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A potential association between *Toxoplasma gondii* infection and schizophrenia in mouse models

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HIGHLIGHTS

- The relative clinical symptom of schizophrenia was tested in mouse models.
- Mice from *Toxoplasma* infection and schizophrenia had a similar impairment.
- Study explored a potential association between *Toxoplasma* and schizophrenia.

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ABSTRACT

Schizophrenia is a serious neuropsychiatric disease of uncertain etiology, which causes human mental disorder and affects about 1% of the population. In recently years, some studies showed that some cases of schizophrenia may be associated with *Toxoplasma gondii* infection. In order to investigate a potential association between *Toxoplasma* infection and schizophrenia, we investigated the relative clinical symptom of schizophrenia such as learning and memory capability, depression and stereotypy to find some useful information by behavioral test in mouse models. Our results demonstrated that mice from *Toxoplasma* infection and MK-801 administration (as the model of schizophrenia) were impaired in learning and memory capability, and they had more serious depression and stereotypy compared with the control mice, especially the mice from congenital *Toxoplasma* infection. In addition, our results clearly showed that the number of cysts in brain tissue of congenital *Toxoplasma* infection mice was significantly low than in acquired *Toxoplasma* infected mice. Collectively, these results suggested a potential association between *Toxoplasma* infection and schizophrenia.

1. Introduction

Schizophrenia is a serious neuropsychiatric disease of uncertain etiology, which causes human mental disorder and affects about 1% of the population (Torrey and Yolken, 2003). An increased occurrence of schizophrenia in family members of affected persons suggests that genetic factors play a role in its etiology (Freedman, 2003). Epidemiological and neuropathological studies have also indicated that some cases of schizophrenia may be associated with environmental factors, especially exposure to infectious agents such as rubella, herpes simplex, polio, varicella zoster virus and *Toxoplasma gondii* (Yolken et al., 2001).

In recent years, *Toxoplasma* has emerged as an interesting candidate as a possible cause of some cases of schizophrenia (Torrey and Yolken, 2003). *Toxoplasma* is a protozoan parasite that can infects a wide variety of warm-blooded vertebrates, including cats, livestock, and humans. Humans are infected with *Toxoplasma* cysts after ingesting cat feces or undercooked meat (Boothroyd, 2009). *Toxoplasma* infection can lead to toxoplasmosis including congenital and acquired toxoplasmosis. Congenital toxoplasmosis can reduce intellectual function of human, and it has been estimated that, in some regions, up to 9% of cases of mental retardation are associated with *Toxoplasma* infection (Flegr et al., 2003).

With respect to *Toxoplasma* as a possible cause of some cases of schizophrenia, many works focused on a large number of epidemiological and serological studies. These studies have indicated that *Toxoplasma* infection has a association with schizophrenia (Torrey and Yolken, 2003; Torrey et al., 2012). In animal models, infection of *Toxoplasma* can lead to change of the host behavior such as moving, rearing, digging and grooming (Hay et al., 1985; Webster, 1994; Berdoy et al., 1995; Bech-Nielsen, 2012). But these neurologic manifestations mostly do not reflect the relative clinical symptom of schizophrenia.

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In order to further investigate a potential association between *Toxoplasma* infection and schizophrenia, we established the mouse model of schizophrenia by MK-801 (a non-competitive and selective NMDA receptor blocker) (Harris et al., 2003; Janac et al., 2008). Meanwhile, we also established the mouse models of *Toxoplasma* infection (congenital infection and acquired infection). Then, we tested the relative clinical symptom of schizophrenia such as learning and memory capability, depression and stereotypy to find some useful information by these mice models.

To date, there is little information about the potential association between *Toxoplasma* infection and schizophrenia in mouse model. Our study will provide a better understanding of defining the possible association between *Toxoplasma* exposure and the risk of schizophrenia.

2. Materials and methods

2.1. Animals and parasites

NIH mice weighing from 35 to 40 g used for establishing animal model and BALB/c mice used for the maintenance of *Toxoplasma* were purchased from the Experimental Animal Centre of Nanchang University, Jiangxi province. The protocol was conducted according to international regulations for animal experimentation.

The Prugniaud (Pru) strain of *Toxoplasma* which was kindly provided by Prof. Sun of Bengbu Medical University, was used throughout the experiment. It was maintained by oral inoculation of cysts from brain tissue of infected BALB/c mice every 3 months. Cysts for the infection of NIH mice were isolated from the brain tissue of infected BALB/c mice and the number of cysts was counted under a microscopy with a 10× objective.

2.2. Animal model establishment

2.2.1. Animal model of Toxoplasma infection

Animal model of *Toxoplasma* infection was performed as described previously (Wang et al., 2011). Female and male NIH mice were caged together at 2:1. Copulation was assessed every 12 h by vaginal suppository. The procedure was repeated until all NIH mice were found to have copulated. In congenital infection group 12 pregnant mice were infected with 5 cysts of *Toxoplasma* by the oral route on the 5th day after gestation. In acquired infection group, 24 mice were infected with 5 cysts of *Toxoplasma* by the oral route.

2.2.2. Animal model of schizophrenia

MK-801 was purchased from the Affiliated Hospital of Jiujiang University. As shown in a previous study (Wu et al., 2005), the injection of MK-801 (0.6 mg/kg, intraperitoneal [i.p.] administration) can induce a similar symptom of schizophrenia in mice. In mouse model of schizophrenia including 12 mice, we selected this dose of MK-801 (0.6 mg/kg) to inject the mice. In the control group, 12 mice were inoculated by the oral route (OR) with 0.2 ml saline solution (0.85%).

2.3. Behavioral test

2.3.1. Step-through passive avoidance test (STPAT)

The learning ability of each mouse was evaluated by step-through passive avoidance test, which was described previously (Zarrindast et al., 2002; Wang et al., 2009). Briefly, the apparatus for the step-through passive avoidance test was an automated shuttle-box which was divided into an illuminated safe compartment and a dark shock compartment of the same size, separated by a wall with a guillotine door. The experiment was divided into learning and memory trials. A mouse was put into the illuminated compartment, facing away from the dark compartment. After 180 s, the door between these two boxes was opened and the mouse was allowed to move into the dark compartment freely. When the mouse stepped into the dark compartment, an inescapable foot-shock (36 V) was delivered through the grid floor and the number of errors within 5 min was recorded. The number of errors was used to assess the learning ability of each mouse. The retention of passive avoidance response, named the memory trial, was measured at 24 h after the learning trial. During the memory trial, each mouse was put into the illuminated compartment and the latency of the first time to enter the dark compartment and the error number within 5 min was recorded. The maximum cut-off time for the latency was 300 s. The latency and number of errors were used to assess the mouse memory. All training and testing were performed between 10:00 am and 3:00 pm.

2.3.2. Forced swimming test (FST)

The experiment was performed according to the procedure described previously, with slight modifications (Piotrowska et al., 2008; Skuza et al., 2009). Briefly, mice were individually forced to swim in a transparent glass cylinder (22-cm high, 14-cm diameter) filled 10-cm high with water (25 ± 0.5 °C). All animals were forced to swim for 6 min, and the duration of immobility was observed and measured during the final 4-min interval of the test. Activity was defined as the swimming, jumping, diving, or scratching of the walls. Immobility was defined when floating motionless or by making only those movements necessary to keep the mouse's head above the water. The water was changed after every other trial. Each test was conducted in a quiet and warm environment. The mice were removed from the water, and then placed into their original cages after being warmed and dried. All training and testing were performed between 10:00 am and 3:00 pm.

2.3.3. Tail suspension test (TST)

Tail suspension test (TST) was performed according to the method described previously with slight modifications (Kwon et al., 2010; Zomkowski et al., 2006). Briefly, the mice were individually suspended by the tail, using medical tape 2 cm away from the tail tip, to a fixed metal rod so that the head of the mouse hung down in the box (30 × 30 × 25 cm); the head was 5 cm away from the bottom of the box. Initially, the mouse would move up and down around his head in an attempt to climb out. Mice were observed for 6 min, and the cumulative immobility time during the final 5-min interval of the test was recorded. 'Immobility' was defined as when they hung passively and were completely motionless. All training and testing were performed between 10:00 am and 3:00 pm.

2.3.4. Stereotyped behavioral test (SBT)

The stereotyped behavior of mice was monitored in a plexiglas box with a CCD camera, recorded on a VCR tape, and analyzed by visual observation. The intensity of stereotyped activity was scored according to an arbitrary four-point scale (1 = normal behavior, 2 = periodic sniffing, 3 = continuous sniffing, 4 = continuous sniffing, periodic licking or gnawing, 4 = continuous licking or gnawing), as described previously (Shen et al., 2010), with slight modifications. Each mouse was assigned a rating score of 1–5, according to the scale. Mice stereotyped behaviors were monitored and scored at 15-min intervals for 1 h. All training and testing were performed between 10:00 am and 3:00 pm.

2.4. Isolation and calculation of cysts from the mouse brain

The calculation of cysts in the mouse brain was performed as described previously (Goodwin et al., 2008). Briefly, mouse infected with *Toxoplasma* was anaesthetized by CO_2 and the whole