ORIGINAL ARTICLE

Psychiatry and Clinical Psychopharmacology 2021;31(4):364-369
DOI:10.5152/pcp.2021.20022

Antipsychotics With Different Chemical Structures Cause Different Degrees of Functional Impairments in the Primary Visual Cortex in a Murine Model: A Pilot Study

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ABSTRACT

Background: Antipsychotic medications can impair vision in patients with schizophrenia. However, little is known regarding the pharmacodynamics of antipsychotics in the primary visual cortex. We aimed to study the pharmacodynamics of antipsychotics in the visual cortex in a murine model.
Methods: We used an adapted 2-photon imaging technique to observe changes in calcium dynamics induced by 4 antipsychotics (olanzapine, risperidone, aripiprazole, and amisulpride) in the primary visual cortex of healthy and schizophrenic C57BL/6 mice. Visual function was further assessed by using a novel object recognition test.
Results: All 4 antipsychotics decreased calcium activity in the primary visual cortex and reduced visual recognition test scores in healthy and schizophrenic mice. The most potent drug was olanzapine, followed by risperidone, aripiprazole, and amisulpride. All drugs showed significant differences between groups.
Conclusion: Our pilot study demonstrated that antipsychotics impair visual cortical function. This finding underscores the importance of monitoring for visual adverse events in patients receiving antipsychotic medications to treat schizophrenia.

ARTICLE HISTORY

Received: March 1, 2020
Accepted: November 21, 2020

KEYWORDS: Antipsychotic agents, visual cortex, calcium signaling, schizophrenia

INTRODUCTION

Impaired visual function is a common side-effect of antipsychotic drugs in patients who received treatment for schizophrenia.[1-3] Retinopathies might be one of the pathological factors which further complicates psychotropic medical therapies.[1] On the other hand, visual hallucinations also occur infrequently in schizophrenic patients during antipsychotic treatment.[4] The disrupted visual perception may distort social recognition of other people, named stigmatization, which is one of the prominent features of schizophrenia.[5] Although visual disturbances may be caused by the underlying disease,[6,7] their potential iatrogenic origin is incompletely understood; antipsychotics may either exacerbate or alleviate visual dysfunction.[8,9] Different lines of evidence have presented the role of multiple brain regions underlying the genetic liability of psychosis.[10] Among those regions, it is imperative to investigate the influences of antipsychotics on the visual cortex's function, to further improve evidence-based therapy.

Two-photon in vivo imaging has been used to characterize the murine cortical function and quantify neural activity; it has been used to investigate multiple diseases with central nervous system complications.[11-13] For example, Chen et al.[14] used 2-photon imaging to observe that

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Cite this article as: Yu H, Ma X, Ji F, et al. Antipsychotics with different chemical structures cause different degrees of functional impairments in the primary visual cortex in a murine model: A pilot study. *Psychiatr Clin Psychopharmacol.* 2021;31(4):.364-369

exercise training improves motor skill learning via selective activation of the mTOR pathway in a murine model.[14] This suggested that we could use 2-photon imaging to investigate the visual cortical effects of antipsychotics in a murine model of schizophrenia.

We conducted the pilot study to investigate the effects of antipsychotics on the visual cortex in a murine model of schizophrenia and healthy mice. We hypothesized that (1) antipsychotics might alter the activity of the visual cortex in healthy and schizophrenic mice and (2) drugs of different chemical classes may have differential effects on neural activity in the visual cortex.

METHODS

Murine Model Development

C57BL/6 mice were housed in an animal facility with food and water available ad libitum. To create a murine model of schizophrenia, a single intraperitoneal injection of MK801 (dizocilpine), a noncompetitive *N*-methyl-D-aspartic acid receptor antagonist with psychotomimetic properties, was administered on the 14th day after birth. When the mice were 4 weeks old, their behavior was assessed by sucrose preference and prepulse inhibition (PPI) tests.[15,16] For PPI, a 120 dB (40 ms) startle (PA) was applied after a 20 ms prepulse (PP) at 75 dB with a time interval of 100 ms. Background noise was controlled at 65 dB. The inter-trial time was set at 30 s. Generally, 3 sessions were used, and the scores were averaged. The PPI was calculated as (PA − PP)/(PA). We defined schizophrenic model mice (SMM) as those exhibiting schizophrenic behavior during the PPI test. In contrast, healthy mice were housed in an animal facility with food and water available ad libitum. All experimental procedures on animals were approved by the Animal Ethics Committee of Jining Medical University, The First Hospital of Shanxi Medical University, Tianjin Mental Health Center (IRB number: JSTEBSRA-001).

Study Groups

We divided SMM into 5 groups of 10 mice. The first group received no antipsychotic agents; groups 2 through 5 were administered antipsychotic agents from different chemical classes: either a thiobenzodiazepine (olanzapine), a benzisoxazole derivative (risperidone), a dihydrocarbostyril derivative (aripiprazole), or a benzamide (amisulpride), respectively. Healthy mice were also divided into 5 groups of 10; the first group received no antipsychotic agents, while groups 2 through 5 were administered either olanzapine, risperidone, aripiprazole, or amisulpride, respectively. Uniform chlorpromazine-equivalent doses were calculated as previous reports. In this pilot study, each group was administrated 600 mg/day of chlorpromazine equivalent to human use dosage.[17] Each group was treated for 8 weeks and observed from 4 to 8 weeks of age. This study was approved by the Ethics Committee of Wenzhou Seventh People's Hospital.

Behavioral Assessment: All behavioral tasks were performed on day 3 after the end of the intervention. The animals were sequentially enrolled in a sucrose preference test with a 24-h interval. The second cohort of mice was tested using the PPI apparatus. The sucrose preference test was adopted, following previously published methodologies.[14] For the PPI test, a 120-dB (40 ms) startle (PA) was applied after a 20-ms prepulse (PP) at 75 dB, with a time interval of 100 ms. The background noise was controlled at 65 dB. The inter-trial time was set to 30 s. In general, 3 sessions were used, and the scores were averaged. PPI was calculated as (PA − PP)/(PA).[15]

The novel object recognition (NOR) test was performed in a 25 × 25 cm plastic chamber, in which the mice were first allowed to freely explore 2 identical objects, followed by a 30-min contextual acclimation. In the recognition session, 1 of these objects was replaced by a novel one with different shapes. The recognition ratio was calculated as the time spent on the novel object divided by the total exploration time on the novel and familiar object, during the recognition phase.

In Vivo Calcium Activity Recordings and Analysis

The neuronal activity of the visual cortex was determined according to previously published methods,[16] with slight modifications. In brief, anesthetized mice were fixed, and a chronic cranial window was created at 4 weeks. A 200 nL inoculum of adeno-associated virus 2/9-syn-GCaMP6s (2×10^{13} genome copies/mL; University of Pennsylvania Vector Core Facility) was injected bilaterally into the visual cortex using the following coordinates: −3.0 mm from the bregma, ±2.5 mm. The imaging window was covered by a circular coverslip, and the cranium was sealed using dental cement. A customized steel bar was embedded into the cranium for fixation of the head during imaging. The calcium activities of pyramidal neurons in layers 2/3 of the primary visual cortex were determined by 2-photon imaging in awake head-fixed mice at 4th and 8th weeks, according to previously reported methods.[17] A 2-photon microscope (LSM780; Zeiss, Germany) was used with a 16 × 0.8 numerical aperture water-immersion objective. Using an excitation wavelength of 950 nm, time-series images were recorded at 1.96 Hz for 150 s periods. Captured images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Regions of interest were selected manually in ImageJ with the FIJI plug-in package,[18] followed by detection and normalization of calcium transients.

Statistical Analysis

All experimental data are presented as the mean±standard error of the mean, unless otherwise specified. The 2-sample Student's *t*-test or the nonparametric Kolmogorov–Smirnov

test was used to compare means between 2 groups. For multi-group comparisons, 1-way analysis of variance was performed, followed by Tukey's post-hoc comparison. GraphPad Prism 7.0 was used for statistical analyses and data plotting. *P* values of <0.05 were considered significant.

RESULTS

In-Study Survival

In the SMM groups, 43 mice survived until the age of 8 weeks, including 9/10 untreated controls, 7/10 receiving olanzapine, 8/10 receiving risperidone, 10/10 receiving aripiprazole, and 9/10 receiving amisulpride. All 50 healthy mice survived.

Dysregulation of Visual Cortical Activities

Time-lapsed recordings of neuronal activity demonstrated changes in calcium activity in the visual cortex of healthy mice and SMM given antipsychotics and controls (Figures 1B and 2A). Quantitative analysis of 2-photon imaging revealed that antipsychotics inhibited calcium activity in SMM in the following order of decreasing activity with significant differences between groups: olanzapine > risperidone > aripiprazole > amisulpride (Figure 1C). The behavioral performance in SMM deteriorated in a corresponding sequence of drug effects with significant differences between groups: olanzapine > risperidone > aripiprasole > amisulpride (Figure 1D).

We further tested the adverse effect of antipsychotic drugs in healthy mice. Using a similar analysis of 2-photon in vivo imaging, we found that the 4 antipsychotics impaired calcium activity in the following order: olanzapine > risperidone > aripiprazole > amisulpride (Figure 2A). There were corresponding effects in the behavioral performance of healthy mice (Figure 2B).

DISCUSSION

This study demonstrated that different antipsychotics impaired visual cortical functions to various degrees as

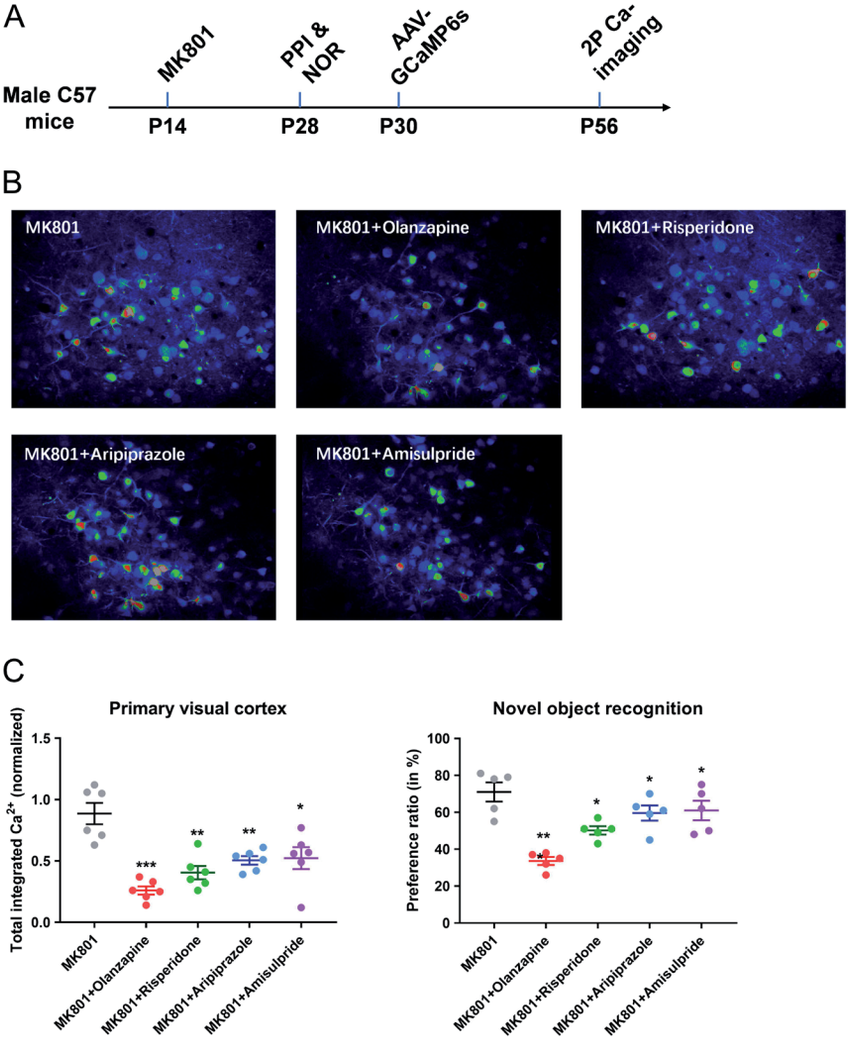

Figure 1.a-c. Effects of antipsychotics on the visual cortex and visual learning in SMM mice.

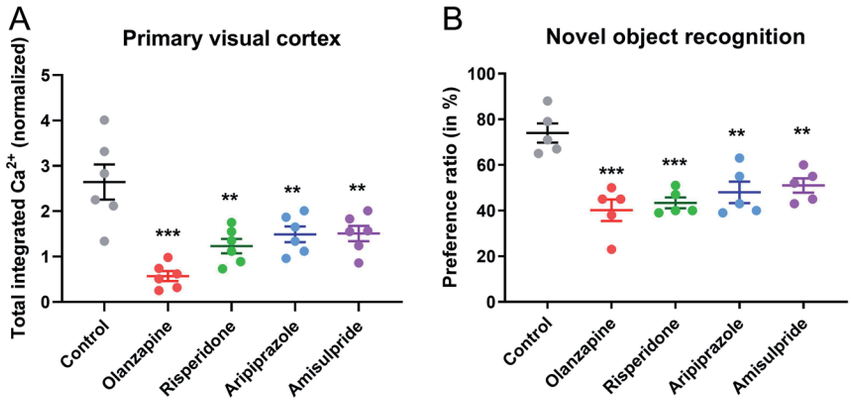

Figure 2.a,b. Effects of antipsychotics on the visual cortex and visual learning in healthy mice.

well as the impairment of NOR function. To the best of our knowledge, this pilot study provides the first evidence obtained by in vivo 2-photon imaging that antipsychotic agents impair visual cortical function in a murine model incorporating both SMM and healthy mice. The impaired behavioral performance was also associated with the altered functional activity of the visual cortex. These 2 observations are worthy of further investigation.

We found that antipsychotic agents impaired neural activity in the primary visual cortex in SMM and healthy mice. These findings provide evidence that antipsychotic agents cause visual cortical dysfunction and underscore the importance of clinical monitoring of visual symptoms in patients treated for schizophrenia. Previous studies of antipsychotic drug activity on the visual tract yielded inconsistent findings of both beneficial and toxic effects.[11] Our study suggests the toxicity of antipsychotic agents on the visual system. Although our study was only a murine pilot study, it provides direction for further investigations.

Second, we found that different chemical classes of antipsychotic agents exhibited differential toxicity (all with significant differences), even though we used uniform dose equivalents.[19] Olanzapine was the most toxic agent, followed by risperidone, while aripiprazole and amisulpride were relatively less toxic.

Visual side-effects of antipsychotics may be of multifactorial origin. Impaired visual accommodation is often ascribed as an anticholinergic side-effect.[2,3] Also, hyperglycemia is a common adverse effect of olanzapine and risperidone,[20-22] and can accelerate cataract formation, cause osmotic water shifts in the lens that change lenticular geometry and alter visual acuity, and lead to retinal microvascular disease, retinopathy, and visual loss.[23-26] However, these are potential indirect effects of antipsychotics rather than direct neurotoxicities to the visual tract. Our pilot study provides direct evidence that antipsychotic agents impair the function of the visual cortex. The pharmacodynamics of antipsychotic agents in the visual cortex may be related to dopaminergic receptor antagonism. Reduced dopaminergic activity in the visual cortex can increase GABAergic neural activity and subsequently inhibit visual cortical neural activity.[27-31] Because olanzapine and risperidone are the most toxic dopamine antagonists of the 4 drugs, it logically follows that they would cause the most severe functional impairments of the visual cortex. However, this is a hypothetical speculation that requires further studies of neurotransmitter activity for clarification.

Limitations

There are several limitations to this pilot study. First, the variable survival rates among the SMM study groups may have introduced survivor bias. Second, the novel subject recognition task's uncertain accuracy for evaluating the murine visual function may have introduced additional errors; however, there were no alternative methods available to us. Third, this pilot study evaluated drugs in only 4 chemical classes that may not represent the entire range of antipsychotic agents. In future studies, other drugs will be used in SMM to explore their effects in the entire visual pathway from the eye to the primary and high-level visual cortex and to examine potential drug-related structural and functional effects. These studies may enable the formulation of prevention and intervention strategies to preserve vision in patients with schizophrenia.

CONCLUSION

This study provides evidence that antipsychotic agents impair the visual cortex's function and may contribute to visual perception disturbances in patients treated for schizophrenia. An especially notable finding was that different chemical classes of antipsychotic agents have differential effects in the visual cortex. In short, frequently used antipsychotics may impair visual cortical functions, implying the necessity of routine functional checks on patients receiving antipsychotic treatment. In clinical practice, such prominently adverse effects can be reduced by neuromodulation approaches, such as transcranial

magnetic stimulation. Moreover, this study provides direction for further studies of the pharmacodynamics of additional antipsychotic drug classes on the visual cortex. These studies can lead to optimal strategies to minimize visual disturbances in schizophrenic patients.

Ethics Committee Approval: Wenzhou Seventh Peoples Hospital Ethics Committee Approved This Study (IRB:2016-01)

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer Review: Chuanjun Zhuo.

Author Contributions: Concept - C.Z.,H.T., H.Y.; Design - S.G., X.M.; Supervision - X.S., F.J., Y.X.; Resource - C.C., D.J.; Materials - W.Q., X.L.; Data Collection and/or Processing - S.G., X.M., C.C.; Analysis and/or Interpretation - C.C., H.T., H.Y.; Literature Search C.C., D.J.; Materials - W.W., F.J.; Writing - S.G., X.M., C.C.; Critical Reviews - C.Z.

Funding: This work was supported by grants from the National Natural Science Foundation of China (81871052 to C.Z., 81801679 and 81571319 to Y.X.); the Key Projects of the Natural Science Foundation of Tianjin, China (17JCZDJC35700 to C.Z.); the Tianjin Health Bureau Foundation (2014KR02 to C.Z.); the National Key Research and Development Program of China (2016YFC1307004 to Y.X.); the Shanxi Science and Technology Innovation Training Team's Multidisciplinary Team for Cognitive Impairment (201705D131027 to Y.X.); the Zhejiang Public Welfare Fund Project (LGF18H090002 to D.J.); the Key Project of the Wenzhou Science and Technology Bureau (ZS2017011 to X.L.); the Tianjin 131 Scholar Project (to C.Z.); and the Tianjin Anding Hospital of 300000 Scholar Award Project (to C.Z.).

Conflicts of Interest: The authors declare no conflicts of interest.

Data Availability Statement: The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

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