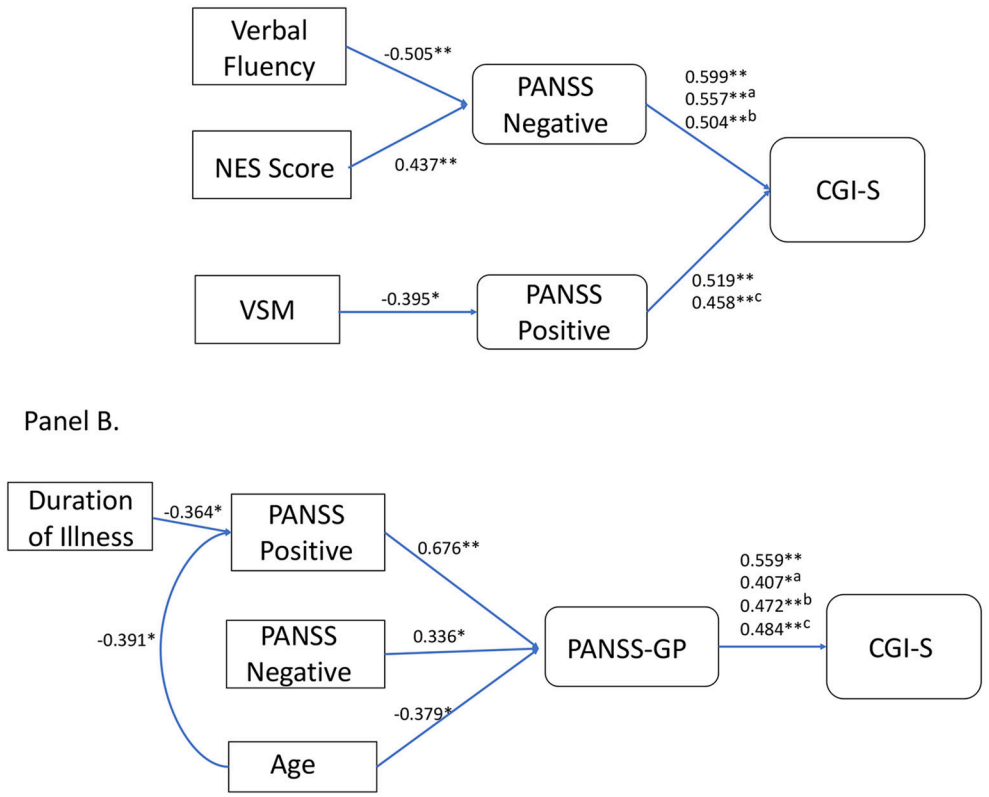


FIGURE 2 | Graphical rendering of mediation analysis. Panel **(A)** reports outputs of mediation analysis for the TRS group. Causal variable is on the left and outcome variable on the right. Significantly associated variables are linked by connection lines. Above connection lines are reported standardized beta values from linear regression analyses, along with p values (*$p < 0.05$; **$p < .005$). For PANSS Negative connection with CGI-S, we reported uncontrolled, Verbal Fluency controlled (a), and NES controlled (b) standardized betas. Verbal Fluency and NES score were significantly predictive of CGI-S (standardized B = −0.34, $p = 0.03$; standardized B = 0.438, $p = 0.005$, respectively), but significance was lost after controlling for PANSS Negative (standardized B = −0.06, $p > 0.05$; standardized B = 0.218, $p > 0.05$). For PANSS Positive connection with CGI-s, we reported uncontrolled and VSM controlled (c) standardized betas. VSM was significantly predictive of CGI-S (standardized B = −0.334, $p = 0.03$), however significance was lost after controlling for PANSS Positive (standardized B = −0.153, $p > 0.05$). Panel **(B)** reports outputs of mediation analysis for the non-TRS group. For PANSS General Psychopathology (GP) connection with CGI-S, we reported uncontrolled, PANSS Positive controlled (a), PANSS Negative controlled (b), and age controlled (c) standardized betas. PANSS Positive, PANSS Negative, age, and duration of illness were significantly predictive of CGI-S (standardized B = 0.500, $p = 0.004$; standardized B = 0.417, $p = 0.02$; standardized B = −0.382, $p = 0.03$; standardized B = −0.362, $p = 0.04$, respectively), but significance was lost after controlling for PANSS-GP (standardized B = 0.225, $p > 0.05$; standardized B = 0.258, $p > 0.05$; standardized B = −0.198, $p > 0.05$, respectively) or PANSS Positive in the case of duration of illness (standardized B = −0.196, $p > 0.05$).

association between negative symptoms and lack of response to antipsychotics had been classically reported (19, 20). Also, it has to be noted that, although being less severe than in TRS patients, the most relevant clinical variable in determining disease severity in non-TRS patients was PANSS General Psychopathology subscale score, which in turn accounts for the effects on disease severity of positive symptoms, negative symptoms, and duration of the illness. These elements let hypothesize a tight and putatively neurobiologically-determined connection between negative symptoms and TRS, affecting disease severity.

Relevance of negative symptoms on disease severity in TRS patients may lead to two alternative explanations: (i) patients with a larger extent of negative symptoms are considered to be TRS since these symptoms may not be impacted by antipsychotic agents; indeed, a large metanalysis of randomized placebo-controlled trials failed to find significant clinical effects of antipsychotics on negative symptoms (21); (ii) patients with a TRS suffer from a neurobiologically distinct form of the disease, which express symptomatically with prominent alterations in cognitive and negative symptoms. Indeed, there is strong evidence that cognitive dysfunctions are strictly interconnected with negative symptoms (22, 23).

The cross-sectional nature of this study does not allow to solve this issue. However, some clarifications may derive from mediation analysis. In TRS patients, mediation analysis showed that negative and positive symptoms directly and independently affected disease severity. Negative symptoms partially mediated the effects on disease severity of verbal fluency deficits and high-level neurological soft signs. Positive symptoms partially mediated the effects of visuospatial memory deficits. These

data imply a strong distal effect of cognitive dysfunctions and neurological soft signs on psychopathology and disease severity in TRS patients. It has been proposed that cognitive deficits in schizophrenia may underlie proper and distinct neurobiology (24). Also, cognitive deficits and severe neurological soft signs may stem from more relevant neurodevelopmental aberrations in schizophrenia patients. Therefore, it should be hypothesized that TRS patients are a subset of schizophrenia patients whose relevant cognitive deficits and high-level neurological soft signs, of putative neurodevelopmental origin, in turn determine severe negative and positive symptoms, affecting disease severity. These theoretical causal inferences need to be demonstrated by means of *ad hoc* designed longitudinal designs.

Notably, determinants of disease severity are sharply divergent and do not involve neurological soft signs or cognitive alterations. Indeed, in non-TRS patients, general psychopathology partially mediated the effects of positive and negative symptoms, age, and duration of illness on disease severity. These results suggest that other clinical variables, not accounted herein, may have a major role in determining disease severity in non-TRS patients.

The results of this study should be interpreted in the light of its limitations: the sample size was relatively small, although TRS is a subpopulation of the whole schizophrenia patients and a representative sample is expected to be lower than that needed to study schizophrenia; rating scale scores may have been partially biased by antipsychotic treatment; selection of non-TRS patients was among patients initially suspected to be non-responsive to antipsychotic regimens and for this reason referred to our specialist unit, which may cause inclusion of severe, albeit non-TRS, patients and may mitigate differences with TRS patients.

AUTHOR CONTRIBUTIONS

FI and AdB designed the study. CA, BA, AB, MM, LD, DN, and ER recruited the patients and administered assessment tools. FI carried out data analysis. FI and AdB wrote the manuscript. All authors read, corrected, and approved the manuscript in its final form.

ACKNOWLEDGMENTS

We thank the staff of the Unit on Treatment Resistant Psychosis of the University of Naples Federico II for all help in recruiting patients for this study.

REFERENCES

1. Kennedy JL, Altar CA, Taylor DL, Degtiar I, Hornberger JC. The social and economic burden of treatment-resistant schizophrenia: a systematic literature review. *Int Clin Psychopharmacol.* (2014) 29:63–76. doi: 10.1097/YIC.0b013e32836508e6
2. Charlson FJ, Ferrari AJ, Santomauro DF, Diminic S, Stockings E, Scott JG, et al. Global epidemiology and burden of schizophrenia: findings from the global burden of disease study 2016. *Schizophr Bull.* (2018) 44:1195–203. doi: 10.1093/schbul/sby058
3. Howes OD, McCutcheon R, Agid O, de Bartolomeis A, van Beveren NJ, Birnbaum ML, et al. Treatment-Resistant Schizophrenia: Treatment Response and Resistance in Psychosis (TRRIP) working group consensus guidelines on diagnosis and terminology. *Am J Psychiatry* (2017) 174:216–29. doi: 10.1176/appi.ajp.2016.16050503
4. Iasevoli F, Giordano S, Balletta R, Latte G, Formato MV, Prinzivalli E, et al. Treatment resistant schizophrenia is associated with the worst community functioning among severely-ill highly-disabling psychiatric conditions and is the most relevant predictor of poorer achievements in functional milestones. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 65:34–48. doi: 10.1016/j.pnpbp.2015.08.010
5. Iasevoli F, Avagliano C, Altavilla B, Barone A, Ciccarelli M, D'Ambrosio L, et al. Evaluation of a few discrete clinical markers may predict categorization of actively symptomatic non-acute schizophrenia patients as treatment resistant or responders: a study by ROC curve analysis and multivariate analyses. *Psychiatry Res.* (2018) 269:481–93. doi: 10.1016/j.psychres.2018.08.109
6. Iasevoli F, D'Ambrosio L, Notar Francesco D, Razzino E, Buonaguro EF, Giordano S, et al. Clinical evaluation of functional capacity in treatment resistant schizophrenia patients: comparison and differences with non-resistant schizophrenia patients. *Schizophr Res.* (2018). doi: 10.1016/j.schres.2018.06.030. [Epub ahead of print].
7. Gillespie AL, Samanaite R, Mill J, Egerton A, MacCabe JH. Is treatment-resistant schizophrenia categorically distinct from treatment-responsive schizophrenia? a systematic review. *BMC Psychiatry* (2017) 17:12. doi: 10.1186/s12888-016-1177-y
8. de Bartolomeis A, Prinzivalli E, Callovini G, D'Ambrosio L, Altavilla B, Avagliano C, et al. Treatment resistant schizophrenia and neurological soft signs may converge on the same pathology: evidence from explanatory analysis on clinical, psychopathological, and cognitive variables. *Prog Neuropsychopharmacol Biol Psychiatry* (2018) 81:356–66. doi: 10.1016/j.pnpbp.2017.09.002
9. Compton MT, Bollini AM, McKenzie Mack L, Kryda AD, Rutland J, Weiss PS, et al. Neurological soft signs and minor physical anomalies in patients with schizophrenia and related disorders, their first-degree biological relatives, and non-psychiatric controls. *Schizophr Res.* (2007) 94:64–73. doi: 10.1016/j.schres.2007.04.002
10. Buchanan RW, Heinrichs DW. The Neurological Evaluation Scale (NES): a structured instrument for the assessment of neurological signs in schizophrenia. *Psychiatry Res.* (1989) 27:335–50. doi: 10.1016/0165-1781(89)90148-0
11. Iasevoli F, Fagiolini A, Formato MV, Prinzivalli E, Giordano S, Balletta R, et al. Assessing patient-rated vs. clinician-rated adherence to the therapy in treatment resistant schizophrenia, schizophrenia responders, and non-schizophrenia patients. *Psychiatry Res.* (2017) 249:159–66. doi: 10.1016/j.psychres.2017.01.015
12. Anselmetti S, Poletti S, Ermoli E, Bechi M, Cappa S, Venneri A, et al. The Brief Assessment of Cognition in Schizophrenia. Normative data for the Italian population. *Neurol Sci.* (2008) 29:85–92. doi: 10.1007/s10072-008-0866-9
13. Galderisi S, Rossi A, Rocca P, Bertolino A, Mucci A, Bucci P, et al. The influence of illness-related variables, personal resources and context-related factors on real-life functioning of people with schizophrenia. *World Psychiatry* (2014) 13:275–87. doi: 10.1002/wps.20167
14. Iasevoli F, Balletta R, Gilardi V, Giordano S, de Bartolomeis A. Tobacco smoking in treatment-resistant schizophrenia patients is associated with impaired cognitive functioning, more severe negative symptoms, and poorer social adjustment. *Neuropsychiatr Dis Treat.* (2013) 9:1113–20. doi: 10.2147/NDT.S47571
15. Hayes AF. Beyond baron and kenny: statistical mediation analysis in the new millennium. *Commun Monogr.* (2009) 76:408–20. doi: 10.1080/03637750903310360
16. Kim E, Howes OD, Veronese M, Beck K, Seo S, Park JW, et al. Presynaptic dopamine capacity in patients with treatment-resistant schizophrenia taking clozapine: an [(18)F]DOPA PET study. *Neuropsychopharmacology* (2017) 42:941–50. doi: 10.1038/npp.2016.258

17. Mouchlianitis E, McCutcheon R, Howes OD. Brain-imaging studies of treatment-resistant schizophrenia: a systematic review. *Lancet Psychiatry* (2016) 3:451–63. doi: 10.1016/S2215-0366(15)00540-4
18. Mouchlianitis E, Bloomfield MA, Law V, Beck K, Selvaraj S, Rasquinha N, et al. Treatment-resistant schizophrenia patients show elevated anterior cingulate cortex glutamate compared to treatment-responsive. *Schizophr Bull.* (2016) 42:744–52. doi: 10.1093/schbul/sbv151
19. Leucht S, Corves C, Arbter D, Engel RR, Li C, Davis JM. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. *Lancet* (2009) 373:31–41. doi: 10.1016/S0140-6736(08)61764-X
20. Leucht S, Komossa K, Rummel-Kluge C, Corves C, Hunger H, Schmid F, et al. A meta-analysis of head-to-head comparisons of second-generation antipsychotics in the treatment of schizophrenia. *Am J Psychiatry* (2009) 166:152–63. doi: 10.1176/appi.ajp.2008.08030368
21. Fusar-Poli P, Papanastasiou E, Stahl D, Rocchetti M, Carpenter W, Shergill S, et al. Treatments of negative symptoms in schizophrenia: meta-analysis of 168 randomized placebo-controlled trials. *Schizophr Bull.* (2015) 41:892–9. doi: 10.1093/schbul/sbu170
22. Sevy S, Khan A, Lindenmayer JP, McGurk S, Paker B, Ljuri I, et al. Cognitive correlates of the negative symptoms expressive and experiential deficit factors in psychosis. *Schizophrenia Bull.* (2018) 44:S244–S. doi: 10.1093/schbul/sby017.594
23. Lim J, Lee SA, Lam M, Rapisarda A, Kraus M, Keefe RS, et al. The relationship between negative symptom subdomains and cognition. *Psychol Med.* (2016) 46:2169–77. doi: 10.1017/S0033291716000726
24. Neill JC, Barnes S, Cook S, Grayson B, Idris NF, McLean SL, et al. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. *Pharmacol Ther.* (2010) 128:419–32. doi: 10.1016/j.pharmthera.2010.07.004

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Iasevoli, Avagliano, Altavilla, Barone, D'Ambrosio, Matrone, Notar Francesco, Razzino and de Bartolomeis. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 04 December 2018
doi: 10.3389/fpsyt.2018.00654

The Representativeness of Participants With Severe Mental Illness in a Psychosocial Clinical Trial

John Lally[1,2,3,4]*, *Rochelle Watkins*[1], *Sarah Nash*[1], *Hitesh Shetty*[5], *Poonam Gardner-Sood*[1], *Shubulade Smith*[6,7], *Robin M. Murray*[1,8,9] and *Fiona Gaughran*[1,9]

[1] Department of Psychosis Studies, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom, [2] Department of Psychiatry, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland, [3] Department of Psychiatry, School of Medicine and Medical Sciences, University College Dublin, Dublin, Ireland, [4] St Vincent's Hospital Fairview, Dublin, Ireland, [5] Biomedical Research Centre (BRC) Case Register, South London and Maudsley NHS Foundation Trust, London, United Kingdom, [6] Department of Forensic and Neurodevelopmental Science, Institute of Psychiatry, Psychology and Neuroscience, Kings College London, London, United Kingdom, [7] Forensic Intensive Care Service, South London and Maudsley NHS Foundation Trust, London, United Kingdom, [8] National Psychosis Service, South London and Maudsley NHS Foundation Trust, London, United Kingdom, [9] Department of Psychiatry, Experimental Biomedicine and Clinical Neuroscience (BIONEC), University of Palermo, Palermo, Italy

OPEN ACCESS

Edited by:
Felice Iasevoli, Università degli Studi di Napoli Federico II, Italy

Reviewed by:
Assen Veniaminov Jablensky, University of Western Australia, Australia
Carmine Tomasetti, Dipartimento di Salute Mentale, SPDC di Giulianova, Italy

***Correspondence:**
John Lally
john.lally@kcl.ac.uk

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *17 September 2018*
Accepted: *16 November 2018*
Published: *04 December 2018*

Citation:
Lally J, Watkins R, Nash S, Shetty H, Gardner-Sood P, Smith S, Murray RM and Gaughran F (2018) The Representativeness of Participants With Severe Mental Illness in a Psychosocial Clinical Trial. Front. Psychiatry 9:654. doi: 10.3389/fpsyt.2018.00654

Introduction: Cardiovascular morbidity and mortality are increased in severe mental illnesses (SMI). Trials of psychosocial health interventions to improve physical health in SMI, including in treatment-resistant schizophrenia, have shown some benefit. However, the representativeness of participants in such trials has not been determined.

Method: We utilized an anonymised case register to determine if participants in a randomized controlled trial (RCT) of a novel psychosocial health intervention aiming to improve physical health in SMI had similar severity of illness to eligible non-participants. A retrospective database analysis was performed, using Health of the Nation Outcome Scale (HoNOS) data from the sample of patients participating in the IMPaCT (Improving Physical health and reducing substance use in Psychosis) RCT ($n = 293$) compared to all eligible participants with a psychotic illness ($n = 774$).

Results: The mean total HoNOS score in the eligible comparator population (Mean = 9.09, $SD = 5.8$, range = 0–30) was significantly greater than that of the IMPaCT RCT participants (Mean = 7.16, SD = 4.7, range =0–26), ($t = 3.810$, $p = 0.006$), as was the degree of overall illness severity and functional impairment, as measured by HoNOS.

Conclusion: This study shows for the first time that the patient population participating in an RCT of a lifestyle intervention for those with SMI had a better mental health status at entry to the trial, than the total eligible population, although there was no difference in physical health needs. This has relevance to the applicability of RCTs of lifestyle interventions in service planning and suggests that when people are more unwell, greater effort may be needed to include them in psychosocial interventions. A more careful and focused recruitment approach should be followed to improve the participation of the more severely ill patients in psychosocial interventions in order to enhance the external validity of such studies.

Keywords: schizophrenia, psychosis, outcomes, cardiovascular, health promotion intervention

INTRODUCTION

People with severe mental illness (SMI), such as schizophrenia, schizoaffective disorder and bipolar affective disorder, have reduced life expectancy compared to those in the general population (1–3). Most of this excess mortality is due to physical illnesses, with cardiovascular disease prominent (4, 5). Meta-analyses have demonstrated that targeted behavioral and non-specific psychosocial interventions can be beneficial in reducing antipsychotic induced weight gain and improving metabolic parameters (6–8). However, in order to determine the external validity of these findings (i.e., the extent to which the results can be generalized to clinical practice), we must determine how the extent to which participants in such studies are representative of people with SMI.

Similar concerns have been raised in relation to inclusion criteria for RCTs investigating the efficacy of clozapine in treatment resistant schizophrenia (TRS), where a broader range of patients are included than those meeting strict criteria for treatment resistance, with inclusion of treatment intolerant and non-refractory cases (9, 10).

Although there are examples of studies that have followed up non-randomized patients in clinical trials making it possible to assess and describe the generalizability of the results (11), few studies involving people with SMI have compared the characteristics of participating and non-participating patients, thus limiting the external validity of study findings. One difficulty in doing so lies in the ethical challenges of obtaining clinical data pertaining to individuals who have not consented to participate in the study (12, 13). The generalisability and real world translation of research in SMI is further limited by non-participation which may be selective (13). For example, at the more severe spectrum of mental illness, people may lack the capacity to consent to research, reducing the representativeness of research samples (14). Participation rates for studies in SMI are thought to be low, although this has not been as widely documented as the high dropout rates in this population (15). A recent survey of a large representative sample of people with psychotic disorders identified that 65% ($n = 773$) of those approached consented to participate in research, with older people less likely to do so (16).

Obstacles to participation include illness severity (17–19), fear of worsening of mental state due to participation (20), and concerns about adverse treatment effects (21). Negative symptomatology in patients with schizophrenia, with its inherent poor motivation and communication difficulties, may further reduce participation and limit the applicability of study findings (13).

While there is extensive work aimed at improving the participation of eligible patients (22) and identifying barriers to patient participation in mental health research (23), little is known about the clinical characteristics of non-participants. In particular, no such data exists for studies of non-pharmacological interventions to improve metabolic parameters in SMI, an important gap in the evidence on which to plan services.

We set out to determine the clinical characteristics of patients eligible to participate in the Improving Physical health and reducing substance use in Psychosis (IMPaCT) randomized controlled trial (RCT) (24) and compare these to the participating group using the Health of the Nations Outcome Scale (HoNOS) scores (25). We hypothesized that overall, individuals who were eligible to participate in the RCT would be more severely ill and functionally impaired as assessed by Health of the Nations Outcome Scale (HoNOS) scores (25), compared to those who agreed to participate.

MATERIALS AND METHODS

This current study is a secondary analysis of the Improving Physical health and reducing substance use in Psychosis (IMPaCT) cluster randomized controlled trial (RCT) (Trial registration: ISRCTN58667926) (24, 26). The IMPaCT study is a multicentre, two arm, parallel cluster randomized controlled trial (RCT) of a psychosocial health promotion intervention (IMPACT Therapy) in people with a diagnosis of SMI (24). The patient-tailored IMPACT Therapy aimed to target one or more health behaviors from a pre-defined list that includes cannabis use; alcohol use; other substance use; cigarette smoking; exercise; diet and diabetic control, prioritizing those identified as problematic by the patient, taking a motivational interviewing (MI) and cognitive behavioral therapy (CBT) approach. Participants were permitted to start the community-based IMPACT Therapy as soon as they were well-enough to attend, even if they were in-patients, to mirror clinical practice (24, 26).

The aim of this current study is to determine whether participants in the IMPaCT RCT were representative of the target population in respect of levels of illness severity and functional impairment.

Setting

The South London and Maudsley NHS Foundation Trust (SLaM) is one of the largest providers of secondary mental health care in Europe. It provides mental healthcare across four London boroughs (Croydon, Lambeth, Lewisham, and Southwark) to a population of 1.2 million. Since 2006, electronic records have been used across all SLAM services. Since 2008 the Case Register Interactive Search (CRIS) system has been developed to allow for the search and retrieval of anonymised electronic clinical records of patient data (27). Over 250,000 cases are currently represented on CRIS in the form of detailed anonymised clinical information (28). The protocol for this case register has been described in an open-access publication (27).

Participants and Recruitment

In the IMPaCT RCT, to maximize inclusiveness, care coordinators from continuing care/recovery teams, community rehabilitation, assertive outreach, and community forensic teams were recruited in random order and the eligible patients of consenting care co-ordinators likewise approached for inclusion in the study in random order. The inclusion criteria for IMPaCT service user participants were as follows: male or female aged between 18 and 65 years old; community patients with a primary diagnosis of a non-affective psychotic disorder (ICD10 diagnostic

criteria: F20.0–F29.0) or an affective psychotic disorder (F30–33). Exclusion criteria included a primary diagnosis of learning disability; a first episode of psychosis; serious physical illness that could impact metabolic measures and substance misuse; pregnant or up to 6 months post-partum; or receiving intensive care for a medical or terminal condition.

In this present study, the comparator population comprised the patients meeting the same inclusion criteria who were on the overall caseload of each of the participating care coordinators. The IMPaCT group were therefore a subset of the comparator population.

Individuals on the comparator caseload were included in the analysis if they had been assessed by a mental health professional using the Health of the Nations Outcome Scale (HoNOS) at least once in a time period of plus or minus 6 months from the date of the recruitment of the care coordinator to the IMPaCT RCT. If the individual had required an acute psychiatric hospital admission during that period they were excluded from the comparator population. Further, if no HoNOS score was completed in the 12 months study period, then the patient was excluded.

Outcome Measures

The primary measure of interest was the total HoNOS scale score, as this was routinely collected clinically and so was available in the pseudoanonymised clinical sample as well as the research sample. We compared HoNOS in patients participating in the IMPaCT RCT compared to that of all patients with SMI in the caseload of their care coordinators. Sociodemographic characteristics were not taken for comparison, as this would have potentially identified study non-participants.

The HoNOS has 12 items and four sections measuring, respectively: behaviors, impairments, symptoms, and social functioning (25).

A total score from the 12 items gives a measure of illness severity. Individual items and subscales in the 4 domains can be analyzed to assess their relative contributions to global functioning. Each item is measured on a 5-point Likert scale where; 0 = no problem within the period rated, 1 = sub-threshold problem, 2 = mild but definitely present, 3 = moderately severe, and 4 = severe to very severe, making 48 the highest possible score on the HoNOS (29).

Individuals in the total caseload who had a HoNOS score assessed during the 12 months study period were included. In the analysis we used the earliest HoNOS completed closest to the recruitment date of the relevant care coordinator to the RCT. HoNOS scores for individuals who had a hospitalization or an admission to a high intensity community support team [such as a Home Treatment Team (HTT)] within 6 months before or after the recruitment date were excluded to reduce the likelihood of us merely demonstrating that a short-term deterioration in mental state reduces participation in community-based trials. We did not exclude participants in the IMPaCT study group who were admitted to hospital or to a Home Treatment Team (HTT) following recruitment to the trial, although trial recruitment solely took place in the community.

The HoNOS scores of interest for this analysis were total HoNOS scale scores, subscale scores and individual itemized HoNOS scale scores. Items 9, 10, 11, and 12 in particular were assessed as a measure of functional impairment.

The means of total HoNOS scores from the overall care coordinator caseload and IMPaCT RCT participants were used as a proxy measure of global illness severity. Secondary outcomes were the "functional impairment" HoNOS subscale, which was composed of: item 9, "impairments in interpersonal relationships" (such as social withdrawal); item 10- "impairments in activities of daily living" (such as washing, dressing, mobility, and use of transport); item 11- "deficits in the quality of living conditions" (including absence of basic necessities such as heat and light); and item 12- "impairments in occupational functioning" (including the ability to engage in occupational and recreational activities).

In the study, HoNOS ratings were conducted by mental health professionals directly involved in the care of patients care for the IMPaCT non-participants and by clinical researchers for the IMPaCT study participants. It was not possible to measure interrater reliability for HoNOS scores between the clinical staff and the IMPaCT clinical researchers.

Statistical Analysis

For this analysis, a paired-samples *t*-test was used to compare:

1. total HoNOS scores from the overall caseload data set and from the IMPaCT RCT participant data set
2. HoNOS subscale scores (items 9, 10, 11, and 12) in the overall caseload data set and the IMPaCT RCT participant data set
3. individual HoNOS item scores between the overall caseload group and the IMPaCT RCT participants group.

The statistical package SPSS version 24 was used for the analyses and all *t*-tests were two-tailed with statistical significance set at an alpha level of $p \leq 0.05$.

RESULTS

The IMPaCT RCT recruited 293 eligible participants from within SLaM, who were on the caseload of 68 care coordinators. In this present study, using CRIS, we identified on the overall caseload of each of the 68 eligible care coordinators, a total comparator population comprising 1,109 patients who met the inclusion criteria, including having a primary diagnosis of a psychotic disorder. Of these 1,109 patients, 19 were excluded due to incomplete HoNOS scoring in the 12 month period from the time of the RCT recruitment, giving a HoNOS completion rate of 98.3%, and leaving a total of 1,090 in the comparator group. A further 316 (21.7% of those with a psychotic disorder) were excluded having had a hospitalization or admission to a HTT over the study period, leaving 774 patients in the comparator group for analysis.

The mean total HoNOS score in the target population ($n = 774$) (Mean = 9.09, $SD = 5.8$, range = 0–30) was significantly greater than the mean total HoNOS score for the IMPaCT RCT participating group ($n = 293$) (Mean = 7.16, $SD = 4.7$, range =0–26), ($t = 3.810$, $p < 0.001$). Comparison

TABLE 1 | Comparison of Mean HoNOS item scores between total caseload and RCT participants.

HoNOS scale item	Mean HoNOS item scores for Comparator group ($n = 293$)	Mean HoNOS item scores for IMPaCT group ($n = 774$)	Mean difference (SD) between total caseload and IMPaCT participants	*T*-test; *p*
Overactivity and aggression	0.49	0.33	0.16 (0.07)	2.284; 0.023*
Non-Accidental self-injury	0.10	0.09	0.01 (0.04)	0.262; 0.794
Problem drinking or drug-taking	0.32	0.33	−0.005 (0.07)	−0.070; 0.945
Cognitive problems	0.74	0.53	0.215 (0.08)	2.855; 0.005*
Physical illness or disability problems	0.92	0.78	0.14 (0.01)	−1.280; 0.201
Hallucinations and delusions	1.22	1.06	0.17 (0.11)	−1.134; 0.258
Depressed mood	0.72	0.71	−0.10 (0.08)	1.469; 0.143
Other symptoms (not delusions or hallucinations)	1.25	0.95	0.29 (0.10)	−2.787; 0.006*
Problems with relationships	1.08	0.78	0.30 (0.09)	−3.388; 0.001*
Problems with Activities of daily living	1.07	0.74	0.34 (0.10)	−3.041; 0.001*
Problems with living conditions	0.74	0.45	0.14 (0.08)	4.225; 0.003*
Problems with occupation and activities	0.75	0.60	0.16(0.10)	−1.641; 0.102
Total HoNOS caseload-Total HoNOS IMPaCT	9.09	7.16	1.93 (0.51)	3.810; 0.001*
HoNOS functional impairment (items 9,10,11, and 12) caseload-HoNOS functional impairment IMPaCT	3.35	2.41	0.94 (0.24)	3.945; 0.001*

*$p < 0.05$.

of the individual HoNOS item scores between those participating in the RCT and those on the care coordinators total caseload is shown in **Table 1**.

There were significantly increased scores for HoNOS item 1 (overactive, aggressive, or agitated behavior regardless of cause), item 4 (cognitive problems), item 8 (symptoms due to other mental or behavioral problems), item 9 (problems with relationships), item 10 (problems with activities of daily living), and item 11 (problems with living conditions) in the comparator group when compared to the IMPaCT participants (see **Table 1**). There was no significant difference in the HoNOS scores on the physical health item between the comparator group and the IMPaCT study group [Mean difference (MD) =0.24; $t = 1.408$, $p = 0.161$].

There was a significant increase in the HoNOS subgroup score for items 9,10,11, and 12 in the comparator group [mean HoNOS score for items 9,10,11, and 12 = 3.35 ($SD = 2.6$)] compared to the IMPaCT group [mean HoNOS score for items 9,10,11, and 12 = 2.41 ($SD = 2.3$) ($t = 3.945$, $p < 0.001$]. This indicates that the comparator group were more functionally impaired than the IMPaCT study participants. The frequency of responses to each of the HoNOS items by group is shown in **Table 2**.

DISCUSSION

To the best our knowledge, this is the first time that comparative levels of illness severity and functional impairment in a large, eligible non-participating group, and participating sample of a psychosocial health intervention RCT in SMI have been explored. This study demonstrates that both illness severity and functional impairment were increased in the non-participating population compared to the participating group. The overall health status was better in the study population, and the less severely ill patients were recruited to this trial.

Of interest, the levels of physical health problems were similar between both groups. This is relevant, as the IMPaCT trial intervention was designed to effect physical health improvements in the study population. The comparability between physical health impairment in both groups suggests that this did not drive participation selection bias, as would have been indicated by either increased severity of physical illness in the non-participating comparator group or indeed, by people with more physical health problems electing to participate. Instead, it appears there was an equivalent physical health need, but that other factors accounted for the difference in uptake of the research opportunity.

Limitations of this study need to be considered. It was not possible to assess for the effect of gender, age, ethnicity, and duration of illness of the participating and non-participating patient populations. Our inability to investigate factors that may be predictive of non-trial participation is a limitation, information that would be informative to improve the design of future RCTs. Studies have indicated that older age (16, 23) and ethnicity (30), specifically black ethnicity (16), are barriers to recruitment in mental health studies, factors which may be related to illness severity. Clinical data that may have impacted on study involvement, such as duration of illness and number of psychiatric hospitalizations, were not available in the pseudoanonymised comparator sample. However, the two study populations were comparable on clinical symptoms such as hallucinations/delusions, and depression, indicating that active symptoms of mental illness were not impacting on the participation rates between the groups. Data were obtained retrospectively, which may limit the generalisability of the findings. However, the recruitment of a patient population for a prospective study would be difficult, and the method used has enabled us to for the first time compare these groups in a behavioral intervention in SMI.

TABLE 2 | Cohort characteristics itemized by HoNOS scale items.

HoNOS items	Total caseload (n = 776) N = (%)	IMPaCT participants (n = 293) N = (%)
OVERACTIVITY AND AGGRESSION		
Not a problem	539 (70)	223 (76)
Subclinical, minor problems requiring no action	153 (20)	53 (18)
Mild to very severe problem	84 (10)	23 (6)
NON-ACCIDENTAL SELF-INJURY		
Not a problem	721 (93)	268 (91)
Subclinical, minor problems requiring no action	46 (6)	26 (8.7)
Mild to very severe problem	9 (1)	1(0.3)
PROBLEM DRINKING OR DRUG-TAKING		
Not a problem	585 (76)	223 (76)
Subclinical, minor problems requiring no action	111 (14)	44 (15)
Mild to very severe problem	79 (10)	28 (9)
COGNITIVE PROBLEMS		
Not a problem	416 (54)	166 (56)
Subclinical, minor problems requiring no action	239 (31)	100 (34)
Mild to very severe problem	121 (15)	29 (10)
PHYSICAL ILLNESS OR DISABILITY PROBLEMS		
Not a problem	357 (46)	155 (52)
Subclinical, minor problems requiring no action	192 (25)	64 (22)
Mild to very severe problem	227 (29)	76 (26)
HALLUCINATIONS AND DELUSIONS		
Not a problem	314 (41)	127 (43)
Subclinical, minor problems requiring no action	171 (22)	47 (16)
Mild to very severe problem	290 (37)	121 (41)
DEPRESSED MOOD		
Not a problem	394 (51)	147 (50)
Subclinical, minor problems requiring no action	264 (34)	96 (33)
Mild to very severe problem	118 (15)	52 (17)
OTHER SYMPTOMS		
Not a problem	94 (24)	124 (42)
Subclinical, minor problems requiring no action	291 (38)	73 (25)
Mild to very severe problem	90 (38)	98 (33)
PROBLEMS WITH RELATIONSHIPS		
Not a problem	79 (36)	136 (46)
Subclinical, minor problems requiring no action	266 (35)	89 (30)
Mild to very severe problem	237 (29)	70 (24)
PROBLEMS WITH ACTIVITIES OF DAILY LIVING		
Not a problem	338 (44)	161 (55)
Subclinical, minor problems requiring no action	176 (23)	69 (23)
Mild to very severe problem	261 (33)	65 (22)
PROBLEMS WITH LIVING CONDITIONS		
Not a problem	545 (71)	221 (75)
Subclinical, minor problems requiring no action	131 (17)	53 (18)
Mild to very severe problem	97 (12)	19 (7)
PROBLEMS WITH OCCUPATION AND ACTIVITIES		
Not a problem	396 (51)	191 (65)
Subclinical, minor problems requiring no action	193 (25)	55 (19)
Mild to very severe problem	183 (24)	48 (16)

In the analysis, we used only a single HoNOS score based on the first HoNOS assessment in the relevant study period. This precludes a more encompassing assessment of fluctuating symptom profiles over time. The use of the HoNOS provides a behavioral assessment of functioning at the level of individual items, but does not allow for assessment of discrepancies between behavior and inner experience. It may be that in a population of individuals with SMI that the significantly increased functional impairment in the inclusive comparator group is related to negative symptomatology (31, 32), but this is something that we were not able to assess.

In this study, HoNOS scores measured by care coordinators for the comparator population were compared with those measured by the IMPaCT RCT researchers. HoNOS is reported to show a moderate inter-rater reliability, but this is improved with training in HoNOS completion (33). Both care coordinators and researchers in the IMPaCT RCT study were trained in the use of the HoNOS, with the expectation that this would support inter-rater reliability.

Strengths of this study include the size and comprehensiveness of the sample. We were able to access the clinical records of over 1,000 community dwelling individuals with psychotic disorders. We looked at data specifically relating to individuals with SMI living in the community and purposefully excluded those who were hospitalized over the study period. This aids the applicability of our study findings to ambulatory research and enhances the generalisability of the study findings. Due to the comprehensiveness of the search tool, we were able to identify a patient population that reflects the characteristics of patients seen in standard clinical practice thus further enhancing the validity of our findings.

An additional important finding in this study was the high rate of HoNOS completion in the sample of community dwelling patients with SMI (98% completion rate). This finding demonstrates the clinical utility of the HoNOS and a high level of acceptability for its use in community mental health settings, although this was likely enhanced in UK practice by HoNOS measures being used for funding models. These findings are however mirrored in other community samples, such as in New Zealand where high completion rates of the HoNOS are

documented, more so than in inpatient settings (95 and 79% respectively) (34).

The greater illness severity in the comparator group as compared to the participating group is unlikely to be a result of the recruitment and randomization method in the IMPaCT RCT. An important recruitment factor in the IMPaCT RCT was the use of relatively wide inclusion criteria, including dual diagnosis, and complex patients.

We cannot tell from these data whether the more ill patients would have participated in the psychosocial health promotion intervention were it not part of a clinical trial. If research itself were the barrier, it raises questions as to whether published research into psychosocial interventions for physical health is applicable to the patients with the greatest impairment in health and social functioning. This mirrors more broad concerns regarding the representativeness of RCTs of treatment interventions in SMI, and how this impacts on translation to real world clinical practice. Observational data can help in this regard. Clinical implementation trials for physical health problems in SMI are required (35), which may be pragmatic large scale trials to ensure broad inclusion criteria, heterogeneous populations and assessment of the real world effectiveness of psychosocial interventions for physical health (36). There remains limited data on what factors predict entry to psychosocial intervention trials in SMI. An increased awareness of this may aid increased knowledge of when medication based and/or psychosocial interventions are preferable to psychosocial interventions alone.

Our findings have implications for future RCTs of psychosocial intervention in SMI. The study finding that individuals with greater functional impairment are less likely to participate in RCTs should lead to focused interventions to increase their participation. This is required in order to ensure that trial results can be confidently translated into clinical practice.

AUTHOR CONTRIBUTIONS

JL and FG conceptualized the study. JL, SS, RM, and FG contributed to the design. JL, RW, SN, and HS conducted the data collection. JL and RW undertook the statistical analysis. JL wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

FUNDING

This paper summarizes independent research funded by the National Institute for Health Research (NIHR) under its IMPACT program (Grant Reference Number RP-PG-0606-1049). Trial registration: The trial was retrospectively registered with ISRCTN registry on 23/4/2010 at ISRCTN58667926.

ACKNOWLEDGMENTS

FG and BS are, in part, funded by the National Institute for Health Research Collaboration for Leadership in Applied Health Research & Care Funding scheme with support from the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust. The views expressed in this publication are those of the authors and not necessarily those of the National Health Service (NHS), the National Institute for Health Research (NIHR) or the Department of Health and Social Care.

REFERENCES

1. Chang CK, Hayes RD, Perera G, Broadbent MT, Fernandes AC, Lee WE, et al. Life expectancy at birth for people with serious mental illness and other major disorders from a secondary mental health care case register in London. *PLoS ONE* (2011) 6:e19590. doi: 10.1371/journal.pone.0019590
2. Oakley P, Kisely S, Baxter A, Harris M, Desoe J, Dziouba A, et al. Increased mortality among people with schizophrenia and other non-affective psychotic disorders in the community: a systematic review and meta-analysis. *J Psychiatr Res.* (2018) 102:245–53. doi: 10.1016/j.jpsychires.2018.04.019
3. Walker E, McGee RE, Druss BG. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. *JAMA Psychiatry* (2015) 72:334–41. doi: 10.1001/jamapsychiatry.2014.2502
4. Correll CU, Solmi M, Veronese N, Bortolato B, Rosson S, Santonastaso P, et al. Prevalence, incidence and mortality from cardiovascular disease in patients with pooled and specific severe mental illness: a large-scale meta-analysis of 3,211,768 patients and 113,383,368 controls. *World Psychiatry* (2017) 16:163–80. doi: 10.1002/wps.20420
5. Westman J, Eriksson SV, Gissler M, Hallgren J, Prieto ML, Bobo WV, et al. Increased cardiovascular mortality in people with schizophrenia: a 24-year national register study. *Epidemiol Psychiatr Sci.* (2017) 27:519–27. doi: 10.1017/S2045796017000166
6. Caemmerer J, Correll CU, Maayan L. Acute and maintenance effects of non-pharmacologic interventions for antipsychotic associated weight gain and metabolic abnormalities: a meta-analytic comparison of randomized controlled trials. *Schizophr Res.* (2012) 140:159–68. doi: 10.1016/j.schres.2012.03.017
7. McGinty EE, Baller J, Azrin ST, Juliano-Bult D, Daumit GL. Interventions to address medical conditions and health-risk behaviors among persons with serious mental illness: a comprehensive review. *Schizophr Bull.* (2016) 42:96–124. doi: 10.1093/schbul/sbv101
8. Taylor J, Stubbs B, Hewitt C, Ajjan RA, Alderson SL, Gilbody S, et al. The effectiveness of pharmacological and non-pharmacological interventions for improving glycaemic control in adults with severe mental illness: a systematic review and meta-analysis. *PLoS ONE* (2017) 12:e0168549. doi: 10.1371/journal.pone.0168549
9. Lally J, Ajnakina O, Di Forti M, Trotta A, Demjaha A, Kolliakou A, et al. Two distinct patterns of treatment resistance: clinical predictors of treatment resistance in first-episode schizophrenia spectrum psychoses. *Psychol Med.* (2016) 46:3231–40. doi: 10.1017/S0033291716002014
10. Taylor DM. Clozapine for treatment-resistant schizophrenia: still the gold standard? *CNS Drugs* (2017) 31:177–80. doi: 10.1007/s40263-017-0411-6
11. Rovers MM, Straatman H, Ingels K, van der Wilt GJ, van den Broek P, Zielhuis GA. Generalizability of trial results based on randomized versus nonrandomized allocation of OME infants to ventilation tubes or watchful waiting. *J Clin Epidemiol.* (2001) 54:789–94. doi: 10.1016/S0895-4356(01)00340-7
12. Haapea M, Miettunen J, Veijola J, Lauronen E, Tanskanen P, Isohanni M. Non-participation may bias the results of a psychiatric survey: an analysis from the survey including magnetic resonance imaging within the Northern Finland 1966 Birth Cohort. *Soc Psychiatry Psychiatr Epidemiol.* (2007) 42:403–9. doi: 10.1007/s00127-007-0178-z

13. Riedel M, Strassnig M, Müller N, Zwack P, Möller HJ. How representative of everyday clinical populations are schizophrenia patients enrolled in clinical trials? *Eur Arch Psychiatr Clin Neurosci.* (2005) 255:143–8. doi: 10.1007/s00406-004-0547-5
14. Whelan PJ, Walwyn R, Gaughran F, Macdonald A. Impact of the demand for 'proxy assent' on recruitment to a randomised controlled trial of vaccination testing in care homes. *J Med Ethics* (2013) 39:36–40. doi: 10.1136/medethics-2011-100119
15. Leucht S, Heres S, Hamann J, Kane JM. Methodological issues in current antipsychotic drug trials. *Schizoph Bull.* (2008) 34:275–85. doi: 10.1093/schbul/sbm159
16. Patel R, Oduola S, Callard F, Wykes T, Broadbent M, Stewart R, et al. What proportion of patients with psychosis is willing to take part in research? A mental health electronic case register analysis. *BMJ Open* (2017) 7:e013113. doi: 10.1136/bmjopen-2016-013113
17. Bowen J, Hirsch S. Recruitment rates and factors affecting recruitment for a clinical trial of a putative anti-psychotic agent in the treatment of acute schizophrenia. *Human Psychopharmacol.* (1992) 7:337–41. doi: 10.1002/hup.470070507
18. Fischer EH, Dornelas EA, Goethe JW. Characteristics of people lost to attrition in psychiatric follow-up studies. *J Nerv Ment Dis.* (2001) 189:49–55. doi: 10.1097/00005053-200101000-00009
19. Hofer A, Hummer M, Huber R, Kurz M, Walch T, Fleischhacker W. Selection bias in clinical trials with antipsychotics. *J Clin Psychopharmacol.* (2000) 20:699–702. doi: 10.1097/00004714-200012000-00019
20. Kaminsky A, Roberts LW, Brody JL. Influences upon willingness to participate in schizophrenia research: an analysis of narrative data from 63 people with schizophrenia. *Ethics Behav.* (2003) 13:279–302. doi: 10.1207/S15327019EB1303_06
21. Zullino D, Conus P, Borgeat F, Bonsack C. Readiness to participate in psychiatric research. *Can J Psychiatry* (2003) 48:480–4. doi: 10.1177/070674370304800709
22. Treweek S, Mitchell E, Pitkethly M, Cook J, Kjeldstrom M, Taskila T, et al. Strategies to improve recruitment to randomised controlled trials. *Cochrane Database Syst Rev.* (2010) 20:MR000013. doi: 10.1002/14651858.MR000013.pub5
23. Woodall A, Morgan C, Sloan C, Howard L. Barriers to participation in mental health research: are there specific gender, ethnicity and age related barriers? *BMC Psychiatry* (2010) 10:103. doi: 10.1186/1471-244X-10-103
24. Gaughran F, Stahl D, Ismail K, Greenwood K, Atakan Z, Gardner-Sood P, et al. Randomised control trial of the effectiveness of an integrated psychosocial health promotion intervention aimed at improving health and reducing substance use in established psychosis (IMPaCT). *BMC Psychiatry* (2017) 17:413. doi: 10.1186/s12888-017-1571-0
25. Wing JK, Beevor AS, Curtis RH, Park SB, Hadden S, Burns A. Health of the nation outcome scales (HoNOS). *Res Dev Br J Psychiatry* (1998) 172:11–8. doi: 10.1192/bjp.172.1.11
26. Gaughran F, Stahl D, Ismail K, Atakan Z, Lally J, Gardner-Sood P, et al. Improving physical health and reducing substance use in psychosis—randomised control trial (IMPACT RCT): study protocol for a cluster randomised controlled trial. *BMC Psychiatry* (2013) 13:263. doi: 10.1186/1471-244X-13-263
27. Stewart R, Soremekun M, Perera G, Broadbent M, Callard F, Denis M, et al. The south london and maudsley NHS foundation trust biomedical research centre (SLAM BRC) case register: development and descriptive data. *BMC Psychiatry* (2009) 9:9–51. doi: 10.1186/1471-244X-9-51
28. Perera G, Broadbent M, Callard F, Chang CK, Downs J, Dutta R, et al. Cohort profile of the south london and maudsley NHS foundation trust biomedical research centre (SLaM BRC) case register: current status and recent enhancement of an electronic mental health record-derived data resource. *BMJ Open* (2016) 6:e008721. doi: 10.1136/bmjopen-2015-008721
29. Orrell M, Yard P, Handysides J, Schapira R. Validity and reliability of the health of the nation outcome scales in psychiatric patients in the community. *Br J Psychiatry* (1999) 174:409–12. doi: 10.1192/bjp.174.5.409
30. Jackson JS, Torres M, Caldwell CH, Neighbors HW, Nesse RM, Taylor RJ, et al. The national survey of american life: a study of racial, ethnic and cultural influences on mental disorders and mental health. *Int J Methods Psychiatr Res.* (2004) 13:196–207. doi: 10.1002/mpr.177
31. Rabinowitz J, Levine SZ, Garibaldi G, Bugarski-Kirola D, Berardo CG, Kapur S. Negative symptoms have greater impact on functioning than positive symptoms in schizophrenia: analysis of CATIE data. *Schizophr Res.* (2012) 137:147–50. doi: 10.1016/j.schres.2012.01.015
32. Ventura J, Hellemann GS, Thames AD, Koellner V, Nuechterlein KH. Symptoms as mediators of the relationship between neurocognition and functional outcome in schizophrenia: a meta-analysis. *Schizophr Res.* (2009) 113:189–99. doi: 10.1016/j.schres.2009.03.035
33. Amin S, Singh SP, Croudace T, Jones P, Medley I, Harrison G. Evaluating the health of the nation outcome scales. Reliability and validity in a three-year follow-up of first-onset psychosis. *Br J Psychiatry* (1999) 174:399–403. doi: 10.1192/bjp.174.5.399
34. Eagar K, Trauer T, Mellsop G. Performance of routine outcome measures in adult mental health care. *Aust N Z J Psychiatry* (2005) 39:713–8. doi: 10.1080/j.1440-1614.2005.01655.x
35. Lally J, Stubbs B, Guerandel A, O'Shea D, Gaughran, F. Pharmacological management of diabetes in severe mental illness: a comprehensive clinical review of efficacy, safety and tolerability. *Exp Rev Clin Pharmacol.* (2018) 11:411–24. doi: 10.1080/17512433.2018.1445968
36. Stroup TS, Geddes JR. Randomized controlled trials for schizophrenia: study designs targeted to distinct goals. *Schizoph Bull.* (2008) 34:266–74. doi: 10.1093/schbul/sbm156

Conflict of Interest Statement: RM has received honoraria for lectures from Lundbeck, Otsuka, Janssen, and Sunovian. FG has received honoraria for advisory work and lectures or CME activity support from Roche, BMS, Lundbeck, Otsuka, Janssen, and Sunovion, is a collaborator on a NHS Innovations project co-funded by Janssen and has a family member with professional links to Lilly and GSK, including shares.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Lally, Watkins, Nash, Shetty, Gardner-Sood, Smith, Murray and Gaughran. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

BRIEF RESEARCH REPORT
published: 28 January 2019
doi: 10.3389/fpsyt.2018.00781

Bone Status in Obese, Non-diabetic, Antipsychotic-Treated Patients, and Effects of the Glucagon-Like Peptide-1 Receptor Agonist Exenatide on Bone Turnover Markers and Bone Mineral Density

*Robert Eriksson[1,2], Brian V. Broberg[3], Pelle L. Ishøy[3], Nikolaj Bak[3], Ulrik B. Andersen[4], Niklas R. Jørgensen[5,6], Filip K. Knop[7,8,9] and Bjørn H. Ebdrup[1,3,7]**

[1] Mental Health Centre Glostrup, Copenhagen University Hospital, Glostrup, Denmark, [2] Department of Disease Systems Biology, Faculty of Health and Medical Sciences, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark, [3] Center for Neuropsychiatric Schizophrenia Research, Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research, Mental Health Centre Glostrup, Copenhagen University Hospital, Glostrup, Denmark, [4] Department of Clinical Physiology, Nuclear Medicine and PET, Rigshospitalet, Copenhagen University Hospital, Glostrup, Denmark, [5] Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Glostrup, Denmark, [6] Odense Patient Data Explorative Network, Odense University Hospital, Institute of Clinical Research, University of Southern Denmark, Odense, Denmark, [7] Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [8] Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentofte Hospital, Hellerup, Denmark, [9] Faculty of Health and Medical Sciences, The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark

OPEN ACCESS

Edited by:
Felice Iasevoli,
University of Naples Federico II, Italy

Reviewed by:
Domenico De Berardis,
Azienda Usl Teramo, Italy
Martino Belvederi Murri,
Università di Genova, Italy
Takao Ando,
Nagasaki University Hospital, Japan

***Correspondence:**
Bjørn H. Ebdrup
bebdrup@cnsr.dk

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: 17 August 2018
Accepted: 27 December 2018
Published: 28 January 2019

Citation:
Eriksson R, Broberg BV, Ishøy PL, Bak N, Andersen UB, Jørgensen NR, Knop FK and Ebdrup BH (2019) Bone Status in Obese, Non-diabetic, Antipsychotic-Treated Patients, and Effects of the Glucagon-Like Peptide-1 Receptor Agonist Exenatide on Bone Turnover Markers and Bone Mineral Density. Front. Psychiatry 9:781. doi: 10.3389/fpsyt.2018.00781

Background: Low bone mineral density (BMD) may constitute an underestimated comorbidity in schizophrenia patients undergoing long-term antipsychotic treatment. Glucagon-like peptide 1 (GLP-1) receptor agonists are antidiabetic drugs, which may also affect bone turnover.

Methods: In planned secondary analyses of a 3 months, double-blind, randomized, placebo-controlled trial ($n = 45$), we explored effects of the GLP-1 receptor agonist exenatide 2 mg once-weekly ($n = 23$), or placebo ($n = 22$) on bone turnover markers (BTMs) and BMD in chronic, obese, antipsychotic-treated patients with schizophrenia spectrum disorder. Baseline BTMs were compared to sex- and age-adjusted reference values from a Danish population cohort, and *T*- and *Z*-scores were calculated for BMD.

Results: In women ($n = 24$), all baseline BTM measurements of procollagen type I N-terminal propeptide (PINP) and C-terminal cross-linking telopeptide of type I collagen (CTX) were within reference values. In men ($n = 21$), 5% displayed lower PINP and 14% displayed lower CTX. One patient displayed BMD *Z*-score < -2, and 23% of patients (17% of women and 29% of men) displayed $-2.5 <$ *T*-scores < -1 indicating osteopenia, but none had osteoporosis. After treatment, PINP decreased at trend level significance ($P = 0.05$), and body mass index BMD increased for L2–L4 ($P = 0.016$). No changes in bone markers were significant after correction for mean prolactin levels.

Conclusions: Sex- and age-adjusted measures of bone status in chronic, obese, antipsychotic-treated patients appeared comparable to the reference population. Subtle changes in bone markers during 3 months exenatide treatment may suggest beneficial effects of GLP-1 receptor agonists on bone status in antipsychotic-treated patients, and further studies should consider the potential influence of prolactin.

Keywords: exenatide, procollagen type I N-terminal propeptide (PINP), C-terminal cross-linking telopeptide of type I collagen (CTX), bone mineral density, randomized controlled trial

INTRODUCTION

Antipsychotic medication is the mainstay of treatment of schizophrenia and other psychotic disorders (1). The drug class is generally effective in treating psychotic symptoms, however around 30% of schizophrenia patients do not respond sufficiently (2). Antipsychotics are widely associated with undesirable effects such as extrapyramidal symptoms and dysmetabolism (3, 4), but more recently, osteoporosis and increased risk of bone fractures have also been linked to antipsychotic treatment (3).

Although studies have not consistently reported associations between bone mineral density (BMD) and prolactin levels in antipsychotic-treated patients (5), antipsychotic-induced hyperprolactinaemia has been suggested a causal factor underlying osteoporosis (6). BMD is commonly assessed by dual-energy X-ray absorptiometry (DXA). According to WHO criteria *T*-scores are used as thresholds for osteopenia and osteoporosis. Osteopenia is defined as 1 to 2.5 standard deviations (*SD*) or more below the average value for young healthy subjects of the same sex ($-1 > T\text{-score} > -2.5$), and osteoporosis is defined as a *T*-score below −2.5 ($T\text{-score} \leq -2.5$) (7). Besides *T*-scores, DXA enables calculation of a *Z*-score, which is a comparison of bone density with a healthy population of the same age and same sex. The reference range for *Z*-scores is ±2. In addition to BMD measurements of bone mass, circulating bone turnover markers (BTMs) can be used to evaluate changes in bone formation and resorption. International consensus guidelines recommend assessment of two BTMs: procollagen type I N-terminal propeptide (PINP) (produced by osteoblasts during bone formation), and C-terminal cross-linking telopeptide of type I collagen (CTX) (released by osteoclasts during bone resorption) (8, 9).

Glucagon-like peptide 1 (GLP-1) receptor agonists are known to induce positive effects on metabolism (10), but the drugs might also affect bone turnover. Animal models have indicated positive effects of the GLP-1 receptor agonists exendin-4 and liraglutide on bone metabolism (11–13). These findings have motivated translational efforts aiming to investigate the potential benefits of GLP-1 receptor agonists on bone status in humans. Treatment with liraglutide has been shown to increase bone formation in body weight-reduced obese women when compared to placebo (14). Conversely, studies of type 2 diabetes patients have indicated that GLP-1 receptor agonists have no effect on bone metabolism or fracture risk (15, 16).

The current study comprises planned secondary analyses of the "TAO study": Treatment of antipsychotic-associated obesity with a GLP-1 receptor agonist (17–20). The TAO study was an investigator-initiated, double-blind, randomized, placebo-controlled trial, investigating the effects of 3 months treatment with the GLP-1 receptor agonist exenatide 2 mg once-weekly in chronic obese, antipsychotic-treated patients with schizophrenia spectrum disorder. First we compared baseline BTMs with the Danish Health 2006 study cohort as reference population (21), and we calculated BMD *T*- and *Z*-scores. Next, we compared baseline PINP, CTX and BMD with end-of-trial measures aiming to unravel potential beneficial effects of exenatide on BTMs and BMDs.

METHODS

Details of the "TAO study" have previously been reported (17–20). Below, key methodology, experimental procedures and analyses are outlined.

Study Population and Procedures

Inclusion criteria included clinically stable schizophrenia spectrum patients (ICD-10 diagnoses F20.x and F25.x); treatment with minimum one antipsychotic drug; age 18 to 65 years; obesity (BMI $\geq$ 30 kg/m^2). Exclusion criteria included substance dependence, diabetes (any type), severe somatic disease, pregnancy and breastfeeding. Patients were randomized to either receive injections of 2 mg exenatide once-weekly (Bydureon®, AstraZeneca AB, Södertälje, Sweden) or placebo. We used the solvent from the Bydureon® kit as placebo. Unblinded trial staff otherwise not involved in the study performed the subcutaneous injections of exenatide or placebo ensuring 100% medication adherence. Both groups were assessed with biochemical analyses and DXA measurements at trial initiation and after 3 months (12–16 weeks) (17).

Biochemical Analyses

All biochemical analyses were performed at the Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark. Since CTX (8) and prolactin (22) are influenced by diurnal variations, all blood samples were collected in the morning before food intake (fasting >8 h).

Bone Mineral Density

Patients underwent DXA examinations on a Lunar Prodigy whole-body scanner (GE Medical Systems, Madison, Wisconsin, USA). As input for statistical analyses, we calculated averages of the left and right femoral neck measurements and the left

and right total femur measurements. *T*-score and *Z*-score were not calculated for patients below 20 years of age. Likewise, we calculated standardized BMD values from the raw data as described by Fan et al. (23).

Statistical Analyses

To enable comparison with the sex and age intervals obtained from the background population cohort (21), we split patients into men and women and compared baseline levels of PINP, CTX, and BMD for each group and age interval, separately. Baseline demographic and clinical variables were tested with independent *t*-tests for continuous data, and χ^2-test for nominal data. Non-normally distributed BTM and BMD values were transformed by logarithm or square root to achieve normal distribution. All outcomes were initially analyzed without covariates by two-way repeated measures ANOVA. Next, analyses were repeated with mean prolactin level [(baseline + follow-up)/2] as a covariate to evaluate the potential effect of prolactin. We *a priori* decided to repeat analyses after excluding patients with baseline values, which could indicate pre-study disturbance of bone metabolism (vitamin D < 30 nmol/L and/or parathyroid hormone (PTH) >7.63 pmol/L) (24). IBM SPSS Statistics Version 22 (IBM Corp. for Windows, Armonk, NY) was used for statistical analyses. The significance level was set to 0.05, and all tests were two-tailed.

RESULTS

Demographic and Clinical Data

In total, 45 patients were included in the baseline analyses. Twenty patients in the exenatide-treated group and 20 patients in the placebo-treated group completed the trial (**Supplementary Figure 1**). At baseline we found no significant group differences in age, sex, ethnicity, illness duration, education, body weight, BMI, diagnosis or antipsychotic medication. However, we found a higher proportion ($p = 0.02$) of current smokers in the exenatide group (**Supplementary Table 1**). After 3 months of exenatide or placebo treatment patients lost 2.3 kg with no significant difference between groups (18, 20).

Comparison of Baseline Bone Turnover Markers to Reference Values

PINP and CTX concentrations were within the age-adjusted reference range (21) for all women (24 of 24). One of 21 male patients (5%) had lower PINP, and three male patients (14%) had lower CTX levels than the corresponding age-adjusted reference range (**Figure 1A**).

Comparison of Baseline Bone Mineral Density to Reference Values

One hundred and fourteen (86%) out of the total 132 DXA measurements corresponded to *Z*-scores between ±2. One patient had a *Z*-score (L2–L4) < −2. In both the L2–L4 and the total femur measurements five patients (11%) had *Z*-scores above 2, whereas in the femoral neck BMD measurement four patients (9%) had *Z*-scores above 2 (**Figure 1B**). Ten patients (23%), [4 women (17%), and 6 men (29%)] had *T*-scores < −1 indicating osteopenia. No patients had osteoporosis (*T*-scores ≤−2.5) (**Figure 1B**).

Effect of Exenatide on Biomarkers of Bone Turnover and Bone Mineral Density

After 3 months we observed numerical reductions in levels of both PINP and CTX in the exenatide-treated group, whereas the levels in the placebo-treated group numerically increased. For PINP, we found a time × group interaction (i.e., a treatment effect) at trend-level significance ($p = 0.05$), however, when prolactin was included as a covariate this trend-level observation was no longer present. For CTX, we observed no significant interactions. Apart from a trend-level increase in osteocalcin over time in both groups, analyses on other bone-related biomarkers were non-significant (**Table 1**).

Analyses of BMD data (**Table 1**) revealed a significant time × group interaction ($p = 0.016$) in the L2–L4, indicating higher BMD after exenatide treatment and lower BMD after placebo. After correction for the mean prolactin level, this interaction was only significant at trend-level ($p = 0.057$). The remaining analyses of BMD were not significant.

Exclusion of four patients with suspected pre-study disturbance of bone metabolism did not change the significance levels of the above results.

DISCUSSION

The current analyses of bone status in chronic, obese, non-diabetic, antipsychotic-treated schizophrenia spectrum patients indicated that most patients had BTMs, i.e., PINP and CTX levels within the reference ranges obtained from a Danish background population (21). On the contrary, our BMD measurements indicated that 23% of patients had osteopenia. However, in men younger than 50 years of age and premenopausal women, *T*-scores are typically not used for BMD (25), rather *Z*-scores are preferred. Nevertheless, a meta-analysis of prevalence of low bone mass in schizophrenia patients reported an even higher prevalence of osteopenia which was present in both patients and controls (around 40%) (26). This could be explained by the fact that the patients included in our trial were markedly obese (mean BMI 38.8 kg/m^2), and obesity is generally associated with increased BMD. Paradoxically, obesity has also been associated with an increase in fracture risk (27). Hence, the seemingly unaffected BMD observed in our patient sample may still render the patients at an increased risk of fractures, but the association between BMI and fracture risk is complex (28), and fracture risk was beyond what could be assessed from the current data.

The mean age of our patients was 35.8 years, and with one exception, all sex and age-adjusted BMD *Z*-score measurements were within the normal range. In fact, five patients (12%) had *Z*-scores above 2 in L2–L4 and total femur measurements, and four patients (9%) had *Z*-scores above 2 in the femoral neck BMD measurement (**Figure 1B**). Therefore, in contrast to our expectations, the current comparative baseline analyses do not lend overall support to the emerging concern of markedly compromised bone status in chronic schizophrenia patients. As

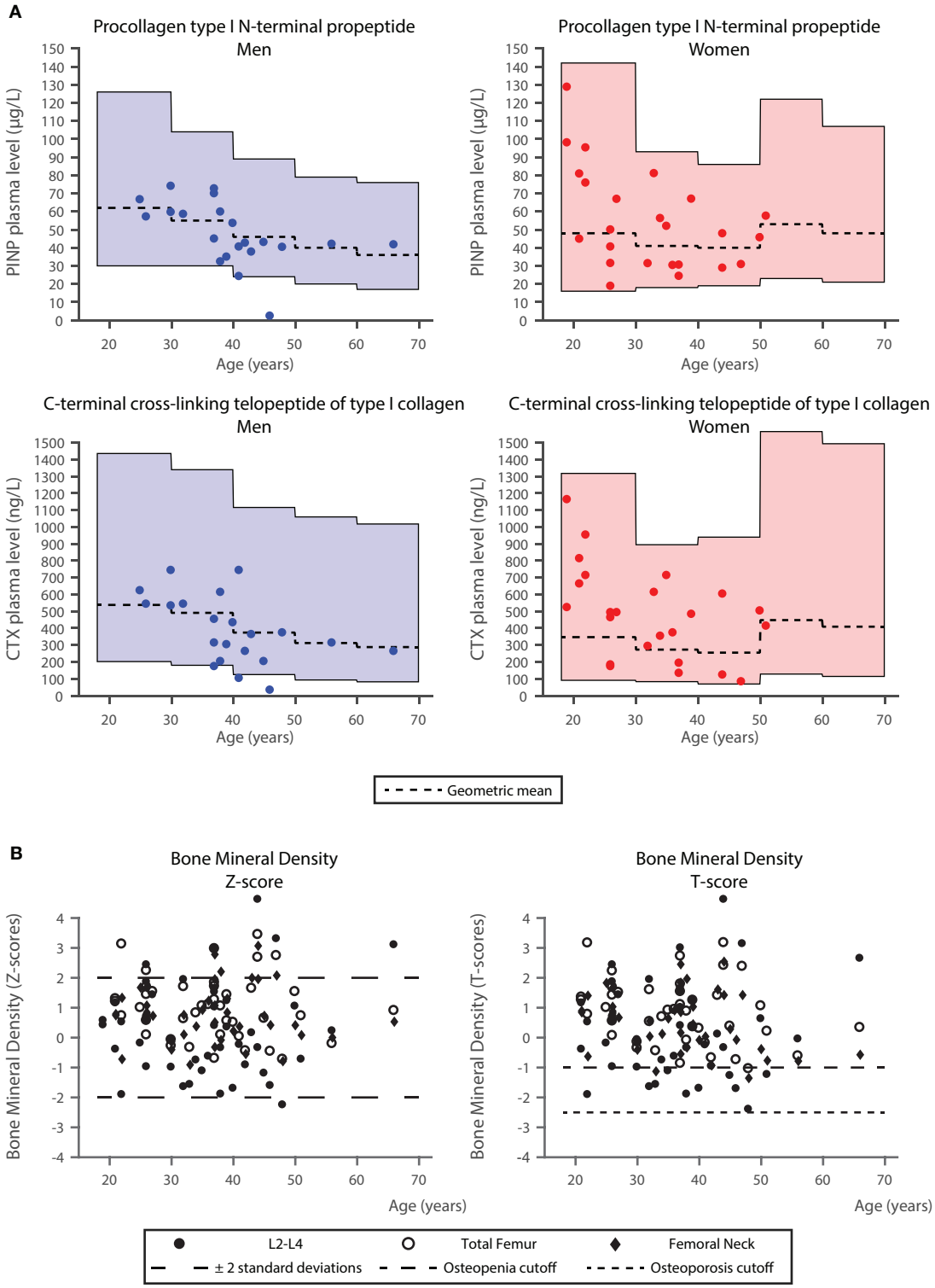


FIGURE 1 | Comparison of bone turnover markers and bone mineral density in the study cohort with the background population. **(A)** Procollagen type I N-terminal propeptide (PINP) and C-terminal cross-linking telopeptide of type I collagen (CTX) levels in the study cohort with reference ranges obtained from the background population (21). Geometric means for the reference intervals are plotted. **(B)** Bone mineral density values of the lumbar spine L2–L4, left and right femoral neck, as well as left and right total femur (acquired using CORE software [version 14.1]). Age is plotted against *Z*-scores (average values for a healthy population of the same age and same sex) (left panel) and *T*-scores (average values for young healthy subjects of the same sex) (right panel). In the plot of *Z*-scores, ±2 standard deviations are presented. In the plot of *T*-scores, the cutoffs for osteopenia and osteoporosis are presented (*T-score* = −1 *SD* and *T-score* = −2.5 *SD*).

TABLE 1 | Effect of exenatide and placebo on biomarkers related to bone metabolism and on bone mineral density.

Blood marker	Exenatide ($n = 20$) Mean ± *SD* [Range]	Placebo ($n = 20$) Mean ± *SD* [Range]	Time *p*-value	Group *p*-value	Time x Group *p*-value (No covariance)	Time x Group *p*-value (Prolactin covariance)
C-terminal cross-linking telopeptide of type I collagen (ng/L) $						
Baseline	409.0 ± 229.6 [170–1160]	475.5 ± 265.7 [30-950]	0.93	0.19	0.15	0.25
End of trial	357.0 ± 171.9 [80–780]	514.5 ± 292.5 [60–1420]				
Procollagen type I N-terminal propeptide (μg/L)						
Baseline	49.3 ± 23.0 [18.5–128.4]	52.4 ± 24.9 [2.0-97.7]	0.52	0.27	0.05	0.39
End of trial	46.1 ± 20.5 [17.2–104.3]	58.7 ± 24.4 [29.2–111.9]				
Bone-specific alkaline phosphatase (μg/L)						
Baseline	20.9 ± 8.3 [11.3–47.1]	22.2 ± 10.3 [8.2–50.8]	0.65	0.55	0.61	0.26
End of trial	20.8 ± 7.3 [11.3–37.5]	22.8 ± 9.7 [8.7–48.6]				
Osteocalcin (μg/L) §			0.05	0.28	0.71	0.40
Baseline	14.3 ± 7.6 [2.0–37.7]	16.1 ± 8.0 [6.2–39.0]				
End of trial	15.4 ± 5.9 [8.0–33.5]	17.8 ± 8.5 [8.7–42.4]				
Osteocalcin/CTX ratio §			0.28	0.86	0.11	0.24
Baseline	40.3 ± 21.1 [4.3–96.5]	51.5 ± 52.4 [19.2–256.7]				
End of trial	54.1 ± 39.2 [18.7–176.7]	47.0 ± 40.7 [14.6–188.3]				
Parathyroid hormone (pmol/L) $						
Baseline	4.30 ± 3.30 [1.0–14.7]	4.15 ± 1.90 [1.1–10.8]#	0.32	0.75	0.30	0.31
End of trial	3.71 ± 1.88 [1.1–9.6]#	4.08 ± 1.81 [1.5–9.3]#				
Prolactin (mIU/L) §						–
Baseline	369.6 ± 397.3 [24.1–1484.0]	435.3 ± 434.9 [34.2–1614.0]	0.11	0.74	0.42	
End of trial	357.7 ± 404.0 [20.9–1416.0]	372.8 ± 393.7 [30.4–1502.0]				
Vitamin D (nmol/L)						
Baseline	62.9 ± 33.8 [8.0–126.0]	70.4 ± 33.6 [14.2–130.0]	0.14	0.59	0.51	0.67
End of trial	69.0 ± 37.7 [16.2–135.0]	72.8 ± 31.3 [17.0–136.0]				
Dual-energy X-ray absorptiometry—Bone mineral density (g/cm^2)	**Exenatide ($n = 20$) Mean ± *SD* [Range]**	**Placebo ($n = 19$) Mean ± *SD* [Range]**	**Time *p*-value**	**Group *p*-value**	**Time x Group *p*-value (No covariance)**	**Time x Group *p*-value (Prolactin covariance)**
L2–L4						
Baseline	1.23 ± 0.19 [0.90–1.68]	1.13 ± 0.16 [0.92–1.49]	0.576	0.055	0.016*	0.057
End of trial	1.24 ± 0.19 [0.93–1.67]	1.12 ± 0.16 [0.88–1.48]				
Femoral neck						
Baseline	1.05 ± 0.13 [0.82–1.28]	1.00 ± 0.10 [0.80–1.21]	0.125	0.223	0.576	0.720
End of trial	1.04 ± 0.13 [0.76–1.26]	1.00 ± 0.10 [0.82–1.16]				
Total femur						
Baseline	1.12 ± 0.12 [0.90–1.38]	1.08 ± 0.11 [0.90–1.35]	0.976	0.212	0.419	0.070
End of trial	1.13 ± 0.11 [0.89–1.40]	1.08 ± 0.11 [0.90–1.35]				

Bone mineral density was measured with dual-energy X-ray absorptiometry scanning. All outcomes were initially analyzed without covariates by two-way repeated measures ANOVA, where the between-subject factor, i.e. exenatide vs placebo, was denoted "Group," and the within-subject factor between time points was denoted "Time." A significant "Time × Group interaction" would indicate a difference in response between the two treatment groups. Results from ANOVA/ANCOVA are corrected for age and sex. P-values are rounded to two decimals and significant p-values are shown with an asterisk (*). The table is based on data from patients, who completed the trial.

 # One observation missing. $ Square root-transformed to obtain normal distribution. § Natural logarithm-transformed to obtain normal distribution. Alkaline phosphatase and parathyroid hormone (PTH) were measured on the Vitros 5.1FS or the Vitros 5600 chemistry analyzer (Ortho Clinical Diagnostics, Raritan, NJ, USA) and total 25-hydroxycholecalciferol (vitamin D) was measured on the Cobas e411 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). All three assays are electro-chemiluminescence binding assays. Prolactin was measured using an immunofluorimetric assay on the BRAHMS Kryptor Compact Plus analyzer (Thermo Scientific, Hennigsdorf, Germany). Plasma PINP, plasma CTX, plasma osteocalcin, and serum bone-specific alkaline phosphatase were measured with chemiluminescence immunoassays using the automated analyzer, iSYS (Immunodiagnostic Systems plc, Tyne and Wear, UK) according to the manufacturer's instructions.

noted above the presence of marked obesity may partly explain these findings.

We observed that treatment with exenatide resulted in a trend-level reduction in PINP, and a significant increase in the BMD measurement of L2–L4. The reduction in PINP in the exenatide-treated group contrasts a previously reported increase of PINP in obese women, who experienced a 12% body weight reduction after 12 months of liraglutide treatment (14). In our 3 months study, patients experienced a weight loss of 2.3 kg corresponding to a subtle reduction in body weight of around 2%. Based on these placebo-controlled studies it could appear that GLP-1 receptor agonists may affect PINP, however, the directionality of this change may be influenced by antipsychotic exposure or by concurrent changes in body weight. Additionally, the two GLP-1 receptor agonists liraglutide and exenatide may also affect levels of PINP differently.

Finally, in our study the potential effect of exenatide on bone markers did not remain significant after correction for mean prolactin levels. The limited sample size and large variability in prolactin level render the impact of this finding unclear. Although a previous study did not find correlation between prolactin levels and BMD measures (5), modulation of the dopamine system by GLP-1 receptor agonism has previously been suggested (29). Nevertheless, our current observation of a potential interplay between prolactin levels and effect of GLP-1 receptor agonists, suggests that correction for prolactin in future studies of antipsychotic-treated patients should be considered.

The current study has some limitations. The 3 months study period, and the relatively young (with respect to bone status), and non-diabetic sample compromise the inferences which can be drawn from the present data. Firstly, our patients and the population cohort were not matched on BMI (30). Moreover, we intentionally included a naturalistic trial population (18), which is reflected in the broad medication profiles (**Supplementary Table 1**). To this end, individual antipsychotic compounds may affect prolactin levels (22, 31, 32), and bone status differentially, but the current data did not allow for separating effects of specific antipsychotics. Finally, patients were not instructed to keep their level of physical activity stable and refrain from taking vitamin D supplements during the trial, and we were therefore unable to control for these potential confounders.

In conclusion, these planned secondary analyses of the TAO study showed that sex and age-adjusted measures of bone status were comparable to the Danish reference population. Subtle changes in bone markers over a 3 months treatment course with the GLP-1 receptor agonist exenatide may suggest beneficial effects of GLP-1 on bone status in antipsychotic-treated, obese patients, which may relate to GLP-1-induced changes in prolactin levels.

ETHICS STATEMENT

This study was approved by the National Committee on Health Research Ethics (project no. 36378), the Danish Health and Medicines Authority (EudraCT no. 2012-005404-17) and The Danish Data Protection Agency (project no. RHP-2012-027). The study was registered at clinicalTrials.gov (NCT01794429). The Good Clinical Practice (GCP) Unit at Copenhagen University Hospital monitored the trial according to ICH-GCP guidelines.

All referred patients received both an oral and a written description of the TAO trial, and all were screened for eligibility by the principal investigator (PLI). All patients approved participation by written informed consent prior to enrolment.

AUTHOR CONTRIBUTIONS

All authors fulfill authorship criteria of the ICMJE by substantial contribution to the conception and design, to acquisition of data, or to the analysis and interpretation of the data. FK and BE contributed conception and design of the study. PI acquired the data. BB, PI, and NB organized the database. RE, BB, NB, and BE performed the statistical analysis. RE and BE wrote the first draft of the manuscript. RE, PI, UA, NJ, and FK wrote Methods section of the manuscript. RE, BB, NB, NJ, and BE wrote Results section of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version. The trial was investigator-initiated and data analysis was conducted without influence from the pharmaceutical industry. We also affirm that there was no editorial direction or censorship from any pharmaceutical company.

FUNDING

This work was supported by grants from the University of Copenhagen (211-0649/11-3012), the Mental Health Services in the Capital Region of Denmark, and the Lundbeck Foundation (R25-A2701). The sponsors had no role in the design, conduct, interpretation or dissemination of the study.

ACKNOWLEDGMENTS

The authors would like to thank Gitte Saltoft Andersen for administering trial medication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00781/full#supplementary-material

REFERENCES

1. Kuipers E, Yesufu-Udechuku A, Taylor C, Kendall T. Management of psychosis and schizophrenia in adults: summary of updated NICE guidance. *BMJ* (2014) 348:g1173. doi: 10.1136/bmj.g1173

2. Howes OD, McCutcheon R, Agid O, de Bartolomeis A, van Beveren NJM, Birnbaum ML, et al. Treatment-resistant schizophrenia: treatment response and resistance in psychosis (TRRIP) working group consensus guidelines on diagnosis and terminology. *Am J Psychiatry* (2017) 174:216–29. doi: 10.1176/appi.ajp.2016.16050503

3. Correll CU, Detraux J, De Lepeleire J, De Hert M. Effects of antipsychotics, antidepressants and mood stabilizers on risk for physical diseases in people with schizophrenia, depression and bipolar disorder. *World Psychiatry* (2015) 14:119–36. doi: 10.1002/wps.20204
4. Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet* (2013) 382:951–62. doi: 10.1016/S0140-6736(13)60733-3
5. Howes OD, Wheeler MJ, Meaney AM, O'Keane V, Fogelman I, Blake G, et al. Bone mineral density and its relationship to prolactin levels in patients taking antipsychotic treatment. *J Clin Psychopharmacol.* (2005) 25:259–61. doi: 10.1097/01.jcp.0000162798.87249.4d
6. Liang Y, Su YA, Zhao ZG, Gao N, Huang JZ, Tang MQ, et al. Acute effects of haloperidol, amisulpride, and quetiapine on bone turnover markers in patients with schizophrenia. *J Clin Psychopharmacol.* (2015) 35:583–6. doi: 10.1097/JCP.0000000000000379
7. World Health Organization. *Prevention and Management of Osteoporosis*. Geneva: World Health Organization (2003).
8. Brown JP, Albert C, Nassar BA, Adachi JD, Cole D, Davison KS, et al. Bone turnover markers in the management of postmenopausal osteoporosis. *Clin Biochem.* (2009) 42:929–42. doi: 10.1016/j.clinbiochem.2009.04.001
9. Eastell R, Szulc P. Use of bone turnover markers in postmenopausal osteoporosis. *Lancet Diabetes Endocrinol.* (2017) 5:908–23. doi: 10.1016/S2213-8587(17)30184-5
10. Trujillo JM, Nuffer W, Ellis SL. GLP-1 receptor agonists: a review of head-to-head clinical studies. *Ther Adv Endocrinol Metab.* (2015) 6:19–28. doi: 10.1177/2042018814559725
11. Ma X, Meng J, Jia M, Bi L, Zhou Y, Wang Y, et al. Exendin-4, a glucagon-like peptide-1 receptor agonist, prevents osteopenia by promoting bone formation and suppressing bone resorption in aged ovariectomized rats. *J Bone Miner Res.* (2013) 28:1641–52. doi: 10.1002/jbmr.1898
12. Pereira M, Jeyabalan J, Jørgensen CS, Hopkinson M, Al-Jazzar A, Roux JP, et al. Chronic administration of Glucagon-like peptide-1 receptor agonists improves trabecular bone mass and architecture in ovariectomised mice. *Bone* (2015) 81:459–67. doi: 10.1016/j.bone.2015.08.006
13. Lu N, Sun H, Yu J, Wang X, Liu D, Zhao L, et al. Glucagon-like peptide-1 receptor agonist Liraglutide has anabolic bone effects in ovariectomized rats without diabetes. *PLoS ONE* (2015) 10:e0132744. doi: 10.1371/journal.pone.0132744
14. Iepsen EW, Lundgren JR, Hartmann B, Pedersen O, Hansen T, Jørgensen NR, et al. GLP-1 receptor agonist treatment increases bone formation and prevents bone loss in weight-reduced obese women. *J Clin Endocrinol Metab.* (2015) 100:2909–17. doi: 10.1210/jc.2015-1176
15. Driessen JHM, Henry RMA, van Onzenoort HAW, Lalmohamed A, Burden AM, Prieto-Alhambra D, et al. Bone fracture risk is not associated with the use of glucagon-like peptide-1 receptor agonists: a population-based cohort analysis. *Calcif Tissue Int.* (2015) 97:104–12. doi: 10.1007/s00223-015-9993-5
16. Mabilleau G, Mieczkowska A, Chappard D. Use of glucagon-like peptide-1 receptor agonists and bone fractures: a meta-analysis of randomized clinical trials. *J Diabetes* (2014) 6:260–6. doi: 10.1111/1753-0407.12102
17. Ishøy PL, Knop FK, Broberg BV, Baandrup L, Fagerlund B, Jørgensen NR, et al. Treatment of antipsychotic-associated obesity with a GLP-1 receptor agonist — protocol for an investigator- initiated prospective , randomised , intervention study : the TAO study protocol. *BMJ Open* (2014) 4:e004158. doi: 10.1136/bmjopen-2014-004158
18. Ishøy PL, Knop FK, Broberg BV, Bak N, Andersen UB, Jørgensen NR, et al. Effect of GLP-1 receptor agonist treatment on body weight in obese antipsychotic-treated patients with schizophrenia: a randomized, placebo-controlled trial. *Diabet Obes Metab.* (2017) 19:162–71. doi: 10.1111/dom.12795
19. Ishøy PL, Fagerlund B, Broberg BV, Bak N, Knop FK, Glenthøj BY, et al. No cognitive-enhancing effect of GLP-1 receptor agonism in antipsychotic-treated, obese patients with schizophrenia. *Acta Psychiatr Scand.* (2017) 136:52–62. doi: 10.1111/acps.12711
20. Ebdrup BH, Broberg BV, Ishøy PL, Bak N, Andersen UB, Jørgensen NR, et al. Erratum: Effect of GLP-1 receptor agonist treatment on body weight in obese antipsychotic-treated patients with schizophrenia: a randomized, placebo-controlled trial. *Diabet Obes Metab.* (2018) 20:1327–8. doi: 10.1111/dom.13204
21. Jørgensen NR, Møllehave LT, Hansen YBL, Quardon N, Lylloff L, Linneberg A. Comparison of two automated assays of BTM (CTX and P1NP) and reference intervals in a Danish population. *Osteoporos Int.* (2017) 28:2103–13. doi: 10.1007/s00198-017-4026-z
22. Coello K, Broberg BV, Bak N, Madsen A, Mortensen HB, Søgaard B, et al. Postprandial prolactin suppression appears absent in antipsychotic-treated male patients. *Psychoneuroendocrinology* (2015) 60:1–6. doi: 10.1016/j.psyneuen.2015.05.014
23. Fan B, Lu Y, Genant H, Fuerst T, Shepherd J. Does standardized BMD still remove differences between Hologic and GE-Lunar state-of-the-art DXA systems? *Osteoporos Int.* (2010) 21:1227–36. doi: 10.1007/s00198-009-1062-3
24. Sempos CT, Heijboer AC, Bikle DD, Bollerslev J, Bouillon R, Brannon PM, et al. Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D. *Br J Clin Pharmacol.* (2018) 84:2194–207. doi: 10.1111/bcp.13652
25. *The International Society for Clinical Densitometry. Official Positions 2015 ISCD Combined*. (2015)
26. Stubbs B, De Hert M, Sepehry AA, Correll CU, Mitchell AJ, Soundy A, et al. A meta-analysis of prevalence estimates and moderators of low bone mass in people with schizophrenia. *Acta Psychiatr Scand.* (2014) 130:470–86. doi: 10.1111/acps.12313
27. Palermo A, Tuccinardi D, Defeudis G, Watanabe M, D'Onofrio L, Lauria AP, et al. BMI and BMD: the potential interplay between obesity and bone fragility. *Int J Environ Res Public Health* (2016) 13:E544. doi: 10.3390/ijerph13060544
28. Johansson H, Kanis JA, Odén A, McCloskey E, Chapurlat RD, Christiansen C, et al. A meta-analysis of the association of fracture risk and body mass index in women. *J Bone Miner Res.* (2014) 29:223–33. doi: 10.1002/jbmr.2017
29. Ebdrup BH, Knop FK, Ishøy PL, Rostrup E, Fagerlund B, Lublin H, Glenthøj B. Glucagon-like peptide-1 analogs against antipsychotic-induced weight gain: potential physiological benefits. *BMC Med.* (2012) 10:92. doi: 10.1186/1741-7015-10-92
30. Iwaniec UT, Turner RT. Influence of body weight on bone mass, architecture and turnover. *J Endocrinol.* (2016) 230:R115–30. doi: 10.1530/JOE-16-0089
31. Berardis D, Fornaro M, Serroni N, Marini S, Piersanti M, Cavuto M, et al. Treatment of antipsychotic-induced hyperprolactinemia: an update on the role of the dopaminergic receptors D2 partial agonist aripiprazole. *Recent Pat Endocr Metab Immune Drug Discov.* (2014) 8:30–7. doi: 10.2174/1872214807666131229125700
32. Orsolini L, Tomasetti C, Valchera A, Vecchiotti R, Matarazzo I, Vellante F, et al. An update of safety of clinically used atypical antipsychotics. *Expert Opin Drug Saf.* (2016) 15:1329–47. doi: 10.1080/14740338.2016.1201475

Conflict of Interest Statement: BB became a full-time employee at Novo Nordisk after completion of the clinical study. NB became a full-time employee at H. Lundbeck A/S after completion of the clinical study. FK has received lecture fees from, is part of Advisory Boards of and/or has consulted for AstraZeneca, Boehringer Ingelheim, Carmot Therapeutics, Eli Lilly, Merck Sharp & Dohme, Novo Nordisk, Norgine, Sanofi, and Zealand Pharma. BE has received lecture fees from and/or is part of Advisory Boards of Bristol-Myers Squibb, Eli Lilly and Company, Janssen-Cilag, Otsuka Pharma Scandinavia, and Takeda Pharmaceutical Company.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Eriksson, Broberg, Ishøy, Bak, Andersen, Jørgensen, Knop and Ebdrup. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

MINI REVIEW
published: 30 January 2019
doi: 10.3389/fpsyt.2018.00757

The Group of Treatment Resistant Schizophrenias. Heterogeneity in Treatment Resistant Schizophrenia (TRS)

*Bruce J. Kinon**

Lundbeck North America, Deerfield, IL, United States

Schizophrenia is composed of a heterogeneous group of patient segments. Our current notion of the heterogeneity in schizophrenia is based on patients presenting with diverse disease symptom phenotypes, risk factors, structural and functional neuropathology, and a mixed range of expressed response to treatment. It is important for clinicians to recognize the various clinical presentations of resistance to treatment in schizophrenia and to understand how heterogeneity across treatment resistant patient segments may potentially inform new strategies for the development of effective treatments for Treatment Resistant Schizophrenia (TRS). The heterogeneity of schizophrenia may be reduced by parsing patient segments based on whether patients demonstrate an adequate or inadequate response to treatment. In our current concept of TRS, TRS is defined as non-response to at least two adequate trials of antipsychotic medication and is estimated to affect about 30% of all patients with schizophrenia. In this narrative review, the author discusses that the demonstration of inadequate response to antipsychotic drugs (APDs) may infer that some TRS patients may be suffering from a non-dopamine pathophysiology since D2 receptor antagonist-based treatment is ineffective. Preliminary neurobiological findings may further support the pathophysiologic distinction of TRS from that of general schizophrenia. Investigation of the basis for heterogeneity in TRS through the systematic investigation of relevant "clusters" of similarly at risk individuals may hopefully bring us closer to realize a precision medicine approach for developing effective therapies for TRS patient segments.

Keywords: schizophrenia, antipsychotic drug, treatment resistant, clozapine, dopamine, first-episode schizophrenia (FES), magnetic resonance spectroscopy (MRS), positron emission tomography–PET

OPEN ACCESS

Edited by:
Felice Iasevoli,
University of Naples Federico II, Italy

Reviewed by:
Chad A. Bousman,
University of Calgary, Canada
Frederick Charles Nucifora,
Johns Hopkins Medicine,
United States

***Correspondence:**
Bruce J. Kinon
bkin@lundbeck.com

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *01 September 2018*
Accepted: *20 December 2018*
Published: *30 January 2019*

Citation:
Kinon BJ (2019) The Group of Treatment Resistant Schizophrenias. Heterogeneity in Treatment Resistant Schizophrenia (TRS). Front. Psychiatry 9:757. doi: 10.3389/fpsyt.2018.00757

Schizophrenia is composed of a heterogeneous group of patient segments. This heterogeneity has been long recognized. In Bleuler's treatise on schizophrenia, *The Group of Schizophrenias,* he writes of the heterogeneity of symptoms, for example primary, secondary or accessory, as well as of the heterogeneity of outcomes; good, fair, and poor (1). Our current notion of the heterogeneity in schizophrenia is similarly based on patients presenting with diverse phenotypes characterized by differing symptoms and signs of illness as well as life course, multiple risk factors leading to disease including a complex genetic loading, a broad spectrum of neurobiological features suggesting a pathophysiology of structure and function that is not necessarily shared by all patients, and a mixed range of expressed response to treatment.

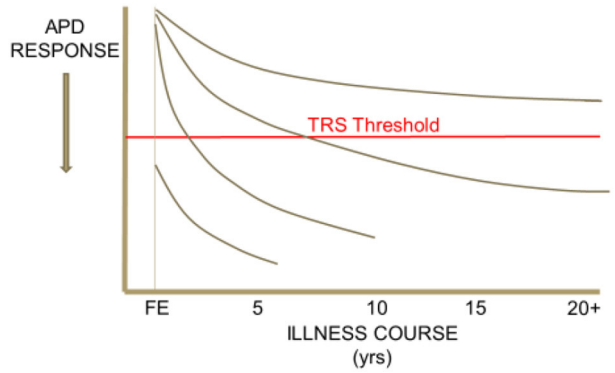


FIGURE 1 | Heterogeneity in the trajectory of response to APD treatment over the illness course of schizophrenia. This schematic drawing illustrates that some patient segments may demonstrate APD responsiveness throughout their illness, others demonstrate resistance to treatment only after an initial period of treatment responsiveness, and others still may be found to respond poorly to APD treatment since their first episode of psychosis.

Heterogeneity in response to antipsychotic drug (APD) treatment is seen across the course of schizophrenia. Some patient segments demonstrate APD responsiveness throughout their illness, others demonstrate resistance to treatment only after many years, or only a few years, of treatment responsiveness. Others still may be found to respond poorly to APD treatment since their first episode of psychosis (**Figure 1**). It is important for clinicians to recognize the various clinical presentations of resistance to treatment in schizophrenia and to understand how heterogeneity across treatment resistant patient segments may potentially inform new strategies for the development of effective treatments for TRS. In addition, it is crucial for clinicians to rule-out "pseudo-TRS" due to inadequacy of APD exposure from either poor adherence (2), under-dosing, ultrarapid drug metabolism (3), or limited length of treatment duration (4).

The heterogeneity of schizophrenia may be reduced by bifurcating patient segments based on whether patients demonstrate an adequate or inadequate response to treatment. In our current concept of TRS, TRS is defined as non-response to at least two adequate trials of antipsychotic medication (4). TRS is estimated to affect about 30% of all patients with schizophrenia (5). As presently defined, TRS reflects the persistence of prominent positive, psychotic symptoms. Other non-psychotic-symptom dominant TRS groups may, possibly, also be identified *IF* we had efficacious treatments for, e.g., negative symptoms, cognitive impairment, or social and vocational dysfunction. TRS infers resistance to dopamine D2 receptor (DAD2R) antagonism (through APD treatment) in relevant central nervous system (CNS) loci which may mediate symptomatic resistance. The specificity of resistance to D2 receptor antagonism to explaining TRS, though compelling, is tentative in view of clozapine, the only APD indicated to treat TRS, still does possess D2 receptor antagonism, though weak, as demonstrated by low *in vivo* human D2 striatal receptor occupancy [61%; (6)].

Are patients with TRS different from treatment responsive patients? Does this distinction reduce some of the heterogeneity in schizophrenia by "carving schizophrenia at a joint?" Unfortunately, much heterogeneity remains in TRS even after parsing it out from general schizophrenia. This persistent heterogeneity is based in part due to TRS patients demonstrating diversity in:

- Factors associated with poor response to treatment
- Onset of TRS in their disease course
- Response to clozapine (CLZ), the only approved treatment for TRS
- Inconsistent manner in which TRS has been defined across clinical research studies to date (4)
- Dominant symptom domains (e.g., positive, negative, cognitive) that are resistant to treatment.

Somewhat opposing views may consider TRS as a disease category distinct from general schizophrenia or perhaps rather as an outlier on a continuum of disease outcome severity, from full and adequate response and recovery to inadequate response to non-response, that characterizes schizophrenia (7). The continuum hypothesis posits that more severe pathophysiology leads to less response to treatment. Conversely, the categorical hypothesis presumes TRS patients suffer from a fundamentally different pathophysiology(s?) from that of the cohort with treatment responsive schizophrenia (8–10). The persistent challenge associated with both hypotheses is that the response/non-response dichotomy in either case is at best an arbitrarily defined boundary across dimensional measures of symptom severity.

A continuum of cumulative factors, or loading of factors, associated with poor response to treatment in schizophrenia may lead to TRS. Many genetic, developmental, behavioral, ethnocultural, and neurobiological factors have been associated with poor response or outcome in schizophrenia (11, 12) (**Figure 2**). TRS may be considered a consequence of diminished likelihood to respond favorably to treatment in the face of such overwhelming factors. Despite these associations, there are no clearly defined predictors of TRS nor even the likelihood to respond to a course of APD treatment.

Early non-response to acute APD treatment may predict subsequent non-response throughout the duration of that treatment episode. Of the few available predictors of APD response, early non-response suggests a plausible categorical distinction between TRS, and non-TRS patients. Early treatment responders, at 2 weeks, demonstrate better symptom improvement than early non-responders after a 12 week course of treatment with risperidone (13, 14). Early non-responders fail to achieve the same level of improvement seen in the early responders. The negative predictive value of early non-response has been reported extensively in the literature (15). Thus, the demonstration of early non-response to acute treatment may be a predictor for TRS as subsequent switching APDS has been shown to offer limited further efficacy for these early non-responders. The demonstration that switching treatment does not appear to be an effective treatment intervention in first episode patients failing their first course of APD treatment supports the observation that failure to respond is quite predictive of subsequent treatment failure (16, 17). Unfortunately, efforts to

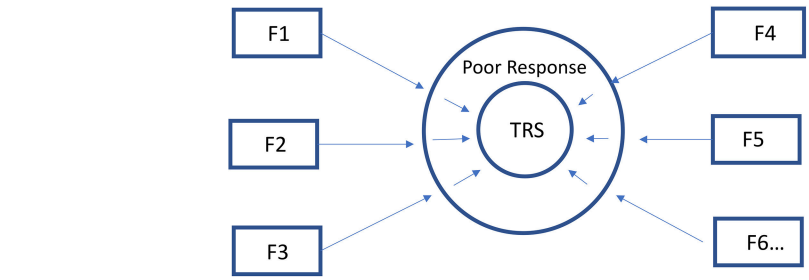


FIGURE 2 | A continuum of cumulative factors (F*n*), as suggested by the above bulleted factors, may additively contribute (i.e., Factor Loading) to compromise response to treatment in schizophrenia. TRS may be considered a consequence of diminished likelihood to respond favorably to treatment in the face of such overwhelming factors. Despite these associations, there may be no clearly defined predictors of TRS.

characterize who may be an early responder or non-responder prior to treatment trial and failure have not been very revealing.

The demonstration of inadequate response to APDs may infer that some TRS patients may be suffering from a non-dopamine pathophysiology since D2 receptor antagonist-based treatment is ineffective. Research has found through L-DOPA positron emission tomography (PET) imaging that patients with TRS may have "normal" rather than hyperactive dopamine synthesis and release in the striatum, whereas APD treatment responsive patients with schizophrenia do reveal significantly greater striatal dopamine activity compared to healthy controls. Conversely, patients with TRS seem to exhibit higher glutamate activity in the anterior cingulate based on glutamate magnetic resonance spectroscopy (MRS) imaging in contrast to treatment responsive patients (18). Therefore, dopamine D2 receptor antagonism may not have a significant influence on TRS symptoms. This provides initial support to consider TRS as a disease state categorically different from treatment responsive schizophrenia based on the apparent absence of a dopamine-based pathophysiology amenable to dopamine D2 receptor blockade. Further support for this neurobiological distinction awaits confirmation in other studies. TRS patients may additionally be distinguished from non-TRS patients by evidence of reduced brain gray matter volume (7, 19, 20), although this may not be a consistent finding across most studies in part due to the heterogeneity of the TRS population studied and inconsistencies in defining TRS (21). Other potentially distinguishing factors of TRS compared to non-TRS, such as gene profiling, polygenic loading, neurocognitive function, and demographics including non-urban residence (9, 10) also require further study and replication before any conclusions can be reached.

The categorical pathophysiologic distinction of TRS from that of general schizophrenia may be further illustrated in patients suffering from Primary TRS, or TRS occurring early in a patient's schizophrenia illness, within 5 years of illness onset (4, 22). Primary TRS is distinguished from Secondary TRS, or TRS occurring late in patient's illness (more than 5 years after illness onset) after a period of years of APD responsiveness. Up to 34% of all TRS may be Primary TRS (22). Many Primary TRS patients may never have demonstrated response to non-clozapine APDs or if so only briefly in the early course of their illness. Primary TRS may be associated with a normal- or hypo-dopaminergic CNS state. Few additional characteristics are presently known to distinguish Primary from Secondary TRS, other than possibly a higher proportion of males in Primary TRS (22).

Through utilization of an algorithm for the treatment of a first episode of schizophrenia, ∼25% of patients were identified to be non-responders to either risperidone or olanzapine during their first treatment period, and of these, >80% again failed to respond to a subsequent second treatment trial when switched to the remaining treatment choice with either olanzapine or risperidone, respectively, (16). Therefore, this algorithm apparently identified Primary TRS patients whose failure to respond to non-clozapine APDs suggests that the symptoms of their first episode psychosis may not be mediated by an increase in dopaminergic activity nor at least improved by blocking the effects of a hyperdopaminergic state. Interestingly, after these 2 APD failures, the overwhelming majority of the non-responding

patients (75%) when treated with clozapine now demonstrated an adequate treatment response, suggesting that clozapine may be mediating a treatment response through a mechanism beyond limited D2 receptor antagonism that may involve a non-dopamine pathophysiology. The limitations of this naturalistic algorithm-based study include no blinding of treatment, the patient's choice of first APD treatment received, and the relatively small number of patients who received clozapine ($n = 28$) in the third treatment trial compared to the number of patients who entered the first treatment trial ($n = 244$). An additional limitation might be that since both olanzapine and risperidone are mainly metabolized through CYP2D6, ultra-rapid metabolizers may have a reduced opportunity to respond to these APDs, whereas response to clozapine, in which CYP2D6 plays a minor metabolic role, may not be similarly disadvantaged. In a somewhat similar but larger and controlled switching clinical trial, (17) have recently reported that first episode patients who failed to achieve remission after an initial open-label trial on amisulpiride (44% non-remitters), later demonstrate a remission rate of <50% regardless of whether they subsequently receive double-blind treatment with either a switch to olanzapine or remaining on amisulpiride (56 and 55% non-remitters, respectively). A small number of these non-remitters went on to receive 12 week open-label clozapine treatment ($n = 28$); 5 of these patients (28%) remitted. These results further support the conclusion that first failure on a D2 antagonist APD may predict subsequent APD treatment failure in first episode schizophrenia patients. More data will be needed before one can conclude on the efficacy of clozapine in these first episode treatment resistant patients.

Therefore, risk factors associated with poor APD response in First Episode Schizophrenia (FES) should be associated with a hypo-dopaminergic state, or at least a "normo-" dopaminergic state, and these risk factors may similarly identify patients at risk for Primary TRS. Some risk factors that have been reported to be associated with poor response in FES include:

- Negative symptoms on illness presentation (23–25)
- Cognitive impairment at baseline (26) or during APD treatment (27)
- Continued substance abuse during early years in treatment (28, 29)
- Extrapyramidal side effects (EPS) during first APD treatment (26)
- Reduced DA activity, as evidenced by diminished frontal DA D2/3 binding potential as compared to APD responding FES patients (30).

These factors may be associated with a hypo-dopaminergic state and may therefore possibly reflect to some degree such state in treatment non-responsive FES patients. It is of course important to note that poor adherence to treatment may also be an overriding factor contributing to poor response in FES.

As first demonstrated in TRS by Demjaha et al. (18), a hyper-glutamatergic state has more specifically been associated with Primary TRS. FES patients with minimal APD exposure have been found to demonstrate an elevated glutamate MRS signal in the anterior cingulate as compared to healthy controls (31). This elevated glutamate signal is also seen in FES non-remitters as compared to remitters to APD treatment (32). These results provide further evidence to suggest that Primary TRS unlike treatment responsive schizophrenia may be a category of schizophrenia characterized more by a hyper-glutamatergic than a hyper-dopaminergic pathology. Of course, these neurotansmitters may be the result of proximal structural and/or genetic factors that may be primarily responsible for these distal distinctions between TRS and non-TRS patients.

Secondary TRS differs from Primary TRS in that the once APD responsive patient now no longer experiences an improvement in psychotic symptoms with APD treatment. This loss of response to APD treatment may conceivably be due to a progressive worsening of the underlying disease state or tolerance to the therapeutic effectiveness of continued DA D2 antagonism. First episode patients have been found to experience a progressive loss of APD response with each subsequent psychotic relapse experienced (33). This suggests that recurrent relapses may have a "neurotoxic" effect that worsens the underlying disease reducing the likelihood of full response to APD treatment (34, 35). Conversely, continuing treatment with APDs may have an iatrogenic effect, perhaps through chronic adaptive alteration of the dopamine receptor [e.g., pharmacologic tolerance due to dopamine receptor supersensitivity (36)] that over time contributes to Secondary TRS [i.e., Supersensitivity Psychosis (37)]. At present, no clear causative mechanism for Secondary TRS has been elucidated.

Clozapine (CLZ) stands as the only approved treatment for TRS. Unfortunately, not all TRS patients respond to an adequate treatment trial with CLZ (38). Thus, CLZ non-responsive as opposed to CLZ responsive patients further divide TRS into at least two additional patient segments. Possible factors associated with CLZ non-response ("Ultra-TRS") include:

- Delayed initiation of CLZ (38–40)
- Cortical (temporal) thinning (41)
- Reduced glutamate activity (42)
- Polygenic factors (43, 44).

Ultra-TRS may reflect a schizophrenia disease state in which dopaminergic, glutamatergic, and perhaps much of the receptor pharmacology of available APDs have a greatly diminished influence on psychosis expression.

CONCLUSIONS

A significant challenge to developing a new and effective treatment for TRS is the heterogeneity in the patient segments that make up what we refer to as *The Group of Treatment Resistant Schizophrenias*. By parsing these patient segments to achieve more homogeneous segments that may share a common pathophysiology, new drug development efforts for TRS may possibly emerge which may be more data driven, or at least hypothesis driven, than a "one size fits all" discovery strategy for a new treatment that may be efficacious in *all* TRS patients. A relevant first step may be to tentatively outline potential patient segments, in order from:

A More Broadly Defined Segment…

- All TRS patients?
- Fewer or greater load of poor response factors?
- Hypo-dopaminergic or hyper-glutamatergic activity?
- Early-in-Disease vs. Late-in-Disease?
- Fewer vs. greater number of failed treatment trials or relapses?
- Enhanced or diminished DAD2R signaling?
- History of response or non-response to clozapine?

…to A More Targeted Segment

At present, this concept has not been substantiated as targets that may mediate illness in specific patient segments have not been validated nor targeted therapies tested. Furthermore, parsing schizophrenia into patient segments based upon response to presently available treatments with all their limitations (e.g., little efficacy to improve such core symptoms as negative symptoms and cognitive impairment) may miss other, more fundamental neurobiological determinants of heterogeneity within schizophrenia. Lastly, consistency in defining TRS and diligence in ruling-out pseudo-TRS will be a requisite in all future studies in order to avoid clouding the pool of bonafide TRS cases.

As TRS remains an area of significant unmet medical need, a systematic effort to find new treatment alternatives must continue. Furthering our understanding of the basis for heterogeneity in TRS through the systematic investigation of relevant "clusters" of similarly at risk individuals for common neuropathology may hopefully bring us closer to realize a precision medicine approach from a clinical drug development strategy to target homogeneous TRS patient segments.

AUTHOR CONTRIBUTIONS

BK contributed to the intellectual content of the work including the conception and design of the work, the acquisition and interpretation of data and literature for the work, the critical drafting and review of the work, and the final approval for this work to be published.

REFERENCES

1. Bleuler E. *Dementia Praecox oder Gruppe der Schizophrenien*. Leipzig, Germany: Deuticke (1911). Trans. J. Zinkin. *Dementia Praecox or the Group of Schizophrenias*. New York, NY: International Universities Press (1950).
2. Kane JM, Kishimoto T, Correll CU. Non-adherence to medication in patients with psychotic disorders: epidemiology, contributing factors, and management strategies. *World Psychiatry* (2013) 12:216–26. doi: 10.1002/wps.20060
3. Spina E, de Leon J. Clinical applications of CYP genotyping in psychiatry. *J Neural Trans*. (2015) 122:5–28. doi: 10.1007/s00702-014-1300-5
4. Howes OD, McCutcheon R, Agid O, de Bartolomeis A, van Beveren NJM, Birnbaum, ML, et al. Treatment-resistantschizophrenia:treatment response and resistance in psychosis (TRRIP) working group consensus guidelines on diagnosis and terminology and resistance in psychosis (TRRIP) working group consensus guidelines on diagnosis and terminology. *Am J Psychiatry* (2016) 174:216–29. doi: 10.1176/appi.ajp.2016.16050503
5. Caspi A, Davidson M, Tamminga CA. Treatment-refractory schizophrenia. *Dialogues Clin Neurosci*. (2004) 6:61–70.
6. Tauscher J, Hussain T, Agid O, Verhoeff, N. P. L. G, Wilson AA, Houle S, et al. Equivalent occupancy of dopamine D1 and D2 receptors with clozapine: differentiation from other atypical antipsychotics. *Am J Psychiatry* (2004) 161:1620–5. doi: 10.1176/appi.ajp.161.9.1620
7. Mouchlianitis E, McCutcheon R, Howes OD. Brain-imaging studies of treatment-resistant schizophrenia: a systematic review. *Lancet Psychiatry* (2016) 3:451–63. doi: 10.1016/S2215-0366(15)00540-4
8. Howes OD, Kapur S. A neurobiological hypothesis for the classification of schizophrenia: type A (hyperdopaminergic) and type B (normodopaminergic). *Br J Psychiatry* (2014) 205:1–3 doi: 10.1192/bjp.bp.113.138578
9. Gillespie AL, Samanaite R, Mill J, Egerton A, MacCabe JH. Is treatment-resistant schizophrenia categorically distinct from treatment responsive schizophrenia? a systematic review. *BMC Psychiatry* (2017) 17:1–14. doi: 10.1186/s12888-016-1177-y
10. Nucifora FC Jr, Woznica E, Lee BJ, Cascella N, Sawa A. Treatment resistant schizophrenia: clinical, biological, and therapeutic perspectives. *Neurobiol Dis*. (2018). doi: 10.1016/j.nbd.2018.08.016. [Epub ahead of print].
11. Kahn RS, Sommer IE, Murray RM, Meyer-Lindenberg A, Weinberger DR, Cannon TD, et al. Schizophrenia. *Nat Rev Dis Primer*. (2015) 1:15067. doi: 10.1038/nrdp.2015.67
12. Radua J, Ramella-Cravaro V, Ioannidis JPA, Reichenberg A, Phiphopthatsanee N, Amir T, et al. What causes psychosis? An umbrella review of risk and protective factors. *World Psychiatry* (2018) 17:49–66. doi: 10.1002/wps.20490
13. Kinon BJ, Chen L, Ascher-Svanum H, Stauffer VL, Kollack-Walker S, Zhou W, et al. Challenging the assumption that improvement in functional outcomes is delayed relative to improvement in symptoms in the treatment of schizophrenia. *Schizophr Res*. (2010) 118:176–82. doi: 10.1016/j.schres.2009.12.013
14. Kinon BJ, Chen L, Ascher-Svanum H, Stauffer VL, Kollack-Walker S, Zhou W, et al. Early response to antipsychotic drug therapy as a clinical marker of subsequent response in the treatment of schizophrenia. *Neuropsychopharmacology* (2010) 35:581–90. doi: 10.1038/npp.2009.164
15. Samara MT, Leucht C, Leeflang MM, Anghelescu I-G, Chung Y-C, Benedicto Crespo-Facorro B, et al. Early improvement as a predictor of later response to antipsychotics in schizophrenia: a diagnostic test review. *Am J Psychiatry* (2015) 172:617–29. doi: 10.1176/appi.ajp.2015.14101329
16. Agid O, Arenovich T, Sajeev G, Zipursky RB, Kapur S, Foussias G, et al. An algorithm-based approach to first-episode schizophrenia: response rates over 3 prospective antipsychotic trials with a retrospective data analysis. *J Clin Psychiatry* (2011) 72:1439–44. doi: 10.4088/JCP.09m05785yel
17. Kahn RS, van Rossum IW, Leucht S, McGuire P, Lewis SW, Leboyer M, et al. Amisulpride and olanzapine followed by open-label treatment with clozapine in first-episode schizophrenia and schizophreniform disorder (OPTiMiSE): a three-phase switching study. *Lancet Psychiatry* (2018) 5:797–807. doi: 10.1016/S2215-0366(18)30252-9
18. Demjaha A, Egertona A, Murray RM, Kapura S, Howesa OD, Stone JM, et al. *Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function*. *Biol Psychiatry* (2014) 75:e11–3. doi: 10.1016/j.biopsych.2013.06.011
19. Anderson VM, Goldstein ME, Kydd RR, Russell BR. Extensive gray matter volume reduction in treatment-resistant schizophrenia. *Int J Neuropsychopharmacol*. (2015) 18:pyv016. doi: 10.1093/ijnp/pyv016
20. Molina V, Reig S, Sanz J, Palomo T, Benito C, Sarramea F, et al. Differential clinical, structural and P300 parameters in schizophrenia patients resistant to conventional neuroleptics. *Prog Neuropsychopharmacol Biol Psychiatry* (2008) 32:257–66. doi: 10.1016/j.pnpbp.2007.08.017
21. Nakajima S, Takeuchi H, Plitman E, Fervaha G, Gerretsena P, Caravaggio F, et al. Neuroimaging findings in treatment-resistant schizophrenia: a systematic review. Lack of neuroimaging correlates of treatment-resistant schizophrenia. *Schizophr Res*. (2015) 164:164–75. doi: 10.1016/j.schres.2015.01.043

22. Lally J, Ajnakina O, Di Forti M, Trotta A, Demjaha A, Kolliakou A, et al. Two distinct patterns of treatment resistance: clinical predictors of treatment resistance in first-episode schizophrenia spectrum psychose. *Psychol Med*. (2016) 46:3231–40. doi: 10.1017/S0033291716002014
23. Ucok A, Serbest S, Kandemiret PE. Remission after first-episode schizophrenia: results of a long-term follow-up. *Psychiatry Res*. (2011) 189:33–7. doi: 10.1016/j.psychres.2010.11.013
24. Downs J, Dean H, Lechler S, Sears N, Patel R, Shetty H, et al. Negative symptoms in early-onset psychosis and their association with antipsychotic treatment failure. *Schizophr Bull*. (2018) 45:69–79. doi: 10.1093/schbul/sbx197
25. Chang WC, Ho RWH, Tang JYM, Wong CSM, Hui CLM, Chan SKW, et al. Early-stage negative symptom trajectories and relationships with 13-year outcomes in first-episode nonaffective psychosis. *Schizophr Bull*. (2018). doi: 10.1093/schbul/sby115. [Epub ahead of print].
26. Robinson DG, Woerner MG, Alvir JMJ, Geisler S, Koreen A, Sheitman B, et al. Predictors of treatment response from a first episode of schizophrenia or schizoaffective disorder. *Am J Psychiatry* (1999) 156:544–9.
27. Bak N, Ebdrup BH, Oranje B, Fagerlund B, Jensen MH, Düring SW, et al. *Two subgroups of* antipsychotic-naive, first-episode schizophrenia patients identified with a Gaussian mixture model on cognition and electrophysiology. *Transl Psychiatry* (2017) 7:e1087. doi: 10.1038/tp.2017.59
28. Weibell MA, Hegelstad WTV, Auestad B, Bramness J, Evensen J, Haahr U, et al. The effect of substance use on 10-year outcome in first-episode psychosis. *Schizophr Bull*. (2017) 43:843–51. doi: 10.1093/schbul/sbw179
29. Awad AG, Voruganti LLNP. Revisiting the 'self-medication' hypothesis in light of the new data linking low striatal dopamine to comorbid addictive behavior. *Ther Adv Psychopharmacol*. (2015) 5:172–8. doi: 10.1177/2045125315583820
30. Nørbak-Emig H, Ebdrup BH, Fagerlund B, Svarer C, Rasmussen H, Friberg L, et al. Frontal D2/3 receptor availability in schizophrenia patients before and after their first antipsychotic treatment: relation to cognitive functions and psychopathology. *Int J Neuropsychopharmacol*. (2016) 19:pyw006. doi: 10.1093/ijnp/pyw006
31. Bustillo JR, Rowland LM, Mullins P, Jung R, Chen H, Qualls C, et al. 1H-MRS at 4 Tesla in minimally treated early schizophrenia. *Mol Psychiatry* (2009) 15:629–36. doi: 10.1038/mp.2009.121
32. Egerton A, Brugger S, Raffin M, Barker GJ, Lythgoe DJ, McGuire PK, et al. Anterior cingulate glutamate levels related to clinical status following treatment in first-episode schizophrenia. *Neuropsychopharmacology* (2012) 37:2515–21. doi: 10.1038/npp.2012.113
33. Wiersma D, Nienhuis FJ, Slooff CJ, Qiel R. Natural course of schizophrenic disorders: a 15-year followup of a dutch incidence cohort. *Schizophr Bull*. (1998) 24:75–85. doi: 10.1093/oxfordjournals.schbul.a033315
34. Lieberman JA, Sheitman BB, Kinon BJ. Neurochemical sensitization in the pathophysiology of schizophrenia: deficits and dysfunction in neuronal regulation and plasticity. *Neuropharmacology* (1997) 17:205–29.
35. Seeman MV, Seeman P. Is schizophrenia a dopamine supersensitivity psychotic reaction? *Prog Neuro Psychopharmacol Biol Psychiatry* (2014) 48:155–60. doi: 10.1016/j.pnpbp.2013.10.003
36. Iyo M, Tadokoro S, Kanahara N, Hashimoto T, Niitsu T, Watanabe H, et al. Optimal extent of dopamine D2 receptor occupancy by antipsychotics for treatment of dopamine supersensitivity psychosis and late-onset psychosis. *J Clin Psychopharmacol*. (2013) 33:398–404. doi: 10.1097/JCP.0b013e31828ea95c
37. Chouinard G. Severe cases of neuroleptic-induced supersensitivity psychosis. Diagnostic criteria for the disorder and its treatment. *Schizophr Res*. (1990) 5:21–33. doi: 10.1016/0920-9964(91)90050-2
38. Üçok A, Çikrikçili U, Karabulut S, Salaj A, Öztürk M, Tabak O, et al. Delayed initiation of clozapine may be related to poor response in treatment-resistant schizophrenia. *Int Clin Psychopharmacol*. (2015) 30:290–5. doi: 10.1097/YIC.0000000000000086
39. Nielsen J, Nielsen RE, Correll CU. Predictors of clozapine response in patients with treatment-refractory schizophrenia. results from a danish register study. *J Clin Psychopharmacol*. (2012) 32:678–83. doi: 10.1097/JCP.0b013e318267b3cd
40. Yoshimura B, Yadaa Y, Soa R, Takakib M, Yamadabet N. The critical treatment window of clozapine in treatment-resistant schizophrenia: Secondary analysis of an observational study. *Psychiatry Res*. (2017) 250:65–70. doi: 10.1016/j.psychres.2017.01.064
41. Molina V, Reig S, Sarramea F, Sanza J, Artaloytia JF, Luque R, et al. Anatomical and functional brain variables associated with clozapine response in treatment-resistant schizophrenia. *Psychiatry Res*. (2003) 124:153–61. doi: 10.1016/S0925-4927(03)00108-2
42. Goldstein ME, Anderson VM, Pillai A, Kydd RR, Russell BR. Glutamatergic neurometabolites in clozapine-responsive and -resistant schizophrenia. *Int J Neuropsychopharmacol*. (2015) 2015: pyu117. doi: 10.1093/ijnp/pyu117
43. Frank J, Lang M, Witt SH, Strohmaier J, Rujescu D, Cichon S, et al. Identification of increased genetic risk scores for schizophrenia in treatment-resistant patients. *Mol Psychiatry* (2015) 20:150–1. doi: 10.1038/mp.2014.56
44. Lin C-C, Wang Y-C, Chen J-y, Liou Y-J, Baid Y-M, Lai I-C, et al. Artificial neural network prediction of clozapine response with combined pharmacogenetic and clinical data. *Comput Methods Programs Biomed*. (2008) 91:91–9. doi: 10.1016/j.cmpb.2008.02.004

Conflict of Interest Statement: BK is an employee of Lundbeck North America, a pharmaceutical company that develops drugs for CNS disorders.

This manuscript though was developed completely by the author BK who has expressly stated in the work that the opinions expressed are solely those of the author and not necessarily those of Lundbeck.

Copyright © 2019 Kinon. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

SYSTEMATIC REVIEW
published: 17 April 2019
doi: 10.3389/fpsyt.2019.00210

Treatment-Resistant to Antipsychotics: A Resistance to Everything? Psychotherapy in Treatment-Resistant Schizophrenia and Nonaffective Psychosis: A 25-Year Systematic Review and Exploratory Meta-Analysis

*Daniela Polese[1,2], Michele Fornaro[1], Mario Palermo[1], Vincenzo De Luca[3,4] and Andrea de Bartolomeis[1]**

[1] Treatment Resistant Psychosis Unit and Laboratory of Molecular and Translational Psychiatry, Section of Psychiatry, University School of Medicine of Naples "Federico II", Naples, Italy, [2] Department of Neuroscience, Psychiatric Unit, Sant'Andrea University Hospital, "Sapienza" University of Rome, Rome, Italy, [3] Centre for Addiction and Mental Health, Toronto, Canada, [4] Department of Psychiatry, University of Toronto, Toronto, ON, Canada

OPEN ACCESS

Edited by:
Frederick Charles Nucifora, Johns Hopkins Medicine, United States

Reviewed by:
Antonio Bruno, University of Messina, Italy
Jose Antonio Apud, National Institute of Mental Health (NIMH), United States

***Correspondence:**
Andrea de Bartolomeis
adebarto@unina.it

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: 15 October 2018
Accepted: 22 March 2019
Published: 17 April 2019

Citation:
Polese D, Fornaro M, Palermo M, De Luca V and de Bartolomeis A (2019) Treatment-Resistant to Antipsychotics: A Resistance to Everything? Psychotherapy in Treatment-Resistant Schizophrenia and Nonaffective Psychosis: A 25-Year Systematic Review and Exploratory Meta-Analysis. Front. Psychiatry 10:210. doi: 10.3389/fpsyt.2019.00210

Background: Roughly 30% of schizophrenia patients fail to respond to at least two antipsychotic trials. Psychosis has been traditionally considered to be poorly sensitive to psychotherapy. Nevertheless, there is increasing evidence that psychological interventions could be considered in treatment-resistant psychosis (TRP). Despite the relevance of the issue and the emerging neurobiological underpinnings, no systematic reviews have been published. Here, we show a systematic review of psychotherapy interventions in TRP patients of the last 25 years.

Methods: The MEDLINE/PubMed, ISI WEB of Knowledge, and Scopus databases were inquired from January 1, 1993, to August 1, 2018, for reports documenting augmentation or substitution with psychotherapy for treatment-resistant schizophrenia (TRS) and TRP patients. Quantitative data fetched by Randomized Controlled Trials (RCTs) were pooled for explorative meta-analysis.

Results: Forty-two articles have been found. Cognitive behavioral therapy (CBT) was the most frequently recommended psychotherapy intervention for TRS (studies, n = 32, 76.2%), showing efficacy for general psychopathology and positive symptoms as documented by most of the studies, but with uncertain efficacy on negative symptoms. Other interventions showed similar results. The usefulness of group therapy was supported by the obtained evidence. Few studies focused on negative symptoms. Promising results were also reported for resistant early psychosis.

Limitations: Measurement and publication bias due to the intrinsic limitations of the appraised original studies.

Conclusions: CBT, psychosocial intervention, supportive counseling, psychodynamic psychotherapy, and other psychological interventions can be recommended for clinical practice. More studies are needed, especially for non-CBT interventions and for all psychotherapies on negative symptoms.

Keywords: treatment-resistant psychosis, dopamine supersensitivity, negative symptoms, psychotherapy, behavioral therapy, group psychotherapy, positive symptoms

INTRODUCTION

Schizophrenia affects approximately 1% of the population, usually starting in adolescence or young adulthood, frequently leading to persistent disability, with a high risk of suicide (8%). Despite the advance in antipsychotics treatment, approximately 30% of patients with schizophrenia show a poor response or no response to antipsychotics (1–7), demonstrating persistent positive symptoms (i.e., hallucinations, delusions). The experience of persistent delusions and hallucinations may result in further disability, poor prognosis, and risk of suicide (8, 9). Finally, treatment-resistant psychosis (TRP) is responsible for increasing health assistance expenditure. For instance, in the United States, treatment-resistant schizophrenia (TRS) adds more than 34 billion dollars in the annual direct medical costs (10).

In the presence of pharmacological treatment resistance, can nonpharmacological, psychotherapy-based interventions significantly overcome the therapeutic response deadlock? Which psychotherapy in combination with antipsychotics does work better? Finally, what are the limitations and the pitfalls of the research on psychotherapy in TRS and TRP?

This review aims to provide a critical, systematic overview covering the last 25 years of published results of all types of psychotherapy, as adjunctive or substitutive therapy, specifically in TRS or TRP patients, including early psychosis and psychotic onset. TRS and TRP for many patients are lifelong mental disorders with significant consequences on most functional domains (11, 12). TRS represents a severe condition with relevant clinical, social, and health costs and consequences (2). In clinical practice, the criteria to define TRS have not been always consistent over time (2). The first complete definition was introduced in the seminal article of Kane and collaborators (13) on clozapine efficacy in TRS. Most of the new proposed criteria require the lack of response to at least two consecutive treatments with antipsychotics; in most cases, one of the two antipsychotics should be an atypical one, of adequate dose and duration (≥6 weeks). An adequate dose of antipsychotic medication in the most recent report is defined as a daily dose of ≥400 mg chlorpromazine equivalence (14–17). The lack of response has been indicated as a relative change in the evaluation scales (i.e., ≥20% decrease in the Positive and Negative Syndrome Scale) (17). Psychotic symptom persistence has been demonstrated to cause distress and serious interference with functioning (18), complicating the clinical course of schizophrenia. Therefore, a large proportion of patients may never reach a functional recovery (19). These patients show poor global functioning and life quality (20, 21), increased drug abuse (6), and reduced cognitive performance compared to patients who respond to the treatment (22). Persistent psychotic symptoms have been observed for 2 years after the initiation of symptoms in 15% of cases (23). In a 15-year follow-up study of patients affected by nonaffective psychosis, every psychotic episode has resulted in raising the probability to experience residual positive symptoms. At least 25% of patients showed persistent positive and negative symptoms after the first episode, while nearly 50% presented persistent symptoms after the fourth episode (24). According to this progression of symptoms persistence, the total number of treatment-resistant patients can increase up to 60% (25). Two forms of treatment resistance have been hypothesized: a type of resistance that is already present at the onset of the pathology, and a second one that develops later on during the trajectory of the disorder and after a period of successful response to antipsychotics (26–28). Remarkably, 82% of TRS had been reported to be resistant since their first episode of psychosis, while 18% of patients with TRS develop resistance after a period of adequate response. It has been reported that the first group could recognize a neurodevelopmental disorder with relatively normal dopaminergic function and prevalent aberrant cortical–subcortical dysfunction (29, 30). Clozapine, the prototypical second-generation antipsychotic, is considered the gold standard of pharmacological treatment for TRS (31–34), even if its superiority in comparison to other second-generation antipsychotics has been challenged in recent meta-analysis (16, 35, 36). Moreover, drug combinations strategies are often used in TRP (32, 37–39) and in the "ultraresistant patients," who do not respond or respond only partially to clozapine. It has been estimated that approximately 30% of patients who are treated with clozapine do not respond adequately (14, 40, 41). Clinical features at diagnosis can only partially predict resistance to the treatment: poorer premorbid functions, an earlier age at onset of positive symptoms, family history of schizophrenia spectrum disorder, longer duration of untreated psychosis (DUP) (26, 42–48), male gender, a history of specific substance abuse, severe negative symptoms, and presence of soft neurological signs (3, 23, 42, 47, 49–51). Functional and structural brain imaging has identified potential brain abnormalities related to treatment response or resistance, specifically at the level of the frontal cortex, basal ganglia, corpus callosum, and anterior cingulate. Nevertheless, correlations with brain abnormalities have still not been consistently replicated (52, 53). In our study, we included an exploratory meta-analysis to provide a quantitative synthesis of data from Randomized Controlled Trials (RCTs). The aim of this latter analysis was to compare the efficacy of an augmentation approach with cognitive behavioral therapy (CBT) versus treatment as usual (TAU) in patients with treatment-resistant schizophrenia.

Psychotherapy Approach to Psychosis

The so-called "Dodo Bird Verdict" has been suggested in many reports to indicate that different psychological therapies are of nonspecific or similar efficacy, but this view is controversial and can be contrasted by meta-analytic studies (54–59). Criteria to define evidence-based psychotherapy (EBP) have been established in youth psychotherapy (60). The comparison between EBP and the usual care has shown a more effective performance in the former but advantages in the latter (61, 62). Some researchers have used befriending (BF), an atheoretical and manualized control therapy (63), as a nonspecific relationship that works as a control group, but it has been shown that this approach could have a therapeutic impact, too (64). Nevertheless, psychological interventions have become more widely accepted over the past two decades (65–67). The majority of recent publications consider CBT the elective psychotherapy for psychosis (68, 70) and other treatments are not frequently studied. In particular, the number of articles on the psychodynamic treatment of schizophrenia was very high from 1966 to 1987, with the decline starting after 1980; however, no one was centered on treatment-resistant schizophrenia (71). Mueser et al. observed that the published studies are "only a crude index of the current therapy in schizophrenia since a small fraction of psychodynamic psychotherapy practitioners publishes their treatment cases." In the history of psychodynamic psychiatry and psychoanalysis, psychosis has been traditionally considered impervious to treatment. However, recent literature points out to the association between environmental factors, such as childhood adversity, and the development of psychotic experiences, psychotic symptoms, and diseases (72–79). In fact, trajectory-based approaches to study clinical consequences to potentially traumatic events (PTEs) have recently emerged. In particular, prototypical trajectories have been found across independent studies, and resilience seems to determine the modal response to adversity (80). Abnormal early-life experience, such as early relationships characterized by a "lack of affectivity" during the first year of life, has been suggested to be potentially pathogenic (81). This aspect should also be evaluated as psychologically determinant in contributing to the development of a psychotic disorder. Furthermore, recent literature has also shown the important role played by the therapeutic relationships in all psychiatric settings in predicting the outcome (82–84). It has also been evidenced how therapist attitude and characteristics in the relationship can influence the outcome specifically in TRS patients (85).

Therefore, in the last 20 years, there has been a growing interest in developing a psychological intervention for people who continue to experience psychotic symptoms despite adequate pharmacological treatment (14, 86–90). In early interventions on psychosis, psychotherapy is a potentially relevant part of the treatment, whereas the medication only might neither be sufficient nor efficient (44, 91–96). Medications can also determine a worse clinical condition and be detrimental, since they can have brain structural effects (97–99). Remarkably, antipsychotic treatment can result in further psychotic symptomatology at this stage, due to a dopaminergic supersensitivity effect, induced by the treatment itself (100–102). It has been observed that early psychosis patients may present treatment resistance. In particular, approximately 20% continue to have significant residual positive symptoms after 12 weeks of comprehensive treatment (103). Nevertheless, in early psychosis, a psychological or an integrated therapy with an adequate dose of medication could be effective, maximize results, prevent relapses, achieve recovery, and overcome drug resistance. Studies on the prodromal phase of psychosis have demonstrated that psychological treatments can be effective in reducing transition to psychosis (103, 104). Also, studies on psychosis onset have shown that, in selected cases, psychological interventions can be more appropriated as the first choice than medications (86, 105–107). The National Institute for Clinical Excellence (NICE) (108) and the Schizophrenia Patient Outcome Report Team (PORT) guidance included cognitive behavioral therapy (CBT) in their preferred list of treatments for schizophrenia (108, 109).

MATERIALS AND METHODS

Aimed at achieving a high standard of reporting, we followed the procedures indicated by the 2009 update of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (http://www.prisma-statement.org/) (see **Figure 1**) (110).

Eligibility Criteria, Information Sources, and Search Strategy

We limited our search to those records related to TRP, TRS and psychotherapy of the last 25 years, from January 1, 1993, until August 1, 2018. Such timeframe owed to methodological considerations aimed at including studies relying on homogeneous diagnostic criteria. A systematic database search was performed on MEDLINE/PubMed, Web of Science/ISI Web of Knowledge, and Scopus. The following combinations of keywords have been used: "treatment resistant psychosis OR treatment resistance psychosis AND treatment-resistant schizophrenia OR treatment resistance schizophrenia AND psychotherapy," "antipsychotic resistant response OR antipsychotic resistance response AND psychotherapy," "clozapine resistance AND psychotherapy OR augmentation strategies," "partial responders antipsychotics AND psychotherapy OR augmentation psychotherapy," "clozapine non responders AND/OR poor responder antipsychotics AND psychotherapy OR augmentation psychotherapy," "psychosis AND antipsychotics psychotherapy augmentation," "medical resistance AND psychosis psychotherapy," "treatment resistant OR treatment-resistant OR treatment resistance OR treatment-resistance AND psychosis AND/OR schizophrenia AND psychotherapy AND/OR psychodynamic psychotherapy AND/OR therapeutic relationship." RCT, meta-analyses relevant open-label trials, significant articles, including case reports, controlled and uncontrolled trials, and ongoing trials of pharmacological treatments, augmented or substituted with psychotherapeutic approaches to TRP and TRS, have been selected. No language restriction was applied, and relevant cross-references were retrieved as necessary. Studies concerning augmentation or

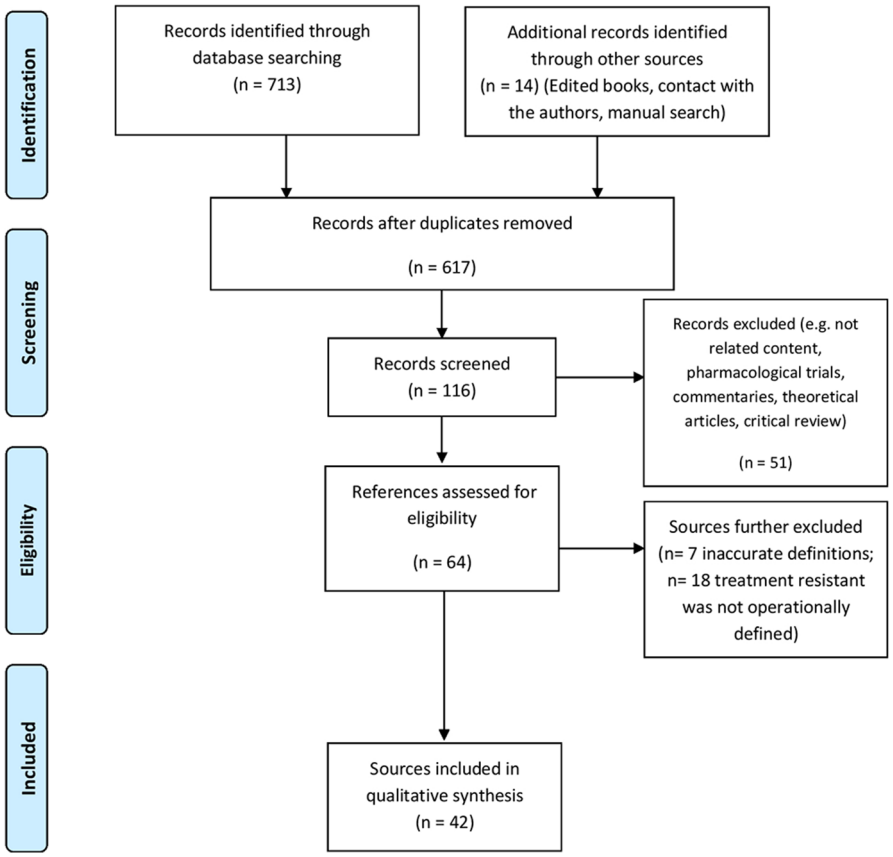


FIGURE 1 | Flow chart of review procedures.

substitution with medication have been excluded. Articles referring to TR in different pathologies from nonaffective psychosis and schizophrenia spectrum disorders have also been excluded. To overcome the problem of nonspecificity in psychotherapy, particular attention has been paid to the psychotherapy method and its details and to the control groups. Critical and systematic reviews on psychological interventions in TRP and TRS have been considered for a further review of literature. The most frequent cluster of symptoms measured by clinical scale assessments that have been included are 1) general psychopathology, 2) positive and negative symptoms, 3) cognitive symptoms, 4) affective symptoms, and 5) social functioning. The following aspects have been considered: 1) the stage of illness, such as the prodromal phase, the onset, any time after the onset and during the chronic phase; 2) the population of patients regarding diagnosis, duration of illness, age, age of onset, and duration of untreated psychosis (DUP); and 3) the type of psychotherapy, such as individual or group, duration of the treatment, frequency and time of the sessions, type of comparison or control group (if present), and blindness of the raters.

About the meta-analysis portion, we performed a fixed-effect meta-analysis aimed at evaluating the efficacy of augmentation therapy with CBT on the positive symptoms of Positive and Negative Syndrome Scale (PANSS) (see **Figure 2**). The same analysis was replicated on the negative symptoms of PANSS (see **Figure 3**). A further meta-analytical random-effect evaluation was carried out in order to evaluate the effectiveness of augmentation therapy with CBT in terms of variation of the total PANSS scores (see **Figure 4**). The estimate uses SMD (standard mean difference pre- vs. posttreatment) as an effect size.

The heterogeneity index of the studies and the publication bias were respectively evaluated with I^2 and Funnel plots (see **Figures 5**, **6**, and **7**).

The inclusion criteria used for the selection of the RCTs suitable for the meta-analysis carried out were as follows:

1. Presence of a uniform control group (patients treated with the usual therapy) (TAU)
2. Measurement of outcome with validated scales (PANSS)
3. Studies only of the RCT type
4. Same type of psychotherapeutic intervention (individual CBT)
5. Evaluation, pre- and posttreatment, with the same type of scale
6. Follow-up to 6 or 9 months

Study Selection

Included papers were those reporting efficacy outcomes about the positive and/or negative symptoms of TRS and TRP exposed to antipsychotic replacement or augmentative psychotherapy, any modality. Outcome measures could be reordered by means of varying standard rating tools or by means of the clinicians' judgment.

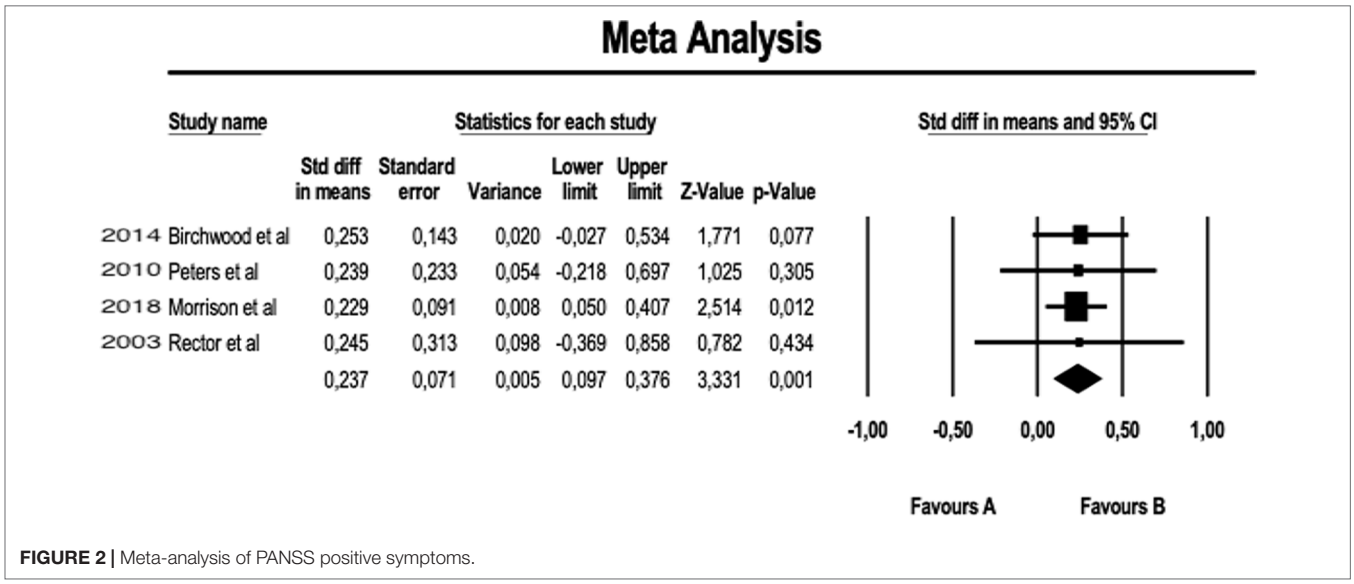


FIGURE 2 | Meta-analysis of PANSS positive symptoms.

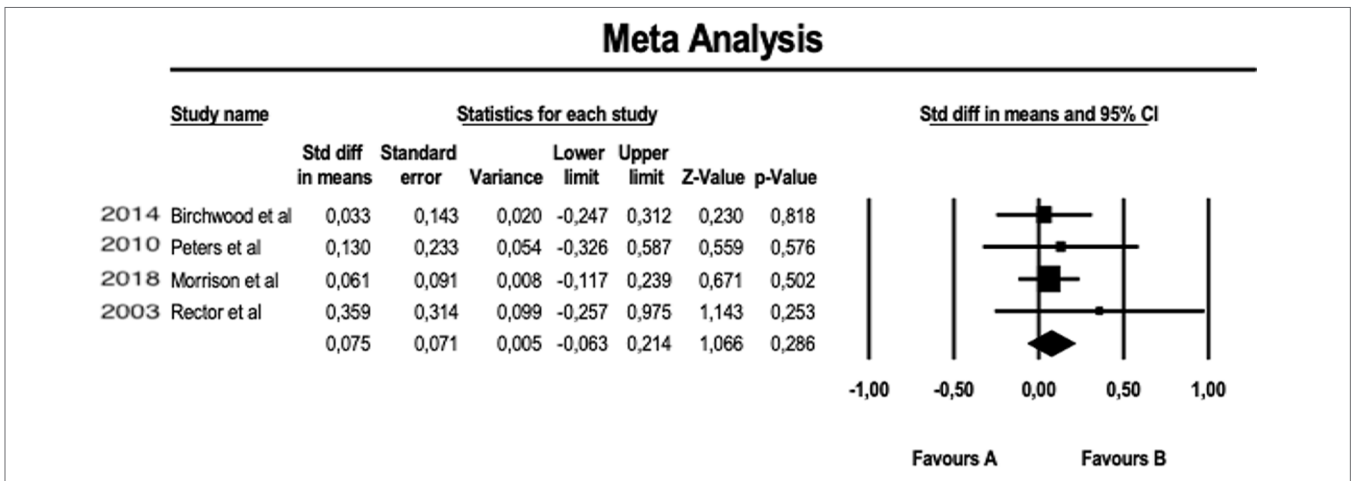


FIGURE 3 | Meta-analysis of PANSS negative symptoms.

Data Collection Process

Two authors (DP and MP) conducted a two-step literature search, examining all titles and abstracts, accessing the full texts of potentially relevant papers. Upon data collection and extraction, the appointed authors compared their results with each other to reach a final consensus based on consensual inclusion and exclusion criteria. Any eventual discrepancy between the principal investigators, blind to each other, was solved by consultation with the senior author (AdB). Finally, the leading senior author with considerable experience on the topic (AdB) assisted in manuscript revision. Data were sought for the following characteristics: participants, interventions, comparisons, outcomes, and study design (PICOS), as well as funding sources. Specifically, the recorded variables for each article included in the review were the following: author(s), year of publication, study design, sample size, eventual follow-up or control group, outcome measures, conclusions, limitations, quality score, and quality differentiation.

Risk of Bias in Individual Studies

Potential major confounding biases in the studies were ascertained at study level focusing on the following: measurement/diagnostic bias (e.g., lack of reliable diagnostic tools to make the diagnosis of TRS or TRP), confounding bias (e.g., lack of stratification and multivariate control for specific sociodemographic, vital, or clinical features), information (especially recall) bias, unrepresentativeness or inhomogeneity of the sample size or lack of control group (where applicable),

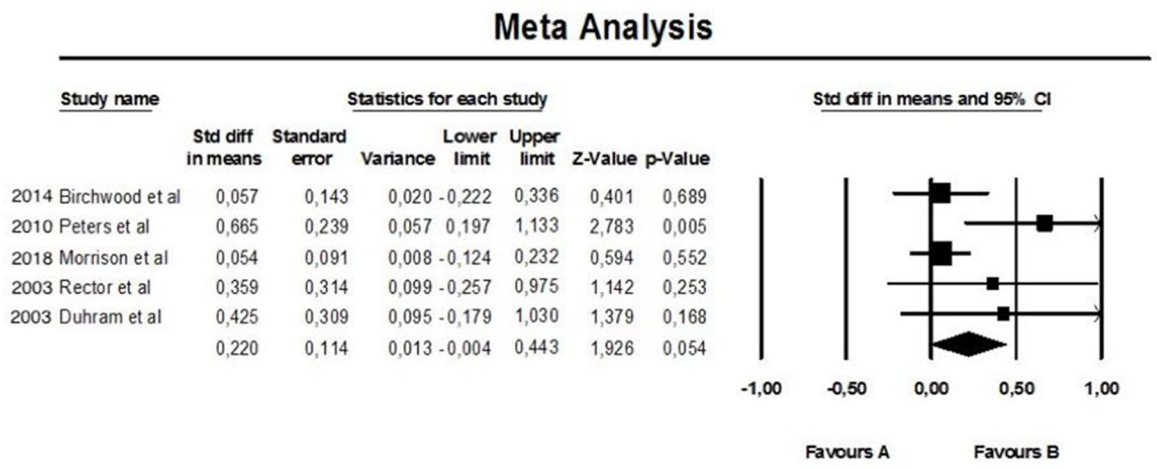


FIGURE 4 | Meta-analysis of PANSS total.

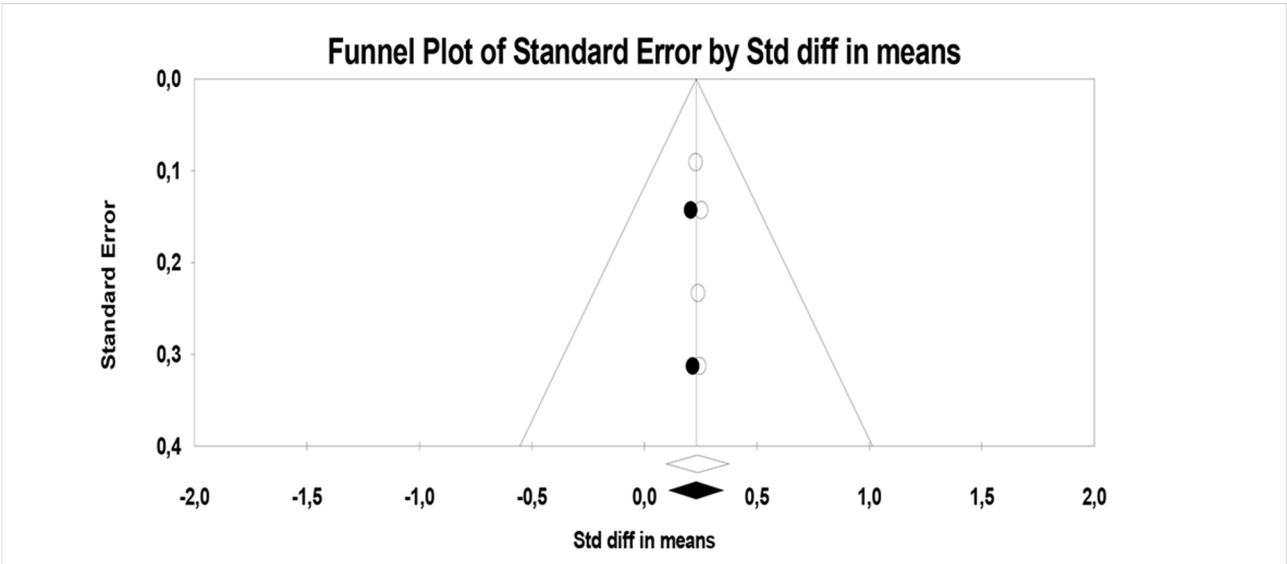


FIGURE 5 | Funnel plot of PANSS positive symptoms.

and selection by indication bias (nonrandom assignment of the exposure where applicable) (111).

Scoring and Ranking of the Studies

The present systematic review purposely encompassed a broad range of records and different types of study designs. To avoid an "apples and oranges" bias, we strived at stratifying the appraised results by discriminating between different quality levels. Specifically, observational case–control reports were appraised by means of the Newcastle–Ottawa Rating Scale (see **Table 1**) (118) and randomized controlled studies were appraised using the Jadad scale (see **Appendix 1**) (119).

Risk of Bias Across the Studies

Any eventual bias affecting cumulative evidence (e.g., publication bias, selective reporting within studies) was assessed through the study evaluation process and accounted in the discussion of the present manuscript.

RESULTS

The process of the literature search is shown in **Figure 1**. The search identified 42 references, of which 18 were RCT articles (see **Table 2** for all the types of studies). **Appendix 1** provides an overview of descriptive information about the 42 studies.

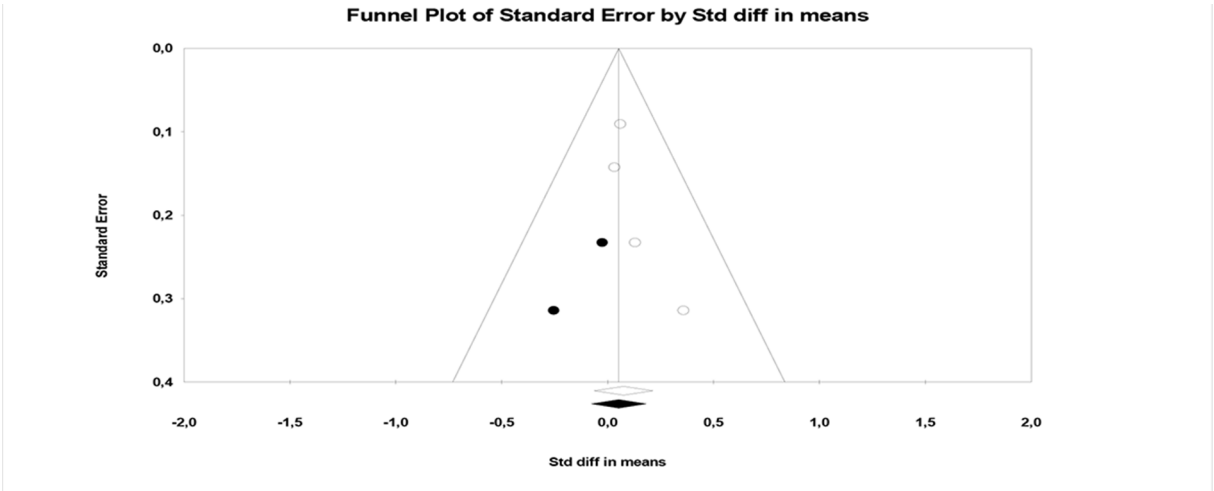


FIGURE 6 | Funnel plot of PANSS negative symptoms.

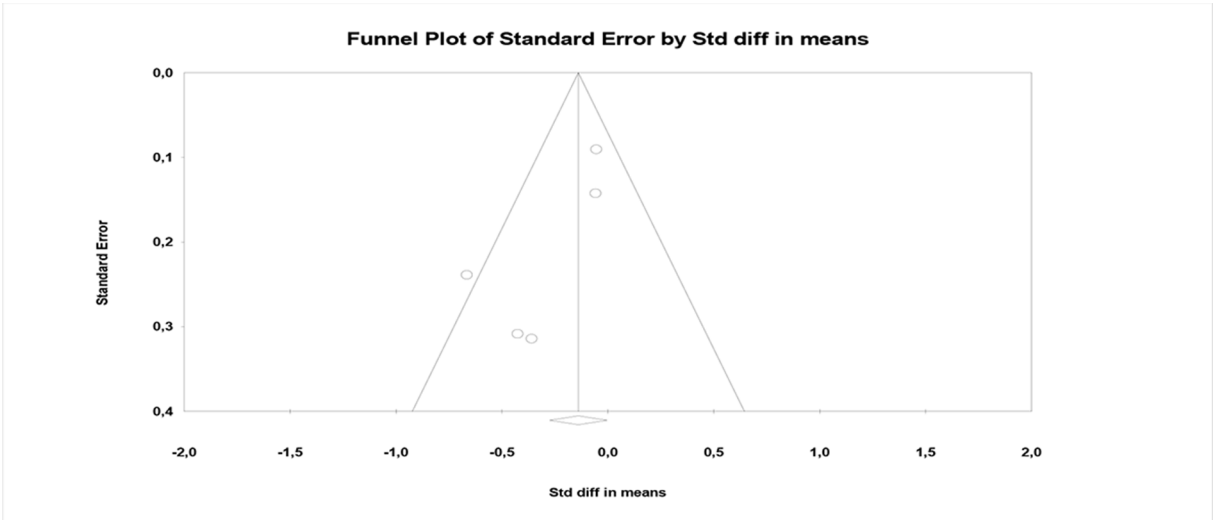


FIGURE 7 | Funnel plot of PANSS total.

Overall Number, Selected Number, and Typology of Psychotherapy Intervention

Only patients who had been stable on medication for a defined period (from 8 weeks to 6 months) were included in the studies. As reported in **Table 3**, CBT works were found in 32 trials: 25 on individual and 7 on group CBT. Social skill training (SST) was studied in adjunction to CBT, and they were compared to supportive counseling (SC) in one trial (120). Works on family interventions (FI), psychosocial intervention (PI), psychoeducation (PE), key-person counseling (KC), cognitive remediation (CR), supportive counseling (SC), and supportive therapy (ST) were studied in comparison with CBT in 12 CBT works. No studies with these interventions alone on TRP patients have been found. In one trial, CBT was compared to SC plus PE (121). Mindfulness was used in adjunction to CBT, acceptance-based intervention (ACT), and treatment of resistant command hallucinations (TORCH) in one study (122), while it was examined alone in another work (123). One study on multimodal individual psychotherapy, including individual CBT, was found (114). Two controlled trials that compared individual CBT to treatment as usual (TAU) have been collected (117, 124). One RCT that compared CBT to enriched TAU (125) has been found. The studies regarding other interventions alone were as follows: reasoning training (RT, $n = 2$) (112, 126), metacognitive therapy (MCT, $n = 2$) (127), cognitive therapy for command hallucinations (CTCH, $n = 1$) (128), art group

TABLE 1 | Newcastle-Ottawa Scale for assessing the quality of the included studies.

Author	Year	Newcastle–Ottawa Scale Case–Control Studies (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)					
		Selection—case definition	Selection—representativeness of the cases	Selection—selection of controls	Selection—definition of controls	Comparability of cases and controls	Exposure/ascertainment of exposure
Ross et al. (112)	2009	*	*	*	*	*	*
Cather et al. (113)	2005	*	*	*	*	*	*
Temple, Ho. (124)	2004	*	*	*	*	**	*
Randal et al. (114)	2003	*	*	*	*	**	*
Durham et al. (115)	2003	*	*	*	*	*	*
Levine et al. (116)	1998	*	*	*	*	*	*
Garety et al. (117)	1994	*	*	*	*	*	*

TABLE 2 | Study design of the included trials.

Type of study	Number of studies	Number of studies with blind assessors
RCTs	18	14 blind studies
Randomized experimental trials	1	0 blind studies
Controlled clinical trials	5	2 blind studies
Uncontrolled clinical trials	6	0 blind studies
Case reports	3	0 blind studies
Pilot studies	2	0 blind studies
Follow-up studies	3	2 blind studies
Meta-analysis	3	2 (1 blind study + 1 blind vs. nonblind study)
Cochrane Intervention Review	1	1 blind vs. nonblind study
Total	42	21

TABLE 3 | Type of psychological intervention in the retrieved studies.

Psychological intervention	Number of studies
Individual or group CBT vs. treatment as usual and/or other nonspecific therapies	17
CBT, Psychosocial Intervention	2
CBT, Supportive Therapy	3
CBT, Psychoeducation (PE)	2
CBT, Supportive Counseling (SC)	1
CBT, SC + PE	1
CBT, Psychoeducation, SC	2
CBT, Family Intervention	1
CBT, Social Skill Training (SST), ST	1
CBT, ACT, TORCH, Mindfulness	1
CBT, Cognitive Remediation (CR)	1
Individual Multimodal Psychotherapy	1
Cognitive Therapy for Command Hallucinations	2
Reasoning Training	1
Mindfulness	1
Metacognitive Therapy	2
Art Group therapy	1
Occupational Therapy	1
Psychodynamic Interpersonal Therapy	1
Total	42

therapy (n = 1) (129), occupational therapy (OT, n = 1) (130), and psychodynamic-interpersonal therapy (PIT, n = 1) (131).

Ten of the 42 studies regarded group therapy (116, 123, 129, 132–138). They are shown in **Table 5**.

The CBT studies represented the majority of articles (32 out of 42). They were generally rigorous, as 22 out of 32 were of the RCT type, including 3 follow-up studies and 2 meta-analyses, while 10 studies included six trials with a control group. Only four CBT studies had no comparison group or control group. Some CBT researchers have used befriending (BF) (122, 139, 140). An RCT study on BF in first episode psychosis has been found and reported in **Appendix 2** (64). The mindfulness study used BF as a comparison group as well. The work on multimodal psychotherapy used a TAU control group. Of the remaining studies, 3 out of 9 included a control group: the brief RT was compared to the Attention Control Activity (141), OT was compared to clozapine alone (130), and the CTCH was compared to TAU (128).

Moreover, a meta-analysis (138) was focused on individual and group FI studies on schizophrenia patients who were both TR and not TR patients and included CBT works in TRS patients, who were accurately described.

For this reason, it has been incorporated in our work. A second phase of the same meta-analysis has been excluded, as it did not pertain to medication resistance (142). The dose of treatment was measured by the total number of sessions and was from 4 to 27, given throughout a period between 12 weeks and 24 months. In five studies, the number of sessions and the time of treatment were not specified.

Therapists and Blindness

Therapists were generally expert, except for one case (143). In two cases, the raters were trained and experienced nurses (140, 141). One study specifically on treatment resistance in early psychosis was found (144). Another study included early psychosis in a heterogeneous group (93). Eighteen articles were trials with blind raters, while blindness could not be used in 21 works. Only one meta-analysis out of three was specifically focused on blind studies (145). The Cochrane review compared blind studies with nonblind studies (146).

Stage of Illness

The stage of illness (initial or chronic) was heterogeneous in 10 studies, where the patients who were enrolled had different ages or very diverse duration of illness. In 11 articles, the duration of

illness was not specified. Twenty trials had a sample of chronic TRP patients. None of the found articles resulted in reporting the DUP.

Pharmacological Co-Treatment

In only two studies were there patients who were not on medication. In the first, there were 5 of 40 patients in an uncontrolled naturalistic study of CBT plus FI (147), while in the second there were 3 of 12 patients on individual CBT in a RCT (148). Remarkably, no study has proposed a psychological treatment as an alternative to medication in the whole sample. No study with regard to music therapy, specifically on medication-resistant psychosis patients, has been found. However, other 18 studies, which did not focus on TR patients and were not included in this research, have been collected in **Appendix 2**.

Clinical Outcome

Assessments used to measure improvement often differed between the various trials. Hence, we pooled study results either based or not based on statistics along with the authors' conclusion to compare them. See **Table 4** for details on the number of works that had statistically significant outcomes. Articles reporting no improvement are also included in **Table 4**. No changes after treatment have been observed in only 2 studies out of 42. Those two trials were on CBT: one on CBT integrated with FI (147) and one on group CBT (132).

Symptoms and Clinical Domains

The symptomatology studied in the retrieved trials is mainly represented by the positive symptoms and, above all, the auditory hallucinations, especially in the CBT studies, while negative symptoms have been rarely evaluated. Ten studies out of 42 reported a decrease of negative symptomatology (see **Table 4**). Efficacy on negative symptoms has been shown in three CBT trials and in ST, CR, CTCH, and OT. Art group therapy, MCT, and PIT trials have also reported a positive outcome on negative symptoms but without control group and not statistically evaluated (see **Appendix 1** and **Table 4**). Affectivity has not been specifically evaluated, except for art group therapy and PIT. Clinical progress has also been observed in other areas, such as social functioning and personal care. Self-esteem and hopelessness have been evaluated, but their improvement has not been shown.

Studies with chronic patients affected by treatment resistance have shown that CBT could be effective, providing positive symptom reduction, which was considered equivalent to a "medium effect size." A trend to effective treatment has been observed as well, in case series with psychosis onset, which was resistant to medication alone: almost three-quarters of patients achieved clinically significant improvement (144). However, results of CBT efficacy compared to other treatments in TRP are not homogeneous in all studies. For instance, when compared to other treatments, similar improvements to the CBT experimental group have been observed in other comparison groups, while a significant difference has been constantly observed only from TAU (132). In particular, in the Cochrane meta-analytical review on schizophrenia including TRS, psychosocial therapies have shown no clear difference from CBT for outcomes relevant to adverse effect/events, global mental state measures, and effects on positive or negative

TABLE 4 | Improvements observed in the different psychological interventions, which were examined in the reviewed studies.

Psychological intervention on TRP patients	Studies with statistically significant improvement	Studies with no statistically significant improvement	Studies with no different improvement between groups	Studies with no improvement	Studies with improvement specifically on negative symptoms
Individual CBT	8	2	0	0	2
Individual CBT vs. Befriending	1	0	1	0	1
Group CBT	3	0	0	1	0
Group CBT vs. Group ST	1	0	1	0	0
CBT vs. Psychosocial Intervention	0	0	1	0	0
CBT, Supportive Therapy	0	0	1	0	0
CBT vs. Psychoeducation	0	0	2	0	1
CBT vs. Supportive Counseling	2	0	3	0	0
CBT, Family Intervention	1	0	0	1	0
CBT, Social Skill Training vs. ST	0	0	1	0	0
CBT, (ACT, TORCH), Mindfulness vs. Befriending	0	0	1	0	1
CBT vs. Cognitive Remediation	0	0	1	0	0
Multimodal Psychotherapy	1	0	0	0	0
Reasoning Training	1	1	0	0	1
Cognitive Therapy for Command Hallucinations (CTCH)	1	0	0	0	1
Mindfulness	0	1	0	0	0
Metacognitive Therapy	2	0	0	0	1
Art Group Therapy	0	1	0	0	0
Occupational Therapy	1	0	0	0	1
Psychodynamic Interpersonal Therapy	0	1	0	0	1
Total	22	6	12	2	10

symptoms (146). Moreover, the studies comparing CBT to another treatment, such as cognitive remediation (149), befriending (122, 140), supportive therapy (115, 120, 134), psychoeducation (113, 150), supportive counseling (121), or family intervention (138), have shown significant clinical improvement in all groups that were studied. Finally, a statistically significant major improvement in supportive therapy has also been observed (135). Two trials have shown a significant improvement in the CBT group when compared to other psychological interventions, such as befriending (139) or supportive counseling (151). Moreover, in two follow-up studies, CBT did not maintain the superiority to SC (152, 153). In particular, after 1 year from the end of treatment, CBT started to decline while SC improved, and this trend continued at 2-year-follow-up. Finally, our results show that group therapy is related to significant improvement for all psychological interventions retrieved, except for family intervention (138), where single family treatment resulted better than the group family one. In six out of seven trials, group CBT presented the same improvement as the comparison group, showing the same results that were observed in the studies on individual CBT.

Meta-Analysis Result

The results obtained in our meta-analysis concerning the domain "POSITIVE SYMPTOMS" of the PANSS scale are as follows:

Fixed-effect meta-analysis: number of studies = 4; number of comparisons (k) = 4; total sample = 800 patients; SMD (standard mean difference) = 0.237 (C.I. = 0.097–0.376).

These preliminary results suggest that, on average, the PANSS score for positive symptoms was reduced by 23.7% more (with a margin between 9.7% and 37.6%) in patients who performed augmentation therapy with CBT compared to patients who received the usual therapy (TAU) (see **Figure 2**). Moreover, this reduction is statistically significant ($p = 0.001$).

Although the number of meta-analyzable studies is small, the heterogeneity index I^2 is 0% (**Figure 5**).

The results obtained in our meta-analysis concerning the domain "NEGATIVE SYMPTOMS" of the PANSS scale are as follows:

Fixed-effect meta-analysis: no. of studies = 4; number of comparisons (k) = 4; total sample = 800 patients; SMD (standard mean difference) = 0.075 (C.I. = −0.063–0.214).

These preliminary results suggest that, on average, the PANSS score for negative symptoms was reduced by 7.5% more (with a margin between −6.3% and 21.4%) in patients performing augmentation therapy with CBT compared to patients receiving the usual therapy (TAU) (see **Figure 3**). However, this reduction is not statistically significant ($p = 0.286$).

Furthermore, it is noteworthy that the "lower limit" of the negative confidence interval (−6.3%) indicates how, at least in a small number of events, the CBT in augmentation to the usual treatment (TAU) could potentially induce even an effect opposite to the therapeutic one.

Although the number of meta-analyzable studies is small, the heterogeneity index I^2 is also 0% in this case (**Figure 6**).

The results obtained in our meta-analysis concerning the "TOTAL Score" domain of the PANSS scale are as follows:

Random-effect meta-analysis: no. of studies = 5; number of comparisons (k) = 5; total sample = 843 patients; SMD (standard mean difference) = 0.220 (C.I. = 0.443–0.004).

These preliminary results suggest that, on average, the total score at the PANSS was reduced by 22% more (with a margin between 44.3% and −0.4%) in patients who performed augmentation therapy with CBT compared to patients who received the usual therapy (TAU) (see **Figure 4**). However, this result is not statistically significant ($p = 0.054$).

Moreover, in this case, the heterogeneity index I^2 is equal to 46% and, being quite high, therefore indicates a poor homogeneity of the analyzed data (**Figure 7**).

DISCUSSION

Psychological Interventions

Psychological interventions in TRP patients have shown a therapeutic effect in 40 out of 42 selected studies. In particular, results demonstrate improvement in positive symptoms for CBT, as well as for other psychological interventions, albeit with different degrees. More specifically, CBT effects in selected studies were not statistically different respectively from psychosocial intervention (146), cognitive remediation (149), befriending (122, 140), supportive therapy (115, 120, 134, 135), psychoeducation (113, 150), supportive counseling (121), and family intervention (138).

CBT has been recognized as more efficient in persistent positive symptoms at follow-up. Supportive counseling (SC) was less effective than CBT at the 9-month follow-up, while it demonstrated the same efficacy as CBT at the following follow-up. Finally, the SC showed its superiority in some measures at 2 years follow-up (140, 153). It has been speculated that supportive counseling may enhance frequent and regular nonthreatening social interaction, which might have worked on self-esteem and helped patients to recuperate their social activity (16). Furthermore, metacognitive therapy has also shown significant improvements in both positive and negative symptoms compared to the baseline (but a control group was not provided) (127). Although art therapy is not strictly considered as a form psychotherapy, it has shown to lead to improvements in a short time in fields that are not easily measured by regular assessments, for example when considering interhuman relationship (129). Moreover, affectivity has not been specifically evaluated, except for art group therapy and psychodynamic interpersonal therapy (131), which were case series. In this work, clinical progress has also been observed in other areas such as social functioning, showing a marked reduction in the severe disturbances presented prior to treatment (131). Occupational therapy has been shown to give a statistically significant improvement compared to clozapine alone in the performance of the activity, in psychotic symptoms, social interaction, and personal care (130). Multimodal psychotherapy, reasoning training, and cognitive therapy for command hallucinations (CTCH) have also shown significant improvements compared to TAU (112, 114, 126, 128). The sample population targeted in the trials included different phases of the illness, showing

that an *integrated* treatment with psychological intervention and pharmacological treatment could be helpful at any point of the disease trajectory. On the contrary, no data on the use of psychological intervention alone on TRP patients are currently available.

Few methodological issues need to be considered, such as the *type of intervention*, *characteristics of the sample*, including *age of patients* as well as *stage of the illness*, and *duration of the treatment*. With regard to the type of intervention, it has already been observed that all psychological therapies, including befriending and supportive therapy, may have a clinically relevant impact, and statistically significant results are reported in more than half of the trials included in this review (22 out of 42, see **Table 4**).

A controversial aspect of psychotherapeutic interventions in TRP is represented by the fact that psychological interventions, including CBT, have an effect mainly on positive symptoms while they seem to be less clearly effective on other main aspects, such as negative and cognitive symptoms. Eighteen CBT trials have shown that CBT, in adjunction to antipsychotics, could produce better outcomes on a variety of measures than medication alone, but target treatment was mainly represented by positive symptoms. In fact, negative symptoms are generally left aside and remained prevalently persistent in the majority of studies. In some trials, negative symptoms have not even been evaluated. In summary, 10 studies out of 42 reported a significant reduction of negative symptomatology: 3 on CBT, 1 on CR, 1 on MCT, 1 on CTCH, 2 on occupational therapy, 1 on art group therapy, and 1 on psychodynamic interpersonal therapy (see **Table 4**). These evidence are also compatible with the result of a recent meta-analysis on psychological treatments of negative symptoms in a population of psychotic patients that were not specifically resistant to treatment (154). In particular, improvement in negative symptoms has been observed after CBT intervention in patients who were at any stage of the disease. This amelioration has resulted in 59% of the studies when CBT was compared to TAU, while none of the analyzed studies suggested a benefit of CBT if compared to active controls. Moreover, another recent meta-analysis for a total of 4,068 patients who were on average moderately ill at baseline has confirmed the efficacy of CBT on positive symptomatology (155). A recent systematic review has newly reported that CR can also have beneficial effects on negative symptoms, compared to TAU and TAU plus active control in schizophrenia patients who were not treatment resistant (68).

Additional researches are needed in order to test "self-disturbance" (156, 157). Consequently, it would be necessary "to tailor" psychological treatment aimed at this symptom. Since the "hyperreflexive attitude" is typical in self-disturbance and in nonaffective psychosis, CBT might not be the most suited psychological intervention on these patients. This is due to the fact that an important feature of this therapeutic approach is the encouragement of "thinking about thinking" (14, 158), which is what the patients already do repeatedly in a pathological fashion (159).

It has been observed that brain dysfunctions, for example, dopaminergic supersensitivity, could be secondary to psychological events (74, 160). Furthermore, studies on brain receptor availability after psychotherapy treatments (both CBT and psychodynamic psychotherapy) have shown that a neurobiological alteration can be modifiable or reversible thanks to psychological interventions (161–164). Further steps in augmentation with psychological therapy of TRP seem to be focusing on the total symptomatology, including positive, negative, and self-disturbance. Considering that symptoms are part of unitary and complex psychopathology, acting on one aspect could be partial. On the other hand, publications on psychodynamic psychotherapy, which is focused on unconscious dimension, are poorly available; only one paper referring specifically to TRP patients has been found in this review (131).

Other critical points are as follows: the characteristics of the sample, age of patients, stage of illness, and duration of the treatment. Some gaps have to be highlighted. Firstly, a marked heterogeneity of the selected sample has been observed in 10 trials, while 11 studies did not take it into account. For instance, patients at different ages or at difference stages of illness (early stage, acute or chronic phase) were located in the same group. For example, 18-year-old patients were in the same group as 40-, 50-, and 60-year-old patients: considering the different psychopathological conditions and the long-term effects of the illness (165), patients respond differently.

Furthermore, it has been observed that factors associated with better outcome include a shorter duration of illness and less severe symptom at pretreatment (151, 166). In addition, in the acute phase of psychosis, CBT can produce durable and substantial clinical benefits (165). Concerning the detailed diagnosis of TR, if two different types of TRP or TRS (at the early and at the chronic stage of illness) have been identified, they should be studied separately and not in the same sample. Secondly, in the majority of studies, the duration of the treatment ranged from 4 weeks to 9 months. Only 2 studies out of 42 (114, 138) used a duration of treatment up to 21–24 months, and in one study (147), the length of intervention was 12 months. In two studies, therapy was administered in one single session (112, 126), and in four trials, duration of treatment was not even specified. A significant recovery could not be expected during a 2-month treatment period, when patients are markedly ill and/or chronic with persistent and expressed negative symptoms of schizophrenia (129). This is supported by the observations of an increased effect over time of CBT on mental state (140). For instance, in the selected articles, a longer duration of treatment can generally show better results on negative symptoms. On the other hand, recent publications on the comparison between short- and long-term psychotherapy have shown contrasting results (167, 168). However, these works were referred to nonpsychotic patients. A short-term duration is insufficient for psychotic onset patients, who need to be treated longer, considering guidelines (169). Finally, according to our results, as reported in **Table 5**, group therapy should also be encouraged, as it is generally well supported by evidence in

TABLE 5 | Comparison between different group psychotherapies.

Author/type of study	Efficacy	Comparison between different group therapy	Type of therapy
Mandić-Gajić G (129) Case reports	Yes	No	Group art therapy
Jacobsen et al. (123) Uncontrolled study	Yes	No	Group Mindfulness
Penn et al. (135) RCT	Yes	Yes, improvement in ST at posttreatment and in both groups at follow-up	Group CBT, Group ST
Johnson et al. (134) RCT	Yes	Yes, improvement in both groups with no significant difference	Group CBT, Group ST
Barrowclough et al. (132) RCT	No	No	Group CBT
Wykes et al. (137) RCT	Yes	No	Group CBT
Pinkham et al. (136) Pilot study	Yes	No	Group CBT
Pilling et al. (138) Meta-analysis (part of the study including heterogeneous population: both TRP and not TRP)	No	No. No comparison has been made with single FI. Single FI became more efficient than group FI (not statistically significant)	Group FI (vs. Individual CBT)
Chadwick et al. (133) Uncontrolled study	Yes	No	Group CBT
Levine et al. (116) Controlled trial	Yes	No	Group CBT

improving persistent positive symptom in both CBT and other psychological interventions.

Exploratory Meta-Analysis of Cognitive Behavioral Therapy Interventions

The results obtained from our meta-analytical extraction have confirmed that cognitive–behavioral psychotherapy is very effective particularly in the treatment of positive symptoms in TRS and/or TRP patients. This result is in line with what has already been found in other studies in the literature. The same efficacy was not found in the treatment of negative symptoms while it was only partial in achieving an improvement in the total scores of patients evaluated in the PANSS. We have also found that CBT in augmentation with the usual treatment (TAU) works well in the initial stages and then gradually loses effectiveness (170). In this regard, we can hypothesize that schizophrenia worsens over time, making treatment with CBT more difficult and therefore less effective; moreover, it could happen that, in the initial stages of treatment, there is a sort of "feeling of well-being" that does not necessarily coincide with a real clinical improvement. However, there are very few studies with a sufficiently long follow-up to clarify these hypotheses. As regards the low incisiveness of CBT on negative symptoms, we can hypothesize that patients with more pronounced negative symptoms and therefore with affective dullness and social withdrawal are less suitable for this type of psychotherapeutic approach or that these symptoms require a longer duration of treatment to be effectively affected. Moreover, given that the few studies in the literature with a longer follow-up have shown an efficacy also on the negative symptoms, we can hypothesize that the patients followed for a longer period may have benefited from therapeutic adjustments over time as well as from the CBT. The limits of these results are in some way superimposable to those already listed above about the systematic review on the same topic. In addition to what has already been said, the incompleteness and the partiality of the data at our disposal are worth noting, as, for example, not all the articles indicated the dropout rates accurately, or at what time of the treatment they occurred, or which group they belonged to (cases or controls).

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Psychotherapy should be considered a potential relevant therapeutic strategy in adjunction to medication in TRP patients. An intervention on psychosis that does not consider an integrative approach could miss a potential effective component of the treatment. However, few questions need to be addressed in the future in order to better understand the role of psychotherapy in TRP. Firstly, it would be appropriate to start with large-scale multicenter, controlled studies based on psychotherapeutic approaches (i.e., CBT) that were shown to be effective in smaller studies and to include patients with homogeneous domains of symptoms, duration and doses of antipsychotic treatment, as well as duration of illness. Secondly, a longer time of treatment should be conceived in such studies in order to get an adequate signal of the response. Finally, even if challenging, an important issue is to consider the inclusion of biological markers (i.e., functional imaging) before and after the introduction of the psychotherapeutic augmentation or of the substitution psychotherapy. Moreover, future studies need to adopt reliable operational outcome measures for non-CBT studies to allow quantitative extraction of information and reliable comparison of efficacy measures for psychological interventions other than cognitive therapy that are currently almost invariably not assessed in a controlled, RCT fashion.

AUTHOR CONTRIBUTIONS

DP designed the study, searched the database, wrote the article, and created the appendices, tables, and figures. MP searched the database and participated in the editing of the manuscript. MF and VD supervised the literature procedure extraction, commented on the last draft, and contributed to the writing of the manuscript. AdB wrote and commented the manuscript, as well as supervised all work including the design of the study and the final draft. All authors have read and approved the final version of the manuscript.

FUNDING

The Open Access has been supported by a grant of the Department of Neuroscience, Reproductive Science and Odontostomatology of the University of Naples "Federico II" to the Section of Psychiatry (AdB and FM).

ACKNOWLEDGEMENTS

DP would like to thank Alessandro Mazzetta MD, Vincenzo Polese MD, Georgios D. Kotzalidis MD PhD, and Professor Paolo Girardi MD for their support in the development of the study and critical discussion.

REFERENCES

1. Jones PB, Barnes TR, Davies L, Dunn G, Lloyd H, Hayhurst KP, et al. Randomized controlled trial of the effect on Quality of Life of second-vs first-generation antipsychotic drugs in schizophrenia: Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS 1). *Arch Gen Psychiatry* (2006) 63(10):1079–87. doi: 10.1001/archpsyc.63.10.1079
2. Howes OD, McCutcheon R, Owen MJ, Murray RM. The role of genes, stress, and dopamine in the development of schizophrenia. *Biol Psychiatry* (2017) 81(1):9–20. doi: 10.1016/j.biopsych.2016.07.014
3. Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Eng J Med* (2005) 353(12):1209–23. doi: 10.1056/NEJMoa051688
4. Elkis H. Treatment-resistant schizophrenia. *Psychiatr Clin North Am* (2007) 30(3):511–33. doi: 10.1016/j.psc.2007.04.001
5. Fowler D, Garety P, Kuipers E. *Cognitive behaviour therapy for psychosis: theory and practice*. Chichester, UK: Wiley (1995).
6. Miller A, McEvoy J, Jeste D, Marder S. Treatment of chronic schizophrenia. In: *Textbook of schizophrenia*. Washington DC: The American Psychiatric Publishing (2006). p. 365–81.
7. Miyamoto S, Jarskog LF, Fleischhacker WW. New therapeutic approaches for treatment-resistant schizophrenia: a look to the future. *J Psychiatr Res* (2014) 58:1–6. doi: 10.1016/j.jpsychires.2014.07.001
8. Tarrier N. An investigation of residual psychotic symptoms in discharged schizophrenic patients. *Br J Clin Psychol* (1987) 26(2):141–3. doi: 10.1111/j.2044-8260.1987.tb00740.x
9. Andreasen NC, Arndt S, Alliger R, Miller D, Flaum M. Symptoms of schizophrenia: methods, meanings, and mechanisms. *Arch Gen Psychiatry* (1995) 52(5):341–51. doi: 10.1001/archpsyc.1995.03950170015003
10. Kennedy JL, Altar CA, Taylor DL, Degtiar I, Hornberger JC. The social and economic burden of treatment-resistant schizophrenia: a systematic literature review. *Int Clin Psychopharmacol* (2014) 29(2):63–76. doi: 10.1097/YIC.0b013e32836508e6
11. de Bartolomeis A, Buonaguro EF, Latte G, Rossi R, Marmo F, Iasevoli F, et al. Immediate-early genes modulation by antipsychotics: translational implications for a putative gateway to drug-induced long-term brain changes. *Front Behav Neurosci* (2017) 11:240. doi: 10.3389/fnbeh.2017.00240
12. Rund BR, Barder HE, Evensen J, Haahr U, Hegelstad WtV, Joa I, et al. Neurocognition and duration of psychosis: a 10-year follow-up of first-episode patients. *Schizophr Bull* (2015) 42(1):87–95. doi: 10.1192/bjp.bp.117.201475
13. Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic: a double- blind comparison with chlorpromazine. *Arch Gen Psychiatry* (1988) 45(9):789–96. doi: 10.1001/archpsyc.1988.01800330013001
14. Elkis H, Buckley PF. Treatment-resistant schizophrenia. *Psychiatr Clin* (2016) 39(2):239–65. doi: 10.1016/j.psc.2007.04.001
15. Ajnakina O, Horsdal HT, Lally J, MacCabe JH, Murray RM, Gasse C, et al. Validation of an algorithm-based definition of treatment resistance in patients with schizophrenia. *Schizophr Res* (2018) 197:294–7. doi: 10.1016/j.schres.2018.02.017
16. Leucht S, Arbter D, Engel R, Kissling W, Davis J. How effective are second-generation antipsychotic drugs? A meta-analysis of placebo-controlled trials. *Mol Psychiatry* (2009) 14(4):429. doi: 10.1038/sj.mp.4002136
17. Remington G, Addington D, Honer W, Ismail Z, Raedler T, Teehan M. Guidelines for the pharmacotherapy of schizophrenia in adults. *Can J Psychiatry* (2017) 62(9):604–16. doi: 10.1177/0706743717720448
18. Garety PA, Fowler D, Kuipers E. Cognitive-behavioral therapy for medication-resistant symptoms. *Schizophr Bull* (2000) 26(1):73–86. doi: 10.1093/oxfordjournals.schbul.a033447
19. Silverstein SM, Bellack AS. A scientific agenda for the concept of recovery as it applies to schizophrenia. *Clin Psychol Rev* (2008) 28(7):1108–24. doi: 10.1016/j.cpr.2008.03.004
20. Davidson L, Stayner D, Haglund KE. Phenomenological perspectives on the social functioning of people with schizophrenia. In KT Mueser, N Tarrier (Eds.), *Handbook of Social Functioning in Schizophrenia*. Needham Heights, MA, US: Allyn & Bacon (1998) p. 97–120.
21. Iasevoli F, Giordano S, Balletta R, Latte G, Formato MV, Prinzivalli E, et al. Treatment resistant schizophrenia is associated with the worst community functioning among severely-ill highly-disabling psychiatric conditions and is the most relevant predictor of poorer achievements in functional milestones. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 65:34–48. doi: 10.1016/j.pnpbp.2015.08.010
22. de Bartolomeis A, Balletta R, Giordano S, Buonaguro EF, Latte G, Iasevoli F. Differential cognitive performances between schizophrenic responders and non-responders to antipsychotics: correlation with course of the illness, psychopathology, attitude to the treatment and antipsychotics doses. *Psychiatry Res* (2013) 210(2):387–95. doi: 10.1016/j.psychres.2013.06.042
23. Manchanda R, Norman RM, Malla AK, Harricharan R, Northcott S. Persistent psychoses in first episode patients. *Schizophr Res* (2005) 80(1):113–6. doi: 10.1016/j.schres.2005.08.005
24. Wiersma D, Nienhuis FJ, Slooff CJ, Giel R. Natural course of schizophrenic disorders: a 15-year followup of a Dutch incidence cohort. *Schizophr Bull* (1998) 24(1):75–85. doi: 10.1093/oxfordjournals.schbul.a033315
25. Meltzer H, Kostacoglu A. Treatment-resistant schizophrenia. In: *Comprehensive care of schizophrenia: a textbook of clinical management*. London: Martin Dunitz (2001). p. 181–203.
26. Lally J, Ajnakina O, Di Forti M, Trotta A, Demjaha A, Kolliakou A, et al. Two distinct patterns of treatment resistance: clinical predictors of treatment resistance in first-episode schizophrenia spectrum psychoses. *Psychol Med* (2016) 46(15):3231–40. doi: 10.1017/S0033291716002014
27. Lee J, Takeuchi H, Fervaha G, Sin GL, Foussias G, Agid O, et al. Subtyping schizophrenia by treatment response: antipsychotic development and the central role of positive symptoms. *Focus* (2016) 14(3):396–402. doi: 10.1176/appi.focus.140306
28. Sheitman B, Lieberman J. The natural history and pathophysiology of treatment resistant schizophrenia. *J Psychiatr Res* (1998) 32(3–4):143–50. doi: 10.1016/S0022-3956(97)00052-6
29. de Bartolomeis A, Sarappa C, Magara S, Iasevoli F. Targeting glutamate system for novel antipsychotic approaches: relevance for residual psychotic symptoms and treatment resistant schizophrenia. *Eur J Pharmacol* (2012) 682(1–3):1–11. doi: 10.1016/j.ejphar.2012.02.033
30. Demjaha A, Egerton A, Murray RM, Kapur S, Howes OD, Stone JM, et al. Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function. *Biol Psychiatry* (2014) 75(5):e11–e3. doi: 10.1016/j.biopsych.2013.06.011
31. Buchanan RW. Clozapine: efficacy and safety. *Schizophr Bull* (1995) 21(4):579–91. doi: 10.1093/schbul/21.4.579
32. Kane JM, Correll CU. Pharmacologic treatment of schizophrenia. *Dialogues Clin Neurosci* (2010) 12(3):345–57.
33. Young CR, Longhurst JG, Bowers MB, Jr., Mazure CM. The expanding indications for clozapine. *Exp Clin Psychopharmacol* (1997) 5(3):216. doi: 10.1037/1064-1297.5.3.216
34. Lewis S, Davies L, Jones P, Barnes T, Murray R, Kerwin R, et al. Randomised controlled trials of conventional antipsychotic versus new atypical drugs,

and new atypical drugs versus clozapine, in people with schizophrenia responding poorly to, or intolerant of, current drug treatment. *Health Technol Assess* (2006) 10(17):iii–iv, ix–xi, 1–165.
35. Kane JM, Correll CU. The role of clozapine in treatment-resistant schizophrenia. *JAMA Psychiatry* (2016) 73(3):187–8. doi: 10.1001/jamapsychiatry.2015.2966
36. Samara MT, Dold M, Gianatsi M, Nikolakopoulou A, Helfer B, Salanti G, et al. Efficacy, acceptability, and tolerability of antipsychotics in treatment-resistant schizophrenia: a network meta-analysis. *JAMA Psychiatry* (2016) 73(3):199–210. doi: 10.1001/jamapsychiatry.2015.2955
37. Bruno A, Zoccali RA, Abenavoli E, Pandolfo G, Scimeca G, Spina E, et al. Augmentation of clozapine with agomelatine in partial-responder schizophrenia: a 16-week, open-label, uncontrolled pilot study. *J Clin Psychopharmacol* (2014) 34(4):491–4. doi: 10.1097/JCP.0000000000000157
38. Bruno A, Pandolfo G, Romeo VM, Mallamace D, D'arrigo C, Spina E, et al. Duloxetine as adjunctive treatment to clozapine in patients with schizophrenia: a randomized, placebo-controlled trial. *Int Clin Psychopharmacol* (2011) 26(6):303–10. doi: 10.1097/YIC.0b013e32834bbc0d
39. Muscatello MRA, Bruno A, De Fazio P, Segura-Garcia C, Pandolfo G, Zoccali R. Augmentation strategies in partial responder and/or treatment-resistant schizophrenia patients treated with clozapine. *Expert Opin Pharmacother* (2014) 15(16):2329–45. doi: 10.1517/14656566.2014.956082
40. Buckley P, Miller A, Olsen J, Garver D, Miller DD, Csernansky J. When symptoms persist: clozapine augmentation strategies. *Schizophr Bull* (2001) 27(4):615–28. doi: 10.1093/oxfordjournals.schbul.a006901
41. Zink M. Augmentation of olanzapine in treatment-resistant schizophrenia. *J Psychiatry Neurosci* (2005) 30(6):409–15.
42. Lindenmayer J-P. Treatment refractory schizophrenia. *Psychiatr Quart* (2000) 71(4):373–84. doi: 10.1023/A:1004640408501
43. Addington J, Van Mastrigt S, Addington D. Duration of untreated psychosis: impact on 2-year outcome. *Psychol Med* (2004) 34(2):277–84. doi: 10.1017/S0033291703001156
44. Barnes TR, Leeson VC, Mutsatsa SH, Watt HC, Hutton SB, Joyce EM. Duration of untreated psychosis and social function: 1-year follow-up study of first-episode schizophrenia. *Br J Psychiatry* (2008) 193(3):203–9. doi: 10.1192/bjp.bp.108.049718
45. Carbon M, Correll CU. Clinical predictors of therapeutic response to antipsychotics in schizophrenia. *Dialogues Clin Neurosci* (2014) 16(4):505–24.
46. Larsen TK, McGlashan TH, Moe LC. First-episode schizophrenia: I. Early course parameters. *Schizophr Bull* (1996) 22(2):241–56. doi: 10.1093/schbul/22.2.241
47. Meltzer HY, Rabinowitz J, Lee MA, Cola PA, Ranjan R, Findling RL, et al. Age at onset and gender of schizophrenic patients in relation to neuroleptic resistance. *Am J Psychiatry* (1997) 154(4):475–82. doi: 10.1176/ajp.154.4.475
48. Singh SP, Merino C. Treatment of first-episode and prodromal signs. *Psychiatry* (2008) 7(11):467–71. doi: 10.1016/j.mppsy.2008.10.001
49. Caspi A, Davidson M, Tamminga CA. Treatment-refractory schizophrenia. *Dialogues Clin Neurosci* (2004) 6(1):61–70.
50. Johnstone E, Owens D, Bydder G, Colter N, Crow T, Frith C. The spectrum of structural brain changes in schizophrenia: age of onset as a predictor of cognitive and clinical impairments and their cerebral correlates. *Psychol Med* (1989) 19(1):91–103. doi: 10.1017/S0033291700011053
51. Kolakowska T, Williams A, Ardern M, Reveley M, Jambor K, Gelder M, et al. Schizophrenia with good and poor outcome. I: early clinical features, response to neuroleptics and signs of organic dysfunction. *Br J Psychiatry* (1985) 146(3):229–39. doi: 10.1192/bjp.146.3.229
52. Nakajima S, Takeuchi H, Plitman E, Fervaha G, Gerretsen P, Caravaggio F, et al. Neuroimaging findings in treatment-resistant schizophrenia: a systematic review: lack of neuroimaging correlates of treatment-resistant schizophrenia. *Schizophr Res* (2015) 164(1–3):164–75. doi: 10.1016/j.schres.2015.01.043
53. Quarantelli M, Palladino O, Prinster A, Schiavone V, Carotenuto B, Brunetti A, et al. Patients with poor response to antipsychotics have a more severe pattern of frontal atrophy: a voxel-based morphometry study of treatment resistance in schizophrenia. *BioMed Res Int* (2014) 2014:1–9. doi: 10.1155/2014/325052
54. Rosenbaum B, Harder S, Knudsen P, Køster A, Lindhardt A, Lajer M, et al. Supportive psychodynamic psychotherapy versus treatment as usual for first-episode psychosis: two-year outcome. *Psychiatry* (2012) 75(4):331–41. doi: 10.1521/psyc.2012.75.4.331
55. Fonagy P. The effectiveness of psychodynamic psychotherapies: an update. *World Psychiatry* (2015) 14(2):137–50. doi: 10.1002/wps.20235
56. Budd R, Hughes I. The Dodo Bird Verdict—controversial, inevitable and important: a commentary on 30 years of meta-analyses. *Clin Psychol Psychother* (2009) 16(6):510–22. doi: 10.1002/cpp.648
57. Gottdiener WH, Haslam N. The benefits of individual psychotherapy for people diagnosed with schizophrenia: a meta-analytic review. *Ethical Hum Sci Serv* (2002) 4(3):163–87. doi: 10.1891/1523-150X.4.3.163
58. Malmberg L, Fenton M, Rathbone J. Individual psychodynamic psychotherapy and psychoanalysis for schizophrenia and severe mental illness. *Cochrane Database Syst Rev* (2001) 3:CD001360. doi: 10.1002/14651858.CD001360
59. Paley G, Shapiro DA. Lessons from psychotherapy research for psychological interventions for people with schizophrenia. *Psychol Psychother Theor Res Pract* (2002) 75(1):5–17. doi: 10.1348/147608302169517
60. Castelnuovo G. Empirically supported treatments in psychotherapy: towards an evidence-based or evidence-biased psychology in clinical settings? *Front Psychol* (2010) 1:27. doi: 10.3389/fpsyg.2010.00027
61. Kazdin AE. Evidence-based treatment and usual care: cautions and qualifications. *JAMA Psychiatry* (2013) 70(7):666–7. doi: 10.1001/jamapsychiatry.2013.2112
62. Weisz JR, Kuppens S, Eckshtain D, Ugueto AM, Hawley KM, Jensen-Doss A. Performance of evidence- based youth psychotherapies compared with usual clinical care: a multilevel meta-analysis. *JAMA Psychiatry* (2013) 70(7):750–61. doi: 10.1001/jamapsychiatry.2013.1176
63. Bendall S, Killackey E, Jackson H, Gleeson J. *Befriending manual*. Melbourne: ORYGEN Research Centre, University of Melbourne (2003).
64. Bendall S, Jackson HJ, Killackey E, Allott K, Johnson T, Harrigan S, et al. The credibility and acceptability of befriending as a control therapy in a randomized controlled trial of cognitive behaviour therapy for acute first episode psychosis. *Behav Cogn Psychother* (2006) 34(3):277–91. doi: 10.1017/S1352465806002815
65. Beck AT. Successful outpatient psychotherapy of a chronic schizophrenic with a delusion based on borrowed guilt. *Psychiatry* (1952) 15(3):305–12. doi: 10.1080/00332747.1952.11022883
66. Shapiro MB, Ravenette A. A preliminary experiment on paranoid delusions. *J Ment Science* (1959) 105(439):295–312. doi: 10.1192/bjp.105.439.295
67. Health NCCfM. *Schizophrenia: core interventions in the treatment and management of schizophrenia in primary and secondary care (update)*. Leicester, UK: British Psychological Society (2009).
68. Grant N, Lawrence M, Preti A, Wykes T, Cella M. Social cognition interventions for people with schizophrenia: a systematic review focussing on methodological quality and intervention modality. *Clin Psychol Rev* (2017) 56:55–64. doi: 10.1016/j.cpr.2017.06.001
69. Hazell CM, Hayward M, Cavanagh K, Strauss C. A systematic review and meta-analysis of low intensity CBT for psychosis. *Clin Psychol Rev* (2016) 45:183–92. doi: 10.1016/j.cpr.2016.03.004
70. Tarrier N, Haddock G, Barrowclough C, Wykes T. Are all psychological treatments for psychosis equal? The need for CBT in the treatment of psychosis and not for psychodynamic psychotherapy. *Psychol Psychother Theor Res Pract* (2002) 75(4):365–74. doi: 10.1348/147608302321151871
71. Mueser KT, Berenbaum H. Psychodynamic treatment of schizophrenia: is there a future? *Psychol Med* (1990) 20(2):253–62. doi: 10.1017/S003329170001758X
72. Cicchetti D, Doyle C. Child maltreatment, attachment and psychopathology: mediating relations. *World Psychiatry* (2016) 15(2):89–90. doi: 10.1002/wps.20337
73. Cristofaro SL, Cleary SD, Wan CR, Broussard B, Chapman C, Haggard PJ, et al. Measuring trauma and stressful events in childhood and adolescence among patients with first-episode psychosis: initial factor structure, reliability, and validity of the Trauma Experiences Checklist. *Psychiatry Res* (2013) 210(2):618–25. doi: 10.1016/j.psychres.2013.06.015
74. Egerton A, Valmaggia LR, Howes OD, Day F, Chaddock CA, Allen P, et al. Adversity in childhood linked to elevated striatal dopamine function in adulthood. *Schizophr Res* (2016) 176(2–3):171–6. doi: 10.1016/j.schres.2016.06.005

75. Fisher HL, Jones PB, Fearon P, Craig TK, Dazzan P, Morgan K, et al. The varying impact of type, timing and frequency of exposure to childhood adversity on its association with adult psychotic disorder. *Psychol Med* (2010) 40(12):1967–78. doi: 10.1017/S0033291710000231
76. Janssen I, Krabbendam L, Bak M, Hanssen M, Vollebergh W, de Graaf R, et al. Childhood abuse as a risk factor for psychotic experiences. *Acta Psychiatr Scand* (2004) 109(1):38–45. doi: 10.1046/j.0001-690X.2003.00217.x
77. Morgan C, Gayer-Anderson C. Childhood adversities and psychosis: evidence, challenges, implications. *World Psychiatry* (2016) 15(2):93–102. doi: 10.1002/wps.20330
78. Murray RM, Mehta M, Di Forti M. Different dopaminergic abnormalities underlie cannabis dependence and cannabis-induced psychosis. *Biol Psychiatry* (2014) 75(6):430–1. doi: 10.1016/j.biopsych.2014.01.011
79. Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W, et al. Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective-and cross-sectional cohort studies. *Schizophr Bull* (2012) 38(4):661–71. doi: 10.1093/schbul/sbs050
80. Galatzer-Levy IR, Huang SH, Bonanno GA. Trajectories of resilience and dysfunction following potential trauma: a review and statistical evaluation. *Clin Psychol Rev* (2018) 63:41–55. doi: 10.1016/j.cpr.2018.05.008
81. Maccari S, Polese D, Reynaert M-L, Amici T, Morley-Fletcher S, Fagioli F. Early-life experiences and the development of adult diseases with a focus on mental illness: the human birth theory. *Neuroscience* (2017) 342:232–51. doi: 10.1016/j.neuroscience.2016.05.042
82. Priebe S, McCabe R. The therapeutic relationship in psychiatric settings. *Acta Psychiatr Scand* (2006) 113:69–72. doi: 10.1111/j.1600-0447.2005.00721.x
83. Priebe S, Mccabe R. Therapeutic relationships in psychiatry: the basis of therapy or therapy in itself? *Int Rev Psychiatry* (2008) 20(6):521–6. doi: 10.1080/09540260802565257
84. Priebe S, Richardson M, Cooney M, Adedeji O, McCabe R. Does the therapeutic relationship predict outcomes of psychiatric treatment in patients with psychosis? A systematic review. *Psychother Psychosom* (2011) 80(2):70–7. doi: 10.1159/000320976
85. Harper Romeo K, Meyer PS, Johnson D, Penn DL. An investigation of the relationship between therapist characteristics and alliance in group therapy for individuals with treatment-resistant auditory hallucinations. *J Ment Health* (2014) 23(4):166–70. doi: 10.3109/09638237.2013.869568
86. Calton T, Ferriter M, Huband N, Spandler H. A systematic review of the Soteria paradigm for the treatment of people diagnosed with schizophrenia. *Schizophr Bull* (2007) 34(1):181–92. doi: 10.1093/schbul/sbm047
87. Ganasen K, Ipser J, Stein D. Augmentation of cognitive behavioral therapy with pharmacotherapy. *Psychiatr Clin North Am* (2010) 33(3):687–99. doi: 10.1016/j.psc.2010.04.008.
88. Lauriello J, Lenroot R, Bustillo JR. Maximizing the synergy between pharmacotherapy and psychosocial therapies for schizophrenia. *Psychiatr Clin North Am* (2003) 26(1):191–211. doi: 10.1016/S0193-953X(02)00017-5
89. Rathod S, Kingdon D, Weiden P, Turkington D. Cognitive-behavioral therapy for medication-resistant schizophrenia: a review. *J Psychiatr Pract* (2008) 14(1):22–33. doi: 10.1097/01.pra.0000308492.93003.db
90. Plakun E. Treatment resistance and psychodynamic psychiatry: concepts psychiatry needs from psychoanalysis. *Psychodyn Psychiatry* (2012) 40(2):183–209. doi: 10.1521/pdps.2012.40.2.183
91. Harder S, Koester A, Valbak K, Rosenbaum B. Five-year follow-up of supportive psychodynamic psychotherapy in first-episode psychosis: long-term outcome in social functioning. *Psychiatry* (2014) 77(2):155–68. doi: 10.1521/psyc.2014.77.2.155
92. Craig TK, Garety P, Power P, Rahaman N, Colbert S, Fornells-Ambrojo M, et al. The Lambeth Early Onset (LEO) Team: randomised controlled trial of the effectiveness of specialised care for early psychosis. *Bmj* (2004) 329(7474):1067. doi: 10.1136/bmj.38246.594873.7C
93. Birchwood M, Michail M, Meaden A, Tarrier N, Lewis S, Wykes T, et al. Cognitive behaviour therapy to prevent harmful compliance with command hallucinations (COMMAND): a randomised controlled trial. *Lancet Psychiatry* (2014) 1(1):23–33. doi: 10.1016/S2215-0366(14)70247-0
94. Haddock G, Tarrier N, Morrison A, Hopkins R, Drake R, Lewis S. A pilot study evaluating the effectiveness of individual inpatient cognitive-behavioural therapy in early psychosis. *Soc Psychiatry Psychiatr Epidemiol* (1999) 34(5):254–8. doi: 10.1007/s001270050141
95. Lewis S, Tarrier N, Haddock G, Bentall R, Kinderman P, Kingdon D, et al. Randomised controlled trial of cognitive-behavioural therapy in early schizophrenia: acute-phase outcomes. *Br J Psychiatry* (2002) 181(S43):s91–s7. doi: 10.1192/bjp.181.43.s91
96. Remington G, Kapur S, Zipursky RB. Pharmacotherapy of first-episode schizophrenia. *Br J Psychiatry* (1998) 172(S33):66–70. doi: 10.1192/S0007125000297687
97. Dorph-Petersen K-A, Pierri JN, Perel JM, Sun Z, Sampson AR, Lewis DA. The influence of chronic exposure to antipsychotic medications on brain size before and after tissue fixation: a comparison of haloperidol and olanzapine in macaque monkeys. *Neuropsychopharmacology* (2005) 30(9):1649. doi: 10.1038/sj.npp.1300710
98. Torres US, Duran FL, Schaufelberger MS, Crippa JA, Louzã MR, Sallet PC, et al. Patterns of regional gray matter loss at different stages of schizophrenia: a multisite, cross-sectional VBM study in first-episode and chronic illness. *Neuroimage Clin* (2016) 12:1–15. doi: 10.1016/j.nicl.2016.06.002
99. Vita A, De Peri L, Deste G, Barlati S, Sacchetti E. The effect of antipsychotic treatment on cortical gray matter changes in schizophrenia: does the class matter? A meta-analysis and meta-regression of longitudinal magnetic resonance imaging studies. *Biol Psychiatry* (2015) 78(6):403–12. doi: 10.1016/j.biopsych.2015.02.008
100. Chouinard G, Samaha A-N, Chouinard V-A, Peretti C-S, Kanahara N, Takase M, et al. Antipsychotic- induced dopamine supersensitivity psychosis: pharmacology, criteria, and therapy. *Psychother Psychosom* (2017) 86(4):189–219. doi: 10.1159/000477313
101. Chouinard G, Chouinard V-A. Atypical antipsychotics: CATIE study, drug-induced movement disorder and resulting iatrogenic psychiatric-like symptoms, supersensitivity rebound psychosis and withdrawal discontinuation syndromes. *Psychother Psychosom* (2008) 77(2):69–77. doi: 10.1159/000112883
102. Samaha A-N, Seeman P, Stewart J, Rajabi H, Kapur S. "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. *J Neurosci* (2007) 27(11):2979–86. doi: 10.1523/JNEUROSCI.5416-06.2007
103. Edwards J, Maude D, Herrmann-Doig T, Wong L, Cocks J, Burnett P, et al. Rehab rounds: a service response to prolonged recovery in early psychosis. *Psychiatr Serv* (2002) 53(9):1067–9. doi: 10.1176/appi.ps.53.9.1067
104. Morrison AP, French P, Walford L, Lewis SW, Kilcommons A, Green J, et al. Cognitive therapy for the prevention of psychosis in people at ultra-high risk: randomised controlled trial. *Br J Psychiatry* (2004) 185(4):291–7. doi: 10.1192/bjp.185.4.291
105. Kaur T, Cadenhead KS. Treatment implications of the schizophrenia prodrome. In: *Behavioral Neurobiology of Schizophrenia and Its Treatment*. Berlin Heidelberg, Germany: Springer (2010). p. 97–121. doi: 10.1007/7854_2010_56
106. McGorry PD, Nelson B, Amminger GP, Bechdolf A, Francey SM, Berger G, et al. Intervention in individuals at ultra-high risk for psychosis: a review and future directions. *J Clin Psychiatry* (2009) 70(9):1206–12. doi: 10.4088/JCP.08r04472
107. McGorry PD, Hickie IB, Yung AR, Pantelis C, Jackson HJ. Clinical staging of psychiatric disorders: a heuristic framework for choosing earlier, safer and more effective interventions. *Austr N Z J psychiatry* (2006) 40(8):616–22. doi: 10.1080/j.1440-1614.2006.01860.x
108. National Collaborating Centre for Mental Health (UK). Schizophrenia: core interventions in the treatment and management of schizophrenia in primary and secondary care (Update). Leicester, UK: British Psychological Society (2009). Available from http://www.ncbi.nlm.nih.gov/books/NBK11681/PubMed.
109. Lehman AF, Kreyenbuhl J, Buchanan RW, Dickerson FB, Dixon LB, Goldberg R, et al. The schizophrenia patient outcomes research team (PORT): updated treatment recommendations 2003. *Schizophr Bull* (2004) 30(2):193–217. doi: 10.1093/oxfordjournals.schbul.a007071
110. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* (2009) 6(7):e1000097. doi: 10.1371/journal.pmed.1000097
111. McGauran N, Wieseler B, Kreis J, Schuler YB, Kolsch H, Kaiser T. Reporting bias in medical research - a narrative review. *Trials* (2010) 11:37. doi: 10.1186/1745-6215-11-37

112. Ross K, Freeman D, Dunn G, Garety P. A randomized experimental investigation of reasoning training for people with delusions. *Schizophr Bull* (2009) 37(2):324–33. doi: 10.1093/schbul/sbn165
113. Cather C, Penn D, Otto MW, Yovel I, Mueser KT, Goff DC. A pilot study of functional Cognitive Behavioral Therapy (fCBT) for schizophrenia. *Schizophr Res* (2005) 74(2–3):201–9. doi: 10.1016/j.schres.2004.05.002
114. Randal P, Simpson AI, Laidlaw T. Can recovery-focused multimodal psychotherapy facilitate symptom and function improvement in people with treatment-resistant psychotic illness? A comparison study. *Austr N Z J psychiatry* (2003) 37(6):720–7. doi: 10.1080/j.1440-1614.2003.01261.x
115. Durham RC, Guthrie M, Morton RV, Reid DA, Treliving LR, Fowler D, et al. Tayside–Fife clinical trial of cognitive–behavioural therapy for medication-resistant psychotic symptoms: results to 3-month follow-up. *Br J Psychiatry* (2003) 182(4):303–11. doi: 10.1192/bjp.182.4.303
116. Levine J, Barak Y, Granek I. Cognitive group therapy for paranoid schizophrenics: applying cognitive dissonance. *J Cogn Psychother* (1998) 12(1):3. doi: 10.1891/0889-8391.12.1.3
117. Garety P, Kuipers L, Fowler D, Chamberlain F, Dunn G. Cognitive behavioural therapy for drug-resistant psychosis. *Br J Med Psychol* (1994) 67(3):259–71. doi: 10.1111/j.2044-8341.1994.tb01795.x
118. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. (2013). [Accessed 2019, April 8, on http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp].
119. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* (1996) 17(1):1–12. doi: 10.1016/0197-2456(95)00134-4
120. Pinto A, Pia SL, Mennella R, Giorgio D, DeSimone L. Rehab rounds: cognitive-behavioral therapy and clozapine for clients with treatment-refractory schizophrenia. *Psychiatr Serv* (1999) 50(7):901–4. doi: 10.1176/ps.50.7.901
121. Valmaggia LR, Van Der Gaag M, Tarrier N, Pijnenborg M, Slooff CJ. Cognitive–behavioural therapy for refractory psychotic symptoms of schizophrenia resistant to atypical antipsychotic medication: randomised controlled trial. *Br J Psychiatry* (2005) 186(4):324–30. doi: 10.1192/bjp.186.4.324
122. Shawyer F, Farhall J, Mackinnon A, Trauer T, Sims E, Ratcliff K, et al. A randomised controlled trial of acceptance-based cognitive behavioural therapy for command hallucinations in psychotic disorders. *Behav Res Ther* (2012) 50(2):110–21. doi: 10.1016/j.brat.2011.11.007
123. Jacobsen P, Morris E, Johns L, Hodkinson K. Mindfulness groups for psychosis; key issues for implementation on an inpatient unit. *Behav Cogn Psychother* (2011) 39(3):349–53. doi: 10.1017/S1352465810000639
124. Temple S, Ho B-C. Cognitive therapy for persistent psychosis in schizophrenia: a case-controlled clinical trial. *Schizophr Res* (2005) 74(2–3):195–9. doi: 10.1016/j.schres.2004.05.013
125. Rector NA, Seeman MV, Segal ZV. Cognitive therapy for schizophrenia: a preliminary randomized controlled trial. *Schizophr Res* (2003) 63(1–2):1–11. doi: 10.1016/S0920-9964(02)00308-0
126. Waller H, Freeman D, Jolley S, Dunn G, Garety P. Targeting reasoning biases in delusions: a pilot study of the Maudsley Review Training Programme for individuals with persistent, high conviction delusions. *J Behav Ther Exp Psychiatry* (2011) 42(3):414–21. doi: 10.1016/j.jbtep.2011.03.001
127. Hutton P, Morrison AP, Wardle M, Wells A. Metacognitive therapy in treatment-resistant psychosis: a multiple-baseline study. *Behav Cogn Psychother* (2014) 42(2):166–85. doi: 10.1017/S1352465812001026
128. Trower P, Birchwood M, Meaden A, Byrne S, Nelson A, Ross K. Cognitive therapy for command hallucinations: randomised controlled trial. *Br J Psychiatry* (2004) 184(4):312–20. doi: 10.1192/bjp.184.4.312
129. Mandić-Gajić G. Group art therapy as adjunct therapy for the treatment of schizophrenic patients in day hospital. *Vojnosanitet Pregl* (2013) 70(11):1065–9. doi: 10.2298/VSP1311065M
130. Buchain PC, Vizzotto ADB, Henna Neto J, Elkis H. Randomized controlled trial of occupational therapy in patients with treatment-resistant schizophrenia. *Rev Bras Psiquiatr* (2003) 25(1):26–30. doi: 10.1590/S1516-44462003000100006
131. Davenport S, Hobson R, Margison F. Treatment development in psychodynamic interpersonal psychotherapy (Hobson's 'Conversational Model') for chronic treatment resistant schizophrenia: two single case studies. *Br J Psychother* (2000) 16(3):287–302. doi: 10.1111/j.1752-0118.2000.tb00520.x
132. Barrowclough C, Haddock G, Lobban F, Jones S, Siddle R, Roberts C, et al. Group cognitive-behavioural therapy for schizophrenia: randomised controlled trial. *Br J Psychiatry* (2006) 189(6):527–32. doi: 10.1192/bjp.bp.106.021386
133. Chadwick P, Sambrooke S, Rasch S, Davies E. Challenging the omnipotence of voices: group cognitive behavior therapy for voices. *Behav Res Ther* (2000) 38(10):993–1003. doi: 10.1016/S0005-7967(99)00126-6
134. Johnson DP, Penn DL, Bauer DJ, Meyer P, Evans E. Predictors of the therapeutic alliance in group therapy for individuals with treatment-resistant auditory hallucinations. *Br J Clin Psychol* (2008) 47(2):171–84. doi: 10.1348/014466507X241604
135. Penn DL, Meyer PS, Evans E, Wirth RJ, Cai K, Burchinal M. A randomized controlled trial of group cognitive-behavioral therapy vs. enhanced supportive therapy for auditory hallucinations. *Schizophr Res* (2009) 109(1–3):52–9. doi: 10.1016/j.schres.2008.12.009
136. Pinkham AE, Gloege AT, Flanagan S, Penn DL. Group cognitive-behavioral therapy for auditory hallucinations: a pilot study. *Cogn Behav Pract* (2004) 11(1):93–8. doi: 10.1016/S1077-7229(04)80011-7
137. Wykes T, Hayward P, Thomas N, Green N, Surguladze S, Fannon D, et al. What are the effects of group cognitive behaviour therapy for voices? A randomised control trial. *Schizophr Res* (2005) 77(2–3):201–10. doi: 10.1016/j.schres.2005.03.013
138. Pilling S, Bebbington P, Kuipers E, Garety P, Geddes J, Orbach G, et al. Psychological treatments in schizophrenia: I. Meta-analysis of family intervention and cognitive behaviour therapy. *Psychol Med* (2002) 32(5):763–82. doi: 10.1017/S0033291702005895
139. de Paiva Barretto EM, Kayo M, Avrichir BS, Sa AR, Camargo MDGM, Napolitano IC, et al. A preliminary controlled trial of cognitive behavioral therapy in clozapine-resistant schizophrenia. *J Nerv Ment Dis* (2009) 197(11):865–8. doi: 10.1097/NMD.0b013e3181be7422
140. Sensky T, Turkington D, Kingdon D, Scott JL, Scott J, Siddle R, et al. A randomized controlled trial of cognitive-behavioral therapy for persistent symptoms in schizophrenia resistant to medication. *Arch Gen Psychiatry* (2000) 57(2):165–72. doi: 10.1001/archpsyc.57.2.165
141. Brabban A, Tai S, Turkington D. Predictors of outcome in brief cognitive behavior therapy for schizophrenia. *Schizophr Bull* (2009) 35(5):859–64. doi: 10.1093/schbul/sbp065
142. Pilling S, Bebbington P, Kuipers E, Garety P, Geddes J, Martindale B, et al. Psychological treatments in schizophrenia: II. Meta-analyses of randomized controlled trials of social skills training and cognitive remediation. *Psychol Med* (2002) 32(5):783–91. doi: 10.1017/S0033291702005640
143. Peters E, Landau S, McCrone P, Cooke M, Fisher P, Steel C, et al. A randomised controlled trial of cognitive behaviour therapy for psychosis in a routine clinical service. *Acta Psychiatr Scand* (2010) 122(4):302–18. doi: 10.1111/j.1600-0447.2010.01572.x
144. Erickson DH. Cognitive-behaviour therapy for medication-resistant positive symptoms in early psychosis: a case series. *Early Interv Psychiatry* (2010) 4(3):251–6. doi: 10.1111/j.1751-7893.2010.00184.x
145. Burns AM, Erickson DH, Brenner CA. Cognitive-behavioral therapy for medication-resistant psychosis: a meta-analytic review. *Psychiatr Serv* (2014) 65(7):874–80. doi: 10.1176/appi.ps.201300213
146. Jones C, Hacker D, Cormac I, Meaden A, Irving CB. Cognitive behavioural therapy versus other psychosocial treatments for schizophrenia. *Cochrane Database Syst Rev* (2012) 4:CD008712. doi: 10.1002/14651858.CD008712.pub2
147. Wiersma D, Jenner J, Van de Willige G, Spakman M, Nienhuis F. Cognitive behaviour therapy with coping training for persistent auditory hallucinations in schizophrenia: a naturalistic follow-up study of the durability of effects. *Acta Psychiatr Scand* (2001) 103(5):393–9. doi: 10.1034/j.1600-0447.2001.00213.x
148. Kuipers E, Garety P, Fowler D, Dunn G, Bebbington P, Freeman D, et al. London–East Anglia randomised controlled trial of cognitive–behavioural

therapy for psychosis: I: Effects of the treatment phase. *Br J Psychiatry* (1997) 171(4):319–27. doi: 10.1192/bjp.171.4.319

149. Klingberg S, Herrlich J, Wiedemann G, Wölwer W, Meisner C, Engel C, et al. Adverse effects of cognitive behavioral therapy and cognitive remediation in schizophrenia: results of the treatment of negative symptoms study. *J Nerv Ment Dis* (2012) 200(7):569–76. doi: 10.1097/NMD.0b013e31825bfa1d
150. Klingberg S, Buchkremer G, Holle R, Mönking HS, Hornung WP. Differential therapy effects of psychoeducational psychotherapy for schizophrenic patients–results of a 2-year follow-up. *Eur Arch Psychiatry Clin Neurosci* (1999) 249(2):66–72. doi: 10.1007/s004060050068
151. Tarrier N, Yusupoff L, Kinney C, McCarthy E, Gledhill A, Haddock G, et al. Randomised controlled trial of intensive cognitive behaviour therapy for patients with chronic schizophrenia. *Bmj* (1998) 317(7154):303–7. doi: 10.1136/bmj.317.7154.303
152. Tarrier N, Witttkowskj A, Kinney C, McCarthy E, Morris J, Humphreys L. Durability of the effects of cognitive–behavioural therapy in the treatment of chronic schizophrenia: 12-month follow-up. *Br J Psychiatry* (1999) 174(6):500–4. doi: 10.1192/bjp.174.6.500
153. Tarrier N, Kinney C, McCarthy E, Humphreys L, Wittkowski A, Morris J. Two–year follow–up of cognitive–behavioral therapy and supportive counseling in the treatment of persistent symptoms in chronic schizophrenia. *J Consult Clin Psychol* (2000) 68(5):917. doi: 10.1037/0022-006X.68.5.917
154. Lutgens D, Gariepy G, Malla A. Psychological and psychosocial interventions for negative symptoms in psychosis: systematic review and meta-analysis. *Br J Psychiatry* (2017) 210(5):324–32. doi: 10.1192/bjp.bp.116.197103
155. Bighelli I, Salanti G, Huhn M, Schneider-Thoma J, Krause M, Reitmeir C, et al. Psychological interventions to reduce positive symptoms in schizophrenia: systematic review and network meta-analysis. *World Psychiatry* (2018) 17(3):316–29. doi: 10.1002/wps.20577
156. Koren D, Scheyer R, Reznik N, Adres M, Apter A, Parnas J, et al. Basic self-disturbance, neurocognition and metacognition: a pilot study among help-seeking adolescents with and without attenuated psychosis. syndrome. *Early Interv Psychiatry* (2017). doi: 10.1111/eip.12500
157. Parnas J, Sass LA. Self, solipsism, and schizophrenic delusions. *Philos Psychiatr Psychol* (2001) 8(2):101–20. doi: 10.1353/ppp.2001.0014
158. Beck AT, Rector NA. Cognitive therapy of schizophrenia: a new therapy for the new millennium. *Am J Psychother* (2000) 54(3):291–300. doi: 10.1176/appi.psychotherapy.2000.54.3.291
159. Nelson B, Yung AR, Bechdolf A, McGorry PD. The phenomenological critique and self-disturbance: implications for ultra-high risk ("prodrome") research. *Schizophr Bull* (2007) 34(2):381–92. doi: 10.1093/schbul/sbm094
160. Mizrahi R, Addington J, Rusjan PM, Suridjan I, Ng A, Boileau I, et al. Increased stress-induced dopamine release in psychosis. *Biol Psychiatry* (2012) 71(6):561–7. doi: 10.1016/j.biopsych.2011.10.009
161. Karlsson H, Hirvonen J, Kajander J, Markkula J, Rasi-Hakala H, Salminen J, et al. Psychotherapy increases brain serotonin 5-HT 1A receptors in patients with major depressive disorder. *Psychol Med* (2010) 40(3):523–8. doi: 10.1017/S0033291709991607
162. Lai C, Daini S, Calcagni ML, Bruno I, De Risio S. Neural correlates of psychodynamic psychotherapy in borderline disorders–a pilot investigation. *Psychother Psychosom* (2007) 76(6):403–5. doi: 10.1159/000107572
163. Lehto SM, Tolmunen T, Joensuu M, Saarinen PI, Valkonen-Korhonen M, Vanninen R, et al. Changes in midbrain serotonin transporter availability in atypically depressed subjects after one year of psychotherapy. *Prog Neuropsychopharmacol Biol Psychiatry* (2008) 32(1):229–37. doi: 10.1016/j.pnpbp.2007.08.013
164. Paquette V, Lévesque J, Mensour B, Leroux J-M, Beaudoin G, Bourgouin P, et al. "Change the mind and you change the brain": effects of cognitive-behavioral therapy on the neural correlates of spider phobia. *Neuroimage* (2003) 18(2):401–9. doi: 10.1016/S1053-8119(02)00030-7
165. Rury V, Birchwood M, Cochrane R. Cognitive therapy and recovery from acute psychosis: a controlled trial: 3. Five-year follow-up. *Br J Psychiatry* (2000) 177(1):8–14. doi: 10.1192/bjp.177.1.8
166. Tarrier N, Wykes T. Is there evidence that cognitive behaviour therapy is an effective treatment for schizophrenia? A cautious or cautionary tale? *Behav Res Ther* (2004) 42(12):1377–401. doi: 10.1016/j.brat.2004.06.020
167. Jyrä K, Knekt P, Lindfors O. The impact of psychotherapy treatments of different length and type on health behaviour during a five-year follow-up. *Psychother Res* (2017) 27(4):397–409. doi: 10.1080/10503307.2015.1112928
168. Knekt P, Lindfors O, Härkänen T, Välikoski M, Virtala E, Laaksonen M, et al. Randomized trial on the effectiveness of long-and short-term psychodynamic psychotherapy and solution-focused therapy on psychiatric symptoms during a 3-year follow-up. *Psychol Med* (2008) 38(5):689–703. doi: 10.1017/S003329170700164X
169. Psychosis N. *Schizophrenia in Adults: the NICE guideline on treatment and management, updated edition*. London: NICE (2014).
170. Morrison AP, Pyle M, Gumley A, Schwannauer M, Turkington D, MacLennan G, et al. Cognitive behavioural therapy in clozapine-resistant schizophrenia (FOCUS): an assessor-blinded, randomised controlled trial. *Lancet Psychiatry* (2018) 5(8):633–64. doi: 10.1016/S2215-0366(18)30184-6
171. Morrison AP, Pyle M, Chapman N, French P, Parker SK, Wells A. Metacognitive therapy in people with a schizophrenia spectrum diagnosis and medication resistant symptoms: a feasibility study. *J Behav Ther Exp Psychiatry* (2014) 45(2):280–4. doi: 10.1016/j.jbtep.2013.11.003
172. Penn DL, Meyer PS, Evans E, Wirth RJ, Cai K, Burchinal M. A randomized controlled trial of group cognitive-behavioral therapy vs. enhanced supportive therapy for auditory hallucinations. *Schizophr Res* (2009) 109(1–3):52–9. doi:10.1016/j.schres.2008.12.009
173. Zimmermann G, Favrod J, Trieu VH, Pomini V. The effect of cognitive behavioral treatment on the positive symptoms of schizophrenia spectrum disorders: a meta-analysis. *Schizophr Res* (2005) 77(1):1–9. doi: 10.1016/j.schres.2005.02.018
174. Hutton P. Cognitive-behavioural therapy for schizophrenia: a critical commentary on the Newton-Howes and Wood meta-analysis. *Psychol Psychother* (2013) 86(2):139–45. doi: 10.1111/papt.12009
175. Newton-Howes G, Wood R. Cognitive behavioural therapy and the psychopathology of schizophrenia: systematic review and meta-analysis. *Psychol Psychother* (2013) 86(2):127–38. doi: 10.1111/j.2044-8341.2011.02048.x
176. Crawford MJ, Killaspy H, Barnes TR, Barrett B, Byford S, Clayton K, et al. Group art therapy as an adjunctive treatment for people with schizophrenia: a randomised controlled trial (MATISSE). *Health Technol Assess* (2012) 16(8):iii–iv, 1–76. doi: 10.3310/hta16080
177. Lynch D, Laws KR, McKenna PJ. Cognitive behavioural therapy for major psychiatric disorder: does it really work? A meta-analytical review of well-controlled trials. *Psychol Med* (2010) 40(1):9–24. doi: 10.1017/S003329170900590X
178. Gold C, Solli HP, Krüger V, Lie SA. Dose-response relationship in music therapy for people with serious mental disorders: systematic review and meta-analysis. *Clin Psychol Rev* (2009) 29(3):193–207. doi: 10.1016/j.cpr.2009.01.001
179. Garety PA, Fowler DG, Freeman D, Bebbington P, Dunn G, Kuipers E. Cognitive--behavioural therapy and family intervention for relapse prevention and symptom reduction in psychosis: randomised controlled trial. *Br J Psychiatry* (2008) 192(6):412–23. doi: 10.1192/bjp.bp.107.043570
180. Talwar N, Crawford MJ, Maratos A, Nur U, McDermott O, Procter S. Music therapy for in-patients with schizophrenia: exploratory randomised controlled trial. *Br J Psychiatry* (2006) 189:405–9. doi: 10.1192/bjp.bp.105.015073
181. Bechdolf A, Knost B, Kuntermann C, Schiller S, Klosterkötter J, Hambrecht M, et al. A randomized comparison of group cognitive-behavioural therapy and group psychoeducation in patients with schizophrenia. *Acta Psychiatr Scand* (2004) 110(1):21–8. *Erratum in: Acta Psychiatr Scand* (2004) 110(6):483. doi: 10.1111/j.1600-0447.2004.00300.x
182. Shahar G, Blatt S, Ford RQ. Mixed anaclitic-introjective psychopathology in treatment-resistant inpatients undergoing psychoanalytic psychotherapy. *Psychoanalytic Psychology* (2003) 20(1):84–102. doi: 10.1037/0736-9735.20.1.84
183. Haddock G, Barrowclough C, Tarrier N, Moring J, O'Brien R, Schofield N, et al. Cognitive-behavioural therapy and motivational intervention for schizophrenia and substance misuse. 18-month outcomes of a randomised controlled trial. *Br J Psychiatry* (2003) 183(5):418–26. doi: 10.1192/bjp.183.5.418
184. Turkington D, Kingdon D, Turner T; Insight into Schizophrenia Research Group. Effectiveness of a brief cognitive-behavioural therapy intervention

in the treatment of schizophrenia. *Br J Psychiatry* (2002) 180(6):523–7. doi: 10.1192/bjp.180.6.523

185. Drury V, Birchwood M, Cochrane R. Cognitive therapy and recovery from acute psychosis: a controlled trial. 3. Five-year follow-up. *Br J Psychiatry* (2000) 177(1):8–14. doi: 10.1192/bjp.177.1.8
186. Hogarty GE, Greenwald D, Ulrich RF, Kornblith SJ, DiBarry AL, Cooley S, et al. Three-year trials of personal therapy among schizophrenic patients living with or independent of family, II: Effects on adjustment of patients. *Am J Psychiatry* (1997) 154(11):1514–24. doi: 10.1176/ajp.154.11.1514
187. Buchkremer G, Klingberg S, Holle R, Schulze Mönking H, Hornung WP. Psychoeducational psychotherapy for schizophrenic patients and their key relatives or care-givers: results of a 2-year follow-up. *Acta Psychiatr Scand* (1997) 96(6):483–91. doi: 10.1111/j.1600-0447.1997.tb09951.x

Conflict of Interest Statement: AdB has received research support from Janssen, Lundbeck, and Otsuka and lecture honoraria from Chiesi, Lundbeck, Roche, Sunovion, and Takeda; he has served on advisory boards for Eli Lilly, Janssen, Lundbeck, Otsuka, Roche, and Takeda. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Polese, Fornaro, Palermo, De Luca and de Bartolomeis. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

APPENDIX 1

Author/type of study	Psychological intervention	Adjunction/ antipsychotic	Number of patients/ comparison groups	Stage of illness/age of patients/diagnosis	Frequency (and time) of sessions/ duration of treatment	Results	Jadad score
Morrison et al. (171) Uncontrolled trial Nonblind study	Metacognitive therapy (MCT)	Yes/atypical	10 patients MCT/ none	Not specified/34.3 years+/ schizophrenia (SKP), schizoaffective disorder (SKA), delusional disorder (DD), other*	6–12 sessions/ 9 months	PANSS total and positive significantly reduced at the end of treatment (E) and at 3 months follow-up (FU) PSYRATS reduction with borderline significance	–
Hutton et al. (127) Case report randomized nonblind study	MCT	Yes/various	3 patients MCT/ none	Chronic SKP/15, 20, and 40 years/KA, DD, with positive symptoms	11–13 1-h weekly sessions/3 months	Clinical worthwhile benefits in all. In 2 patients, significant PANSS reduction and increased recovery. At 3 months, FU reduction in positive and negative symptoms	–
Birchwood et al. (93) RCT Single blind	Individual CBT for command hallucinations	Yes/atypical	98 patients CBT+ Treatment as usual (TAU)/99 TAU	Heterogeneous/≥16 years/ SKP, SKA, psychosis (P), bipolar disorder (BD), with self-harm	Up to 25 sessions/ 9 months	Reduction of compliance to voices but not significant	3
Burns et al. (145) Meta-analysis Blind study	Individual CBT	Yes/various	552 patients/waiting list, TAU or other	Not specified/not specified/ SKP, SKA, DD	10–24 sessions/ 6 weeks to 9 months	Statistically significant beneficial effects of CBT at E and FU for positive and general symptoms	–
Jones et al. (132) Cochrane Intervention review Blind vs. nonblind studies	CBT vs. other psychosocial therapies	Yes/various	Not specified; 20 trials/CBT/ psychosocial therapies/TAU	Heterogeneous/18–65 years/SKP	Various	No advantage for CBT over other treatments, including less sophisticated therapies	–
Mandić-Gajić G (129) Case report Blind study	Group art therapy	Yes/various	2 patients/no control group	Chronic/31 years, 27 years/ paranoid and simplex SKP with severe negative symptoms	Not specified/2 months	Improvement in all symptoms, but not statistically significant. Intervention has helped to understand the inner world of patients	–
Klingberg et al. (149) RCT Blind study	Individual CBT, cognitive remediation (CR)	Yes/not specified	99 CBT+TAU/99 CR+TAU	Chronic/18–55 years/ SKP outpatients at least with moderate negative symptom	16.6+ CBT sessions vs. 13.7+ CR sessions/9 months	No suicide, at the E and at 3 months FU adverse events (AEs) in 10 CBT patients, including suicidal attempts, and in 5 CR patients. Depression more frequent in CBT patients. Not statistically significant results	3

(Continued)

Continued

Author/type of study	Psychological intervention	Adjunction/ antipsychotic	Number of patients/ comparison groups	Stage of illness/age of patients/diagnosis	Frequency (and time) of sessions/ duration of treatment	Results	Jadad score
Shawyer et al. (122) RCT Blind study	CBT with acceptance-based intervention (ACT), treatment of resistant command hallucinations (TORCH), mindfulness, befriending (BF)	Yes/not specified	21 CBT/21 CBT/22 BF/17 control group (waiting list)	Chronic/18–65 years (39 years+)/SKF (72%), SKA (21%) and affective P (7%)	1,550-min weekly sessions/4–6 months. 10 minutes mindfulness exercises, with home practice	Subjective greater improvement in CBT vs. BF, but not significant results. CBT here has more modest effect that in early studies	4
Waller et al. (126) Pilot study Nonblind study	Reasoning training Maudley review training program	Yes/not specified	13 patients/no control group	Chronic/44.6 years (mean)/P with low levels of belief flexibility, with jump to conclusion	Single session. One-off computerized training package, lasting approximately 1.5 h	Significant improvement at post-intervention in belief flexibility and improved reasoning	–
Erickson (144) Uncontrolled study Nonblind study	Individual CBT	Yes/according to the early psychosis program	14 patients/no control group	Early psychosis patient/≥18 years/SKP spectrum outpatient	15–25 sessions/not specified period	Significant reduction of positive symptoms and not significant reduction of PANSS negative scale	–
Peters et al. (143) RCT Nonblind study	CBT by nonexpert therapists (supervised)	Yes/various; unmedicated: 6% in the therapy group, 3% in the control group	36 CBT (in 2 CBT groups)/38 TAU	Not specified/18–65 years/P with persistent positive symptoms	16 (mean) weekly or forthrightly sessions lasting up to 1 h/ 6 months	Significant main result in depression, in CBT. At PANSS, positive improvement only in one CBT group	3
Jacobsen et al. (109) Uncontrolled study Nonblind study	Mindfulness Group	Yes/Not specified	8 patients/No control group	Chronic/ 21–43 years/ Complex Psychosis inpatients	1-hour weekly session with 3–5 people/6 weeks	Improvements in PSYRATS, SMQ and a stress scale, but a statistical analysis of results was not provided	
de Paiva Barretto EM et al. (139) RCT Blind study	Individual CBT, befriending (BF)	Yes/clozapine	12 CBT/9 BF	Chronic (CBT 15++ years, BF 10++ years)/CBT 39.8+ BF 33.2+/TRS	20 sessions/21 weeks	Statistically significant improvement in positive symptoms in CBT. Reduced negative symptoms in both groups but not significant	1
Penn et al. (135) RCT Blind study	Group CBT/group supportive therapy (ST)	Yes/at least two trials, one atypical for 8 weeks prior to randomization	32 group CBT/ 33 group ST	Non specified/18–65 years/ SKP or SKA outpatients	Twelve 1-h weekly CBT sessions/3 months; 12 weeks of enhanced ST (172)	Statistically significant improvement only in ST group at E. At 12 months FU significant reduction also in CBT group at PANSS. ST had more specific impact on hallucinations	4
Brabban et al. (141) RCT Blind study Nurses	Brief CBT	Yes/not specified	226 CBT/128 TAU	Not specified/CBT 40+, TAU 41.2+/SKP	From three to six 1-h sessions/2–3 months	Improvement, but not significant	3

(Continued)

Continued

Author/type of study	Psychological intervention	Adjunction/ antipsychotic	Number of patients/ comparison groups	Stage of illness/age of patients/diagnosis	Frequency (and time) of sessions/ duration of treatment	Results	Jadad score
Ross et al. (112) Randomized experimental trial Nonblind study	Reasoning training (RT), attention control activity (ACA)	Yes/not specified	1st stage: 34 RT/34 healthy volunteer 2nd stage: 17 RT/17 ACA	Chronic/16.2 years+ RT, 10.8 years+ controls/SKP spectrum disorder	45-min reasoning intervention in 3 tasks	After training, 24% showed greater belief flexibility and 18% showed a reduction in delusional conviction. Not statistically significant.	–
Johnson et al. (134) RCT Blind study	Group CBT/Group ST	Yes/2 trials, one of which atypical for 8 weeks	58 patients randomly assigned to either group	Chronic/42.1+ years/SKP, SKA outpatients	Twelve 1-h weekly sessions, 1–2 therapists for 4–7 patients over 12 weeks	No difference in ratings between groups.	3
Barrowclough et al. (132) RCT Nonblind study	Group CBT	Yes/not specified	12 patients CBT group/12 TAU	Not specified/age of illness: 13.67++ years; 18–55+ years/SKP, SKA	18 sessions of 2 h including breaks over 6 months	No difference between groups at PANSS, SFS, HADS, BHS, RSE, GAF	1
Cather et al. (113) Controlled trial blind study	Functional CBT (fCBT), psychoeducation (PE)	Yes/olanzapine	15 CBT/15 psychoeducation (PE)	Not specified/age of illness: 24.88++ years; 18–65+ years/SKP, SKA	16 weekly sessions over 4 months	Greater benefit for fCBT on positive symptoms at PSYRATS voices subscale. Not statistically significant	–
Zimmermann et al. (173) Meta-analysis Blind studies vs. nonblind studies	CBT (mainly individual)	Yes/various	1484 patients in 14 studies with at least one CBT group with a control group	Heterogeneous; 10 studies on chronic condition and TRP plus 3 studies on acute; 36.02+ years/SKP spectrum	Weekly sessions/5 weeks to 9 months	Significant reduction of positive symptoms in CBT	–
Wykes et al. (137) RCT Blind study	Group CBT	Yes/typical and atypical antipsychotic	45 CBT + TAU/40 TAU (10 people had specific individual psychotherapy, contaminating the sample)	Chronic/39.7+ years/SKP	Seven sessions/10 weeks	Significant improvement for group CBT patients in social functioning at FU. (Effects could be influenced by extra psychological help and change of medication). No improvement in the severity at PSYRATS	4
Valmaggia et al. (121) RCT Nonblind study	Individual CBT supportive counseling (SC) plus psychoeducation (PE)	Yes/atypical antipsychotic	36 CBT/26 SC, PE	Chronic/18–70+ years/TRS	16 1-h sessions: 12 weekly sessions, 3 fortnightly sessions and last session after 4 weeks/22 weeks	No significant differences between the group at PANNS and PSYRATS, except for the factor 2 of the hallucination subscale	2
Pinkham et al. (136) Pilot study	Group CBT	Yes/atypical antipsychotics	11 patients in two CBT groups/	Chronic/39.6+ years/SKP, SKA inpatients	1-h weekly sessions/ 7 weeks, 11 weeks	Significant changes in both groups in the participants' beliefs	
Nonblind study			No control			Reduction at PANSS and PSYRATS, but not significant	–

(Continued)

Continued

Author/type of study	Psychological intervention	Adjunction/ antipsychotic	Number of patients/ comparison groups	Stage of illness/age of patients/diagnosis	Frequency (and time) of sessions/ duration of treatment	Results	Jadad score
Trower et al. (128) Single-blind RCT	Cognitive therapy for command hallucinations (CTCH)	Yes/typical or atypical	18 CHTC+ TAU/20 TAU	Heterogeneous; 17–60+ years/SKP spectrum disorder with "severe commands"	On average 16 sessions/6 months	Significant reduction of the compliance with voices with maintained results at 12 months FU. Small reduction in negative symptoms	4
Temple and Ho (124) Controlled trial Nonblind study	Individual CBT	Yes/not specified	8 CBT/9 TAU	Not specified. Age of illness onset 21+ years CBT, 24.2+ TAU, 28.8+ years CBT, 35,9+ years TAU/SKP	19–20 sessions/not specified timing and frequency	CBT showed a statistically significant decline in delusions and hallucinations. Trend of reduction in negative symptoms ($p = 0.06$)	–
Randal et al. (114) Controlled trial Nonblind study	Individual multimodal psychotherapy (individual, flexible and recovery-focused)	Yes/minimum dose of atypical antipsychotics	9 Multimodal psychotherapy + TAU/12 TAU group retrospectively considered	Chronic/age of onset 18.9–19.3 years; duration of illness 8.6–11.2 years; 29–30+ years/SKP, SKA inpatients (rehabilitation)	15 min to 1 h, twice weekly, reduced to weekly and to fortnightly or monthly/ up to 21 months	Clinically significant improvements in the overall PANSS, as well as scores for deviant behavioral RCS	–
Rector et al. (125) RCT Blind study	Individual CBT + ETAU (enriched TAU)	Yes/typical, atypical antipsychotic, anti depressants	24 CBT + ETAU/18 ETAU	Chronic/age of onset 21+ CBT-ETAU, 19.2+ ETAU, 37.5+ years CBT-ETAU, 41.2+ ETAU/SKP	20 sessions/6 months	Significant effects for positive, negative, and overall symptom severity at E, but nonsignificant reduction of negative symptoms at 6 months FU	4
Durham et al. (115) Controlled trial Blind study	Individual CBT, supportive psychotherapy (SPT)+	Yes/typical or atypical antipsychotics	22 CBT/23 SPT + TAU/21 TAU	Chronic/duration of illness 15++ CBT, 14++ SPT;10 TAU; 36+ years/SKP, SKA, DD	Up to 20 sessions of approximately 30 min/over 9 months	Significant improvement in CBT and SPT groups vs. TAU, at 3 months FU, but nonsignificant differences between CBT and SPT at E	–
Buchain et al. (130) RCT Nonblind study	Occupational therapy	Yes/clozapine	14 Occupational therapy/12 clozapine	Chronic/age of onset 20.9+ years, 19.67+ years; 33.71+ years, 36.58+ years/TRS	Nonspecified sessions/6 months	Statistically significant difference at EOITO	2
Pilling et al. (138) Meta-analysis RCT Nonblind study	Family intervention (FI) or CBT	Yes/various	1,467 of 18 FI trials/other treatments or TAU or no control group; 528 of 8 CBT studies/ several other treatments	Chronic/Duration of illness: 6 ± 3++ years FI, 11+ + years CBT; 31.2+ years FI, 33.9 years CBT/SKP	8 sessions over a short time period, fortnightly for 2 years, then monthly for 4 years for FI; weekly, monthly sessions 6 weeks to years for CBT	Significant benefit more in FI than standard care, nonsignificant when compared to other treatments More improvement in single FI than in group FI. CBT shows clear positive effects at 9 months FU	–

(Continued)

Continued

Author/type of study	Psychological intervention	Adjunction/ antipsychotic	Number of patients/ comparison groups	Stage of illness/age of patients/diagnosis	Frequency (and time) of sessions/ duration of treatment	Results	Jadad score
Wiersma et al. (147) Uncontrolled naturalistic study Nonblind study	CBT and coping training in an integrated single family treatment program	Yes/typical (65%) or atypical (8%) antipsychotics, antidepressants, benzodiazepines, and/or other medication (17%); 5 patients used no medication at all	40 patients/no control group	Heterogeneous/duration of auditory hallucinations: 8+ years; 37+ years/SKP	Average number of contacts 15 (varying from 2 to 51)/1–32 months	Worsening or no improvements at PANSS Subjective improvement at the end of the study and at 2 or 4 years FU in patients and family Statistically significant reduction at <2 years, but not at 4 years Disappearance of hallucination in 18% of patients. At discharge, 20% of the patients left without any antipsychotic medication	–
Tarrier et al. (153) 2 years follow-up [Tarrier et al. (151), RCT]	CBT, SC Individual	Yes/various	CBT/SC/TAU At this FU, 61 out of 72 patients were available	See Tarrier et al. (151)	See Tarrier et al. (151)	No significant differences During the 2nd year, CBT continued to decline, whereas SC improved	–
Sensky et al. (140) RCT Blind study Nurses	CBT, befriending	Yes/various	46 CBT/44 BF	Chronic/duration of illness: 14–15++ years; 39–40+ years/SKP	18 45-min weekly sessions/9 months and less frequent after	Significant clinical improvement in both groups at E	3
Davenport et al. (131) Two case reports Nonblind study	Interpersonal therapy (conversational, model of Hobson)	Yes/various	2 patients/no control group	Chronic/onset at 18 years; F, 38 years; M, 43 years/SKP	Weekly community group, twice daily staff handover meetings	Improvement at the Krawiecka Goldberg Vaughan scale for schizophrenia social behavior schedule	–
Chadwick et al. (133) Uncontrolled study Nonblind study	Group CBT	Yes/not specified	22 CBT no control group	Not specified/SKP and SKA TRP	Eight 1-h weekly sessions over 8 weeks	Significant improvement in mean conviction scores and in the three beliefs	–
Klingberg et al. (150) 2 years follow-up of an RCT Nonblind study	Psychoeducational medication management training (PMT), CBT	Yes/various	191 patients PMT/ PMT+ CBT/ PMT+ Key person counseling (KC)/ PMT+ CBT+KC/TAU	Chronic/age at onset 22.9+ years; 31.3+ years/SKP	10 h of PMT combined with 15 h of CBT and with 15 h of KC/8 months	Not significant difference in BPRS, SANS.	–

(Continued)

Continued

Author/type of study	Psychological intervention	Adjunction/ antipsychotic	Number of patients/ comparison groups	Stage of illness/age of patients/diagnosis	Frequency (and time) of sessions/ duration of treatment	Results	Jadad score
Pinto et al. (120) RCT Nonblind study	CBT, SST, ST	Yes/clozapine	20 CBT + SST + clozapine/21 supportive therapy (ST) + clozapine	Not specified/duration of illness: 11.6–11.7++ years/33.9+ CBT, 35.8 ST/ SKP	1-h weekly sessions/6 months. Monthly family support	Statistically significant improvement in both groups but no significant difference for negative symptoms.	3
Tarrier et al. (152) One-year follow-up (Tarrier et al. (151), RCT)	CBT, SC	Yes/various	CBT/SC/RC	See Tarrier et al. (137)	See Tarrier et al. (137)	Significant difference in CBT vs. RC for positive symptoms. Nonsignificant improvement for negative symptoms	–
Tarrier et al. (151) RCT Single-blind study	CBT, SC	Yes/typical and atypical antipsychotics	33intensive CBT+ routine care (RC)/26 SC + RC/28 RC	Heterogeneous/duration of illness 11++ years; 38.6+ years/SKP, SKA, DD	Twenty sessions/10 weeks, 4 booster (B) sessions/4 months; 6 h SC session/10 weeks, 4 B sessions; 20RC/10 weeks	Significant improvement for positive symptoms in CBT vs. SC and RC, and in SC vs. RC. RC showed slight deterioration. 18 patients achieved 50% improvement in psychotic symptoms: 11 CBT, 4 SC, 3RC	3
Levine et al. (116) Controlled trial Nonblind study	Group CBT	Yes/typical antipsychotics	6 group CBT/6 control group	Chronic; duration of illness: at least 5 years 20–45 years/paranoid SKP	Six 50-min weekly sessions, with a 4-week follow-up	Significant result at PANSS score at 4th and at 6th week and at 4 weeks follow-up in group CBT	–
Kuipers et al. (148) RCT Blind study	Individual CBT	Yes/various Three patients did not assume medication (2 in CBT group,1 in control group)	28 of 60 CBT plus standard care/32 TAU	Heterogeneous/duration of illness: 12.1++ years CBT, 14++ years TAU; 38.5+, CBT, 41.8+ TAU/SKP, SKA, DD	Mean number of 1-h session (flexible)/15 given over 9 months	Significant improvement only in CBT group, who showed a 25% reduction on the BPRS. Three people became worse and one committed suicide	3
Garety et al. (117) Controlled trial Nonblind study	Individual CBT	Yes/not specified	13 CBT/7 TAU (waiting list group)	Chronic/duration of illness 16.5++ years CBT, 10.9++ years TAU; 39,6+ years CBT, 37.6+ years TAU/SKP or SKA	Weekly or fortnightly sessions, up to 22 sessions, with an average of 16 sessions/6 months	Significant improvement in delusions, preoccupation and action at MADS, BPRS in CBT group. No variations in self-esteem, distress, and insight.	–

** According to the entry criteria for an early intervention for psychosis service, defined using PANSS scores of at least 4 on hallucinations or delusions or at least 5 on conceptual disorganization, grandiosity, or suspiciousness, in the context of initial presentation to services with psychotic experiences.*

+ Mean age of patients (yrs); ++ Mean duration of illness (yrs).

APPENDIX 2

Author/type of study	Psychological intervention	Adjunction/ antipsychotic	Number of patients/ control group	Stage of illnesses and/or age of patients/diagnosis	Frequency and time sessions/duration of treatment	Results
Hutton, (174) Simple meta-analysis	CBT	See Newton-Howes and Wood, (175)	See Newton-Howes and Wood, (175)	Not specified; 18–65 years/ SKP	See Newton-Howes and Wood (175)	Group significant differences at 8–18 months, CBT is more effective
Crawford et al. (176) RCT	Group art therapy	Yes/Various	649/activity groups plus TAU/TAU	417 of 41+ years/17++ years/SKP	Weekly sessions 90 min/12 months	No statistically significant difference
Newton-Howes and Wood (175) Meta-analysis	CBT	Yes/not specified	602/placebo group	Not specified SKA/18–65 years	9 Studies/7–22 weeks/4, 6, 9 months	No significant differences
Lynch et al. (177) Meta-analytical review of well-controlled trial	CBT	Yes/not specified	310 CBT/291 control groups	Acute and chronic adult SKP	From 5 weeks to 9 months	CBT is no better than nonspecific controls and does not reduce relapse rates
Gold et al. (178) Meta-analysis RCT, CCT and pre–post study	Music therapy	Yes/not specified	15 studies (n = 691 patients): 8 RCTs, 3 CCTs, 4 uncontrolled studies	Psychotic and nonpsychotic severe mental illness patients	3–51 sessions	Significant effects on general and negative symptoms, with dose effect
Garety et al. (179) RCT	CBT, FI with and without carers	Yes/various	301 (1) 27 without carers TAU or CBT+TA U, (2) 106 with carers TAU, CBT + TAU or FI + TAU	Non affective psychosis/ 18–65 years, at least moderate severity for one symptom at PANSS	CBT and FI focusing on relapse prevention, 12–20 1-h sessions/9 months	The CBT and FI had no effects at 12 or 24 months. CBT showed effects on depression at 24 months
Bendall et al. (64) RCT	BF	Yes/various	30 ACE/30 BF	Acute first episode psychosis	Up to 20 sessions, 45 min/ 14 weeks	BF was comparable to CBT
Talwar et al. (180) RCT	Music therapy	Yes/not specified	33 music therapy + T AU/48 TAU	Inpatients, SKA spectrum	45-min weekly sessions/ 12 weeks	Significant reduction in PANSS total score
Bechdolf et al. (181) RCT	Group CBT, group PE	Yes/not specified	88/40 CBT/48 PE	One episode of SKP or related disorder, 18–64 years	16 sessions group CBT or 18 sessions group PE/8 weeks	Significant less re-hospitalization at 6 months FU in CBT group
Tarrier et al. (166) RCT	CBT, supportive counseling (SC)	Yes/TAU	101 of 309 CBT + TAU/106 SC + TAU/102 TAU	SKA spectrum or delusional disorder	An 18-month follow-up; 15–20 h plus four "booster" sessions treatment/5 weeks	Improvement at PANSS in both groups for positive and negative symptoms
Shahar et al. (182) Retrospective study	Psychoanalytically oriented treatment	No	29 anaclitic/34 introjective/27 mixed type	Inpatients with psychosis (30%), severe personality disorders (60%) and severe depression (10%)	Treatment including psychoanalytic psychotherapy 4 times a week/15 months	Significant improvement only in the mixed type (anaclitic–introjective) at WAIS, Rorschach, and TAT

(Continued)

Continued

Author/type of study	Psychological intervention	Adjunction/ antipsychotic	Number of patients/ control group	Stage of illnesses and/or age of patients/diagnosis	Frequency and time sessions/duration of treatment	Results
Haddock et al. (183) RCT	Individual and family-oriented CBT combined with motivational intervention for substance use problems	Yes/neuroleptics	18 patients and 18 carers. Individual intervention (II) with CBT + motivational intervention combined with FI + TAU/TAU	Schizophrenia spectrum disorder or delusional disorder, 18–35 years and face-to-face contact with a carer for a minimum of 10 h per week.	9 months of motivational intervention with 18-month FU period/II: around 29 sessions. FI: 10–16 sessions use	There was no difference between the two groups for PANSS general or total subscale scores. SFS total scores at 18 months II had significantly superior GAF scores at the 18-month follow-up
Turkington et al. (184) RT	Brief CBT	Yes/not specified antipsychotics	257 of 422 patients CBT/165 standard care	Patients with schizophrenia in secondary care settings	6-h-long sessions over 2–3 months	Improvements at CPRS, IRS, BCQ, and MRS, in overall symptomatology, carer burden, insight into CBT group
Pilling et al. (142) Meta-analysis	Social skill training (SST), cognitive remediation (CR)	Yes/various	SST/CR	Chronic SKP/mean duration of illness: 6 ± 3 years (specified in 7 studies)	1-h session, weekly–fortnightly–monthly	No clear evidence on improvements of SST. No benefits of CR
Lewis et al. (95) RCT Early psychosis in acute phase	CBT	Yes/not specified	101 of 309 patients CBT/106 of supportive counseling/102 routine care	Acute phase of first and second episode within 2 years of treatment/DSM schizophrenia spectrum	15–20 h in 5 weeks plus 1–2 weeks and 1–3 months	PANSS total and positive showed "trend" for the CBT to improve fastest; in 60% hallucination resolution in CBT > SC; TAU > SC
Drury et al. (185) RCT	CBT/recreational activities and support	Yes/various	20 of 40 adjunction CBT/20 with social recreational program	Hospitalized patients suffering from acute episode of nonaffective psychosis	8 h for week treatment for a maximum of 6 months	PAS and PBIQ scores showed no significant variation in positive and negative symptoms
Hogarty et al. (186) Clinical trial	Personal therapy	Yes/minimum effective dose (not specified)	151 randomly assigned to 1) personal therapy, 2) FI PE, 3) mixed therapy 4) ST, 54 patients randomly assigned to 1) personal therapy 2)FI	SKP or SKA disorder patients after hospital discharge	3 years	Personal therapy improves the social adjustment in the 2^{nd} and 3^{rd} years. ST, with or without FI, effective with peak at 12 months. Long-term therapy is more effective
Buchkremer et al. (187) RC intervention study	Psychoeducational medication management training (PMT), cognitive psychotherapy (CP), key-person counseling (KC)	Yes/4,639 ± 680 (mean dose) of chlorpromazine equivalents 40% depot 49% oral 11% combined oral and depot	132 patients/5 group: 32 PMT+ regular leisure-time group (LGT)/ 35 PMT + CP/34 PMT + LG T + KC/33 PMT + CP + KC/57 LGT	SKP 31.3+ years, 22.9+ at onset years, the mean number of hospitalizations: 4.7 ± 3.6, total duration of hospitalization: 56.4 ± 52.5 weeks	PMT: 10 group sessions, the first 5 at weekly interval, then at fortnightly. (6–8 persons per group)	Favorable result in PMT + CP + KC Best results in PMT + CP + KC with 24% lower rehospitalization at 1 year follow-up and 26% at 2 years follow-up

frontiers in Psychiatry

REVIEW
published: 28 May 2019
doi: 10.3389/fpsyt.2019.00314

Hypofunctional Dopamine Uptake and Antipsychotic Treatment-Resistant Schizophrenia

Davide Amato[1], Anna Kruyer[1], Anne-Noël Samaha[2] and Andreas Heinz[3]*

[1] Department of Neuroscience, Medical University of South Carolina, Charleston, SC, United States, [2] Department of Pharmacology and Physiology, Faculty of Medicine, Université de Montréal, Montreal, QC, Canada, [3] Department of Psychiatry, Charité University Medicine Berlin, Campus Charité Mitte, Berlin, Germany

Antipsychotic treatment resistance in schizophrenia remains a major issue in psychiatry. Nearly 30% of patients with schizophrenia do not respond to antipsychotic treatment, yet the underlying neurobiological causes are unknown. All effective antipsychotic medications are thought to achieve their efficacy by targeting the dopaminergic system. Here we review early literature describing the fundamental mechanisms of antipsychotic drug efficacy, highlighting mechanistic concepts that have persisted over time. We then reconsider the original framework for understanding antipsychotic efficacy in light of recent advances in our scientific understanding of the dopaminergic effects of antipsychotics. Based on these new insights, we describe a role for the dopamine transporter in the genesis of both antipsychotic therapeutic response and primary resistance. We believe that this discussion will help delineate the dopaminergic nature of antipsychotic treatment-resistant schizophrenia.

Keywords: schizophrenia, drug addiction, antipsychotic efficacy, antipsychotic-resistant schizophrenia, dopamine transporter, dopamine synthesis, dopamine release

OPEN ACCESS

Edited by:
Felice Iasevoli, University of Naples Federico II, Italy

Reviewed by:
Mette Ødegaard Nielsen, Center for Neuropsychiatric Schizophrenia Research (CNSR), Denmark
Mark Slifstein, Stony Brook University, United States
Hiroyoshi Takeuchi, Keio University, Japan

****Correspondence:***
Davide Amato
amatod@musc.edu
amatodavide@gmail.com

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *30 January 2019*
Accepted: *23 April 2019*
Published: *28 May 2019*

Citation:
Amato D, Kruyer A, Samaha A-N and Heinz A (2019) Hypofunctional Dopamine Uptake and Antipsychotic Treatment-Resistant Schizophrenia. Front. Psychiatry 10:314. doi: 10.3389/fpsyt.2019.00314

INTRODUCTION

Schizophrenia is a psychiatric condition often involving a complex genetic predisposition (1–3) as well as vulnerability to certain environmental factors (4), eventually culminating in symptoms clinically defined as positive (emergent symptoms, including hallucinations and delusions) or negative (characterized by loss of a particular function, including apathy and lack of motivation) (5–7). Additionally, a proportion of patients with schizophrenia are impaired on standard neurocognitive tasks (8), and this is considered an important correlate of disease severity (9–12). The fundamental neurobiological maladaptations underlying the symptoms of schizophrenia are not completely understood. Regardless, sub-chronic blockade of a proportion (60–80%) of dopamine $D_{2/3}$ receptors (which we will refer to as "D_2") is considered to underlie treatment efficacy in schizophrenia (13). Previous and recent literature supports the effectiveness of D_2 antagonism compared to any alternative pharmacological intervention (14–17). However, blocking dopamine receptors is not an effective therapeutic mechanism for all individuals with schizophrenia (18–24). For example, some patients with first-episode psychosis do not respond to antipsychotic treatment (25). Lack of response to antipsychotic treatment can also be "acquired" and can develop over time with long-term treatment regimens (23, 26) or can develop after a period of treatment abstinence, such as that which occurs during medication nonadherence (27–30). In many of these cases, patients unresponsive to first-line antipsychotic treatments are instead responsive to clozapine (18, 31, 32). Furthermore, there

exists an additional group of patients with schizophrenia who will not respond to clozapine or to any other antipsychotic drug. This category of patients is defined as "ultra-resistant" (18, 33).

Whether all instances of antipsychotic resistance share a common neurobiological mechanism is not clear (10, 24, 34–39), nor is there a precise behavioral signature indicating its clinical manifestation, since criteria to define resistance to antipsychotic treatment were standardized only recently (40). It is not within the scope of this review to contribute to the behavioral definition of treatment resistance in schizophrenia. Rather the focus here is narrowed onto the putative role of dopamine clearance in the expression of primary antipsychotic-resistant schizophrenia (i.e., patients with first episode psychosis who never responded to treatment). We do not exclude the possibility that alterations in other neurotransmitter systems might also be involved, nor do we exclude that the dopaminergic mechanisms described here will also apply to other forms of antipsychotic resistance. Simply, we focus on dopamine, because clinical observations emphasize the importance of this neurotransmitter in the pathophysiology of psychosis (41–43) and its treatment (44). Our attention on dopamine clearance is motivated by recent data from *ex vivo* and *in vivo* studies with animal models demonstrating that antipsychotic failure is accompanied by tolerance to antipsychotic-induced increases in basal dopamine and dopamine turnover, and that the dopamine transporter (DAT) is a key moderator of both extracellular dopamine and antipsychotic response (35, 38, 45). The link between preserved, or slightly elevated, dopaminergic tone and antipsychotic responsiveness has also been observed in humans with schizophrenia (46). Recent interpretations of these data suggest that a preserved extracellular dopaminergic tone might have an important pharmacological role in the therapeutic efficacy of antipsychotics (24). These observations have been directly and indirectly supported by independent studies (38, 47–50). Due to space limitations, we will only briefly outline dopaminergic biomarkers described in the literature that appear relevant to understanding antipsychotic responsiveness. We will then conclude with the suggestion that DAT could be a more powerful moderator of antipsychotic efficacy and failure than currently recognized. Changes in DAT expression and/or function alone can alter the expected response to antipsychotic medications, making DAT a highly relevant protein when considering the dopaminergic nature of antipsychotic-resistant schizophrenia.

DOPAMINERGIC DYSREGULATION IN SCHIZOPHRENIA

Before discussing dopaminergic mechanisms of antipsychotic efficacy, it is important to describe the dopaminergic signaling abnormalities in schizophrenia that are targeted by antipsychotic drugs. As described in the Introduction, the underlying etiology and neuropathology of schizophrenia symptoms are still unclear. Genetic studies point to associations with genes regulating neurodevelopment, the immune system, and dopaminergic and glutamatergic transmission (2, 51), while other studies demonstrate a potential role for disruption of multiple intracellular signaling pathways in schizophrenia (52). Furthermore, environmental factors linked to schizophrenia such as migration or obstetric infection can change dopamine neurotransmission (4), in addition to other neurobiological systems (53–58). Despite the many factors that appear to contribute to schizophrenia, treatment has focused on correcting a dysregulated dopaminergic system by inhibiting dopaminergic transmission. However, it should be noted that the efficacy of pharmacologically targeting the dopaminergic system in schizophrenia does not definitively prove a dopaminergic dysregulation. Dopamine has a powerful neuromodulatory role in the brain and in the basal ganglia in particular and it can regulate motor activity as well as motivation and cognition. Since all of these functions are impacted in schizophrenia, it should not be surprising that many antidopaminergic drugs are effective (or deleterious) for schizophrenia symptoms, even though the observable symptoms may have some other underlying cause(s). Thus, the dopaminergic system should be seen as a treatment pathway capable of affecting behavioral features that appear to be disrupted in schizophrenia, but that may be caused by alterations in other neurotransmitter systems.

MECHANISMS OF ANTIPSYCHOTIC RESPONSIVENESS

Brain dopamine receptor blockade has been embraced as a mechanism for the therapeutic efficacy of antipsychotic drugs for over 60 years (9, 59). Thus, very frequently, researchers have focused on the interactions between molecule(s) and receptor(s) to describe antipsychotic mechanisms. Although this approach is correct in principle, practically it may be too simplistic. Receptors do not act in isolation. Receptors on neurons are connected *via* synapses and organized into networks within neuronal circuitries. Receptors are also functionally linked with intracellular molecular networks that control membrane excitability, as well as neurotransmitter synthesis, release, and metabolism, and by these mechanisms, neurons can regulate their own activity. Due to the nature of neural signaling, changes in the inactivation or activation of neural receptors with antipsychotic drugs, or with any other compound, which cause local intracellular changes, will affect other cell populations through signal propagation along neural pathways. Thus, antipsychotic medications can impact neurotransmitter synthesis, release, and metabolism not only in neurons that directly interact with antipsychotics but also in those neurons that are part of the same neural circuitry. Therefore, a proper understanding of the mechanisms underlying antipsychotic responsiveness should not simply describe the chemical interactions between antipsychotic drugs and their target receptors, but should consider modifications induced by antipsychotics at the cellular and circuit levels. We will focus on neuroadaptations occurring at the cellular level that link receptors to synthesis, release, and uptake of extracellular dopamine.

STRIATAL D_2 RECEPTOR BLOCKADE IN TREATMENT-RESPONSIVE SCHIZOPHRENIA

Striatal D_2 receptor blockade is considered the most effective mechanism to reduce psychotic symptoms in schizophrenia

(60, 61). Extra-striatal mechanisms of antipsychotics have been debated previously (62) and will not be discussed here. The general theory of the therapeutic efficacy of antipsychotics builds on two main observations. First, clinical potency of antipsychotics, including clozapine, is directly related to their affinity for the dopamine D_2 receptor *in vitro* (14, 15). This is substantiated by evidence that therapeutic concentrations of antipsychotics in the plasma or in the spinal fluid accurately match the antipsychotic dissociation constant (K_d) at D_2 receptors (63). Secondly, therapeutic concentrations of all antipsychotics (typical and atypical) produce a similar D_2 receptor occupancy (13, 59, 64). Although this observation does not strictly apply for clozapine (21) or quetiapine (65), it has been shown that D_2 receptor occupancy in the human brain ranges between 70% and 80% within 2 h of treatment and remains elevated for over 24 h for both typical and atypical antipsychotics (21, 66, 67). D_2 receptor occupancy with clozapine (20) and quetiapine (68, 69), on the other hand, decreases significantly within 24 h. Based on these findings, Seeman and Tallerico (63) suggested that the main difference between typical and atypical antipsychotics is the temporal decay of antipsychotic binding to the D_2 receptor when challenged by endogenous dopamine. In fact, antipsychotics compete with endogenous dopamine within the synaptic space and the presence of dopamine would theoretically affect the concentration of antipsychotic required to reach a particular range of D_2 receptor occupancy. Subsequently, it was observed that the dissociation rate constant, k_{off} (rather than association rate constant, k_{on}), largely accounts for the difference in binding affinity when comparing typical and atypical antipsychotics (70). This also implies that measurements of D_2 receptor occupancy with antipsychotics can be affected by the chemistry of the radioligands used (i.e., lipid-soluble spiperone, nemonapride versus water-soluble dopamine, raclopride) (71–73). D_2 receptor occupancy by atypical antipsychotics such as clozapine and quetiapine will be reduced by (^{11}C)raclopride less so than if lipid-soluble radioligands such as (^{11}C)methylspiperone were used (63, 73, 74). Therefore, differences in D_2 receptor occupancy between clozapine, quetiapine, and other antipsychotics could be influenced by the chemistry of the radioligands used (75). This intriguing interpretation, developed using *in vitro* assays, has not been confirmed functionally. Typical and atypical antipsychotics dissociate with similar temporal kinetics in electrophysiological evaluations, suggesting that the reversal of D_2 receptor antagonism by typical and atypical antipsychotics does not differ markedly (76, 77). These contradictory results point to the possibility that mechanisms other than receptor occupancy may also be involved in the outcomes of these assays, although we cannot dismiss the relevance of ligand binding kinetics at D_2 receptors for achieving antipsychotic efficacy (24, 38).

STRIATAL D_2 RECEPTOR DENSITY AND BLOCKADE IN TREATMENT-RESISTANT SCHIZOPHRENIA

As already mentioned above, the blockade (or occupancy) of a proportion of D_2 receptors is not a working antipsychotic mechanism for a significant number of patients with schizophrenia (31). In fact, roughly one-third of individuals with schizophrenia are resistant to treatment with first-line antipsychotics despite sufficient D_2 receptor occupancy (19). Clozapine, which works at a relatively low (~40%) striatal D_2 receptor occupancy (20, 21, 78, 79), is the most effective antipsychotic in the majority of patients refractory to other antipsychotic medications (18, 32, 80). If we hypothetically accept the suggestion that this outcome is not attributable to D_2 receptor binding kinetics (77), we begin to consider other dopaminergic mechanism that may account for this apparent discrepancy. A growing literature supports the idea that additional dopaminergic mechanisms may underlie therapeutic efficacy of antipsychotic drugs (24, 38). Some patients who respond to first-line antipsychotic treatment experience diminished treatment efficacy over time (23), which can lead to treatment non-compliance and relapse (81). Diminished antipsychotic efficacy may also occur despite stable D_2 receptor occupancy (82). These dynamics are depicted in **Figure 1**. The opposite has also been observed with long-term antipsychotic efficacy occurring despite decreasing D_2 receptor occupancy (89–85).

Acquired resistance to antipsychotics (tolerance) could involve antipsychotic-induced dopamine receptor supersensitivity, potentially resulting from D_2 receptor upregulation, consequent to chronic D_2 receptor blockade (34, 86, 87). In patients with schizophrenia, antipsychotic-induced dopamine supersensitivity is thought to impair treatment efficacy, promote relapse to psychosis, and also worsen psychotic symptoms (88–90). In laboratory animals, antipsychotic-induced dopamine supersensitivity produces loss of antipsychotic efficacy (35, 91, 92) and an exaggerated behavioral response to dopamine agonists (35, 93–95). However, the link to antipsychotic-induced striatal D_2 upregulation is complex. Changes in levels of dopamine receptor expression in patients have not been replicated reliably by independent research groups (96, 97). Recent studies using animal models also show tolerance to antipsychotics despite

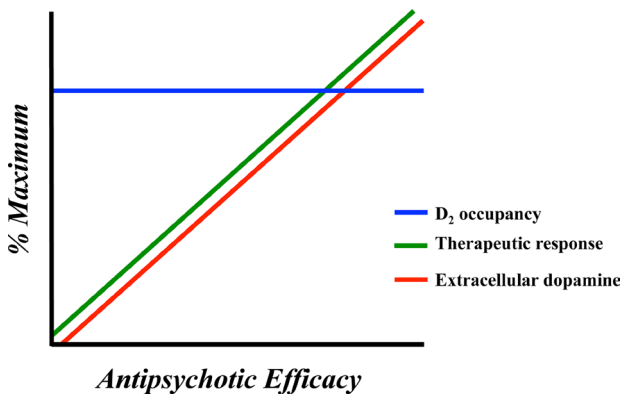


FIGURE 1 | Representation of the neurochemical factors affecting antipsychotic response in humans and animal models. Antipsychotic response is optimal in concert with elevated extracellular dopamine levels. D_2 receptor occupancy is less dynamic and appears stable during time periods characterized by both therapeutic efficacy and antipsychotic failure.

clinically representative levels of striatal D_2 receptor blockade, as measured either with *in vivo* imaging (38) or *ex vivo* receptor autoradiography (35, 91). Antipsychotic-induced dopamine supersensitivity and tolerance to antipsychotics can also be dissociable from changes in striatal D_2 receptor density (35). Thus, changes in striatal D_2 receptor expression are not always predictive of either changes in antipsychotic efficacy or the emergence of antipsychotic-induced dopamine supersensitivity (24, 35, 38, 98, 99), although high doses of antipsychotics may upregulate striatal D_2 receptors (100).

Beyond changes in striatal D_2 receptor density, chronic antipsychotic treatment can also increase D_2 receptor function, and this has been linked to dopamine supersensitivity and acquired antipsychotic tolerance. When D_2 receptors are coupled to $G_{i/o}$ proteins, they are in a functional, high affinity state for dopamine (referred to as D_2^{HIGH}). When D_2 receptors are uncoupled to $G_{i/o}$ proteins, they are in a functionally inert, low affinity state for dopamine (D_2^{LOW}). As such, the proportion of D_2^{HIGH} can modulate dopamine signaling *via* D_2 receptors. The link between antipsychotic tolerance and changes in striatal D_2^{HIGH} sites comes largely from work in animal models showing that chronic antipsychotic treatment increases striatal D_2^{HIGH} levels (35, 91, 101). Antipsychotic treatment regimens that promote behavioral dopamine supersensitivity and antipsychotic treatment tolerance produce an even greater increase in D_2^{HIGH} sites (91). D_2^{HIGH} receptor elevation and antipsychotic-induced dopamine supersensitivity also follow a similar time course (35). However, D_2^{HIGH} sites can increase early in antipsychotic treatment, before any behavioral evidence of dopamine supersensitivity or treatment tolerance (35). In addition, antipsychotic dosing regimens that do not produce dopamine supersensitivity can still increase striatal D_2^{HIGH} sites (91, 101). Furthermore, there is no conclusive evidence of elevated D_2^{HIGH} receptors in patients with schizophrenia [see (102)]. Thus, there is likely a link between changes in D_2^{HIGH} sites and acquired antipsychotic treatment tolerance, but this requires further study.

DOPAMINE D_2 RECEPTOR ISOFORMS AND SCHIZOPHRENIA

The majority of the cells expressing D_2 receptors in the striatum are neurons with medium-sized cell bodies and spiny dendrites (medium spiny neurons, MSNs, about 95% of all cells in this region), which are postsynaptic to dopaminergic terminals projecting from the midbrain, among other regions for an overview, see Refs. (24, 103). The striatum also contains presynaptic D_2 receptors expressed on dopaminergic axon terminals, which represent only a small percentage of the total D_2 receptor pool found in the striatum and may have a different molecular structure (104). Accordingly, there are two isoforms of dopamine D_2 receptors deriving from alternative splicing of exon 6 to produce the long (D_{2L}) and the short (D_{2S}) forms of the protein (105–107) (**Figure 2A–C**). Both isoforms appear to regulate dopaminergic firing (108), but only D_{2S} controls Ca^{2+}-mediated autoinhibition (109, 110). Furthermore, post-synaptic D_{2S}, but not D_{2L}, controls MSN excitability in rodents (111) and likely in humans (112), despite its pre-dominant presynaptic localization. These effects are likely a consequence of the distinct molecular mechanisms linked to D_2 receptor isoforms (113–116) (**Figure 2C**).

The expression of D_2 isoforms in the mammalian brain is distributed unevenly (**Figure 2A**). Genomic studies of human and rodent D_2 mRNA, which share ~95–99% homology (117), report that while D_{2L} and D_{2S} mRNA are widely expressed in the brain, D_{2L} mRNA is highly expressed in the striatum (i.e., caudate nucleus and putamen) relative to D_{2S} mRNA (117–120). Investigation of D_2 protein expression in primates shows that D_{2L} is highly expressed in the striatum and found specifically on MSNs and cholinergic interneurons, while D_{2S} is instead expressed on dopaminergic axons (121). In the cortex and midbrain, D_{2L} is mostly expressed on neuronal somata, while D_{2S} is found on somata, dendrites, and axon terminals (121). Interestingly, high potency antipsychotics (with high affinity

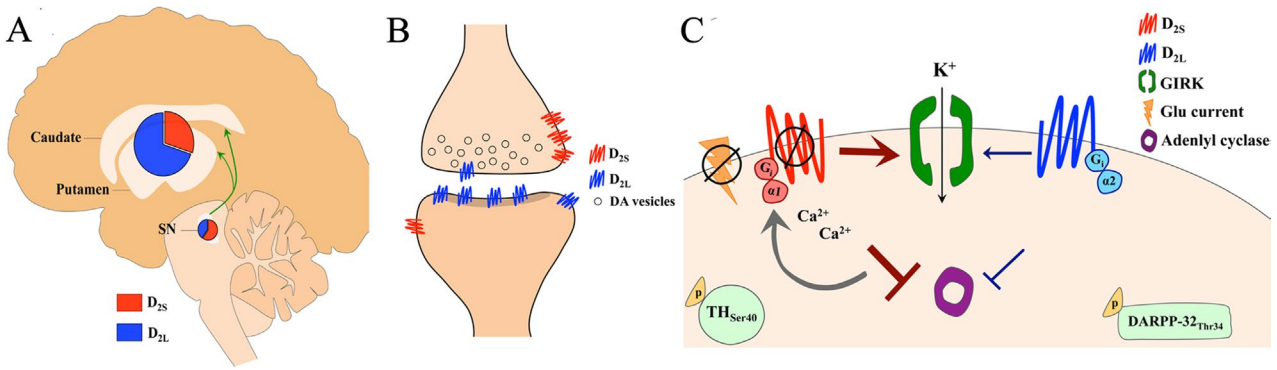


FIGURE 2 | (A) Uneven expression of dopamine D_2 receptor isoforms (short, D_{2S} and long, D_{2L}) in the human midbrain (substantia nigra, SN) and striatum (caudate nucleus and putamen). D_{2L} is predominant in the striatum, while D_{2S} is prevalently expressed in the midbrain. This unbalanced D_{2L}/D_{2S} ratio is observed across species. **(B)** Schematic of a synaptic contact between a dopaminergic terminal projecting from SN and a somatodendritic spine in the striatum shows the unbalanced D_{2L}/D_{2S} ratio on midbrain and striatal neurons. **(C)** Distinct physiological effects are mediated by the two D_2 receptor isoforms. Both D_{2S} and D_{2L} receptors inhibit adenylyl cyclase, though D_{2L}-mediated inhibition is weaker, *via* $G_i\alpha_1$ and $G_i\alpha_2$, respectively. D_{2S} stimulation leads to phosphorylation of tyrosine hydroxylase (TH) at serine 40 in nigrostriatal dopaminergic neurons, whereas D_{2L} stimulation leads to phosphorylation of dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) at threonine 34, in medium spiny neurons. D_{2S}, but not D_{2L}, activates G protein-gated inwardly rectifying potassium (GIRK) conductance, which is Ca^{2+} sensitive. D_{2S}, but not D_{2L}, inhibits excitation in response to glutamate (Glu) currents.

for the D_2 receptor) appear to selectively bind those receptors expressed in the striatum (a structure with high D_{2L}/D_{2S} ratio) (122, 123), supporting the notion that antipsychotics could bind both D_2 isoforms, but that effective antipsychotic doses would bind largely D_{2L} and only a small proportion of total D_{2S} receptors in the brain. Although this possibility is not completely supported from binding studies using cloned D_2 receptors in cultured cells (124–127), saturation binding studies and *in vivo* studies with ED50 antipsychotics using transgenic mice (i.e., D_{2L} receptor knockout mice) appear to confirm an antipsychotic selectivity for D_{2L} (128–131). Consistent with these observations, humans studies have shown that more effective antipsychotics have higher D_2 receptor occupancy in the striatum than in the midbrain (SN) (132, 133).

Postmortem studies using brain tissue from patients with schizophrenia that received antipsychotic treatment prior to death demonstrate a significant increase in D_{2L} mRNA in the caudate nucleus (134), arguing in favor of specific adaptations of D_{2L} in response to chronic blockade with antipsychotics. Studies have reported that D_2 receptor mRNA adaptations with chronic D2 blockade might (135, 136) or might not (137) associate with membrane receptor expression suggesting that post-transcriptional mechanisms might more robustly control D_2 receptor trafficking (138). Other studies instead demonstrate direct links between gene transcription and D_2 receptor expression selectively in the striato-pallidal pathway (139). Currently, the precise action of antipsychotics on the D_2 receptor isoforms is still inconclusive despite strong evidence from these studies with transgenic rodents.

DOPAMINE SYNTHESIS, RELEASE, AND UPTAKE

Dopamine levels in schizophrenia are thought to be higher than in healthy individuals especially during psychotic episodes (140) and antipsychotics are intended to reduce this increased dopamine signaling (13). But it is unclear how this could occur when narrowly considering only D_2 receptor occupancy (24). D_2 receptors are expressed in the dendrites, somata, and terminals of dopaminergic neurons (autoreceptors) and in postsynaptic neurons (heteroreceptors). Dopamine stimulation of D_2 autoreceptors at terminals decreases synaptic dopamine release, while stimulation of somatic D_2 autoreceptors instead decreases the firing activity of these cells (141). Acute application of antipsychotics with high affinity for the D_2 receptor has been found to increase dopamine release in projection areas (142), and this increase in dopamine is only minimally driven by increased dopamine neuron firing (143, 144), since application of antipsychotics directly onto somatic autoreceptors of midbrain dopamine neurons causes only modest dopamine release (145). Also, postsynaptic D_2 heteroreceptors can moderately regulate extracellular dopamine in the striatum *via* GABA transmission, especially if autoreceptors are hypofunctional (131). Altogether, these seminal studies suggest that antipsychotics most effectively control dopamine transmission by targeting receptors in terminals found in the striatum. Interestingly, since most of the striatal receptors are heteroreceptors and only modestly control dopamine release, increases or decreases in extracellular dopamine levels (35, 45) are likely mediated by other mechanisms impacted by antipsychotics (38). These regulatory mechanisms include modifications to dopamine synthesis, release, and uptake.

Synthesis: Early studies demonstrated that acute antipsychotic treatment increased dopamine synthesis in *in vitro* (146, 147) and *ex vivo* preparations (148) as well as *in vivo* in rodents (149, 150). This was thought to be mediated by direct modification of the enzyme tyrosine hydroxylase (TH) (151, 152). However, later studies could not find changes in dopamine synthesis *in vivo* in human striatum, while comparable doses of antipsychotics appeared to increase dopamine synthesis in animals (153), thus only partially confirming previous work (149). While this discrepancy between rodents and human data was not clarified, a different enzymatic pathway for the synthesis of dopamine (TH vs. aromatic amino acid decarboxylase, AAAD) in rats and humans seemed a plausible explanation (153, 154). The regulation of extracellular dopamine through an autoreceptor-based mechanism of dopamine synthesis using antipsychotics is complex. In fact, studies have shown that decreasing dopamine synthesis has no therapeutic antipsychotic efficacy (155), and though antipsychotic treatment can either increase or decrease dopamine synthesis capacity (DSC, DOPA decarboxylase mediated L-DOPA conversion to dopamine) independently from D_2 receptor blockade (156), both effects are associated with an improvement of symptomatology (157–159). These contrasting findings may result in part from the very complex molecular machinery that co-regulates DAT, TH, and D_2 autoreceptors (160–163), making it unlikely that antipsychotic medications will affect this machinery in a predictable manner.

We previously found that TH expression was not changed by effective doses of typical and atypical antipsychotics in animal models (38). However, TH expression increased when antipsychotics were no longer effective, and this was positively correlated with increased DAT expression (38). Interestingly, although TH expression did not change during antipsychotic efficacy, extracellular dopamine increased and vesicular release of dopamine decreased, suggesting that antipsychotics contributed to modulation of extracellular dopamine *via* reduced uptake rather than modified synthesis. Thus, changes in extracellular dopamine levels can be independent from the synthesis rate and may rely more on autoinhibition and uptake (38, 164), and/or a compensatory activity of TH (160).

Release: The idea that antipsychotics control dopamine release primarily by D_2 autoreceptor blockade first emerged with the results of early molecular pharmacology experiments (146, 147, 165–167) showing that antipsychotics revert the inhibitory effects of apomorphine. Subsequent microdialysis (142) and electrophysiological (144) studies supported these early molecular findings. However, most of the results from these early studies have been obtained with limited experimental preparations such as synaptosomes (146, 165, 167) or have involved the use of neurotoxins to destroy post-synaptic neurons in freely moving microdialysis (142), which incurs severe brain lesions. Thus, the significant interaction of antipsychotics with D_2 autoreceptors found in these early studies should be considered in light of the fact that these manipulations can disrupt the natural organization of structures within the brain. Therefore, whether

therapeutic doses of antipsychotics *in vivo* control dopamine release uniquely through D_2 autoreceptors is not completely clear (141). Contemporary researchers working when these early studies were conducted acknowledged that this mechanism was only partially plausible (146, 147, 165). Furthermore, the fact that clozapine, which has moderate binding affinity for D_2 receptors relative to other antipsychotics (78), is as effective as high potency antipsychotics at increasing depolarization by D_2 autoreceptor blockade (144) likely suggests that mechanisms other than D_2 autoreceptor antagonism may be involved in the regulation of dopamine output by antipsychotics. One mechanistic possibility is that, at least for atypical antipsychotics, dopamine release is modified by serotonergic mechanisms. But this is unlikely to fully account for antipsychotic-induced dopamine release, since both typical and atypical antipsychotics evoke release of dopamine (38), but typical antipsychotics have much lower affinities at 5-HT receptors compared to second-generation therapeutics [for an overview, see Refs. (168, 169)].

Another possibility as to how antipsychotics regulate striatal dopamine output is through their direct impact on the vesicular exocytosis at active zones linked with Ca^{2+} channels (170, 171). We previously reported that typical and atypical antipsychotics can accumulate in synaptic vesicles of cultured hippocampal neurons through an acidic trapping mechanism and inhibit Na^+ channels upon release. The inhibition of Na^+ channels leads to feedback inhibition of Ca^{2+} influx and reduced vesicular dopamine release (171). We tested this mechanism using antipsychotic treatment regimens reflecting clinically relevant outcomes of antipsychotic efficacy and resistance and found that exocytosis-mediated dopamine release was regulated in distinct ways at different points during haloperidol treatment (38). Specifically, haloperidol inhibited dopamine exocytosis in sub-chronic regimens, i.e., ≤6 days and during treatment efficacy, while dopamine exocytosis was enhanced during chronic antipsychotic treatment associated with loss of behavioral efficacy (38). This distinct regulation of vesicular release of dopamine during sub-chronic versus chronic haloperidol might reflect the involvement of two different mechanisms in which K^+ channels mediate the inhibition of vesicular release, while Na^+ channels counteract this inhibition (38). Antipsychotics can regulate dopamine release by directly binding the open state of K^+ channels (i.e. Kv4.3) during depolarization and accelerating the decay rate of inactivation (172–174). This mechanism of action can regulate dopamine release over time independent of depolarization blockade by modifying the intrinsic excitability of dopaminergic neurons (175). Further, changes in K^+ conductance can shunt the effects of innervating signals onto dopaminergic neurons, preventing changes in dopamine release. One additional mechanism through which antipsychotics may impact dopamine release involves elevation in extracellular dopamine as a consequence of antipsychotic-induced DAT blockade (38), which may activate GIRK currents at axon terminals through an interaction between D2 autoreceptors (24, 38) and Kv1 channels (176). We found that K^+-mediated release of dopamine is differentially affected during antipsychotic efficacy and failure in freely moving mice undergoing treatment, although it is not yet known if this is due to a direct action of antipsychotics on K^+ channels or is instead mediated indirectly by elevated endogenous dopamine. Thus, multiple lines of evidence point to the capacity of antipsychotics to impact dopamine release, even though they may not necessarily impact dopamine synthesis.

Uptake: In order to appreciate the core mechanism of antipsychotics, it is essential to understand how antipsychotics influence the temporal dynamics of dopamine signaling in the extracellular space within the striatum, the locus of psychosis (9). Data from early studies described above provided copious evidence that antipsychotics block D_2 receptors and that this is sufficient to restore dysregulated dopamine signaling in many human patients, at least for some period of time. However, these early studies did not distinguish appropriately between antipsychotic action on pre- and post-synaptic D_2 receptors (141), and it is therefore unclear which D_2 receptor type accounts for the clinical outcomes generated by antipsychotics (24). Likewise, it is not clear what happens to dopamine released into the extracellular space when antipsychotic drugs prevent its binding to D_2 receptors (24, 38, 169). Under normal physiological conditions, most extracellular dopamine is recycled by means of re-uptake by DAT and remaining transmitter diffuses away (177). Dopamine re-uptake terminates dopaminergic signaling and prevents toxic consequences of excessive dopamine (178). Accordingly, extracellular dopamine concentration and DAT availability are directly correlated (179). In the absence of DAT-mediated dopamine re-uptake, no other mechanism can maintain homeostatic control of presynaptic function (180), although dopamine spillover also appears to play crucial role in deactivation of dopamine signaling (181). Once dopamine is collected into presynaptic terminals, most of it is recycled and packaged into vesicles (182). The remainder is metabolized enzymatically within the cytosol (180, 183). Therefore, extracellular dopamine concentration is the outcome of dopamine release and clearance (184, 185), and it is of therapeutic relevance to understand how antipsychotics modify this balance (38).

ANTIPSYCHOTIC ACTION ON DAT

Previous meta-analytical studies have found no consistent evidence for DAT changes in schizophrenia (186), and autoradiographic studies found no antipsychotic-induced changes in DAT density labeled with [^{125}I]RTI-121 ([125I]2 beta-carboxylic acid isopropyl ester-3 beta-(4-iodophenyl)tropane) (187, 188). However, other investigations discussed above report that direct blockade of dopamine uptake contributed to the elevated extracellular dopamine in response to acute antipsychotics (146, 147, 165, 189), although the technology at the time did not allow for a clear distinction between release and uptake kinetics. More recent studies using fast scan voltammetry demonstrated that antipsychotics with high affinity for D_2 receptors enhanced dopamine half-life by nearly 50% *via* direct DAT blockade and antagonism of D_2 autoreceptors (190–192). Accordingly, a delayed dopamine half-life results from direct inhibition of DAT, since the decay phase of stimulated dopamine overflow entirely depends on uptake (193). In support of this, striatal slice recordings showed that antipsychotics do not enhance dopamine release after the first stimulation (192), contradicting the idea that D_2 autoreceptor antagonism by

antipsychotics blocks autoinhibition in slices. The direct inhibition of DAT with antipsychotics occurs at low affinity and antipsychotics are less potent than more selective DAT blockers like nomifensine (194–196). This helps us interpret the apparent lack of association between antipsychotics and DAT changes reported by previous studies with low sensitivity methods (187, 188). Since uptake is the main route of elimination of extracellular dopamine (180, 197) and the kinetics of diffusion are independent from release and uptake (177), then DAT blockade by antipsychotics could explain the increase in dopamine and dopamine metabolites observed in previous microdialysis studies (198–202) as well as the prolonged half-life of dopamine stimulated by K^+ (189).

Additional findings from *ex vivo* studies support a direct interaction between antipsychotics and the DAT. Under normal physiological conditions, increased dopamine release rapidly upregulates DAT membrane expression (203, 204). Effective doses of antipsychotics given sub-chronically (i.e., 2–6 days) inhibits the production of DAT mRNA, but does not alter striatal DAT membrane expression (38). These effects are reversed (i.e., upregulation of DAT mRNA and protein) during chronic antipsychotic treatments associated with loss of behavioral efficacy (38). We and others (205) have found similar DAT adaptations *in vivo* (38). MicroPET imaging using [18F]FP-CMT ([18F] N-3-fluoropropyl-2-beta-carbomethoxy-3-beta-(4' methylphenyl)) nortropane, with superior properties for imaging the DAT in the living brain (38, 206), was applied to rats at baseline and follow-up (i.e., during loss of antipsychotic efficacy). Rats show an increase in DAT availability (binding potential; BP_{ND}) during antipsychotic failure, suggesting the putative relevance of dopamine clearance for achieving antipsychotic therapeutic response, at least in animal models. Interestingly, changes affecting DAT expression and corresponding behavioral responses to antipsychotics are accompanied by a stable and clinically relevant D_2 receptor blockade (69%) and by increased or decreased extracellular dopamine in the striatum, during the expression of antipsychotic efficacy and failure, respectively (35, 45). Furthermore, the importance of DAT function in antipsychotic efficacy is supported by genetic studies showing an association between clozapine efficacy and DAT gene polymorphism (207). Regarding the question of where dopamine goes when both presynaptic and postsynaptic D_2 receptors are blocked, these studies suggest that it might be captured by DAT, which is upregulated by clinical doses of antipsychotics (38). However, contrary to the obvious theoretical expectation that reduced dopamine would optimize antipsychotic therapeutic response, we found that it coincided with loss of antipsychotic efficacy. This counterintuitive result has been elaborated elsewhere (24, 38), but it will be briefly recapitulated in the next section and discussed in the context of antipsychotic-resistant schizophrenia.

DOPAMINE AUTOINHIBITION AS A FEATURE OF ANTIPSYCHOTIC RESPONSIVENESS

We have previously proposed a model of antipsychotic efficacy, based on the potential therapeutic properties of endogenous dopamine, by taking into account a number of factors encountered in the clinic and in experimental studies with humans and animals (24, 38). We suggested that antipsychotic efficacy, as observed in animals treated with continuous doses of antipsychotics reaching clinically relevant D_2 receptor blockade, is driven by dynamic interactions between endogenous dopamine and presynaptic D_2 receptors. This suggestion is justified by independent but related findings showing that antipsychotic efficacy occurs in conjunction with high striatal extracellular dopamine in humans and animals (35, 38, 45, 46), while only a proportion of the total striatal D_2 receptors are blocked with antipsychotics in human patients (13, 62) and animals (35, 38). On the other hand, antipsychotic treatment failure is observed when extracellular dopamine (35, 38, 45), but not D_2 receptor blockade (38), is decreased (**Figure 1**). This fluctuation in extracellular dopamine and antipsychotic response over continuous treatment regimens characterized by stable D_2 receptor blockade led us to hypothesize that antipsychotics impact the interaction between endogenous dopamine and the D_2 receptor pool available for binding. Under physiological conditions, spontaneous release of dopamine stimulates a greater proportion of D_2 than D_1 receptors (208, 209) and antipsychotics can bind to all dopamine receptors (24, 210). Therefore, when therapeutic doses of antipsychotics reach the brain, about 70% of D_2 receptors will be blocked along with a modest proportion of D_1 receptors. As a consequence, endogenous dopamine will interact with spare dopamine receptors and particularly with D_2 receptors, since this type, relative to D_1 receptors, is stimulated by low levels of dopamine (209). The resulting neuronal response will be dictated by the molecular characteristics of the D_2 receptors (i.e., $G_{i/o}$ inhibitory coupled protein). During phasic dopamine release (i.e., that which would be expected to induce a psychotic episode in schizophrenia), dopamine reaches presynaptic autoreceptors, producing antipsychotic-dependent dopamine-mediated autoinhibition and a corresponding antipsychotic efficacy (24, 38).

This autoinhibition might be mediated by the D_{2S} isoform since the two splice variants have distinct functions and are unevenly distributed within the striatonigral dopaminergic circuitry (**Figure 2A**, **C**). Furthermore, antipsychotics appear to preferentially bind dopamine receptors in the striatum (123), a brain structure with predominant expression of D_{2L} as discussed above, and dopamine exhibits higher binding affinity for D_{2S} in transgenic mice (130) and in cell culture (113). Together these data suggest that therapeutic doses of antipsychotics in the brain cause a functional segregation of D_{2S} and D_{2L}, which based on the data available until now could overlap with a functional segregation of pre- and post-synaptic D_2 receptors (**Figure 2A**, **C**). It should be noted that both isoforms are expressed in pre- and post-synaptic neurons and the functional segregation might also occur within the same cells (**Figure 2B**). In support of this theory are studies with human schizophrenia patients demonstrating selective reduction in expression of D_{2S} mRNA (211), potentially indicative of a desensitization of the short isoform in response to increased dopamine activity on this receptor. On the other hand, postmortem studies also show that D_{2L} mRNA is upregulated in patients with schizophrenia (212), which may indicate an adaptive response to chronic blockade (119).

Since phasic discharge leads to large extracellular increases in dopamine (213) and is thought to underlie psychotic experiences (9, 41, 46, 140, 214–217), we propose that a therapeutic antipsychotic response is obtained by antipsychotic drugs when an adequate proportion of D_2 receptors is blocked and extracellular dopamine levels are sufficiently elevated to trigger autoinhibition. This crucial combination of effects is achieved by the direct blockade of DAT by antipsychotics (38, 146, 147, 165, 189), which allows for an accumulation of synaptic dopamine that reduces the threshold at which phasic dopamine activates homeostatic autoinhibition. The antipsychotic-induced facilitation of dopamine autoinhibition, mediated by DAT blockade and D_2 autoreceptor stimulation, which may serve as an antipsychotic mechanism is depicted in **Figure 3**. Although we have arrived at this hypothesis by analyzing multiple experimental observations, which sometimes lack corresponding human studies, our functional predictions on the association between extracellular dopamine and antipsychotic therapeutic responsiveness in humans and animals have been observed by a number of independent groups (35, 38, 45–48, 49, 218). In the following section, we will provide naturalistic examples of the potential importance of functional DAT to understanding antipsychotic-resistant schizophrenia.

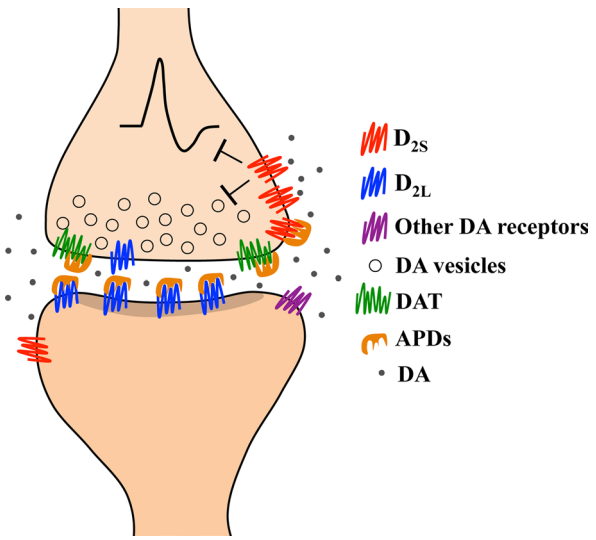


FIGURE 3 | Representation of the hypothesized pharmacological mechanism underlying a therapeutic response in schizophrenia based on human and animal studies. Therapeutic doses of antipsychotic drugs (APDs) block about 70% of striatal D_2 receptors. APDs mostly block heteroreceptors, which are more often D_{2L} than D_{2S}, as well as a smaller proportion of autoreceptors (which are more often D_{2S} than D_{2L}). APDs also block the dopamine transporter (DAT). The combined blockade of D_{2L} heteroreceptors and DAT causes synaptic accumulation of dopamine that allows stimulation of spare D_{2S} receptors. Phasic release of dopamine in response to environmental changes will trigger an enduring autoinhibition since extracellular dopamine levels are already elevated. We hypothesize that the autoinhibition triggered by a phasic discharge of dopamine during antipsychotic treatment is the mechanism underlying a therapeutic antipsychotic response.

THE ROLE OF DAT IN ANTIPSYCHOTIC-RESISTANT SCHIZOPHRENIA: LESSONS FROM AGING AND DRUG ADDICTION

If extracellular dopamine levels contribute to the generation of a therapeutic antipsychotic response and DAT is the main physiological regulator of extracellular dopamine levels, then DAT should have a role in the expression of antipsychotic-resistant schizophrenia. Furthermore, if DAT activity quickly adapts to changes in extracellular dopamine, then it would be surprising if DAT was unaltered in schizophrenia, a disorder with symptoms attributed to dysregulated dopamine neurotransmission. We have described above how blockade of DAT may be a critical factor in antipsychotic efficacy, since DAT blockade allows accumulation of extracellular dopamine and consequently dopamine-mediated autoinhibition upon phasic transmitter release. We have also described research showing that antipsychotics given to rodents at therapeutic doses induce DAT upregulation during loss of behavioral efficacy (38). The loss of efficacy in this scenario coincides with a reduction in extracellular dopamine, which we predict reduces the capacity of dopaminergic terminals to undergo autoinhibition upon phasic release. On the other hand, we introduce below an additional scenario in which reduced expression of DAT may also prove deleterious in terms of antipsychotic therapeutic efficacy. Although theoretically low DAT expression would allow accumulation of extracellular dopamine, which we hypothesize is essential for therapeutic efficacy (**Figure 3**), proteins regulating extracellular dopamine levels including DAT, D_2 autoreceptors, ion channels, and dopamine synthesis machinery appear to be co-regulated (131, 160–163, 172). Thus, DAT downregulation at the expression level may also negatively impact the capacity of dopaminergic terminals to undergo autoinhibition. We predict that downregulation of proteins regulating physiological dopamine neurotransmission at baseline (i.e., tonic neurotransmission) could be the underlying neurobiology of primary antipsychotic treatment-resistant schizophrenia. **Figure 4** depicts a scenario in which the absence of autoinhibition due to ablated DAT expression and autoreceptor co-regulation allows for an enduring stimulation of free unbound post-synaptic receptors, leading to psychosis despite a reduction in dopamine release overall. We can characterize this condition as a form of dopamine supersensitivity driven entirely by presynaptic adaptations. Although DAT expression has been found to change in animal models of antipsychotic responsivity, it cannot be assumed that the same mechanism applies in humans with schizophrenia. Indeed, data may differ across species as already shown with D_2 receptor binding (219) and dopamine synthesis (153). Therefore, why should this principle of species incompatibility not also apply for dopamine uptake? We can gain a better understanding of this issue only after testing it in human patients.

Aging

Meta-analytical studies report that DAT levels in schizophrenia are mostly decreased, unchanged, and sometimes increased (186). These data were obtained mostly with untreated patients

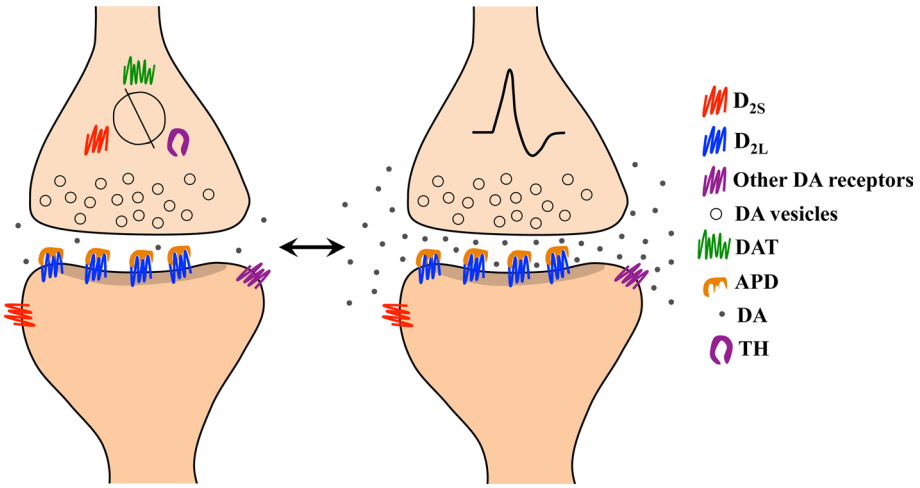


FIGURE 4 | Representation of the pharmacological mechanism underlying the absence of therapeutic response in antipsychotic-resistant schizophrenia based on our model. (Left) Aging and/or addictive drugs consumed before antipsychotic treatment begins (i.e., in first episode psychosis) lead to reduced expression of the dopamine transporter (DAT), D_2 autoreceptors, and tyrosine hydroxylase (TH), as these proteins appear to be co-regulated, at least in rodents. (Right) During environmentally evoked phasic dopamine release, impaired capacity for autoinhibition results from low levels of DAT and D_2 autoreceptors. The resulting post-synaptic stimulation contributes to psychosis despite a significant blockade of D_2 receptors by antipsychotic drugs (APDs).

and therefore we hypothesize (24, 38) that the variability of these results was consequent to genetic factors (220–222) and age. For example, DAT density can decrease with age (223). Based on our proposal, reduced DAT expression as a result of aging can decrease autoinhibition mediated by antipsychotics, due to the co-regulation of autoreceptors described above, and thus reduce antipsychotic response. Interestingly, many of the patients that participated in the aforementioned study were ~40 years old, the age associated with a decline in DAT density (186). It would have been of interest to administer antipsychotics to these individuals and measure their responsiveness. Perhaps, they would have been non-responsive or less responsive than younger individuals and/or those with higher DAT availability. However, these were not the aims of those studies. In support of this suggestion, a previous study (19) showed that the average age of patients with treatment-resistant schizophrenia was 42 years old, while patients responsive to treatment were 25 on average. Interestingly, the treatment with antipsychotics yielded similar levels of D_2 receptor occupancy (19).

Aging is an important factor underlying neuropharmacological responsiveness mediated by the dopaminergic system, since D_2 receptors and DAT expression decline naturally in healthy aging individuals (224–229). The reduction in D_2 receptor and DAT expression is unrelated to dopamine neuron loss (229) and has profound consequences on the antipsychotic therapeutic dosing required to obtain therapeutic responsiveness in schizophrenia (230). Aging can also reveal genetic predisposition to suboptimal DAT and D_2 receptor functions affecting cognitive performance in healthy individuals (231), and it can trigger degeneration of dopaminergic neurons through increased nitrative damage resulting from excess cytosolic dopamine due to an imbalance in DAT/VMAT (vesicular monoamine transporter-2) expression (232). This form of toxicity, deriving from an excess of cytosolic dopamine, has relevance to understand some of the extrapyramidal symptoms (232) and the loss of brain tissue in patients with schizophrenia (233). Although it is not clear if DAT changes are a main player in maladaptive functional and structural changes, both are often observed in schizophrenia and might affect antipsychotic response in elderly patients with schizophrenia (234, 235). While aging could explain the expression of antipsychotic treatment resistance in older patients, it is not yet clear why DAT function would affect antipsychotic responsiveness in younger individual with schizophrenia. A theoretical suggestion is provided in the following section.

Drug Addiction in Schizophrenia

Epidemiological studies report that nearly half of patients with schizophrenia also suffer from drug addiction (236, 237). This is about four times more prevalent than in the general population (238). If we consider that the recreational consumption of addictive drugs is common in the general population (i.e., 84% for alcohol consumption), but only a small proportion of individuals exposed to drugs of abuse become drug addicted (239, 240) and that this happens about four times more often in patients with schizophrenia, then it is possible that many of the remaining ~50% of patients with schizophrenia without formal diagnosis for drug addiction likely consume at least some class of addictive drugs as well. The most commonly consumed drugs in patients with schizophrenia include alcohol, psychostimulants, cannabis, and tobacco (236–238). It has been suggested that patients with schizophrenia may use illicit substances to self-medicate their symptoms (236, 238, 241) as well as the side effects of antipsychotic medications (242), as self-medication with addictive drugs is indeed common in patients with mental illness (243).

All addictive drugs impact the dopaminergic system in the midbrain and in striatal structures (244, 245), a main

component of the brain reward circuitry (246), and likely will also impact the DAT (221, 247–253). We theorize that consumption of substances of abuse to medicate pre-psychotic symptoms during the prodromal period is very likely to trigger psychotic episodes, and importantly, to weaken (or blunt) antipsychotic response since repeated exposure to addictive substances (including psychostimulants, cannabis, tobacco, alcohol and heroin) can decrease DAT membrane expression (248–253). This suggestion is based on our model describing the importance of functional DAT to facilitate antipsychotic mediated autoinhibition (**Figure 3**).

Although reduced DAT expression might be assumed to promote the effectiveness of antipsychotics, since uptake blockade with antipsychotics results in synaptic accumulation of dopamine and facilitates autoinhibition upon phasic dopamine release, receptor desensitization due to a corresponding downregulation (or phosphorylation) of autoreceptors may prevent the occurrence (or reduce the likelihood) of autoinhibition altogether (**Figure 4**). Not only are the DAT and D_2 autoreceptors co-regulated, along with ion channels and the dopamine synthesis machinery (131, 160–163, 172), but reduced DAT, reduced D_2 receptor expression, and reduced dopamine release can all be found in human psychostimulant users (254) and are linked to blunted striatal dopaminergic transmission in human patients with co-morbid schizophrenia and drug addiction (255).

It should be noted that the mechanisms described here and depicted in **Figure 4** apply to the primary form of antipsychotic-resistant schizophrenia and not to acquired antipsychotic resistance (i.e., tolerance) observed in humans (23) and in animal models (35, 38, 45, 91). This distinction is fundamental since DAT plasticity underlying the acquired resistance to antipsychotics is different than what is described here. In fact, based on our own findings from animal models, chronic antipsychotic treatment up-regulates DAT (38), while other studies with humans and animals show that repeated exposure to addictive drugs reduce DAT (254, 256) and both conditions can lead to lack of antipsychotic response [see Ref. (38) for an expanded discussion of acquired antipsychotic resistance and **Figure 4** for a depiction of primary resistance]. The description of several forms of DAT plasticity induced by psychotropic drugs is beyond the scope of this paper, but it should be acknowledged that the reduction of DAT expression with chronic addictive drug use is not absolute and is sensitive to several factors including treatment regimen, drug class, among others, as summarized in these interesting studies (257–259).

Antipsychotic-Resistant Schizophrenia: A Hypothetical Example

A young person who may not be aware of an underlying genetic predisposition to psychosis who becomes exposed to substances of abuse at the same rate as other non-predisposed individuals may risk impacting his or her capacity to buffer excess extracellular dopamine *via* drug-induced downregulation of DAT expression. This individual may seek medical intervention upon first experience of psychosis, at which time he or she will receive antipsychotic treatment and may already face reduced therapeutic efficacy due to the drug-related changes in DAT expression. On the other hand, if patients have no history of addictive substance consumption before starting antipsychotic treatment and begin using moderate doses of addictive drugs thereafter, we speculate that the effects of antipsychotics and certain categories of addictive substances on the expression and function of the dopaminergic machinery (DAT, TH, D_2 receptors) may counterbalance one another (24), producing some therapeutic efficacy for a period of time. This might explain the high rate of smoking and use of illicit substances among patients with schizophrenia.

In summary, we propose that antipsychotic efficacy in patients with schizophrenia and particularly the contribution of DAT expression to antipsychotic response may be influenced by genetic factors as well as environmental factors such as age or history of drug use/abuse. We hypothesize that a history of drug use prior to onset of schizophrenia could be a potential risk factor to becoming antipsychotic treatment resistant, since previous exposure to addictive substances may decrease DAT expression and impair the synaptic machinery required for autoinhibition, which we theorize underlies antipsychotic responsiveness during medical intervention in schizophrenia. Antipsychotic-resistant schizophrenia patients may still respond to clozapine despite reduced DAT expression, because clozapine in particular stimulates serotonin release [for an overview, see Refs. (168, 169)], which suppresses dopaminergic firing (259–262) and thus may compensate for the absence of dopamine-mediated autoinhibition. Though based on a breadth of clinical and bench research, this theoretical suggestion is speculative and requires validation. A more thorough evaluation of this possibility might entail assessment of patient demographics, including history of drug use or abuse, as well as the drug classes used and frequency of use, along with a history of therapeutic responsiveness or resistance when treated with typical or atypical antipsychotics.

CONCLUSION

Although we acknowledge the genetic and neurobiological complexity of schizophrenia and its relevance for the efficacy of pharmacological treatment, we propose that sufficient DAT expression in the brains of patients with schizophrenia may be necessary for an adequate antipsychotic response in first episode psychosis. Particularly, we suggest that the antipsychotic-mediated reduction in dopamine re-uptake by direct DAT blockade allows accumulation of dopamine in the synaptic cleft, which increases the efficiency by which phasically discharged dopamine triggers presynaptic autoinhibition. Furthermore, given the apparent selectivity of antipsychotics for the D_{2L} isoform and the predominant presynaptic expression of D_{2S} in the midbrain, phasic dopamine is likely to activate D_{2S}, which specifically reduces neuronal excitability. Thus, the functional and spatial segregation of the D_2 receptor isoforms within the striatum and midbrain may contribute to the generation of an antipsychotic response. We further propose that consumption of addictive drugs prior to onset of schizophrenia symptoms might reduce expression of both DAT and D_2 autoreceptors and

will increase the risk of antipsychotic resistance upon treatment. Similarly, since DAT and D_2 receptor expression decline with age, aging itself may serve as a risk factor for antipsychotic resistance. Although these hypotheses require further validation, our theory points to the importance of a functional level of membrane DAT expression in patients with schizophrenia in order to gain therapeutic benefit from antipsychotics.

AUTHOR CONTRIBUTIONS

DA conceptualized the ideas presented and wrote the first draft. DA, AK, A-NS, and AH wrote the final manuscript. AK made the figures. All authors have read and approved the final version of the manuscript.

FUNDING

DA is supported by the Deutsche Forschungsgemeinschaft (AM 488/1-1) and by the Brain & Behavior Research Foundation (NARSAD Young Investigator Award 2018). AK is supported by the National Institutes of Health (DA044782). A-NS is supported by a salary award from the Fonds de la Recherche du Québec-Santé (28988).

REFERENCES

1. Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* (2014) 511(7510):421–7. doi: 10.1038/nature13595
2. Sekar A, Bialas AR, de Rivera H, Davis A, Hammond TR, Kamitaki N, et al. Schizophrenia risk from complex variation of complement component 4. *Nature* (2016) 530(7589):177–83. doi: 10.1038/nature16549
3. Avramopoulos D. Recent advances in the genetics of schizophrenia. *Mol Neuropsychiatry* (2018) 4(1):35–51. doi: 10.1159/000488679
4. van Os J, Kenis G, Rutten BP. The environment and schizophrenia. *Nature* (2010) 468(7321):203–12. doi: 10.1038/nature09563
5. Awad AG, Voruganti LN, Heslegrave RJ. A conceptual model of quality of life in schizophrenia: description and preliminary clinical validation. *Qual Life Res* (1997) 6(1):21–6.
6. Wong AH, Voruganti LN, Heslegrave RJ, Awad AG. Neurocognitive deficits and neurological signs in schizophrenia. *Schizophr Res* (1997) 23(2):139–46. doi: 10.1016/S0920-9964(96)00095-3
7. Fusar-Poli P, Borgwardt S, Bechdolf A, Addington J, Riecher-Rossler A, Schultze-Lutter F, et al. The psychosis high-risk state: a comprehensive state-of-the-art review. *JAMA Psychiatry* (2013) 70(1):107–20. doi: 10.1001/jamapsychiatry.2013.269
8. Heinrichs RW, Zakzanis KK. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology* (1998) 12(3):426–45. doi: 10.1037//0894-4105.12.3.426
9. Heinz A. Dopaminergic dysfunction in alcoholism and schizophrenia—psychopathological and behavioral correlates. *Eur Psychiatry* (2002) 17(1):9–16. doi: 10.1016/S0924-9338(02)00628-4
10. de Bartolomeis A, Balletta R, Giordano S, Buonaguro EF, Latte G, Iasevoli F. Differential cognitive performances between schizophrenic responders and non-responders to antipsychotics: correlation with course of the illness, psychopathology, attitude to the treatment and antipsychotics doses. *Psychiatry Res* (2013) 210(2):387–95. doi: 10.1016/j.psychres.2013.06.042
11. Green MF, Harvey PD. Cognition in schizophrenia: past, present, and future. *Schizophr Res Cogn* (2014) 1(1):e1–e9. doi: 10.1016/j.scog.2014.02.001
12. Radua J, Ramella-Cravaro V, Ioannidis JPA, Reichenberg A, Phiphopthatsanee N, Amir T, et al. What causes psychosis? An umbrella review of risk and protective factors. *World Psychiatry* (2018) 17(1):49–66. doi: 10.1002/wps.20490
13. Kapur S, Zipursky R, Jones C, Remington G, Houle S. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. *Am J Psychiatry* (2000a) 157(4):514–20. doi: 10.1176/appi.ajp.157.4.514
14. Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* (1976) 192(4238):481–3. doi: 10.1126/science.3854
15. Seeman P, Lee T, Chau-Wong M, Wong K. Antipsychotic drug doses and neuroleptic/dopamine receptors. *Nature* (1976) 261(5562):717–9. doi: 10.1038/261717a0
16. Johnstone EC, Crow TJ, Frith CD, Carney MW, Price JS. Mechanism of the antipsychotic effect in the treatment of acute schizophrenia. *Lancet* (1978) 1(8069):848–51. doi: 10.1016/S0140-6736(78)90193-9
17. Howes OD, Egerton A, Allan V, McGuire P, Stokes P, Kapur S. Mechanisms underlying psychosis and antipsychotic treatment response in schizophrenia: insights from PET and SPECT imaging. *Curr Pharm Des* (2009) 15(22):2550–9. doi: 10.2174/138161209788957528
18. Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch Gen Psychiatry* (1988) 45(9):789–96. doi: 10.1001/archpsyc.1988.01800330013001
19. Wolkin A, Barouche F, Wolf AP, Rotrosen J, Fowler JS, Shiue CY, et al. Dopamine blockade and clinical response: evidence for two biological subgroups of schizophrenia. *Am J Psychiatry* (1989) 146(7):905–8. doi: 10.1176/ajp.146.7.905
20. Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G. Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. *Arch Gen Psychiatry* (1992) 49(7):538–44. doi: 10.1001/archpsyc.1992.01820070032005
21. Pilowsky LS, Costa DC, Ell PJ, Murray RM, Verhoeff NP, Kerwin RW. Clozapine, single photon emission tomography, and the D2 dopamine receptor blockade hypothesis of schizophrenia. *Lancet* (1992) 340(8813):199–202. doi: 10.1016/0140-6736(92)90467-H
22. Pilowsky LS, Costa DC, Ell PJ, Murray RM, Verhoeff NP, Kerwin RW. Antipsychotic medication, D2 dopamine receptor blockade and clinical response: a 123I IBZM SPET (single photon emission tomography) study. *Psychol Med* (1993) 23(3):791–7. doi: 10.1017/S0033291700025575
23. Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Engl J Med* (2005) 353(12):1209–23. doi: 10.1056/NEJMoa051688
24. Amato D, Vernon AC, Papaleo F. Dopamine, the antipsychotic molecule: a perspective on mechanisms underlying antipsychotic response variability. *Neurosci Biobehav Rev* (2017) 85:146–59. doi: 10.1016/j.neubiorev.2017.09.027
25. Demjaha A, Lappin JM, Stahl D, Patel MX, MacCabe JH, Howes OD, et al. Antipsychotic treatment resistance in first-episode psychosis: prevalence, subtypes and predictors. *Psychol Med* (2017) 47(11):1981–9. doi: 10.1017/S0033291717000435
26. Beasley CM, Jr., Stauffer VL, Liu-Seifert H, Taylor CC, Dunayevich E, Davis JM. All-cause treatment discontinuation in schizophrenia during treatment with olanzapine relative to other antipsychotics: an integrated analysis. *J Clin Psychopharmacol* (2007) 27(3):252–8. doi: 10.1097/JCP.0b013e3180582426
27. Wyatt RJ. Neuroleptics and the natural course of schizophrenia. *Schizophr Bull* (1991) 17(2):325–51. doi: 10.1093/schbul/17.2.325
28. Sullivan G, Wells KB, Morgenstern H, Leake B. Identifying modifiable risk factors for rehospitalization: a case-control study of seriously mentally ill persons in Mississippi. *Am J Psychiatry* (1995) 152(12):1749–56. doi: 10.1176/ajp.152.12.1749

29. Morken G, Widen JH, Grawe RW. Non-adherence to antipsychotic medication, relapse and rehospitalisation in recent-onset schizophrenia. *BMC Psychiatry* (2008) 8:32. doi: 10.1186/1471-244X-8-32
30. Takeuchi H, Siu C, Remington G, Fervaha G, Zipursky RB, Foussias G, et al. Does relapse contribute to treatment resistance? Antipsychotic response in first- vs. second-episode schizophrenia. *Neuropsychopharmacology* (2018) 44(6):1036–42. doi: 10.1038/s41386-018-0278-3
31. Meltzer HY. Treatment-resistant schizophrenia—the role of clozapine. *Curr Med Res Opin* (1997) 14(1):1–20. doi: 10.1185/03007999709113338
32. McEvoy JP, Lieberman JA, Stroup TS, Davis SM, Meltzer HY, Rosenheck RA, et al. 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* (2006) 163(4):600–10. doi: 10.1176/appi.ajp.163.4.600
33. Lieberman JA, Safferman AZ, Pollack S, Szymanski S, Johns C, Howard A, et al. Clinical effects of clozapine in chronic schizophrenia: response to treatment and predictors of outcome. *Am J Psychiatry* (1994) 151(12):1744–52. doi: 10.1176/ajp.151.12.1744
34. Chouinard G, Jones BD. Neuroleptic-induced supersensitivity psychosis: clinical and pharmacologic characteristics. *Am J Psychiatry* (1980) 137(1):16–21. doi: 10.1176/ajp.137.1.16
35. Samaha AN, Seeman P, Stewart J, Rajabi H, Kapur S. "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. *J Neurosci* (2007) 27(11):2979–86. doi: 10.1523/JNEUROSCI.5416-06.2007
36. Molina V, Reig S, Sanz J, Palomo T, Benito C, Sarramea F, et al. Differential clinical, structural and P300 parameters in schizophrenia patients resistant to conventional neuroleptics. *Prog Neuropsychopharmacol Biol Psychiatry* (2008) 32(1):257–66. doi: 10.1016/j.pnpbp.2007.08.017
37. Demjaha A, Egerton A, Murray RM, Kapur S, Howes OD, Stone JM, et al. Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function. *Biol Psychiatry* (2014) 75(5):e11–13. doi: 10.1016/j.biopsych.2013.06.011
38. Amato D, Canneva F, Cumming P, Maschauer S, Groos D, Wrosch JK, et al. A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter. *Molecular Psychiatry* (2018). doi: 10.1038/s41380-018-0114-5
39. Iwata Y, Nakajima S, Plitman E, Caravaggio F, Kim J, Shah P, et al. Glutamatergic neurometabolite levels in patients with ultra-treatment-resistant schizophrenia: a cross-sectional 3T proton magnetic resonance spectroscopy study. *Biol Psychiatry* (2018) 85(7):596–605. doi: 10.1016/j.biopsych.2018.09.009
40. Kane JM, Agid O, Baldwin ML, Howes O, Lindenmayer JP, Marder S, et al. Clinical guidance on the identification and management of treatment-resistant schizophrenia. *J Clin Psychiatry* (2019) 80(2):e1–9. doi: 10.4088/JCP.18com12123
41. Kapur S. Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. *Am J Psychiatry* (2003) 160(1):13–23. doi: 10.1176/appi.ajp.160.1.13
42. Abi-Dargham A. From "bedside" to "bench" and back: a translational approach to studying dopamine dysfunction in schizophrenia. *Neurosci Biobehav Rev* (2018) S0149-7634(18)30314-2. doi: 10.1016/j.neubiorev.2018.12.003
43. Slifstein M, Abi-Dargham A. Is it pre- or postsynaptic? Imaging striatal dopamine excess in schizophrenia. *Biol Psychiatry* (2018) 83(8):635–7. doi: 10.1016/j.biopsych.2018.02.015
44. Huang E, Zai CC, Lisoway A, Maciukiewicz M, Felsky D, Tiwari AK, et al. Catechol-O-methyltransferase Val158Met polymorphism and clinical response to antipsychotic treatment in schizophrenia and schizo-affective disorder patients: a meta-analysis. *Int J Neuropsychopharmacol* (2016) 19(5):1–12. doi: 10.1093/ijnp/pyv132
45. Amato D, Natesan S, Yavich L, Kapur S, Muller CP. Dynamic regulation of dopamine and serotonin responses to salient stimuli during chronic haloperidol treatment. *Int J Neuropsychopharmacol* (2011) 14(10):1327–39. doi: 10.1017/S1461145711000010
46. Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, et al. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci U S A* (2000) 97(14):8104–9. doi: 10.1073/pnas.97.14.8104
47. Scheggia D, Mastrogiacomo R, Mereu M, Sannino S, Straub RE, Armando M, et al. Variations in Dysbindin-1 are associated with cognitive response to antipsychotic drug treatment. *Nat Commun* (2018) 9(1):2265. doi: 10.1038/s41467-018-04711-w
48. Swerdlow NR, Bhakta SG, Talledo JA, Franz DM, Hughes EL, Rana BK, et al. Effects of amphetamine on sensorimotor gating and neurocognition in antipsychotic-medicated schizophrenia patients. *Neuropsychopharmacology* (2018) 43(4):708–17. doi: 10.1038/npp.2017.285
49. Caravaggio F, Iwata Y, Kim J, Shah P, Gerretsen P, Remington G, et al. What proportion of striatal D2 receptors are occupied by endogenous dopamine at baseline? A meta-analysis with implications for understanding antipsychotic occupancy. *Neuropharmacology* (2019) S0028-3908(19)30114-5. doi: 10.1016/j.neuropharm.2019.03.034
50 Tamminga CA, Schaffer MH, Smith RC, Davis JM. Schizophrenic symptoms improve with apomorphine. Science (1978) 200(4341):567–8. doi: 10.1126/science.347574
51. Weinberger DR. Thinking about schizophrenia in an era of genomic medicine. *Am J Psychiatry* (2019) 176(1):12–20. doi: 10.1176/appi.ajp.2018.18111275
52. Karam CS, Ballon JS, Bivens NM, Freyberg Z, Girgis RR, Lizardi-Ortiz JE, et al. Signaling pathways in schizophrenia: emerging targets and therapeutic strategies. *Trends Pharmacol Sci* (2010) 31(8):381–90. doi: 10.1016/j.tips.2010.05.004
53. Gilmore JH, Jarskog LF. Exposure to infection and brain development: cytokines in the pathogenesis of schizophrenia. *Schizophr Res* (1997) 24(3):365–7. doi: 10.1016/S0920-9964(96)00123-5
54. Nicodemus KK, Marenco S, Batten AJ, Vakkalanka R, Egan MF, Straub RE, et al. Serious obstetric complications interact with hypoxia-regulated/vascular-expression genes to influence schizophrenia risk. *Mol Psychiatry* (2008) 13(9):873–7. doi: 10.1038/sj.mp.4002153
55. Moreno JL, Kurita M, Holloway T, Lopez J, Cadagan R, Martinez-Sobrido L, et al. Maternal influenza viral infection causes schizophrenia-like alterations of 5-HT(2)A and mGlu(2) receptors in the adult offspring. *J Neurosci* (2011) 31(5):1863–72. doi: 10.1523/JNEUROSCI.4230-10.2011
56. Holloway T, Moreno JL, Umali A, Rayannavar V, Hodes GE, Russo SJ, et al. Prenatal stress induces schizophrenia-like alterations of serotonin 2A and metabotropic glutamate 2 receptors in the adult offspring: role of maternal immune system. *J Neurosci* (2013) 33(3):1088–98. doi: 10.1523/JNEUROSCI.2331-12.2013
57. Jiang Z, Rompala GR, Zhang S, Cowell RM, Nakazawa K. Social isolation exacerbates schizophrenia-like phenotypes *via* oxidative stress in cortical interneurons. *Biol Psychiatry* (2013) 73(10):1024–34. doi: 10.1016/j.biopsych.2012.12.004
58. Mizrahi R. Social stress and psychosis risk: common neurochemical substrates? *Neuropsychopharmacology* (2016) 41(3):666–74. doi: 10.1038/npp.2015.274
59. Kapur S, Mamo D. Half a century of antipsychotics and still a central role for dopamine D2 receptors. *Prog Neuropsychopharmacol Biol Psychiatry* (2003) 27(7):1081–90. doi: 10.1016/j.pnpbp.2003.09.004
60. van Rossum JM. The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. *Arch Int Pharmacodyn Ther* (1966) 160(2):492–4.
61. Miyamoto S, Duncan GE, Marx CE, Lieberman JA. Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol Psychiatry* (2005) 10(1):79–104. doi: 10.1038/sj.mp.4001556
62. Nord M, Farde L. Antipsychotic occupancy of dopamine receptors in schizophrenia. *CNS Neurosci Ther* (2011) 17(2):97–103. doi: 10.1111/j.1755-5949.2010.00222.x
63. Seeman P, Tallerico T. Antipsychotic drugs which elicit little or no parkinsonism bind more loosely than dopamine to brain D2 receptors, yet occupy high levels of these receptors. *Mol Psychiatry* (1998) 3(2):123–34. doi: 10.1038/sj.mp.4000336
64. Suhara T, Okauchi T, Sudo Y, Takano A, Kawabe K, Maeda J, et al. Clozapine can induce high dopamine D(2) receptor occupancy *in vivo*. *Psychopharmacology (Berl)* (2002) 160(1):107–12. doi: 10.1007/s00213-001-0967-0

65. Gefvert O, Lundberg T, Wieselgren IM, Bergstrom M, Langstrom B, Wiesel F, et al. D(2) and 5HT(2A) receptor occupancy of different doses of quetiapine in schizophrenia: a PET study. *Eur Neuropsychopharmacol* (2001) 11(2):105–10. doi: 10.1016/S0924-977X(00)00133-4
66. Farde L, Wiesel FA, Nordstrom AL, Sedvall G. D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics. *Psychopharmacology (Berl)* (1989) 99:S28–31. doi: 10.1007/BF00442555
67. Nordstrom AL, Farde L, Halldin C. Time course of D2-dopamine receptor occupancy examined by PET after single oral doses of haloperidol. *Psychopharmacology (Berl)* (1992) 106(4):433–8. doi: 10.1007/BF02244811
68. Gefvert O, Bergstrom M, Langstrom B, Lundberg T, Lindstrom L, Yates R. Time course of central nervous dopamine-D2 and 5-HT2 receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel) in patients with schizophrenia. *Psychopharmacology (Berl)* (1998) 135(2):119–26. doi: 10.1007/s002130050492
69. Kapur S, Zipursky R, Jones C, Shammi CS, Remington G, Seeman P. A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D2 receptor occupancy. *Arch Gen Psychiatry* (2000b) 57(6):553–9. doi: 10.1001/archpsyc.57.6.553
70. Kapur S, Seeman P. Antipsychotic agents differ in how fast they come off the dopamine D2 receptors. Implications for atypical antipsychotic action. *J Psychiatry Neurosci* (2000) 25(2):161–6.
71. Seeman P, Guan HC, Niznik HB. Endogenous dopamine lowers the dopamine D2 receptor density as measured by [3H]raclopride: implications for positron emission tomography of the human brain. *Synapse* (1989) 3(1):96–7. doi: 10.1002/syn.890030113
72. Seeman P. Therapeutic receptor-blocking concentrations of neuroleptics. *Int Clin Psychopharmacol* (1995) 10(Suppl 3):5–13. doi: 10.1097/00004850-199509000-00002
73. Seeman P, Van Tol HH. Deriving the therapeutic concentrations for clozapine and haloperidol: the apparent dissociation constant of a neuroleptic at the dopamine D2 or D4 receptor varies with the affinity of the competing radioligand. *Eur J Pharmacol* (1995) 291(2):59–66. doi: 10.1016/0922-4106(95)90125-6
74. Seeman P, Tallerico T. Rapid release of antipsychotic drugs from dopamine D2 receptors: an explanation for low receptor occupancy and early clinical relapse upon withdrawal of clozapine or quetiapine. *Am J Psychiatry* (1999) 156(6):876–84. doi: 10.1176/ajp.156.6.876
75. Seeman P. Atypical antipsychotics: mechanism of action. *Can J Psychiatry* (2002) 47(1):27–38. doi: 10.1177/070674370204700105
76. Sahlholm K, Marcellino D, Nilsson J, Ogren SO, Fuxe K, Arhem P. Typical and atypical antipsychotics do not differ markedly in their reversibility of antagonism of the dopamine D2 receptor. *Int J Neuropsychopharmacol* (2014) 17(1):149–55. doi: 10.1017/S1461145713000801
77. Sahlholm K, Zeberg H, Nilsson J, Ogren SO, Fuxe K, Arhem P. The fast-off hypothesis revisited: a functional kinetic study of antipsychotic antagonism of the dopamine D2 receptor. *Eur Neuropsychopharmacol* (2016) 26(3):467–76. doi: 10.1016/j.euroneuro.2016.01.001
78. Heinz A, Knable MB, Weinberger DR. Dopamine D2 receptor imaging and neuroleptic drug response. *J Clin Psychiatry* (1996) 57(Suppl 11):84–8; discussion 89-93.
79. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am J Psychiatry* (1999) 156(2):286–93. doi: 10.1176/ajp.156.2.286
80. Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet* (2013) 382(9896):951–62. doi: 10.1016/S0140-6736(13)60733-3
81. Emsley R, Chiliza B, Asmal L, Harvey BH. The nature of relapse in schizophrenia. *BMC Psychiatry* (2013) 13:50. doi: 10.1186/1471-244X-13-50
82. Mamo D, Kapur S, Keshavan M, Laruelle M, Taylor CC, Kothare PA, et al. D2 receptor occupancy of olanzapine pamoate depot using positron emission tomography: an open-label study in patients with schizophrenia. *Neuropsychopharmacology* (2008) 33(2):298–304. doi: 10.1038/sj.npp.1301409
83. Nyberg S, Farde L, Halldin C, Dahl ML, Bertilsson L. D2 dopamine receptor occupancy during low-dose treatment with haloperidol decanoate. *Am J Psychiatry* (1995) 152(2):173–8. doi: 10.1176/ajp.152.2.173
84. Remington G, Mamo D, Labelle A, Reiss J, Shammi C, Mannaert E, et al. A PET study evaluating dopamine D2 receptor occupancy for long-acting injectable risperidone. *Am J Psychiatry* (2006) 163(3):396–401. doi: 10.1176/appi.ajp.163.3.396
85. Uchida H, Suzuki T. Dose and dosing frequency of long-acting injectable antipsychotics: a systematic review of PET and SPECT data and clinical implications. *J Clin Psychopharmacol* (2014) 34(6):728–35. doi: 10.1097/JCP.0000000000000065
86. Silvestri S, Seeman MV, Negrete JC, Houle S, Shammi CM, Remington GJ, et al. Increased dopamine D2 receptor binding after long-term treatment with antipsychotics in humans: a clinical PET study. *Psychopharmacology (Berl)* (2000) 152(2):174–80. doi: 10.1007/s002130000532
87. Iyo M, Tadokoro S, Kanahara N, Hashimoto T, Niitsu T, Watanabe H, et al. Optimal extent of dopamine D2 receptor occupancy by antipsychotics for treatment of dopamine supersensitivity psychosis and late-onset psychosis. *J Clin Psychopharmacol* (2013) 33(3):398–404. doi: 10.1097/JCP.0b013e31828ea95c
88. Chouinard G, Jones BD, Annable L. Neuroleptic-induced supersensitivity psychosis. *Am J Psychiatry* (1978) 135(11):1409–10. doi: 10.1176/ajp.135.11.1409
89. Fallon P, Dursun S, Deakin B. Drug-induced supersensitivity psychosis revisited: characteristics of relapse in treatment-compliant patients. *Ther Adv Psychopharmacol* (2012) 2(1):13–22. doi: 10.1177/2045125311431105
90. Suzuki T, Kanahara N, Yamanaka H, Takase M, Kimura H, Watanabe H, et al. Dopamine supersensitivity psychosis as a pivotal factor in treatment-resistant schizophrenia. *Psychiatry Res* (2015) 227(2–3):278–82. doi: 10.1016/j.psychres.2015.02.021
91. Samaha AN, Reckless GE, Seeman P, Diwan M, Nobrega JN, Kapur S. Less is more: antipsychotic drug effects are greater with transient rather than continuous delivery. *Biol Psychiatry* (2008) 64(2):145–52. doi: 10.1016/j.biopsych.2008.01.010
92. Gill KM, Cook JM, Poe MM, Grace AA. Prior antipsychotic drug treatment prevents response to novel antipsychotic agent in the methylazoxymethanol acetate model of schizophrenia. *Schizophr Bull* (2014) 40(2):341–50. doi: 10.1093/schbul/sbt236
93. Smith RC, Davis JM. Behavioral supersensitivity to apomorphine and amphetamine after chronic high dose haloperidol treatment. *Psychopharmacol Commun* (1975) 1(3):285–93.
94. Bedard AM, Maheux J, Levesque D, Samaha AN. Continuous, but not intermittent, antipsychotic drug delivery intensifies the pursuit of reward cues. *Neuropsychopharmacology* (2011) 36(6):1248–59. doi: 10.1038/npp.2011.10
95. Ericson H, Radesater AC, Servin E, Magnusson O, Mohringe B. Effects of intermittent and continuous subchronic administration of raclopride on motor activity, dopamine turnover and receptor occupancy in the rat. *Pharmacol Toxicol* (1996) 79(6):277–86. doi: 10.1111/j.1600-0773.1996.tb00009.x
96. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry* (1991) 148(11):1474–86. doi: 10.1176/ajp.148.11.1474
97. Laruelle M. Imaging dopamine transmission in schizophrenia. A review and meta-analysis. *Q J Nucl Med* (1998) 42(3):211–21.
98. Pierce RC, Rowlett JK, Bardo MT, Rebec GV. Chronic ascorbate potentiates the effects of chronic haloperidol on behavioral supersensitivity but not D2 dopamine receptor binding. *Neuroscience* (1991) 45(2):373–8. doi: 10.1016/0306-4522(91)90234-F
99. Flores G, Barbeau D, Quirion R, Srivastava LK. Decreased binding of dopamine D3 receptors in limbic subregions after neonatal bilateral lesion of rat hippocampus. *J Neurosci* (1996) 16(6):2020–6. doi: 10.1523/JNEUROSCI.16-06-02020.1996
100. Ginovart N, Wilson AA, Hussey D, Houle S, Kapur S. D2-receptor upregulation is dependent upon temporal course of D2-occupancy: a longitudinal [11C]-raclopride PET study in cats. *Neuropsychopharmacology* (2009) 34(3):662–71. doi: 10.1038/npp.2008.116

101. Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, Grandy DK, et al. Dopamine supersensitivity correlates with D2High states, implying many paths to psychosis. *Proc Natl Acad Sci U S A* (2005) 102(9):3513–8. doi: 10.1073/pnas.0409766102
102. Graff-Guerrero A, Mizrahi R, Agid O, Marcon H, Barsoum P, Rusjan P, et al. The dopamine D2 receptors in high-affinity state and D3 receptors in schizophrenia: a clinical [11C]-(+)-PHNO PET study. *Neuropsychopharmacology* (2009) 34(4):1078–86. doi: 10.1038/npp.2008.199
103. Smith AD, Bolam JP. The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. *Trends Neurosci* (1990) 13(7):259–65. doi: 10.1016/0166-2236(90)90106-K
104. Levey AI, Hersch SM, Rye DB, Sunahara RK, Niznik HB, Kitt CA, et al. Localization of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. *Proc Natl Acad Sci U S A* (1993) 90(19):8861–65. doi: 10.1073/pnas.90.19.8861
105. Dal Toso R, Sommer B, Ewert M, Herb A, Pritchett DB, Bach A, et al. The dopamine D2 receptor: two molecular forms generated by alternative splicing. *EMBO J* (1989) 8(13):4025–34. doi: 10.1002/j.1460-2075.1989.tb08585.x
106. Giros B, Sokoloff P, Martres MP, Riou JF, Emorine LJ, Schwartz JC. Alternative splicing directs the expression of two D2 dopamine receptor isoforms. *Nature* (1989) 342(6252):923–6. doi: 10.1038/342923a0
107. Monsma FJ, Jr., McVittie LD, Gerfen CR, Mahan LC, Sibley DR. Multiple D2 dopamine receptors produced by alternative RNA splicing. *Nature* (1989) 342(6252):926–9. doi: 10.1038/342926a0
108. Jang JY, Jang M, Kim SH, Um KB, Kang YK, Kim HJ, et al. Regulation of dopaminergic neuron firing by heterogeneous dopamine autoreceptors in the substantia nigra pars compacta. *J Neurochem* (2011) 116(6):966–74. doi: 10.1111/j.1471-4159.2010.07107.x
109. Gantz SC, Robinson BG, Buck DC, Bunzow JR, Neve RL, Williams JT, et al. Distinct regulation of dopamine D2S and D2L autoreceptor signaling by calcium. *Elife* (2015) 4:1–19. doi: 10.7554/eLife.09358
110. Robinson BG, Condon AF, Radl D, Borrelli E, Williams JT, Neve KA. Cocaine-induced adaptation of dopamine D2S, but not D2L autoreceptors. *Elife* (2017) 6:1–8. doi: 10.7554/eLife.31924
111. Centonze D, Gubellini P, Usiello A, Rossi S, Tscherter A, Bracci E, et al. Differential contribution of dopamine D2S and D2L receptors in the modulation of glutamate and GABA transmission in the striatum. *Neuroscience* (2004) 129(1):157–66. doi: 10.1016/j.neuroscience.2004.07.043
112. Zhang Y, Bertolino A, Fazio L, Blasi G, Rampino A, Romano R, et al. Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. *Proc Natl Acad Sci U S A* (2007) 104(51):20552–7. doi: 10.1073/pnas.0707106104
113. Montmayeur JP, Borrelli E. Transcription mediated by a cAMP-responsive promoter element is reduced upon activation of dopamine D2 receptors. *Proc Natl Acad Sci U S A* (1991) 88(8):3135–9. doi: 10.1073/pnas.88.8.3135
114. Senogles SE. The D2 dopamine receptor isoforms signal through distinct Gi alpha proteins to inhibit adenylyl cyclase. A study with site-directed mutant Gi alpha proteins. *J Biol Chem* (1994) 269(37):23120–7. doi: 10.1073/pnas.0730708100
115. Guiramand J, Montmayeur JP, Ceraline J, Bhatia M, Borrelli E. Alternative splicing of the dopamine D2 receptor directs specificity of coupling to G-proteins. *J Biol Chem* (1995) 270(13):7354–8. doi: 10.1074/jbc.270.13.7354
116. Lindgren N, Usiello A, Goiny M, Haycock J, Erbs E, Greengard P, et al. Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites. *Proc Natl Acad Sci U S A* (2003) 100(7):4305–9. doi: 10.1073/pnas.0730708100
117. Mack KJ, O'Malley KL, Todd RD. Differential expression of dopaminergic D2 receptor messenger RNAs during development. *Brain Res Dev Brain Res* (1991) 59(2):249–51. doi: 10.1016/0165-3806(91)90105-R
118. O'Malley KL, Mack KJ, Gandelman KY, Todd RD. Organization and expression of the rat D2A receptor gene: identification of alternative transcripts and a variant donor splice site. *Biochemistry* (1990) 29(6):1367–71. doi: 10.1021/bi00458a003
119. Neve KA, Neve RL, Fidel S, Janowsky A, Higgins GA. Increased abundance of alternatively spliced forms of D2 dopamine receptor mRNA after denervation. *Proc Natl Acad Sci U S A* (1991) 88(7):2802–6. doi: 10.1073/pnas.88.7.2802
120. Gandelman KY, Harmon S, Todd RD, O'Malley KL. Analysis of the structure and expression of the human dopamine D2A receptor gene. *J Neurochem* (1991) 56(3):1024–9. doi: 10.1111/j.1471-4159.1991.tb02024.x
121. Khan ZU, Mrzljak L, Gutierrez A, de la Calle A, Goldman-Rakic PS. Prominence of the dopamine D2 short isoform in dopaminergic pathways. *Proc Natl Acad Sci U S A* (1998) 95(13):7731–6. doi: 10.1073/pnas.95.13.7731
122. Meador-Woodruff JH, Mansour A, Civelli O, Watson SJ. Distribution of D2 dopamine receptor mRNA in the primate brain. *Prog Neuropsychopharmacol Biol Psychiatry* (1991) 15(6):885–93. doi: 10.1016/0278-5846(91)90016-T
123. Fishburn CS, David C, Carmon S, Fuchs S. The effect of haloperidol on D2 dopamine receptor subtype mRNA levels in the brain. *FEBS Lett* (1994) 339(1–2):63–6. doi: 10.1016/0014-5793(94)80385-4
124. Castro SW, Strange PG. Differences in the ligand binding properties of the short and long versions of the D2 dopamine receptor. *J Neurochem* (1993) 60(1):372–5. doi: 10.1111/j.1471-4159.1993.tb05863.x
125. Malmberg A, Jackson DM, Eriksson A, Mohell N. Unique binding characteristics of antipsychotic agents interacting with human dopamine D2A, D2B, and D3 receptors. *Mol Pharmacol* (1993) 43(5):749–54.
126. Schotte A, Janssen PF, Gommeren W, Luyten WH, Van Gompel P, Lesage AS, et al. Risperidone compared with new and reference antipsychotic drugs: *in vitro* and *in vivo* receptor binding. *Psychopharmacology (Berl)* (1996) 124(1–2):57–73. doi: 10.1007/BF02245606
127. Kongsamut S, Roehr JE, Cai J, Hartman HB, Weissensee P, Kerman LL, et al. Iloperidone binding to human and rat dopamine and 5-HT receptors. *Eur J Pharmacol* (1996) 317(2–3):417–23. doi: 10.1016/S0014-2999(96)00840-0
128. Usiello A, Baik JH, Rouge-Pont F, Picetti R, Dierich A, LeMeur M, et al. Distinct functions of the two isoforms of dopamine D2 receptors. *Nature* (2000) 408(6809):199–203. doi: 10.1038/35041572
129. Wang Y, Xu R, Sasaoka T, Tonegawa S, Kung MP, Sankoorikal EB. Dopamine D2 long receptor-deficient mice display alterations in striatum-dependent functions. *J Neurosci* (2000) 20(22):8305–14. doi: 10.1523/JNEUROSCI.20-22-08305.2000
130. Xu R, Hranilovic D, Fetsko LA, Bucan M, Wang Y. Dopamine D2S and D2L receptors may differentially contribute to the actions of antipsychotic and psychotic agents in mice. *Mol Psychiatry* (2002) 7(10):1075–82. doi: 10.1038/sj.mp.4001145
131. Anzalone A, Lizardi-Ortiz JE, Ramos M, De Mei C, Hopf FW, Iaccarino C, et al. Dual control of dopamine synthesis and release by presynaptic and postsynaptic dopamine D2 receptors. *J Neurosci* (2012) 32(26):9023–34. doi: 10.1523/JNEUROSCI.0918-12.2012
132. Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B, et al. Occupancy of striatal and extrastriatal dopamine D2 receptors by clozapine and quetiapine. *Neuropsychopharmacology* (2006) 31(9):1991–2001. doi: 10.1038/sj.npp.1301108
133. Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B, et al. Occupancy of striatal and extrastriatal dopamine D2/D3 receptors by olanzapine and haloperidol. *Neuropsychopharmacology* (2005) 30:2283–9. doi: 10.1038/sj.npp.1300836
134. Roberts DA, Balderson D, Pickering-Brown SM, Deakin JF, Owen F. The abundance of mRNA for dopamine D2 receptor isoforms in brain tissue from controls and schizophrenics. *Brain Res Mol Brain Res* (1994) 25(1–2):173–5. doi: 10.1016/0169-328X(94)90296-8
135. Le Moine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B. Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proc Natl Acad Sci U S A* (1990) 87(1):230–4. doi: 10.1073/pnas.87.1.230
136. Angulo JA, Coirini H, Ledoux M, Schumacher M. Regulation by dopaminergic neurotransmission of dopamine D2 mRNA and receptor levels in the striatum and nucleus accumbens of the rat. *Brain Res Mol Brain Res* (1991) 11(2):161–6. doi: 10.1016/0169-328X(91)90117-G
137. van Tol HH, Riva M, Civelli O, Creese I. Lack of effect of chronic dopamine receptor blockade on D2 dopamine receptor mRNA level. *Neurosci Lett* (1990) 111(3):303–8. doi: 10.1016/0304-3940(90)90279-I
138. Srivastava LK, Morency MA, Bajwa SB, Mishra RK. Effect of haloperidol on expression of dopamine D2 receptor mRNAs in rat brain. *J Mol Neurosci* (1990) 2(3):155–61. doi: 10.1007/BF02896840

139. Fox CA, Mansour A, Watson SJ, Jr. The effects of haloperidol on dopamine receptor gene expression. *Exp Neurol* (1994) 130(2):288–303. doi: 10.1006/exnr.1994.1207
140. Laruelle M, Abi-Dargham A. Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. *J Psychopharmacol* (1999) 13(4):358–71. doi: 10.1177/026988119901300405
141. Roth RH. CNS dopamine autoreceptors: distribution, pharmacology, and function. *Ann N Y Acad Sci* (1984) 430:27–53. doi: 10.1111/j.1749-6632.1984.tb14497.x
142. Westerink BH, de Vries JB. On the mechanism of neuroleptic induced increase in striatal dopamine release: brain dialysis provides direct evidence for mediation by autoreceptors localized on nerve terminals. *Neurosci Lett* (1989) 99(1–2):197–202. doi: 10.1016/0304-3940(89)90289-9
143. Bunney BS, Walters JR, Roth RH, Aghajanian GK. Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. *J Pharmacol Exp Ther* (1973) 185(3):560–71.
144. Chiodo LA, Bunney BS. Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. *J Neurosci* (1983) 3(8):1607–19. doi: 10.1523/JNEUROSCI.03-08-01607.1983
145. Santiago M, Westerink BH. The regulation of dopamine release from nigrostriatal neurons in conscious rats: the role of somatodendritic autoreceptors. *Eur J Pharmacol* (1991) 204(1):79–85. doi: 10.1016/0014-2999(91)90838-H
146. Seeman P, Lee T. The dopamine-releasing actions of neuroleptics and ethanol. *J Pharmacol Exp Ther* (1974) 190(1):131–40.
147. Iversen LL, Rogawski MA, Miller RJ. Comparison of the effects of neuroleptic drugs on pre- and postsynaptic dopaminergic mechanisms in the rat striatum. *Mol Pharmacol* (1976) 12(2):251–62.
148. Carlsson A, Lindqvist M. Effect of chlorpromazine or haloperidol on formation of 3methoxytyramine and normetanephrine in mouse brain. *Acta Pharmacol Toxicol (Copenh)* (1963) 20:140–4. doi: 10.1111/j.1600-0773.1963.tb01730.x
149. Cumming P, Ase A, Laliberte C, Kuwabara H, Gjedde A. *In vivo* regulation of DOPA decarboxylase by dopamine receptors in rat brain. *J Cereb Blood Flow Metab* (1997) 17(11):1254–60. doi: 10.1097/00004647-199711000-00014
150. Danielsen EH, Smith D, Hermansen F, Gjedde A, Cumming P. Acute neuroleptic stimulates DOPA decarboxylase in porcine brain *in vivo*. *Synapse* (2001) 41(2):172–5. doi: 10.1002/syn.1071
151. Lazar MA, Mefford IN, Barchas JD. Tyrosine hydroxylase activation. Comparison of *in vitro* phosphorylation and *in vivo* administration of haloperidol. *Biochem Pharmacol* (1982) 31(16):2599–607. doi: 10.1016/0006-2952(82)90706-7
152. Hakansson K, Pozzi L, Usiello A, Haycock J, Borrelli E, Fisone G. Regulation of striatal tyrosine hydroxylase phosphorylation by acute and chronic haloperidol. *Eur J Neurosci* (2004) 20(4):1108–12. doi: 10.1111/j.1460-9568.2004.03547.x
153. Mamo D, Remington G, Nobrega J, Hussey D, Chirakal R, Wilson AA, et al. Effect of acute antipsychotic administration on dopamine synthesis in rodents and human subjects using 6-[18F]-L-m-tyrosine. *Synapse* (2004) 52(2):153–62. doi: 10.1002/syn.20016
154. Cumming P, Ase A, Kuwabara H, Gjedde A. [3H]DOPA formed from [3H]tyrosine in living rat brain is not committed to dopamine synthesis. *J Cereb Blood Flow Metab* (1998) 18(5):491–9. doi: 10.1097/00004647-199805000-00004
155. Magelund G, Gerlach J, Casey DE. Neuroleptic-potentiating effect of alpha-methyl-p-tyrosine compared with haloperidol and placebo in a double-blind cross-over trial. *Acta Psychiatr Scand* (1979) 60(2):185–9. doi: 10.1097/00004647-199805000-00004
156. Ito H, Takano H, Takahashi H, Arakawa R, Miyoshi M, Kodaka F, et al. Effects of the antipsychotic risperidone on dopamine synthesis in human brain measured by positron emission tomography with L-[beta-11C]DOPA: a stabilizing effect for dopaminergic neurotransmission? *J Neurosci* (2009) 29(43):13730–4. doi: 10.1111/j.1600-0447.1979.tb03587.x
157. Grunder G, Vernaleken I, Muller MJ, Davids E, Heydari N, Buchholz HG, et al. Subchronic haloperidol downregulates dopamine synthesis capacity in the brain of schizophrenic patients *in vivo*. *Neuropsychopharmacology* (2003) 28(4):787–94. doi: 10.1523/JNEUROSCI.4172-09.2009
158. Vernaleken I, Kumakura Y, Cumming P, Buchholz HG, Siessmeier T, Stoeter P, et al. Modulation of [18F]fluorodopa (FDOPA) kinetics in the brain of healthy volunteers after acute haloperidol challenge. *Neuroimage* (2006) 30(4):1332–9. doi: 10.1038/sj.npp.1300103
159. Eisenberg DP, Yankowitz L, Ianni AM, Rubinstein DY, Kohn PD, Hegarty CE, et al. Presynaptic dopamine synthesis capacity in schizophrenia and striatal blood flow change during antipsychotic treatment and medication-free conditions. *Neuropsychopharmacology* (2017) 42(11):2232–41. doi: 10.1016/j.neuroimage.2005.11.014
160. Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, Caron MG. Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proc Natl Acad Sci U S A* (1998) 95(7):4029–34. doi: 10.1038/npp.2017.67
161. Rocha BA, Fumagalli F, Gainetdinov RR, Jones SR, Ator R, Giros B, et al. Cocaine self-administration in dopamine-transporter knockout mice. *Nat Neurosci* (1998) 1(2):132–7. doi: 10.1073/pnas.95.7.4029
162. Dickinson SD, Sabeti J, Larson GA, Giardina K, Rubinstein M, Kelly MA, et al. Dopamine D2 receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. *J Neurochem* (1999) 72(1):148–56. doi: 10.1038/381
163. Salvatore MF, Calipari ES, Jones SR. Regulation of tyrosine hydroxylase expression and phosphorylation in dopamine transporter-deficient mice. *ACS Chem Neurosci* (2016) 7(7):941–51. doi: 10.1046/j.1471-4159.1999.0720148.x
164. Schmitz Y, Schmauss C, Sulzer D. Altered dopamine release and uptake kinetics in mice lacking D2 receptors. *J Neurosci* (2002) 22(18):8002–9. doi: 10.1021/acschemneuro.6b00064
165. Miller JC, Friedhoff AJ. Effects of haloperidol and apomorphine on the K+-depolarized overflow of [3H] dopamine from rat striatal slices. *Biochem Pharmacol* (1979) 28(5):688–90. doi: 10.1523/JNEUROSCI.22-18-08002.2002
166. McElvain JS, Schenk JO. Blockade of dopamine autoreceptors by haloperidol and the apparent dynamics of potassium-stimulated endogenous release of dopamine from and reuptake into striatal suspensions in the rat. *Neuropharmacology* (1992) 31(7):649–59. doi: 10.1016/0006-2952(79)90158-8
167. Seeman P, Staiman A, Lee T, Chau-Wong M. The membrane actions of tranquilizers in relation to neuroleptic-induced parkinsonism and tardive dyskinesia. *Adv Biochem Psychopharmacol* (1974) 9(0):137–48. doi: 10.1016/0028-3908(92)90143-D
168. Amato D. Serotonin in antipsychotic drugs action. *Behav Brain Res* (2015) 277:125–35.
169. Amato D, Beasley CL, Hahn MK, Vernon AC. Neuroadaptations to antipsychotic drugs: insights from pre-clinical and human post-mortem studies. *Neurosci Biobehav Rev* (2016a) 74(5):830–4. doi: 10.1016/j.bbr.2014.07.025
170. Rayport S, Sulzer D. Visualization of antipsychotic drug binding to living mesolimbic neurons reveals D2 receptor, acidotropic, and lipophilic components. *J Neurochem* (1995) 65(2):691–703. doi: 10.1016/j.neubiorev.2016.10.004
171. Tischbirek CH, Wenzel EM, Zheng F, Huth T, Amato D, Trapp S, et al. Use-dependent inhibition of synaptic transmission by the secretion of intravesicularly accumulated antipsychotic drugs. *Neuron* (2012) 74(5):830–44. doi: 10.1046/j.1471-4159.1995.65020691.x
172. Yang SB, Proks P, Ashcroft FM, Rupnik M. Inhibition of ATP-sensitive potassium channels by haloperidol. *Br J Pharmacol* (2004) 143(8):960–7. doi: 10.1371/journal.pone.0020402
173. Yang SB, Major F, Tietze LF, Rupnik M. Block of delayed-rectifier potassium channels by reduced haloperidol and related compounds in mouse cortical neurons. *J Pharmacol Exp Ther* (2005) 315(1):352–62. doi: 10.1038/sj.bjp.0706017
174. Lee HJ, Sung KW, Hahn SJ. Effects of haloperidol on Kv4.3 potassium channels. *Eur J Pharmacol* (2014) 740:1–8. doi: 10.1124/jpet.105.086561
175. Hahn J, Tse TE, Levitan ES. Long-term K+ channel-mediated dampening of dopamine neuron excitability by the antipsychotic drug haloperidol. *J Neurosci* (2003) 23(34):10859–66. doi: 10.1016/j.ejphar.2014.06.043
176. Martel P, Leo D, Fulton S, Berard M, Trudeau LE. Role of Kv1 potassium channels in regulating dopamine release and presynaptic D2 receptor function. *PLoS One* (2011) 6(5):e20402. doi: 10.1016/j.neuron.2012.04.019
177. van Horne C, Hoffer BJ, Stromberg I, Gerhardt GA. Clearance and diffusion of locally applied dopamine in normal and 6-hydroxydopamine-lesioned

rat striatum. *J Pharmacol Exp Ther* (1992) 263(3):1285–92. doi: 10.1523/JNEUROSCI.23-34-10859.2003

178. Nishino H, Kumazaki M, Fukuda A, Fujimoto I, Shimano Y, Hida H, et al. Acute 3-nitropropionic acid intoxication induces striatal astrocytic cell death and dysfunction of the blood–brain barrier: involvement of dopamine toxicity. *Neurosci Res* (1997) 27(4):343–55.
179. Heinz A, Saunders RC, Kolachana BS, Jones DW, Gorey JG, Bachevalier J, et al. Striatal dopamine receptors and transporters in monkeys with neonatal temporal limbic damage. *Synapse* (1999) 32(2):71–9. doi: 10.1016/S0168-0102(97)01170-X
180. Torres GE, Gainetdinov RR, Caron MG. Plasma membrane monoamine transporters: structure, regulation and function. *Nat Rev Neurosci* (2003) 4(1):13–25. doi: 10.1002/(SICI)1098-2396(199905)32:2<71::AID-SYN1>3.3.CO;2-H
181. Cragg SJ, Rice ME. DAncing past the DAT at a DA synapse. *Trends Neurosci* (2004) 27(5):270–7. doi: 10.1038/nrn1008
182. Sulzer D, Cragg SJ, Rice ME. Striatal dopamine neurotransmission: regulation of release and uptake. *Basal Ganglia* (2016) 6(3):123–48.
183. Flatmark T, Almas B, Ziegler MG. Catecholamine metabolism: an update on key biosynthetic enzymes and vesicular monoamine transporters. *Ann N Y Acad Sci* (2002) 971:69–75. doi: 10.1016/j.tins.2004.03.011
184. Wightman RM, Amatore C, Engstrom RC, Hale PD, Kristensen EW, Kuhr WG, et al. Real-time characterization of dopamine overflow and uptake in the rat striatum. *Neuroscience* (1988) 25(2):513–23. doi: 10.1111/j.1749-6632.2002.tb04436.x
185. Wightman RM, Zimmerman JB. Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. *Brain Res Brain Res Rev* (1990) 15(2):135–44. doi: 10.1016/0306-4522(88)90255-2
186. Fusar-Poli P, Meyer-Lindenberg A. Striatal presynaptic dopamine in schizophrenia, Part I: meta-analysis of dopamine active transporter (DAT) density. *Schizophr Bull* (2013) 39(1):22–32. doi: 10.1016/0165-0173(90)90015-G
187. Rothblat DS, Schneider JS. Regionally specific effects of haloperidol and clozapine on dopamine reuptake in the striatum. *Neurosci Lett* (1997) 228(2):119–22. doi: 10.1093/schbul/sbr111
188. Reader TA, Ase AR, Huang N, Hebert C, van Gelder NM. Neuroleptics and dopamine transporters. *Neurochem Res* (1998) 23(1):73–80. doi: 10.1016/S0304-3940(97)00377-7
189. Meiergerd SM, Patterson TA, Schenk JO. D2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies *in vitro* and *in vivo*. *J Neurochem* (1993) 61(2):764–67. doi: 10.1023/A:1022405621365
190. Parsons LH, Schad CA, Justice JB, Jr. Co-administration of the D2 antagonist pimozide inhibits up-regulation of dopamine release and uptake induced by repeated cocaine. *J Neurochem* (1993) 60(1):376–9. doi: 10.1111/j.1471-4159.1993.tb02185.x
191. Cass WA, Gerhardt GA. Direct *in vivo* evidence that D2 dopamine receptors can modulate dopamine uptake. *Neurosci Lett* (1994) 176(2):259–63. doi: 10.1111/j.1471-4159.1993.tb05864.x
192. Benoit-Marand M, Ballion B, Borrelli E, Boraud T, Gonon F. Inhibition of dopamine uptake by D2 antagonists: an *in vivo* study. *J Neurochem* (2011) 116(3):449–58. doi: 10.1016/0304-3940(94)90096-5
193. Benoit-Marand M, Suaud-Chagny MF, Gonon F. Presynaptic regulation of extracellular dopamine as studied by continuous amperometry in anesthetized animals. In: Michael AC, Borland LM, editors. *Electrochemical methods for neuroscience*. Boca Raton (FL): CRC Press/Taylor & Francis (2007). doi: 10.1111/j.1471-4159.2010.07125.x
194. Suaud-Chagny MF, Dugast C, Chergui K, Msghina M, Gonon F. Uptake of dopamine released by impulse flow in the rat mesolimbic and striatal systems *in vivo*. *J Neurochem* (1995) 65(6):2603–11. doi: 10.1201/9781420005868.ch3
195. Lee SH, Oh DY, Jung SC, Kim YM, Cho HK, Koh JK, et al. Neuroleptic drugs alter the dopamine transporter-mediated uptake and release of dopamine: a possible mechanism for drug-induced tardive dyskinesia. *Pharmacol Res* (1997) 35(5):447–50. doi: 10.1046/j.1471-4159.1995.65062603.x
196. Siebert GA, Pond SM, Bryan-Lluka LJ. Further characterisation of the interaction of haloperidol metabolites with neurotransmitter transporters in rat neuronal cultures and in transfected COS-7 cells. *Naunyn Schmiedebergs Arch Pharmacol* (2000) 361(3):255–64. doi: 10.1006/phrs.1997.0159
197. Ewing AG, Wightman RM. Monitoring the stimulated release of dopamine with *in vivo* voltammetry. II: Clearance of released dopamine from extracellular fluid. *J Neurochem* (1984) 43(2):570–7. doi: 10.1007/s002109900202
198. Zetterstrom T, Sharp T, Ungerstedt U. Effect of neuroleptic drugs on striatal dopamine release and metabolism in the awake rat studied by intracerebral dialysis. *Eur J Pharmacol* (1984) 106(1):27–37. doi: 10.1111/j.1471-4159.1984.tb00936.x
199. Imperato A, Di Chiara G. Dopamine release and metabolism in awake rats after systemic neuroleptics as studied by trans-striatal dialysis. *J Neurosci* (1985) 5(2):297–306. doi: 10.1016/0014-2999(84)90674-5
200. Hernandez L, Hoebel BG. Haloperidol given chronically decreases basal dopamine in the prefrontal cortex more than the striatum or nucleus accumbens as simultaneously measured by microdialysis. *Brain Res Bull* (1989) 22(4):763–9. doi: 10.1523/JNEUROSCI.05-02-00297.1985
201. Invernizzi R, Morali F, Pozzi L, Samanin R. Effects of acute and chronic clozapine on dopamine release and metabolism in the striatum and nucleus accumbens of conscious rats. *Br J Pharmacol* (1990) 100(4):774–8. doi: 10.1016/0361-9230(89)90097-X
202. Meltzer HY, Chai BL, Thompson PA, Yamamoto BK. Effect of scopolamine on the efflux of dopamine and its metabolites after clozapine, haloperidol or thioridazine. *J Pharmacol Exp Ther* (1994) 268(3):1452–61. doi: 10.1111/j.1476-5381.1990.tb14091.x
203. Zahniser NR, Sorkin A. Rapid regulation of the dopamine transporter: role in stimulant addiction? *Neuropharmacology* (2004) 47(Suppl 1):80–91.
204. Furman CA, Chen R, Guptaroy B, Zhang M, Holz RW, Gnegy M. Dopamine and amphetamine rapidly increase dopamine transporter trafficking to the surface: live-cell imaging using total internal reflection fluorescence microscopy. *J Neurosci* (2009) 29(10):3328–36. doi: 10.1016/j.neuropharm.2004.07.010
205. Nikolaus S, Antke C, Kley K, Beu M, Wirrwar A, Muller HW. Pretreatment with haloperidol reduces (123)I-FP-CIT binding to the dopamine transporter in the rat striatum: an *in vivo* imaging study with a dedicated small-animal SPECT camera. *J Nucl Med* (2009) 50(7):1147–52. doi: 10.1523/JNEUROSCI.5386-08.2009
206. Cumming P, Maschauer S, Riss PJ, Tschammer N, Fehler SK, Heinrich MR, et al. Radiosynthesis and validation of (1)(8)F-FP-CMT, a phenyltropane with superior properties for imaging the dopamine transporter in living brain. *J Cereb Blood Flow Metab* (2014) 34(7):1148–56. doi: 10.2967/jnumed.109.061952
207. Xu M, Xing Q, Li S, Zheng Y, Wu S, Gao R, et al. Pharmacogenetic effects of dopamine transporter gene polymorphisms on response to chlorpromazine and clozapine and on extrapyramidal syndrome in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* (2010) 34(6):1026–32. doi: 10.1038/jcbfm.2014.63
208. Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: single spike firing. *J Neurosci* (1984) 4(11):2866–76. doi: 10.1016/j.pnpbp.2010.05.017
209. Marcott PF, Mamaligas AA, Ford CP. Phasic dopamine release drives rapid activation of striatal D2-receptors. *Neuron* (2014) 84(1):164–76. doi: 10.1523/JNEUROSCI.04-11-02866.1984
210. Bueschbell B, Barreto CAV, Preto AJ, Schiedel AC, Moreira IS. A complete assessment of dopamine receptor–ligand interactions through computational methods. *Molecules* (2019) 24(7):1–26. doi: 10.1016/j.neuron.2014.08.058
211. Bertolino A, Fazio L, Caforio G, Blasi G, Rampino A, Romano R, et al. Functional variants of the dopamine receptor D2 gene modulate prefronto-striatal phenotypes in schizophrenia. *Brain* (2009) 132(Pt 2):417–25. doi: 10.3390/molecules24071196
212. Roberts DA, Balderson D, Pickering-Brown SM, Deakin JF, Owen F. The relative abundance of dopamine D4 receptor mRNA in post mortem brains of schizophrenics and controls. *Schizophr Res* (1996) 20(1–2):171–4. doi: 10.1093/brain/awn248
213. Benoit-Marand M, Borrelli E, Gonon F. Inhibition of dopamine release *via* presynaptic D2 receptors: time course and functional characteristics *in vivo*. *J Neurosci* (2001) 21(23):9134–41. doi: 10.1016/0920-9964(96)88526-4
214. Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia.

Neuroscience (1991) 41(1):1–24. doi: 10.1523/JNEUROSCI.21-23-09134.2001

215. Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdos J, et al. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc Natl Acad Sci U S A* (1996) 93(17):9235–40. doi: 10.1016/0306-4522(91)90196-U
216. Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M, et al. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry* (1998) 155(6):761–7. doi: 10.1073/pnas.93.17.9235
217. Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R. Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol Psychiatry* (1999) 46(1):56–72. doi: 10.1176/ajp.155.6.761
218. Amato D, Canneva F, Nguyen HP, Bauer P, Riess O, von Horsten S, et al. Capturing schizophrenia-like prodromal symptoms in a spinocerebellar ataxia-17 transgenic rat. *J Psychopharmacol* (2016b) 31(4):461–73. doi: 10.1016/S0006-3223(99)00067-0
219. Creese I, Stewart K, Snyder SH. Species variation in dopamine receptor binding. *Eur J Pharmacol* (1979) 60(1):55–66. doi: 10.1177/0269881116675510
220. Bannon MJ, Whitty CJ. Age-related and regional differences in dopamine transporter mRNA expression in human midbrain. *Neurology* (1997) 48(4):969–77. doi: 10.1016/0014-2999(79)90052-9
221. Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, et al. Genotype influences *in vivo* dopamine transporter availability in human striatum. *Neuropsychopharmacology* (2000) 22(2):133–9. doi: 10.1212/WNL.48.4.969
222. Faraone SV, Spencer TJ, Madras BK, Zhang-James Y, Biederman J. Functional effects of dopamine transporter gene genotypes on *in vivo* dopamine transporter functioning: a meta-analysis. *Mol Psychiatry* (2014) 19(8):880–9. doi: 10.1016/S0893-133X(99)00099-8
223. Volkow ND, Ding YS, Fowler JS, Wang GJ, Logan J, Gatley SJ, et al. Dopamine transporters decrease with age. *J Nucl Med* (1996) 37(4):554–9. doi: 10.1038/mp.2013.126
224. Antonini A, Leenders KL. Dopamine D2 receptors in normal human brain: effect of age measured by positron emission tomography (PET) and [11C]-raclopride. *Ann N Y Acad Sci* (1993) 695:81–5.
225. Volkow ND, Fowler JS, Wang GJ, Logan J, Schlyer D, MacGregor R, et al. Decreased dopamine transporters with age in health human subjects. *Ann Neurol* (1994) 36(2):237–9. doi: 10.1111/j.1749-6632.1993.tb23033.x
226. Volkow ND, Gur RC, Wang GJ, Fowler JS, Moberg PJ, Ding YS, et al. Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. *Am J Psychiatry* (1998a) 155(3):344–9. doi: 10.1002/ana.410360218
227. Volkow ND, Wang GJ, Fowler JS, Ding YS, Gur RC, Gatley J, et al. Parallel loss of presynaptic and postsynaptic dopamine markers in normal aging. *Ann Neurol* (1998b) 44(1):143–7. doi: 10.1176/ajp.155.3.344
228. Harada N, Nishiyama S, Satoh K, Fukumoto D, Kakiuchi T, Tsukada H. Age-related changes in the striatal dopaminergic system in the living brain: a multiparametric PET study in conscious monkeys. *Synapse* (2002) 45(1):38–45. doi: 10.1002/ana.410440125
229. Troiano AR, Schulzer M, de la Fuente-Fernandez R, Mak E, McKenzie J, Sossi V, et al. Dopamine transporter PET in normal aging: dopamine transporter decline and its possible role in preservation of motor function. *Synapse* (2010) 64(2):146–51. doi: 10.1002/syn.10082
230. Uchida H, Suzuki T, Graff-Guerrero A, Mulsant BH, Pollock BG, Arenovich T, et al. Therapeutic window for striatal dopamine D(2/3) receptor occupancy in older patients with schizophrenia: a pilot PET study. *Am J Geriatr Psychiatry* (2014) 22(10):1007–16. doi: 10.1002/syn.20708
231. Li SC, Papenberg G, Nagel IE, Preuschhof C, Schroder J, Nietfeld W, et al. Aging magnifies the effects of dopamine transporter and D2 receptor genes on backward serial memory. *Neurobiol Aging* (2013) 34358(1):e351–310. doi: 10.1016/j.jagp.2013.01.045
232. Kanaan NM, Kordower JH, Collier TJ. Age-related changes in dopamine transporters and accumulation of 3-nitrotyrosine in rhesus monkey midbrain dopamine neurons: relevance in selective neuronal vulnerability to degeneration. *Eur J Neurosci* (2008) 27(12):3205–15. doi: 10.1016/j.neurobiolaging.2012.08.001
233. Rieckmann A, Hedden T, Younger AP, Sperling RA, Johnson KA, Buckner RL. Dopamine transporter availability in clinically normal aging is associated with individual differences in white matter integrity. *Hum Brain Mapp* (2016) 37(2):621–31. doi: 10.1111/j.1460-9568.2008.06307.x
234. Reis Marques T, Taylor H, Chaddock C, Dell'acqua F, Handley R, Reinders AA, et al. White matter integrity as a predictor of response to treatment in first episode psychosis. *Brain* (2014) 137(Pt 1):172–82. doi: 10.1002/hbm.23054
235. Szeszko PR, Robinson DG, Ikuta T, Peters BD, Gallego JA, Kane J, et al. White matter changes associated with antipsychotic treatment in first-episode psychosis. *Neuropsychopharmacology* (2014) 39(6):1324–31. doi: 10.1093/brain/awt310
236. Blanchard JJ, Brown SA, Horan WP, Sherwood AR. Substance use disorders in schizophrenia: review, integration, and a proposed model. *Clin Psychol Rev* (2000) 20(2):207–34. doi: 10.1038/npp.2013.288
237. Chambers RA, Krystal JH, Self DW. A neurobiological basis for substance abuse comorbidity in schizophrenia. *Biol Psychiatry* (2001) 50(2):71–83. doi: 10.1016/S0272-7358(99)00033-1
238. Volkow ND. Substance use disorders in schizophrenia–clinical implications of comorbidity. *Schizophr Bull* (2009) 35(3):469–72. doi: 10.1016/S0006-3223(01)01134-9
239. Muller CP, Schumann G. Drugs as instruments: a new framework for non-addictive psychoactive drug use. *Behav Brain Sci* (2011) 34(6):293–310. doi: 10.1093/schbul/sbp016
240. Lopez-Quintero C, Anthony JC. Drug use disorders in the polydrug context: new epidemiological evidence from a foodborne outbreak approach. *Ann N Y Acad Sci* (2015) 1349:119–26. doi: 10.1017/S0140525X11000057
241. Khantzian EJ. The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. *Am J Psychiatry* (1985) 142(11):1259–64. doi: 10.1111/nyas.12868
242. Schneier FR, Siris SG. A review of psychoactive substance use and abuse in schizophrenia. Patterns of drug choice. *J Nerv Ment Dis* (1987) 175(11):641–52. doi: 10.1176/ajp.142.11.1259
243. Swendsen J, Conway KP, Degenhardt L, Glantz M, Jin R, Merikangas KR, et al. Mental disorders as risk factors for substance use, abuse and dependence: results from the 10-year follow-up of the National Comorbidity Survey. *Addiction* (2010) 105(6):1117–28. doi: 10.1097/00005053-198711000-00001
244. Nestler EJ. Is there a common molecular pathway for addiction? *Nat Neurosci* (2005) 8(11):1445–9. doi: 10.1111/j.1360-0443.2010.02902.x
245. Volkow ND, Koob GF, McLellan AT. Neurobiologic advances from the brain disease model of addiction. *N Engl J Med* (2016) 374(4):363–71. doi: 10.1038/nn1578
246. Haber SN. Corticostriatal circuitry. *Dialogues Clin Neurosci* (2016) 18(1):7–21. doi: 10.1056/NEJMra1511480
247. Zhu J, Reith ME. Role of the dopamine transporter in the action of psychostimulants, nicotine, and other drugs of abuse. *CNS Neurol Disord Drug Targets* (2008) 7(5):393–409. doi: 10.1007/978-1-4614-6434-1_135-1
248. Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler M, et al. Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *J Neurosci* (2001) 21(23):9414–8. doi: 10.2174/187152708786927877
249. Leroy C, Karila L, Martinot JL, Lukasiewicz M, Duchesnay E, Comtat C, et al. Striatal and extrastriatal dopamine transporter in cannabis and tobacco addiction: a high-resolution PET study. *Addict Biol* (2012) 17(6):981–90. doi: 10.1523/JNEUROSCI.21-23-09414.2001
250. Liu Y, Han M, Liu X, Deng Y, Li Y, Yuan J, et al. Dopamine transporter availability in heroin-dependent subjects and controls: longitudinal changes during abstinence and the effects of Jitai tablets treatment. *Psychopharmacology (Berl)* (2013) 230(2):235–44. doi: 10.1111/j.1369-1600.2011.00356.x
251. Yen CH, Yeh YW, Liang CS, Ho PS, Kuo SC, Huang CC, et al. Reduced dopamine transporter availability and neurocognitive deficits in male patients with alcohol dependence. *PLoS One* (2015) 10(6):e0131017. doi: 10.1007/s00213-013-3148-z
252. Xu S, Liu Y, Li Y, Deng Y, Yuan J, Lv R, et al. Availability of dopamine transporters in heroin-dependent subjects: a (18)F-FECNT PET imaging study. *Psychiatry Res Neuroimaging* (2017) 263:121–6. doi: 10.1371/journal.pone.0131017

253. Yuan J, Liu XD, Han M, Lv RB, Wang YK, Zhang GM, et al. Comparison of striatal dopamine transporter levels in chronic heroin-dependent and methamphetamine-dependent subjects. *Addict Biol* (2017) 22(1):229–34. doi: 10.1016/j.pscychresns.2017.03.011
254. Volkow ND, Wang GJ, Fischman MW, Foltin RW, Fowler JS, Abumrad NN, et al. Relationship between subjective effects of cocaine and dopamine transporter occupancy. *Nature* (1997) 386(6627):827–30. doi: 10.1111/adb.12271
255. Thompson JL, Urban N, Slifstein M, Xu X, Kegeles LS, Girgis RR, et al. Striatal dopamine release in schizophrenia comorbid with substance dependence. *Mol Psychiatry* (2013) 18(8):909–15. doi: 10.1038/386827a0
256. Peraile I, Torres E, Mayado A, Izco M, Lopez-Jimenez A, Lopez-Moreno JA, et al. Dopamine transporter down-regulation following repeated cocaine: implications for 3,4-methylenedioxymethamphetamine-induced acute effects and long-term neurotoxicity in mice. *Br J Pharmacol* (2010) 159(1):201–11. doi: 10.1038/mp.2012.109
257. Ferris MJ, Calipari ES, Mateo Y, Melchior JR, Roberts DC, Jones SR. Cocaine self-administration produces pharmacodynamic tolerance: differential effects on the potency of dopamine transporter blockers, releasers, and methylphenidate. *Neuropsychopharmacology* (2012) 37(7):1708–16. doi: 10.1111/j.1476-5381.2009.00522.x
258. Calipari ES, Ferris MJ, Zimmer BA, Roberts DC, Jones SR. Temporal pattern of cocaine intake determines tolerance vs sensitization of cocaine effects at the dopamine transporter. *Neuropsychopharmacology* (2013) 38(12):2385–92. doi: 10.1038/npp.2012.17
259. Kawa AB, Allain F, Robinson TE, Samaha AN. The transition to cocaine addiction: the importance of pharmacokinetics for preclinical models. *Psychopharmacology (Berl)* (2019). doi: 10.1038/npp.2013.136
260. Kapur S, Remington G. Serotonin-dopamine interaction and its relevance to schizophrenia. *Am J Psychiatry* (1996) 153(4):466–76. doi: 10.1007/s00213-019-5164-0
261. Guiard BP, El Mansari M, Merali Z, Blier P. Functional interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions. *Int J Neuropsychopharmacol* (2008) 11(5):625–39. doi: 10.1176/ajp.153.4.466
262. Dremencov E, El Mansari M, Blier P. Effects of sustained serotonin reuptake inhibition on the firing of dopamine neurons in the rat ventral tegmental area. *J Psychiatry Neurosci* (2009) 34(3):223–9. doi: 10.1017/S1461145707008383

Conflict of Interest Statement: A-NS is a consultant for H. Lundbeck A/S. This had no influence on the manuscript's content. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Amato, Kruyer, Samaha and Heinz. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Psychiatry

REVIEW
published: 03 September 2019
doi: 10.3389/fpsyt.2019.00601

Clinical Course, Neurobiology and Therapeutic Approaches to Treatment Resistant Schizophrenia. Toward an Integrated View

Cheryl Cheuk-Yan Leung[1,2], Romayne Gadelrab[2], Chukwuma Uchenna Ntephe[2], Philip K. McGuire[3,4] and Arsime Demjaha[3,4]**

[1] Department of Old Age Psychiatry, Institute of Psychiatry, Psychology, and Neuroscience (IoPPN), King's College London, London, United Kingdom, [2] South London and Maudsley NHS Foundation Trust, London, United Kingdom, [3] Department of Psychosis Studies, Institute of Psychiatry, Psychology, and Neuroscience (IoPPN), King's College London, London, United Kingdom, [4] National Institute for Health Research (NIHR) Biomedical Research Centre (BRC), South London and Maudsley NHS Foundation Trust, London, United Kingdom

OPEN ACCESS

Edited by:
Vincenzo De Luca,
University of Toronto,
Canada

Reviewed by:
Mette Ødegaard Nielsen,
Center for Neuropsychiatric Schizophrenia Research (CNSR),
Denmark
Hiroyoshi Takeuchi,
Keio University,
Japan

***Correspondence:**
Cheryl Cheuk-Yan Leung
cheryl.leung@kcl.ac.uk
Arsime Demjaha
arsime.demjaha@kcl.ac.uk

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: 15 October 2018
Accepted: 29 July 2019
Published: 03 September 2019

Citation:
Leung CC-Y, Gadelrab R, Ntephe CU, McGuire PK and Demjaha A (2019) Clinical Course, Neurobiology and Therapeutic Approaches to Treatment Resistant Schizophrenia. Toward an Integrated View. Front. Psychiatry 10:601. doi: 10.3389/fpsyt.2019.00601

Despite considerable psychotherapeutic advancement since the discovery of chlorpromazine, almost one third of patients with schizophrenia remain resistant to dopamine-blocking antipsychotics, and continue to be exposed to unwanted and often disabling side effects, but little if any clinical benefit. Even clozapine, the superior antipsychotic treatment, is ineffective in approximately half of these patients. Thus treatment resistant schizophrenia (TRS), continues to present a major therapeutic challenge to psychiatry. The main impediment to finding novel treatments is the lack of understanding of precise molecular mechanisms leading to TRS. Not only has the neurobiology been enigmatic for decades, but accurate and early detection of patients who are at risk of not responding to dopaminergic blockade remains elusive. Fortunately, recent work has started to unravel some of the neurobiological mechanisms underlying treatment resistance, providing long awaited answers, at least to some extent. Here we focus on the scientific advances in the field, from the clinical course of TRS to neurobiology and available treatment options. We specifically emphasize emerging evidence from TRS imaging and genetic literature that implicates dysregulation in several neurotransmitters, particularly dopamine and glutamate, and in addition genetic and neural alterations that concertedly may lead to the formation of TRS. Finally, we integrate available findings into a putative model of TRS, which may provide a platform for future studies in a bid to open the avenues for subsequent development of effective therapeutics.

Keywords: schizophrenia, treatment-resistant, neurobiology, neuroimaging, clozapine

INTRODUCTION

Almost one third of patients with schizophrenia do not respond to dopamine (DA) blocking antipsychotic medication and are described as being treatment-resistant (1). Although clozapine can be effective in these patients, there is usually a long delay before it is used, and what is more around half of treatment-resistant patients do not respond to clozapine (2, 3). Treatment-resistant schizophrenia (TRS), is thus associated with particularly poor clinical outcomes (4), and presents a major therapeutic challenge to psychiatry. One of the main impediments to finding novel treatments

for TRS patients is the lack of understanding of the molecular basis of TRS, despite over 50 years of scientific work in this field. Moreover, biomarkers that can identify patients who are unlikely to respond to conventional treatment remain elusive. Fortunately, recent work has started to unravel some of the mechanisms underlying treatment resistance. Here we describe these scientific advances and propose an integrated model of TRS that may facilitate the identification of biomarkers for TRS and provide a rationale for the development of novel therapeutic approaches.

Defining Treatment-Resistant Schizophrenia

Prior to embarking on finding reliable biomarkers and conduct promising clinical trials, it is of crucial importance to precisely stratify patients according to their response to treatment. The literature, however, has been limited by inconsistent TRS definitions. In the absence of a universally accepted definition, studies have opted for different TRS criteria according to their aims and population studied. This has resulted in marked heterogeneity in results and disparity in response rates. For instance, in Suzuki and colleagues' systematic review, 33 studies reported treatment response rates ranging from 0% to 76% (5). Studies recruiting patients for novel antipsychotic drug trials may use more stringent criteria than those testing psychosocial interventions, thus reporting lower prevalence of TRS (5, 6).

Furthermore, the lack of precise and universal operational definitions of TRS may have important clinical and scientific implications. For instance, it hinders early detection of treatment resistance and, subsequently, may delay initiation of clozapine, and in research settings, it complicates comparisons and interpretation of results. To address these issues, International Treatment Response and Resistance in Psychosis (TRRIP) group has developed operationalized TRS definition criteria and reached consensus on "minimum requirements." The group emphasizes that any definition of treatment resistance should indicate that the patient has received an adequate trial of antipsychotic medication in terms of dosage (equivalent to or greater than 600 mg of chlorpromazine per day), trial of two different antipsychotics for a duration of 6 weeks each at a therapeutic dose, strong advocation for acquiring treatment adherence measures (≥80% of prescribed doses), as well as the use of structured clinical assessments to ascertain symptom presence and severity (7). However, there are limitations to these criteria, such as the use of dichotomous classification, which does not account for the continuum of treatment response. As authors acknowledge, future revisions incorporating novel neurobiological findings are required prior to criteria being fully standardized and more applicable across research and clinical settings.

HETEROGENEITY OF CLINICAL COURSE OF TRS

For decades, researchers in the field of TRS debated whether treatment resistance is a stable phenotype, or whether it is a consequence of neurodegenerative process, evolving over time in the context of multiple episodes and repeated exposure to antipsychotic treatment.

In favor of the first notion were reports that emerged even prior to the existence of psychopharmacology, and indirectly suggested that unfavorable clinical outcomes may be related to more severe and enduring subtype of schizophrenic illness. Kraepelin, referring in his textbook to Albrecht's observations that one third of his cases with hebephrenia reached terminal state within a year of onset, concluded: "Often enough the unmistakable symptoms of dementia appear already within the first year" (8). Decades later, Kolakowska and colleagues demonstrated that the majority of "poor responders" were unresponsive throughout their illness and remarked that treatment resistance was related to the "type," rather than the "stage" of schizophrenia. (9). Analogously, two prospective studies observed that resistance to treatment was apparent in early stages of illness (10, 11). Furthermore, longitudinal first episode psychosis (FEP) studies (12) observed that between 5% and 25% of patients were unresponsive and had persistent positive symptoms during the initial phase of illness (12, 13). Such variability again, might be a consequence of the different TRS criteria employed in these studies.

Other authors, however, considered neurodegenerative hypothesis, attributing treatment resistance to chronicity of illness. Wyatt (1991) reviewed the evidence derived from 22 studies of predominantly FEP patients and concluded that early psychopharmacological intervention could improve the outcomes and prognosis of the disorder (14). It was proposed that a neurodegenerative process might be inherent to psychosis and thus adversely affect the clinical course in those who were non-compliant with treatment and subjected to multiple relapses.

More recently, the largest to date, a 10-year follow-up FEP study, designed to address these inconsistencies in literature, found that over 80% of treatment-resistant patients were persistently resistant from the very early stage of their illness (15).

This work, however, identified a small proportion of patients (16%), who although initially responded to medication, ultimately developed treatment resistance. These patients showed higher number of relapses associated with more inpatient admissions. The reasons for this remain elusive and warrant further exploration. As suggested by animal studies, it can be that chronic treatment with DA blocking agents may induce D2 receptor up-regulation leading to breakthrough DA supersensitivity, which may predispose some patients to treatment resistance (16, 17). Accordingly, it has been shown that in a proportion of patients not only the time to remission is longer in subsequent episodes, but less, if at all, achievable (18, 19). Furthermore, most recently, a study by Takeuchi et al. (20) has implicated that treatment response is unfavorably affected by symptomatic relapse following initial response. This finding could be particularly relevant to this subgroup of patients (20).

On the other hand, some treatment-resistant patients may achieve spontaneous remission or begin responding to treatment later in life (21), which is in line with previous observations that older patients with schizophrenia require much less intensive maintenance antipsychotic treatment than those who are younger in age (22–24). This can perhaps be explained by the fact that DA system is age-dependent, with significant reductions in dopaminergic transmission in older patients being observed (24, 25). This notion is intriguing and contradicts the recent findings of unaltered DA levels in TRS, but it can be that this sub-group of patients have different

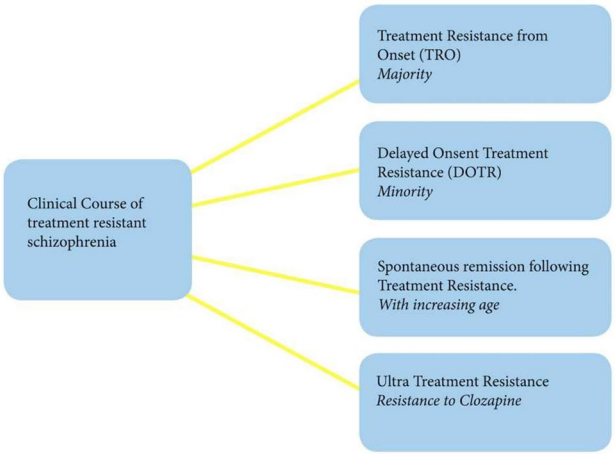


FIGURE 1 | Clinical course of treatment-resistant schizophrenia.

neurobiology altogether, which remains to be determined in larger and more stratified studies. Finally, up to 50% of treatment-resistant patients are resistant to clozapine recently termed as "ultra-treatment resistance" (26). Such non-response to clozapine, a last treatment resort for those who do not respond to first-line antipsychotics, is the major unmet clinical need in schizophrenia **(Figure 1)**.

Putative Predictors of TRS

Several studies have identified younger age at onset, longer duration of untreated psychosis, and negative symptomatology to be associated with treatment resistance (12, 15, 27, 28). Furthermore, severe cognitive impairment, poorer premorbid functioning (21, 29), obstetric complications (30), as well as neurological soft signs (31), have, in addition, shown significant associations with treatment resistance. Additionally, family history and, thus, increased genetic burden (32, 33) have been linked to poor prognosis of illness, and finally, a study comparing first-degree relatives of patients with and without TRS showed higher morbidity risk of schizophrenia in relatives of TRS patients (34).

The findings and observations, to date, indicate that treatment resistance in schizophrenia is heterogenous, as a disorder itself, assuming at least four different trajectories. However, a significant majority of patients with TRS appear to be resistant at the time of their first presentation. This form of treatment resistance may represent an enduring phenotype of schizophrenic illness, which is particularly associated with younger age at onset and negative symptoms (15). Such high prevalence at the early phase of illness should alert clinicians to commence clozapine as soon as possible. However, larger FEP studies are needed to delineate reliable predictors to facilitate early and accurate detection of patients who are not likely to respond to first-line antipsychotic treatment.

NEUROBIOLOGY OF TREATMENT-RESISTANT SCHIZOPHRENIA

Until recently, the underlying neurobiology of treatment-resistant schizophrenia remained elusive. Emerging evidence from TRS imaging and genetic literature implicates dysregulation in several neurotransmitters, particularly DA and glutamate, and in addition genetic and neural alterations that concertedly may lead to the formation of treatment resistance in schizophrenia. The presented literature here is not exhaustive. Instead, we predominantly focus on the most robust and high-impact evidence and neurobiological aspects that may predispose to treatment resistance.

Neurotransmitters in TRS

The DA hypothesis remains an important part of our understanding of psychosis. DA blocking antipsychotics are effective in a majority of patients with schizophrenia, and illicit drugs that induce acute psychotic symptoms increase DA levels. Although this hypothesis does hold true for the patients who are responsive to treatment, it fails to provide explanations for patients with TRS.

To understand the dopaminergic mechanisms underlying treatment resistance, scientists have first focused on striatal DA D2 receptors. Positron emission tomography (PET) studies revealed significant associations between striatal DA D2 occupancy and prediction of short-term clinical response to antipsychotic treatment (35) and suggested that at least 60% of DA D2 receptor occupancy is necessary to reach adequate therapeutic response. This is true for both typical and atypical antipsychotics (31, 35, 36), excluding clozapine (37). Hypothesizing that the lack of response may result from inadequate DA D2 receptor blockade, Wolkin et al. (38), using PET, examined DA D2 receptor occupancy in patients with TRS schizophrenia and intriguingly demonstrated almost identical striatal DA D2 receptor occupancies in both treatment-responsive and treatment-resistant patients (38). Correspondingly, a [123I] IBZM Single Photon Emission Tomography (SPET) study reported a similar degree of DA D2 occupancy in both groups (39). Moreover, Kane et al. (40) in their seminal trial included the most severely ill and treatment-resistant patients with schizophrenia who failed a prospective trial of haloperidol at doses of at least 60 mg/day, which indirectly suggests that DA receptor occupancy was sufficiently achieved (40).

It became apparent that although DA D2 blockade may be necessary, it does not guarantee a response. Thus, the focus shifted to investigating presynaptic DA synthesis capacity (DSC). Increased DSC in patients with schizophrenia is considered one of the most replicated finding in dopaminergic studies of schizophrenia (41–43), and therefore, DSC anomalies are considered critical in the formation of positive psychotic symptoms. The biochemistry of DA synthesis is presented in a schematic diagram **(Figure 2)**.

The first study to directly examine DSC in specifically treatment-resistant patients with schizophrenia, using PET and stringent criteria for TRS, demonstrated unaltered DSC in treatment-resistant patients. In a subgroup of these patients, authors measured glutamate levels using proton magnetic resonance spectroscopy (^{1}H-MRS) and documented increased glutamatergic levels in anterior cingulate cortex (ACC) in TRS patients (44). Analogously, two subsequent studies reported increased glutamate levels in the ACC in treatment-resistant patients, but decreased levels in treatment responders (45, 46). To address the potential effects of medication or chronicity on these findings, the same group prospectively investigated

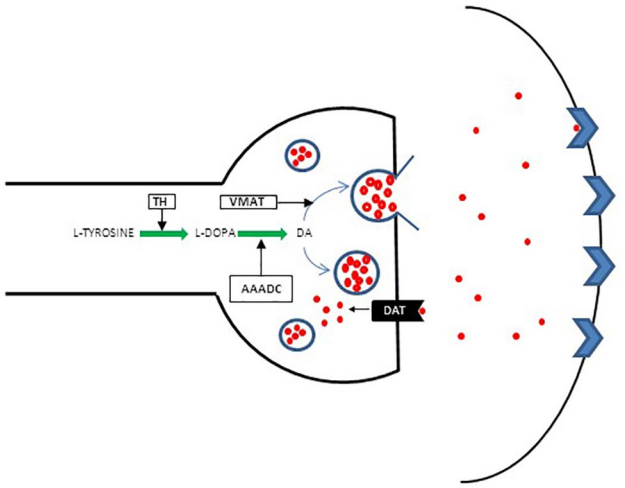


FIGURE 2 | Schematic presentation of presynaptic DA regulation. Conversion of l-tyrosine (4-hydroxyphenylalanin) to l-3, 4 dihydroxyphenylalanine [l-DOPA] constitutes the first step in a complex pathway of DA synthesis. l-tyrosine is derived mainly from dietary sources, although a small quantity originates from l-phenylalanine converted to l-tyrosine by phenylalanine hydroxylase (PHA). l-tyrosine is converted to l-DOPA by tyrosine hydroxylase (TH). Aromatic L-amino acid decarboxylase (AAADC) then acts on l-DOPA to convert it to DA. The DA uptake transporter (DAT) plays an additional role in increasing cytoplasmic DA levels via the reuptake of extracellular DA and thus maintains extracellular DA homeostasis. From the cytoplasm, the majority of DA is stored in specialized synaptic vesicles by the vesicular monoamine transporter (VMAT) and is ready for release upon arrival of the action potential.

DSC and ACC glutamate levels in initially medication-naïve FEP patients and confirmed that although striatal DSC is unaltered, ACC glutamate levels are increased in patients who subsequently do not respond to treatment (47). Most recently, in a multicenter longitudinal study of either minimally treated or medication naïve patients, higher levels of glutamate in the ACC were associated with treatment non-response to amisulpiride (48).

However, not all studies have observed glutamatergic alterations in relation to treatment response as discussed in recent systematic reviews (49, 50). The discrepancy in results may be related to differing methodology and, in particular, different TRS criteria studies. Thus, some studies may have misclassified TRS patients as responders or vice versa, which may lead to different outcomes and complicate comparisons as we discussed in previous sections.

Taken together, the neurochemical evidence to date supports the hypothesis that distinct neurochemical abnormalities, such as normal striatal DSC and increased ACC glutamate function, may underlie TRS. What is more, the demonstrated lack of DA abnormality in this subgroup of patients raises the possibility that other neurotransmitters, such as GABAaergic, glutamatergic, and endocannabinoid systems may be a promising target for novel antipsychotics.

However, the neurobiological underpinning of schizophrenia in general as well as that of TRS may involve complex interactions of these neurotransmitters. Carlsson and colleagues (2000, 2001) proposed that alterations in cortical glutamate levels, either acting directly as an "accelerator" or *via* GABA interneuron projections as a "brake," modulates the firing of dopaminergic neurons that can in turn lead to either decrease or increase in dopaminergic activity (51, 52). Thus, for instance, the reduced glutamate activity enhances DA release in dopaminergic pathways, which then *via* negative feedback, mediated, at least in part, *via* the striatum and the thalamus, regulates glutamate release that would then act as "a brake" on cortical DA production (51, 52). How this mechanism operates in TRS remains to be determined in precise future pre-clinical models. At this stage, and based on available neurochemical imaging evidence, we could only speculate that in TRS, this mechanism involves the indirect pathway that involves GABA interneurons that exerts a "brake" effect on DA production, which may explain the absence of DSC increase in TRS. In turn, the absence of feedback from normal striatal DA status may lead to cortical hyperglutamatergia. In line with this, studies have reported an inverse correlation between cortical glutamate and striatal DSC (53, 54).

Genetic data also support to some degree the distinct neurobiology of TRS by suggesting a specific heritable vulnerability in TRS sub-group of patients. It has been suggested that TRS may be related to increased genetic burden (32). For instance, family history of psychosis has been shown to be associated with TRS (33). Studies that investigated several candidate genes, such as ABCB1, ABCC1, and ABCB11, demonstrated associations with response to antipsychotics as summarized by Vita et al. (50). Subsequent studies have examined polygenic risk scores (PRS) representing aggregate score of risk loci, that have been identified from genome-wide association studies (GWAS) in schizophrenia patients, to determine whether this approach can detect treatment non-response, but both chronic and medication-naive FEP studies have been negative (55, 56).

Functional and Structural Neuroimaging

Evidence from structural magnetic resonance imaging (MRI) studies indicates that patients with limited response to treatment have increased cortical atrophy in comparison with responders (57, 58). Reduced gyrification was observed across multiple brain regions at illness onset in FEP patients who subsequently do not respond to treatment (59). In addition, cortical thinning generally, but particularly in dorsolateral prefrontal cortex (DLPFC) was reported in TRS (60). Recent review has revealed that patients with TRS have larger number of regions with decreased GM when compared with responders (49).

Functional MRI studies have similarly been able to distinguish between responders and non-responders. Most recently, global functional connectivity decrease, particularly in frontotemporal and occipital regions, was reported to be associated with treatment resistance in several studies (61, 62). Two comprehensive reviews have demonstrated decreased metabolism in the prefrontal and frontotemporal regions and hypermetabolism in the basal ganglia in TRS patients (63, 64).

THERAPEUTIC APPROACHES OF TREATMENT-RESISTANT SCHIZOPHRENIA

Clozapine—A Gold Standard

The discovery of chlorpromazine has stimulated the discovery of numerous DA-blocking antipsychotics that in most patients are effective. However, first-line antipsychotic treatment in considerable proportion of patients does not alleviate symptoms, but instead exposes these patients to unwanted and often disabling side effects. The only antipsychotic, to date, that has an adequate therapeutic effect in this subgroup of patients is clozapine, and as such remains superior to other antipsychotics for TRS patients (65–67).

Clozapine has been actualized by Kane and colleagues in their seminal work (40). They have shown clozapine to be more effective than chlorpromazine at symptomatic reduction (30% vs. 4%, respectively) in participants who failed a trial of haloperidol treatment (40). It is, however, underutilized (68) with documented delay of its initiation approximating 5 years (69). This delay has important clinical implications associated with reduced effectiveness, increased number of hospital admissions, and more frequent use of concurrent electroconvulsive therapy (ECT) (68, 70–72). Recent scientific reports advocate its use at much earlier stages of illness (15, 73). This is compounded by the fact that a great majority of TRS patients seem to be destined to non-response to medication at the earliest stages of their illness necessitating much earlier use of clozapine (15). A meta-analysis by Okhuijsen-Pfeifer and colleagues (2018) comparing clozapine with a number of conventional antipsychotics found significant benefit for early clozapine use (Hedges' $g = 0.220$; $P = 0.026$; 95% CI = 0.026–0.414) (74), whereas a large three-phase switching clinical trial conducted by the OPTiMiSe study group found that following a failed initial response to amisulpiride switching to olanzapine resulted in no additional benefit, whereas switching to clozapine did improve clinical outcomes (73).

The precise psychopharmacology of clozapine is yet to be unraveled. Its efficacy in TRS may be related to the fact that clozapine is a weak DA blocker and that its action may be mediated *via* glutamatergic and serotonergic pathway as indicated by recent neurochemical imaging literature (45, 47, 75–79).

TREATMENT STRATEGIES IN ULTRA-TREATMENT RESISTANCE

Clozapine Augmentation With Other Psychotropic Agents

Almost half of TRS patients do not respond to clozapine (2, 3, 40, 80) and are termed ultra-treatment resistant. When faced with such treatment challenge, clinicians tend to resort to augmentation with other psychotropic agents, although there is limited evidence to support this therapeutic approach (32, 81). Antipsychotics are the most frequently utilized and studied agent, and of these, risperidone is the most frequently researched (82). A meta-analysis has shown no increased benefit to augmentation with risperidone (83) and another of 14-placebo controlled RCTs showed that augmentation with antipsychotic medication is of little benefit (effect size, −0.239; 95% CI, −0.45 to −0.026; $P = 0.028$) (84). Furthermore, augmentation with antipsychotics seems to be associated with a worsening of side effects (83). Similarly, the augmentation with mood stabilizers and SSRIs has yielded limited evidence for efficacy (85). Lamotrigine has garnered conflicting evidence (83, 86). While topiramate has some evidence supporting its effect at curtailing weight gain in patients taking clozapine, there is limited evidence for its reduction in psychotic symptoms (85). Augmentation, in theory, may be a useful approach to adopt in managing TRS as it utilizes already existing medication, whose mechanisms of action and side effect profiles have been well studied. Unfortunately, the evidence does not currently support their effectiveness.

Other Treatment Strategies

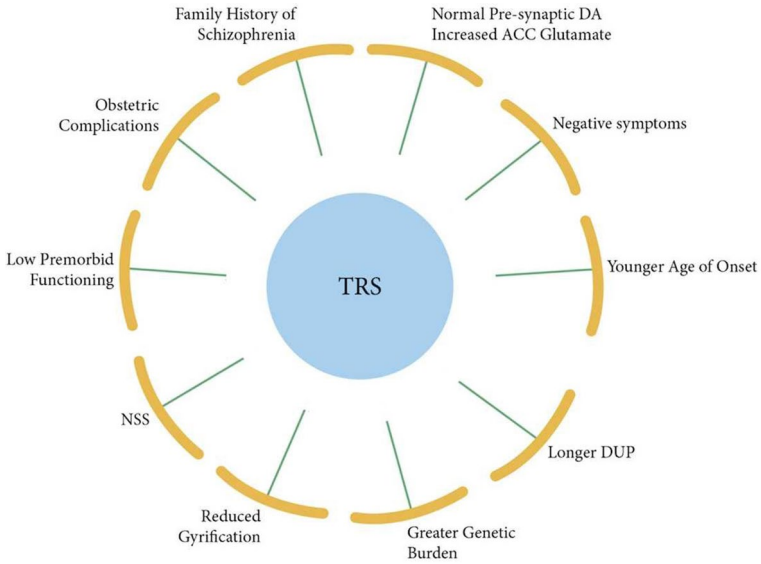
Evidence investigating the effectiveness of ECT in combination with clozapine has shown positive results (87). A recent meta-analysis by Wang and colleagues (2018) who analyzed data from 18 randomized control trials (n = 1769) found that adjunctive ECT was more beneficial for short-term recovery, compared with clozapine alone (standard mean difference = −0.54; 95% CI, −0.88 to −0.20; I2 = 77%, $P = 0.002$) (88).

Repetitive trans-magnetic stimulation (89) and transcranial direct stimulation (90) may be effective at reducing auditory hallucinations, though some of their effects may be short-lived (91). Their low-cost and mild side effect profile (92, 93) make them attractive options to treat schizophrenia and, more specifically, TRS, though with a scarcity of large clinical trials, more research is needed to delineate their effectiveness as sole or adjunct agents (81).

In summary, clozapine remains a gold standard treatment for patients with TRS (74). Research has shown that delay in clozapine initiation leads to a poorer response to treatment (72) and, worse outcomes (70, 71). However, there are significant issues with its tolerability, and there is still a significant subgroup of non-responders to clozapine who see, a modest, if any improvement with pharmacological (32, 81) and non-pharmacological augmentation (87, 89, 90). This strongly supports a need for new therapeutic targets. Recent meta-analytic work has demonstrated significant effects of glutamatergic agents, such as glycine/D-serine site antagonists, on negative symptoms (94) that are generally resistant to DA-blocking antipsychotics. In view of complex interplay of neurotransmitters governing schizophrenia and particularly treatment resistance, other promising therapeutic approaches include the stimulation of GABA receptors to overcome glutamatergic deficits, which is yet to be tested in clinical trials (95), as well as the use of cannabinoids, which have shown promising therapeutic effect in recent drug trials (96–98).

CONCLUSION

Taken together, the findings to date suggest that TRS is a distinct, more severe, and enduring subtype of schizophrenic illness,



TRS- Treatment Resistant Schizophrenia, DA- Dopamine, DUP- Duration of Untreated Psychosis, PRS- Polygenic Risk Score, NSS- Neurological Soft Signs

FIGURE 3 | Putative model integrating factors that are associated with treatment resistance in schizophrenia.

marked by greater neuroanatomical abnormalities and different molecular mechanisms. Such complex and intractable condition requires a more fine-grained conceptualization of underlying neurobiology, which may consequently lead to much-needed novel biologically determined treatments. It is crucial to develop clinical tools that will enable clinicians to predict whether a patient will or will not respond to DA blockade, so that clozapine or other novel alternatives can be commenced as early as possible. Here, we integrate the available findings into a putative predictive model of TRS (**Figure 3**), which may provide a platform for impending scientific developments. Carefully designed studies that address rigorously the heterogeneity of the disorder and that of the antipsychotic treatment response (97, 99) are urgently needed so that patients may be stratified accurately according to their likely therapeutic responses.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to the conception and drafting of the manuscript. AD critically reviewed the manuscript and all authors approved the final version of the manuscript.

REFERENCES

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. *PLoS Med* (2005) 2:e141. doi: 10.1371/journal.pmed.0020141
2. Chakos M, Lieberman J, Hoffman E, Bradford D, Sheitman B. Effectiveness of second-generation antipsychotics in patients with treatment-resistant schizophrenia: a review and meta-analysis of randomized trials. *Am J Psychiatry* (2001) 158:518–26. doi: 10.1176/appi.ajp.158.4.518
3. Lieberman JA, Safferman AZ, Pollack S, Szymanski S, Johns C, Howard A, et al. Clinical effects of clozapine in chronic schizophrenia: response to treatment and predictors of outcome. *Am J Psychiatry* (1994) 151:1744–52. doi: 10.1176/ajp.151.12.1744
4. Lasevoli F, Giordano S, Balletta R, Latte G, Formato MV, Prinzivalli E, et al. Treatment resistant schizophrenia is associated with the worst community functioning among severely-ill highly-disabling psychiatric conditions and is the most relevant predictor of poorer achievements in functional milestones. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 65:34–48. doi: 10.1016/j.pnpbp.2015.08.010
5. Suzuki T, Remington G, Mulsant BH, Rajji TK, Uchida H, Graff-Guerrero A, et al. Treatment resistant schizophrenia and response to antipsychotics: a review. *Schizophr Res* (2011) 133:54–62. doi: 10.1016/j.schres.2011.09.016
6. Caspi A. Treatment-refractory schizophrenia. *Dialogues Clin Neurosci* (2004) 6:61–70.
7. Leifker FR, Patterson TL, Heaton RK, Harvey PD. Validating measures of real-world outcome: the results of the VALERO expert survey and RAND panel. *Schizophr Bull* (2011) 37:334–43. doi: 10.1093/schbul/sbp044
8. Kraepelin E, Barclay RM, Robertson GM. *Dementia praecox and paraphrenia*. Edinburgh: E S Livingston (1919).
9. Kolakowska T, Williams AO, Ardern M, Reveley MA, Jambor K, Gelder MG, et al. Schizophrenia with good and poor outcome. I: Early clinical features, response to neuroleptics and signs of organic dysfunction. *Br J Psychiatry* (1985) 146:229–39. doi: 10.1192/bjp.146.3.229
10. Bleuler M. *The schizophrenic disorders: long-term patient and family studies*. New Haven: Yale University Press (1978).
11. Huber G, Gross G, Schuttler R. A long-term follow-up study of schizophrenia: psychiatric course of illness and prognosis. *Acta Psychiatr Scand* (1975) 52:49–57. doi: 10.1111/j.1600-0447.1975.tb00022.x

12. Robinson DG, Woerner MG, Alvir JM, Geisler S, Koreen A, Sheitman B, et al. Predictors of treatment response from a first episode of schizophrenia or schizoaffective disorder. *Am J Psychiatry* (1999) 156:544–9. doi: 10.1176/ajp.156.4.544
13. MacMillan JF, Crow TJ, Johnson AL, Johnstone EC. Short-term outcome in trial entrants and trial eligible patients. *Br J Psychiatry* (1986) 148:128–33. doi: 10.1192/bjp.148.2.128
14. Wyatt RJ. Neuroleptics and the natural course of schizophrenia. *Schizophr Bull* (1991) 17:325–51. doi: 10.1093/schbul/17.2.325
15. Demjaha A, Lappin JM, Stahl D, Patel MX, MacCabe JH, Howes OD, et al. Antipsychotic treatment resistance in first-episode psychosis: prevalence, subtypes and predictors. *Psychol Med* (2017) 47:1981–9. doi: 10.1017/S0033291717000435
16. Ginovart N, Wilson AA, Hussey D, Houle S, Kapur S. D2-receptor upregulation is dependent upon temporal course of D2-occupancy: a longitudinal [11C]-raclopride PET study in cats. *Neuropsychopharmacology* (2009) 34:662–71. doi: 10.1038/npp.2008.116
17. Samaha AN, Seeman P, Stewart J, Rajabi H, Kapur S. "Breakthrough" dopamine supersensitivity during ongoing antipsychotic treatment leads to treatment failure over time. *J Neurosci* (2007) 27:2979–86. doi: 10.1523/JNEUROSCI.5416-06.2007
18. Lieberman JA. Prediction of outcome in first-episode schizophrenia. *J Clin Psychiatry* (1993) 54(suppl):13–7.
19. Sheitman BB, Lee H, Strous R, Lieberman JA. The evaluation and treatment of first-episode psychosis. *Schizophr Bull* (1997) 23:653–61. doi: 10.1093/schbul/23.4.653
20. Takeuchi H, Siu C, Remington G, Fervaha G, Zipursky RB, Foussias G, et al. Does relapse contribute to treatment resistance? Antipsychotic response in first- vs. second-episode schizophrenia. *Neuropsychopharmacology* (2019) 44:1036–42. doi: 10.1038/s41386-018-0278-3
21. Meltzer HY. Treatment-resistant schizophrenia: the role of clozapine. *Curr Med Res Opin* (1997) 14:1–20. doi: 10.1185/03007999709113338
22. Fenton WS, McGlashan TH. Sustained remission in drug-free schizophrenic patients. *Am J Psychiatry* (1987) 144:1306–9. doi: 10.1176/ajp.144.10.1306
23. Cohen CI, Freeman K, Ghoneim D, Vengassery A, Ghezelaiagh B, Reinhardt MM. Advances in the conceptualisation and study of schizophrenia in later life. *Psychiatr Clin North Am* (2018) 41:39–53. doi: 10.1016/j.psc.2017.10.004
24. Dreher JC, Meyer-Lindenberg A, Kohn P, Berman KF. Age-related changes in midbrain dopaminergic regulation of the human reward system. *Proc Natl Acad Sci U S A* (2008) 105:15106–11. doi: 10.1073/pnas.0802127105
25. Reeves S, Bench C, Howard R. Ageing and the nigrostriatal dopaminergic system. *Int J Geriatr Psychiatry* (2002) 17:359–70. doi: 10.1002/gps.606
26. Remington G, Agid O, Takeuchi H, Lee J, Chintoh A. Ultra-treatment resistance. In: Howes O, editor. *Treatment Response and Resistance in Schizophrenia*. Oxford University Press (2018). p. 144–62. doi: 10.1093/med/9780198828761.003.0012
27. McCreadie RG, Wiles D, Grant S, Crockett GT, Mahmood Z, Livingston MG, et al. The Scottish first episode schizophrenia study. VII. Two-year follow-up. Scottish Schizophrenia Research Group. *Acta Psychiatr Scand* (1989) 80:597–602. doi: 10.1111/j.1600-0447.1989.tb03032.x
28. Malaspina D, Goetz RR, Yale S, Berman A, Friedman JH, Tremeau F, et al. Relation of familial schizophrenia to negative symptoms but not to the deficit syndrome. *Am J Psychiatry* (2000) 157:994–1003. doi: 10.1176/appi.ajp.157.6.994
29. Lindenmayer JP. Treatment refractory schizophrenia. *Psychiatry Q* (2000) 71:373–84. doi: 10.1023/A:1004640408501
30. Cannon M, Jones PB, Murray RM. Obstetric complications and schizophrenia: historical and meta-analytic review. *Am J Psychiatry* (2002) 159:1080–92. doi: 10.1176/appi.ajp.159.7.1080
31. Smith RC, Kadewari RP, Rosenberger JR, Bhattacharyya A. Nonresponding schizophrenia: differentiation by neurological soft signs and neuropsychological tests. *Schizophr Bull* (1999) 25:813–25. doi: 10.1093/oxfordjournals.schbul.a033421
32. Nucifora FC, Woznica E, Lee BJ, Cascella N, Sawa A. Treatment resistant schizophrenia: clinical, biological, and therapeutic perspectives. *Neurobiol Dis* (2018) 18:30494–7. doi: 10.1016/j.nbd.2018.08.016
33. Crespo-Facorro B, de la Foz VO, Ayesa-Arriola R, Perez-Iglesias R, Mata I, Suarez-Pinilla P, et al. Prediction of acute clinical response following a first episode of non affective psychosis: results of a cohort of 375 patients from the Spanish PAFIP study. *Prog Neuropsychopharmacol Biol Psychiatry* (2013) 44:162–7. doi: 10.1016/j.pnpbp.2013.02.009
34. Joober R, Rouleau GA, Lal S, Bloom DM, Lalonde P, Labelle A, et al. Increased prevalence of schizophrenia spectrum disorders in relatives of neuroleptic-nonresponsive schizophrenic patients. *Schizophr Res* (2005) 77:35–41. doi: 10.1016/j.schres.2005.01.008
35. Kapur S, Zipursky R, Jones C, Remington G, Houle S. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first episode schizophrenia. *Am J Psychiatry* (2000) 157:514–20. doi: 10.1176/appi.ajp.157.4.514
36. Zipursky RB, Christensen BK, Daskalakis Z, Epstein I, Roy P, Furimsky I, et al. Treatment response to olanzapine and haloperidol and its association with dopamine D receptor occupancy in first-episode psychosis. *Can J Psychiatry* (2005) 50:462–9. doi: 10.1177/070674370505000806
37. Nordstrom AL, Farde L, Nyberg S, Karlsson P, Halldin C, Sedvall G. D2, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients. *Am J Psychiatry* (1995) 152:1444–9. doi: 10.1176/ajp.152.10.1444
38. Wolkin A, Barouche F, Wolf AP, Rotrosen J, Fowler JS, Shiue CY, et al. Dopamine blockade and clinical response: evidence for two biological subgroups of schizophrenia. *Am J Psychiatry* (1989) 146:905–8. doi: 10.1176/ajp.146.7.905
39. Pilowsky LS, Costa DC, Ell PJ, Murray RM, Verhoeff NP, Kerwin RW. Antipsychotic medication, D2 dopamine receptor blockade and clinical response: a 123I IBZM SPECT (single photon emission tomography) study. *Psychol Med* (1993) 23:791–7. doi: 10.1017/S0033291700025575
40. Kane K, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch Gen Psychiatry* (1988) 45:789–96. doi: 10.1001/archpsyc.1988.01800330013001
41. Demjaha A. *Biological and clinical determinants of treatment resistant schizophrenia.* [dissertation]. London (UK): King's College London (2014).
42. Fusar-Poli P, Meyer-Lindenberg A. Striatal presynaptic dopamine in schizophrenia, Part II: Meta-analysis of [18F/11C]-DOPA PET studies. *Schizophr Bull* (2012) 39:33–42. doi: 10.1093/schbul/sbr180
43. Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry* (2012) 69:776–86. doi: 10.1001/archgenpsychiatry.2012.169
44. Demjaha A, Murray RM, McGuire PK, Kapur S, Howes OD. Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. *Am J Psychiatry* (2012) 169:1203–10. doi: 10.1176/appi.ajp.2012.12010144
45. Demjaha A, Egerton A, Murray RM, Kapur S, Howes OD, Stone JM, et al. Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function. *Biol Psychiatry* (2014) 75:e11–13. doi: 10.1016/j.biopsych.2013.06.011
46. Mouchlianitis E, Bloomfield MA, Law V, Beck K, Selvaraj S, Rasquinha N, et al. Treatment-resistant schizophrenia patients show elevated anterior cingulate cortex glutamate compared to treatment-responsive. *Schizophr Bull* (2015) 42:744–52. doi: 10.1093/schbul/sbv151
47. Jauhar S, Veronese M, Nour MM, Rogdaki M, Hathway P, Turkheimer FE, et al. Determinants of treatment response in first-episode psychosis: an 18F-DOPA PET study. *Mol Psychiatry* (2018) doi: 10.1038/s41380-018-0042-4
48. Egerton A, Broberg BV, Van Haren N, Merritt K, Barker GJ, Lythgoe DJ, et al. Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre ^{1}H-MRS study (OPTiMiSE). *Mol Psychiatry* (2018) 23(11):2145–55. doi: 10.1038/s41380-018-0082-9
49. Gillespie AL, Samanaite R, Mill J, Egerton A, MacCabe JH. Is treatment-resistant schizophrenia categorically distinct from treatment-responsive schizophrenia? A systematic review. *BMC Psychiatry* (2017) 17(1):12. doi: 10.1186/s12888-016-1177-y
50. Vita A, Minelli A, Barlati S, Deste G, Giacopuzzi E, Valsecchi P, et al. Treatment-resistant schizophrenia: genetic and neuroimaging correlates. *Front Pharmacol* (2019) 10:402. doi: 10.3389/fphar.2019.00402

51. Carlsson A, Waters N, Hom-Waters S, Tedroff K, Nilsson M, Carlsson ML. Interactions between monoamines, glutamate, and GABA in schizophrenia: new evidence. *Annu Rev Pharmacol Toxicol* (2001) 41:237– 60. doi: 10.1146/annurev.pharmtox.41.1.237
52. Carlsson A, Waters N, Waters S, Carlsson ML. Network interactions in schizophrenia: therapeutic implications. *Brain Res Rev* (2000) 31:342–9. doi: 10.1016/S0165-0173(99)00050-8
53. Gleich T, Deserno L, Lorenz RC, Boehme R, Pankow A, Buchert R, et al. Prefrontal and striatal glutamate differently relate to striatal dopamine: potential regulatory mechanisms of striatal presynaptic dopamine function? *J Neurosci* (2015) 35(26):9615–21. doi: 10.1523/JNEUROSCI.0329-15.2015
54. Jauhar S, McCutcheon R, Borgan F, Veronese M, Nour M, Pepper F, et al. The relationship between cortical glutamate and striatal dopamine in first-episode psychosis: a cross-sectional multimodal PET and magnetic resonance spectroscopy imaging study. *Lancet Psychiatry* (2018) 5(10):816–23. doi: 10.1016/S2215-0366(18)30268-2
55. Frank J, Lang M, Witt SH, Strohmaier J, Rujescu D, Cichon S, et al. Identification of increased genetic risk scores for schizophrenia in treatment-resistant patients. *Mol Psychiatry* (2015) 20:150–1. doi: 10.1038/mp.2014.56
56. Santoro ML, Ota V, de Jong S, Noto C, Spindola LM, Talarico F, et al. Polygenic risk score analyses of symptoms and treatment response in an antipsychotic-naïve first episode of psychosis cohort. *Transl Psychiatry* (2018) 8:174. doi: 10.1038/s41398-018-0230-7
57. Bilder RM, Wu H, Chakos MH, Bogerts B, Pollack S, Aronowitz J, et al. Cerebral morphometry and clozapine treatment in schizophrenia. *J Clin Psychiatry* (1994) 55(suppl B):53–6.
58. Stern RG, Kahn RS, Davidson M. Predictors of response to neuroleptic treatment in schizophrenia. *Psychiatr Clin North Am* (1993) 16:313–38. doi: 10.1016/S0193-953X(18)30176-X
59. Palaniyappan L, Marques TR, Taylor H, Handley R, Mondelli V, Bonaccorso S, et al. Cortical folding defects as markers of poor treatment response in first-episode psychosis. *JAMA Psychiatry* (2013) 70:1031–40. doi: 10.1001/jamapsychiatry.2013.203
60. Zugman A, Gadelha A, Assuncao I, Sato J, Ota VK, Rocha DL, et al. Reduced dorso-lateral prefrontal cortex in treatment resistant schizophrenia. *Schizophr Res* (2013) 148:81–6. doi: 10.1016/j.schres.2013.05.002
61. Ganella EP, Bartholomeusz CF, Seguin C, Whittle S, Bousman C, Phassouliotis C, et al. Functional brain networks in treatment-resistant schizophrenia. *Schizophr Res* (2017) 184:73–81. doi: 10.1016/j.schres.2016.12.008
62. Vanes LD, Mouchlianitis E, Collier T, Averbeck BB, Shergill SS. Differential neural reward mechanisms in treatment-responsive and treatment-resistant schizophrenia. *Psychol Med* (2018) 48(14):2418–27. doi: 10.1017/S0033291718000041
63. Nakajima S, Takeuchi H, Plitman E, Fervaha G, Gerretsen P, Caravaggio F, et al. Neuroimaging findings in treatment-resistant schizophrenia: a systematic review. *Schizophr Res* (2015) 164:164–75. doi: 10.1016/j.schres.2015.01.043
64. Mouchlianitis E, McCutcheon R, Howes OD. Brain-imaging studies of treatment-resistant schizophrenia: a systematic review. *Lancet Psychiatry* (2016) 3(5):451–63. doi: 10.1016/S2215-0366(15)00540-4
65. Taylor DM, Duncan-McConnell D. Refractory schizophrenia and atypical antipsychotics. *J Psychopharmacol* (2000) 14:409–18. doi: 10.1177/026988110001400411
66. McEvoy JP, Lieberman JA, Stroup TS, Davis SM, Meltzer HY, Rosenheck RA, et al. 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* (2006) 163:600–10. doi: 10.1176/appi.ajp.163.4.600
67. Lewis SW, Barnes TR, Davies L, Murray RM, Dunn G, Hayhurst KP, et al. Randomized controlled trial of effect of prescription of clozapine versus other second-generation antipsychotic drugs in resistant schizophrenia. *Schizophr Bull* (2006) 32:715–23. doi: 10.1093/schbul/sbj067
68. Shah P, Iwata Y, Plitman E, Brown EE, Caravaggio F, Kim J, et al. The impact of delay in clozapine initiation on treatment outcomes in patients with treatment-resistant schizophrenia: a systematic review. *Psychiatry Res* (2018) 268:114–22. doi: 10.1016/j.psychres.2018.06.070
69. Patel MX, Bishara D, Jayakumar S, Zalewska K, Shiers D, Crawford MJ, et al. Quality of prescribing for schizophrenia: evidence from a national audit in England and Wales. *Eur Neuropsychopharmacol* (2014) 24:499–509. doi: 10.1016/j.euroneuro.2014.01.014
70. Harrison J, Janlov M, Wheeler AJ. Patterns of clozapine prescribing in a mental health service in New Zealand. *Pharm World Sci* (2010) 32:503–11. doi: 10.1007/s11096-010-9398-5
71. Yoshimura B, Yada Y, So R, Takaki M, Yamada N. The critical treatment window of clozapine in treatment-resistant schizophrenia: secondary analysis of an observational study. *Psychiatry Res* (2017) 250:65–70. doi: 10.1016/j.psychres.2017.01.064
72. Ucok A, Cikrikcili U, Karabulut S, Salaj A, Ozturk M, Tabak O, et al. Delayed initiation of clozapine may be related to poor response in treatment-resistant schizophrenia. *Int Clin Psychopharmacol* (2015) 30:290–5. doi: 10.1097/YIC.0000000000000086
73. Kahn RS, Winter van Rossum I, Leucht S, McGuire P, Lewis SW, Leboyer M, et al. Amisulpride and olanzapine followed by open-label treatment with clozapine in first-episode schizophrenia and schizophreniform disorder (OPTiMiSE): a three-phase switching study. *Lancet Psychiatry* (2018) 5(10):797–807. doi: 10.1016/S2215-0366(18)30252-9
74. Okhuijsen-Pfeifer C, Huijsman EAH, Hasan A, Sommer IEC, Leucht S, Kahn RS, et al. Clozapine as a first- or second-line treatment in schizophrenia: a systematic review and meta-analysis. *Acta Psychiatr Scand* (2018) 138:281–8. doi: 10.1111/acps.12954
75. Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, et al. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci U S A* (2000) 97:8104–9. doi: 10.1073/pnas.97.14.8104
76. Wulff S, Pinborg LH, Svarer C, Jensen LT, Nielsen MO, Allerup P, et al. Striatal D(2/3) binding potential values in drug-naïve first-episode schizophrenia patients correlate with treatment outcome. *Schizophr Bull* (2015) 41:1143–52. doi: 10.1093/schbul/sbu220
77. Gellman RL, Aghajanian GK. Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors antagonism by atypical antipsychotic drugs. *Neuroscience* (1994) 58:515–25. doi: 10.1016/0306-4522(94)90077-9
78. Javitt DC. Glutamate as a therapeutic target in psychiatric disorders. *Mol Psychiatry* (2004) 9:984–97. doi: 10.1038/sj.mp.4001551
79. Tanahashi S, Yamamura S, Nakagawa M, Motomura E, Okada M. Clozapine, but not haloperidol, enhances glial d-serine and l-glutamate release in rat frontal cortex and primary cultured astrocytes. *Br J Pharmacol* (2012) 165:1543–55. doi: 10.1111/j.1476-5381.2011.01638.x
80. Meltzer HY. Antidepressive drug treatment of the schizophrenic patient. *Schizophr Bull* (1992) 18:515–42. doi: 10.1093/schbul/18.3.515
81. Miyamoto S, Jarskog LF, Fleischhacker WW. New therapeutic approaches for treatment-resistant schizophrenia: a look to the future. *J Psychiatr Res* (2014) 58:1–6. doi: 10.1016/j.jpsychires.2014.07.001
82. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. *Am J Psychiatry* (1999) 156:286–93. doi: 10.1176/ajp.156.2.286
83. Porcelli S, Balzarro B, Serretti A. Clozapine resistance: augmentation strategies. *Eur Neuropsychopharmacol* (2012) 22:165–82. doi: 10.1016/j.euroneuro.2011.08.005
84. Taylor DM, Smith L, Gee SH, Nielsen J. Augmentation of clozapine with a second antipsychotic—a meta-analysis. *Acta Psychiatr Scand* (2012) 125:15–24. doi: 10.1111/j.1600-0447.2011.01792.x
85. Sommer IE, Begemann MJ, Temmerman A, Leucht S. Pharmacological augmentation strategies for schizophrenia patients with insufficient response to clozapine: a quantitative literature review. *Schizophr Bull* (2012) 38:1003–11. doi: 10.1093/schbul/sbr004
86. Tiihonen K, Wahlbeck K, Kiviniemi V. The efficacy of lamotrigine in clozapine-resistant schizophrenia: a systematic review and meta-analysis. *Schizophr Res* (2009) 109:10–4. doi: 10.1016/j.schres.2009.01.002
87. Petrides G, Malur C, Braga RJ, Bailine SH, Schooler NR, Malhotra AK, et al. Electroconvulsive therapy augmentation in clozapine-resistant schizophrenia: a prospective, randomized study. *Am J Psychiatry* (2015) 172:52–8. doi: 10.1176/appi.ajp.2014.13060787
88. Wang G, Zheng W, Li XB, Wang SB, Cai DB, Yang XH, et al. ECT augmentation of clozapine for clozapine-resistant schizophrenia: a

meta-analysis of randomized controlled trials. *J Psychiatr Res* (2018) 105:23–32. doi: 10.1016/j.jpsychires.2018.08.002
89. De Jesus DR, Gil A, Barbosa L, Lobato MI, Magalhaes PV, Favalli GP, et al. A pilot double-blind sham-controlled trial of repetitive transcranial magnetic stimulation for patients with refractory schizophrenia treated with clozapine. *Psychiatry Res* (2011) 188:203–7. doi: 10.1016/j.psychres.2010.11.022
90. Brunelin J, Mondino M, Gassab L, Haesebaert F, Gaha L, Suaud-Chagny MF, et al. Examining transcranial direct-current stimulation (tDCS) as a treatment for hallucinations in schizophrenia. *Am J Psychiatry* (2012) 169:719–24. doi: 10.1176/appi.ajp.2012.11071091
91. Slotema CW, Aleman A, Daskalakis ZJ, Sommer IE. Meta-analysis of repetitive transcranial magnetic stimulation in the treatment of auditory verbal hallucinations: update and effects after one month. *Schizophr Res* (2012) 142:40–5. doi: 10.1016/j.schres.2012.08.025
92. Andrade C. Transcranial direct current stimulation for refractory auditory hallucinations in schizophrenia. *J Clin Psychiatry* (2013) 74:e1054–1058. doi: 10.4088/JCP.13f08826
93. Slotema CW, Blom JD, Hoek HW, Sommer IE. Should we expand the toolbox of psychiatric treatment methods to include Repetitive Transcranial Magnetic Stimulation (rTMS)? A meta-analysis of the efficacy of rTMS in psychiatric disorders. *J Clin Psychiatry* (2010) 71:873–84. doi: 10.4088/JCP.08m04872gre
94. Tsai GE, Lin PY. Strategies to enhance *N*-methyl-d-aspartate receptor-mediated neurotransmission in schizophrenia, a critical review and meta-analysis. *Curr Pharm Des* (2010) 16:522–37. doi: 10.2174/138161210790361452
95. Kantrowitz JT, Javitt DC. Research on glutamatergic dysfunction may lead to therapies targeting negative and cognitive symptoms. *Curr Psychiatry* (2010) 10:68–74.
96. Leweke FM, Mueller JK, Lange B, Fritze S, Topor CE, Koethe D, et al. Role of the endocannabinoid system in the pathophysiology of schizophrenia: implications for pharmacological intervention. *CNS Drugs* (2018) 32:605–19. doi: 10.1007/s40263-018-0539-z
97. Demjaha A, Morgan K, Morgan C, Landau S, Dean K, Reichenberg A, et al. Combining dimensional and categorical representation of psychosis: the way forward for DSM-V and ICD-11? *Psychol Med* (2009) 39:1943–55. doi: 10.1017/S0033291709990651
98. McGuire P, Robson P, Cubala WJ, Vasile D, Morrison PD, Barron R, et al. Cannabidiol (CBD) as an adjunctive therapy in schizophrenia: a multicentre randomised controlled trial. *Am J Psychiatry* (2018) 175(3):225–31. doi: 10.1176/appi.ajp.2017.17030325
99. Demjaha A. On the brink of precision medicine for psychosis: treating the patient, not the disease: a commentary on: association between serum levels of glutamate and neurotrophic factors and response to clozapine treatment by Krivoy et al. 2017. *Schizophr Res* (2018) 193:487–8. doi: 10.1016/j.schres.2017.08.011

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Leung, Gadelrab, Ntephe, McGuire and Demjaha. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership