



# The myelinated fiber loss in the corpus callosum of mouse model of schizophrenia induced by MK-801



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## ABSTRACT

Previous magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) investigations have shown that the white matter volume and fractional anisotropy (FA) were decreased in schizophrenia (SZ), which indicated impaired white matter integrity in SZ. However, the mechanism underlying these abnormalities has been less studied. The current study was designed to investigate the possible reasons for white matter abnormalities in the mouse model of SZ induced by NMDA receptor antagonist using the unbiased stereological methods and transmission electron microscope technique. We found that the mice treated with MK-801 demonstrated a series of schizophrenia-like behaviors including hyperlocomotor activity and more anxiety. The myelinated fibers in the corpus callosum (CC) of the mice treated with MK-801 were impaired with splitting lamellae of myelin sheaths and segmental demyelination. The CC volume and the total length of the myelinated fibers in the CC of the mice treated with MK-801 were significantly decreased by 9.4% and 16.8% when compared to those of the mice treated with saline. We further found that the loss of the myelinated fibers length was mainly due to the marked loss of the myelinated nerve fibers with the diameter of 0.4–0.5  $\mu\text{m}$ . These results indicated that the splitting myelin sheaths, demyelination and the loss of myelinated fibers with small diameter might provide one of the structural bases for impaired white matter integrity of CC in the mouse model of SZ. These results might also provide a baseline for further studies searching for the treatment of SZ through targeting white matter.

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## 1. Introduction

In schizophrenia (SZ) research, a growing body of evidences have provided support for the hypothesis that diminished connectivity referring to abnormal functional integration between different brain regions might be involved in the pathophysiology of SZ, and white matter lesions could be the basis of this disconnectivity (Konrad and Winterer, 2007; Field, 2008; Stephan et al., 2009; Schmitt et al., 2011). Earlier structural magnetic resonance imaging (MRI) and postmortem studies have demonstrated

smaller white matter volumes in diverse brain regions of SZ, including temporal lobe (Okugawa et al., 2002), caudate nucleus (Takase et al., 2004), anterior limb of the internal capsule (Zhou et al., 2003) and corpus callosum (CC) (Highley et al., 1999; Chaim et al., 2010). Diffusion tensor imaging (DTI) studies, indicators of the coherence, organization and density of the fibers within the white matter fiber bundles, have also demonstrated impaired white matter integrity in SZ (Kubicki et al., 2007; Lee et al., 2013; Mori et al., 2007; Skelly et al., 2008). Genetic and postmortem studies have further demonstrated that SZ might be related to myelin sheath abnormalities of myelinated fibers (Hof et al., 2002; Dracheva et al., 2006; Takahashi et al., 2011).

White matter is mainly composed of myelinated nerve fibers. Changes in white matter integrity may be related to alterations in the density of fibers, the coherence of fiber tracts, the number and

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the diameter of fibers as well as the degree of myelination (Kubicki et al., 2007; Peters et al., 2010). It is a more direct measure to investigate connectivity of myelinated fibers by postmortem microscopy. However, due to the difficulties in collection of brain samples, to our knowledge, up to now, there have been only a few studies focused on evaluating the fibers in schizophrenics (Casanova et al., 1989; Chance et al., 1999; Highley et al., 1999; Marner and Pakkenberg, 2003; Uranova et al., 2011). Moreover, those results were inconsistent. For example, Highley et al. (1999) have reported decreased total fiber number and fiber density in the CC in women with SZ, but other investigators have reported that there was no significant difference in the total number of fibers or density of fibers in the CC between schizophrenic patients and controls (Casanova et al., 1989). Such confounders were likely to be influenced by sample selection (sample size, first-episode vs. chronic schizophrenia patients, age, gender) in individual studies (Kuswanto et al., 2012), methodology adopted and neuroimaging techniques used (Marner and Pakkenberg, 2003). In addition, the majority of schizophrenic subjects in these studies have been in illness for long time and have been treated with antipsychotic agent for years, which can influence the results, too (Chen et al., 2013). In fact, the need for models is greater in psychiatry than in other fields (Hyman, 2012), in which the disease tissue is often removed by biopsy or resection and made available for study, however, the brain samples are difficult in collection.

In the current study, we assessed the connectivity of white matter with the stereological method and transmission electron microscope technique (Tang et al., 1997; Tang and Nyengaard, 1997). Firstly, to avoid the confounders such as age, gender, age at onset, duration of illness, antipsychotic medication and so on, we chose the animal model of SZ based on NMDA receptor antagonism. The “NMDA receptor antagonism” theory of schizophrenia emerged from the observation that phencyclidine (PCP), an open channel antagonist to the NMDA subtype of glutamate receptor, induced schizophrenia-like behaviors in humans (Javitt and Zukin, 1991). Many researchers have developed animal models of schizophrenia with NMDA receptor antagonists such as PCP, ketamine, and dizocilpine MK-801 (Jentsch et al., 1997; Jentsch and Roth, 1999; Mouri et al., 2007; Neill et al., 2010; Guo et al., 2010). To date, the animal model of SZ induced by NMDA receptor antagonists such as MK-801 has been widely applied to investigate the pathogenesis of the schizophrenia (Elhardt et al., 2010; Kocerha et al., 2009; Okamura et al., 2010; Yu et al., 2011). To our knowledge, however, there have been few studies investigating the abnormalities of white matter in animal model of SZ based on NMDA receptor antagonism (Zhang et al., 2012; Xiu et al., 2014). Therefore, the aim of the current study was to investigate the white matter deficits in animal model of SZ treated with MK-801. With the stereological methods, the accurate quantitative data of microstructure/ultrastructure of myelinated fibers could be provided and the possible mechanism underlying white matter abnormalities in SZ would be interpreted.

## 2. Materials and methods

### 2.1. Animals

Adult (8-week-old, weighing 20–25 g) male C57BL/6J mice ( $n = 30$ ) that were supplied by Chongqing Medical University, P. R. China, were randomly divided into two groups. The control group ( $n = 15$ ) was sub-chronically treated with saline solution, and the SZ group ( $n = 15$ ) was treated intraperitoneally with MK-801. Animals were maintained on a light-controlled room (12 h light/dark cycle, light on 8:00 AM) at  $(22 \pm 1)^\circ\text{C}$  and  $(45 \pm 1)\%$  humidity with free access to standard lab chow and tap water. Animal care and

treatment followed National Institute of Health guide for the Care and Use of Laboratory Animals. These ethical guidelines were followed throughout the experiment.

### 2.2. Drugs and drug treatments

The non-competitive NMDA receptor antagonist, dizocilpine hydrogen maleate (MK-801), was purchased from Sigma–Aldrich (St. Louis, MO, USA). MK-801 was dissolved in a saline solution (0.1 mg/mL) and prepared fresh every day. Mice in the control group were intraperitoneally administered with saline (10 ml/kg), and mice in the SZ group were intraperitoneally administered with MK-801 (10 ml/kg) at 11:00–12:00 AM. The drug treatments last for 14 consecutive days. The procedure for the chronic exposure of rodents to MK-801 was reported previously (Yu et al., 2011). The dose of 1 mg/kg MK-801 was selected based on other studies (Ahn et al., 2006, 2009; Seo et al., 2007).

### 2.3. Behavioral test

All animals were tested on the 15th day on the open field (OF) test and the elevated plus maze (EPM). **OF test** Effects of MK-801 treatment on locomotor activity were measured using the OF test. This test was conducted as previously described (Mutlu et al., 2011). Briefly, the testing apparatus consists of four Perspex boxes ( $50 \times 50 \times 40 \text{ cm}^3$ ) with video devices (Shanghai Mobil datum Information Technology Co., RD 1112-OF-M-4). The test animal was placed in the center of the test box, and the total distance moved throughout the area as well as the time spent in the center zone were recorded for 10 min. Center zone of the open field was a square with the side length of 15 cm. **EPM** The EPM is a commonly employed test of animals' anxiety. The test setting consisted of a plus-shaped apparatus with two open arms ( $30 \times 5 \text{ cm}^2$ ) and two enclosed arms ( $30 \times 5 \times 15 \text{ cm}^3$ ). Each arm was elevated 70 cm above the floor (Shanghai Mobil datum Information Technology Co., RD 1108-EPM-M1). Mice were placed in the center of the maze facing an open arm at the start of observation and their behavior was observed and quantified for 5 min. The number of open and closed arm entries and the time spent on them were recorded, and the corresponding percentages were interpreted as measures of anxiety-like behavior. After each trial, the apparatus was cleaned with a water solution containing ethanol at 20%, in order to prevent any olfactory-induced behavioral modifications.

### 2.4. Tissue preparation

After behavioral tests, six mice from each group were put to death for stereological studies. The animals were deeply anaesthetized with 4% chloral hydrate (10 ml/kg body weight) and perfused with 0.9% NaCl firstly, followed by 2% paraformaldehyde plus 2.5% glutaraldehyde in 0.1 M phosphate-buffered saline. Brains were removed and post-fixed overnight in the same fixative. The two hemispheres of the cerebrum were separated from each other along the cerebral longitudinal fissure, and they were then cut coronally into 1-mm-thick slabs, starting randomly at the rostral pole. On average, 10 slabs ( $\text{CV} = 0.07$ ) were obtained from each hemisphere.

### 2.5. Estimation of the total volume of CC and the total volume of cerebrum

A transparent counting grid with an area of  $0.39 \text{ mm}^2$  associated with each point was placed randomly on the caudal surface of each slab under the anatomy microscopy, and the total number of points hitting the CC was counted (Fig. 1A). The total volume of CC,  $V_{(\text{CC})}$ ,

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