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Schizophrenia-like olfactory dysfunction induced by acute and postnatal phencyclidine exposure in rats

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ABSTRACT

Deficits in olfactory abilities are frequently observed in schizophrenia patients. However, whether olfactory dysfunction is found in animal models is not known. Here, we examined whether two well-established schizophrenia rat models exhibit olfactory-relevant dysfunction that is similar to schizophrenia patients. Olfactory sensitivity was tested in rats that were acutely (3.3 mg/kg) or postnatally (10 mg/kg, at postnatal day 7, 9 and 11) treated with phencyclidine (PCP) as schizophrenia models. Electrophysiological recordings were conducted to measure the olfactory-relevant local field potential after acute PCP treatment. Olfactory-relevant neural connections were tested via virus tracing in rats postnatally treated with PCP. We also assessed the reversal effects of olanzapine (OLZ) treatment on both models. We found that acute PCP treatment induced a decline in olfactory sensitivity ($p = 0.01$) and significantly lower beta- and higher gamma-band oscillations ($p = 0.03$, and $p = 0.00$ respectively) which were partly attenuated by OLZ treatment (2 mg/kg and 4 mg/kg). Postnatal PCP exposure also resulted in an olfactory sensitivity deficit during adulthood ($p = 0.012$ for males and $p = 0.009$ for females), and an abnormal development of neural circuits ($p = 0.000$). Together, our research indicated that olfactory dysfunction found in schizophrenia patients can also be observed on animal models.

1. Introduction

Serious mental illnesses such as schizophrenia affect a large number of people worldwide. In addition to the cognitive symptoms that have been extensively studied (Kalmady et al., 2014; Ross et al., 2006), there is compelling evidence that schizophrenia patients also exhibit functional and structural abnormalities in the olfactory system (Kiparizoska and Ikuta, 2017; Turetsky et al., 2009). These deficits include decreased olfactory sensitivity (Serby et al., 1990), reduced volumes of the entorhinal cortex, perirhinal cortex and bilateral olfactory bulbs (Turetsky et al., 2000), reduced evoked potentials (Kettenmann et al., 1997), and dysregulation of synaptic efficacy (Egbujo et al., 2015).

Animals acutely or postnatally treated with NMDA receptor (NMDAR) antagonists such as phencyclidine (PCP) are extensively used as schizophrenia models. Contrary to the well-established studies concerning cognitive symptoms in schizophrenia models (Harms et al., 2018; Young et al., 2009), studies regarding to olfactory-related deficits are relative scanty. Some existing findings have revealed neuronal apoptosis in the olfactory bulbs and piriform cortex upon treatment with NMDAR antagonists (Fiske and Brunjes, 2001; Johnson et al., 1998; Wang et al., 2001), however, it remains to be determined whether schizophrenia animal models show functional and developmental abnormalities in the olfactory system that are similar to those observed in schizophrenia patients.

Here, we examine whether olfactory dysfunction, as demonstrated via abnormalities in behaviors, electrophysiology and neural circuits, is present in rats that are acutely or postnatally treated with PCP as acute and developmental model of schizophrenia.

2. Methods

2.1. Animals and drugs

All Sprague-Dawley rats and pups were purchased from Vital River (Beijing, China) and housed under a controlled 12–12 h light-dark cycle (9:00–21:00) with suitable temperature and humidity. The experiments were approved by the Animal Experimental Committee, Kunming Institute of Zoology, Chinese Academy of Sciences. All animal

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experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Guidelines). PCP (Chemsky International Co. Ltd., Shanghai, China), Olanzapine (Eli Lilly, Indianapolis, USA). See also Supplement Information.

2.2. Buried food task and no-bedding food task

As previously described (Yang and Crawley, 2009), rats were allowed to find a piece (1.5 cm in length) of fresh rat food buried under the bedding in one corner of the experimental cage. The time until food discovery was recorded to assess its olfactory sensitivity. See also Supplement Information.

The procedure of *no-bedding food task* was the same for the *buried food task*. The only difference was that the food was exposed and could been seen directly during the experiments.

2.3. Local field potential (LPF) recording in olfactory bulbs (OB)

As previously described (Li et al., 2011), two microelectrodes were implanted to the granule cell layer of bilateral OBs (±1.0 mm lateral to midline, −8.0 mm anterior to Bregma, −3.0 mm from the skull) of adult male rats. The recording site was identified via histological detection after the experiments. A screw was implanted in the parietal lobe to serve as the recording reference.

After 1 week of recovery, the baseline of LFP signals of awake rats were recorded after saline, PCP (3.3 mg/kg) or PCP + olanzapine (OLZ, 2 mg/kg or 4 mg/kg) injection. The LFP signals were amplified (×2000, Dagan) prior to digitization (μ-1401, CED) and filtered between 0.1 and 100 Hz with a 2000-Hz sampling frequency.

The original data were exported as Matlab files and analyzed using a Matlab procedure developed in our laboratory. The signal between 30 min and 40 min was extracted for further analysis. See also Supplement information.

2.4. Virus tracing of olfactory neural circuits

Retrograde-specific pseudorabies virus expressing GFP (PRV152, 0.67×10^8 pfu/ml, 300 nl) was microinjected into the granule cell layer of right OB (+1.0 mm lateral to midline, −8.0 mm anterior to the bregma, −3.0 mm from the skull) of male rats postnatally treated with PCP (10 mg/kg, at postnatal day 7, 9 and 11). At 48 h after infection, the brain was serially sectioned into 40-mm-thick coronal sections. After immunohistochemistry, target areas of the brain sections were scanned sequentially under a 10× objective and the fluorescence intensity of each section was further analyzed. For each rat, six sections (approximate intervals of 320 μm) from approximately −0.12 mm to −2.00 mm from the bregma were used for further analysis. See also Supplement information.

The *difference coefficient* of fluorescence intensity between the homolateral and contralateral olfactory cortices was calculated using the following equation: (homolateral intensity − contralateral intensity) / (homolateral intensity + contralateral intensity). This coefficient reflected the magnitude of the differences in the centrifugal connection from PC/AIP to OB between the homolateral and contralateral cortices.

2.5. Statistical analysis

Statistical comparisons were performed using the independent sample *t*-test and paired samples *t*-test as described above. All values are presented as the mean ± SEM. The null hypothesis was rejected at $p < 0.05$.

3. Results

3.1. Acute PCP exposure induces impairments in olfactory sensitivity

To investigate whether acute PCP treatment can induce the olfactory dysfunction observed in schizophrenia patients (Turetsky et al., 2009), adult male rats were hypodermically injected (i.h.) with saline ($n = 8$) or PCP (3.3 mg/kg, $n = 8$), and their olfactory sensitivity was tested in the *buried food task* 30 min after treatment (Fig. 1A). We found that the rats that were acutely treated with PCP showed a significant increase in time until discovery of the buried food compared with the controls [$p = 0.01$, independent sample *t*-test] (Fig. 1B). However, in *no-bedding food task*, the rats' performance showed no differences (Fig. 1C). Together, these findings indicate that acute PCP treatment in adult rats can lead to a decline in olfactory sensitivity. However, the disruptive effect of acute PCP challenge was reversible at 60 h after injection (Fig. S1) (Table 1).

To verify the validity of our rats as animal models of schizophrenia, we tested their locomotor abilities using open field test and got the same results with previous research (Maia-de-Oliveira et al., 2015), which indicated that our acute-exposed rats have the same ability to model the symptoms of schizophrenia (Fig. S2).

3.2. Acute PCP exposure induces impairments in local field potential and the reversal effects of olanzapine

To examine whether acute PCP exposure can also lead to abnormal oscillation in olfactory regions of conscious rats, adult male rats were hypodermic injected with PCP (3.3 mg/kg, i.h.), and their baseline local field potentials (LFPs) in the granule cell layer of bilateral OBs were recorded from 30 min to 40 min after PCP injection (Fig. 2A). The recorded starting time, duration and dose of PCP were chosen in accordance with the *buried food task* described above. The administration of PCP produced a significant increase in the baseline power (Spike 2) of the low-(36–60 Hz), high-(61–90 Hz), and total gamma (36–90 Hz) oscillations [$n = 10$, $p = 0.00$, $p = 0.02$ and $p = 0.00$ respectively, paired samples *t*-test], whereas the power of the beta oscillation (13–35 Hz) was significantly reduced compared with controls [$p = 0.03$, paired samples *t*-test]. The power of the theta oscillation (0.1–12 Hz) showed no difference between groups (Fig. 2B, see also Fig. S3).

We next investigated whether combined treatment with antipsychotic drug olanzapine (OLZ) could attenuate the PCP-induced abnormality in neural synchrony. OLZ treatment (2 mg/kg, i.h.) restored the PCP (3.3 mg/kg, i.h.)-induced decrease in beta oscillation and increase in high-gamma oscillation to normal levels. However, the powers of the low-gamma and total gamma oscillation were still significantly higher [$n = 7$, $p = 0.012$ and $p = 0.016$ respectively, paired samples *t*-test]. The power of theta oscillation was significantly increased [$p = 0.047$, paired samples *t*-test], possibly due to the enhancement in respiration from the calming effect of OLZ as an antipsychotic drug (Fig. 2C).

We next doubled the dose of OLZ to 4 mg/kg and tested the LFP again. The power of the beta, low-gamma and total gamma oscillation showed no significant difference compared with control. However, the high-gamma oscillation was over-inhibited by a double dose of OLZ [$n = 10$, $p = 0.045$, paired samples *t*-test]. The power of the theta oscillations increased continuously with the effect of high-dose OLZ [$p = 0.003$, paired samples *t*-test] (Fig. 2D).

3.3. Postnatal PCP exposure induces impairments in olfactory sensitivity and the reversal effects of olanzapine

To examine whether postnatal PCP exposure could lead to abnormal olfactory ability in adult stages, rat pups were intraperitoneally injected (i.p.) with saline or PCP (10 mg/kg) at postnatal day (PD) 7, 9 and 11, and their olfactory sensitivity was tested with the *buried food task* at adulthood (8 weeks old, Fig. 3A). Both male ($n = 9$) and female

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