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NMDA RECEPTOR ANTAGONISM BY REPETITIVE MK801 ADMINISTRATION INDUCES SCHIZOPHRENIA-LIKE STRUCTURAL CHANGES IN THE RAT BRAIN AS REVEALED BY VOXEL-BASED MORPHOMETRY AND DIFFUSION TENSOR IMAGING

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Abstract—*Background:* Animal models of N-methyl-D-aspartate receptor (NMDAR) antagonism have been widely used for schizophrenia research. Less is known whether these models are associated with macroscopic brain structural changes that resemble those in clinical schizophrenia. *Methods:* Magnetic resonance imaging (MRI) was used to measure brain structural changes in rats subjected to repeated administration of MK801 in a regimen (daily dose of 0.2 mg/kg for 14 consecutive days) known to be able to induce schizophrenia-like cognitive impairments. *Results:* Voxel-based morphometry (VBM) revealed significant gray matter (GM) atrophy in the hippocampus, ventral striatum (vStr) and cortex. Diffusion tensor imaging (DTI) demonstrated microstructural impairments in the corpus callosum (cc). Histopathological results corroborated the MRI findings. *Limitations:* Treatment-induced behavioral abnormalities were not measured such that correlation between the brain structural changes observed and schizophrenia-like behaviors could not be established. *Conclusion:* Chronic MK801 administration induces MRI-observable brain structural changes that are comparable to those observed in schizophrenia patients, supporting the notion that NMDAR hypofunction contributes to the pathology of schizophrenia. Imaging-derived brain structural changes in animal models of NMDAR antagonism may be useful measurements for studying the effects of treatments and interventions targeting schizophrenia.

Key words: N-methyl-D-aspartate receptor, schizophrenia, magnetic resonance imaging, rat, brain.

INTRODUCTION

Schizophrenia is a severe mental illness affecting about 1% population worldwide (McGrath et al., 2008). More recent data suggest that the lifetime risk could be even higher (Pedersen et al., 2014). Schizophrenia is characterized by extremely heterogeneous symptoms including positive symptoms, negative symptoms and cognitive dysfunctions, making the diagnosis and treatment problematic (Millan et al., 2014). Positive symptoms tend to be more treatment-responsive than negative symptoms and cognitive dysfunctions (Schobel et al., 2013; Millan et al., 2014). Treatment of the negative and cognitive symptoms is a pivotal clinical need for the recovery of schizophrenia patients and their re-integration into society.

Developing valid, reliable and predictive animal models for schizophrenia is important to improve our understanding of the pathogenesis of the disorder and for treatment development. To this end, many animal models of schizophrenia have been established, including neurodevelopmental models (Flagstad et al., 2004), genetic models (Jaaro-Peled et al., 2010) and pharmacological models (Neill et al., 2010). N-methyl-D-aspartate receptor (NMDAR) hypofunction has been postulated to play a central role in the pathogenesis of schizophrenia, with relevance to drug discovery (Stone, 2009; Schobel et al., 2013; Egerton et al., 2014). As a result, animal models of acute/chronic administration of NMDAR antagonist, such as phencyclidine (PCP), ketamine and dizocilpine (MK801), have been widely used for schizophrenia research. Treated animals develop both positive and negative symptoms, as well as cognitive

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Abbreviations: ANOVA, analysis of variance; cc, corpus callosum; DA, axial diffusivity; DG, dentate gyrus; DR, radial diffusivity; DTI, diffusion tensor imaging; ec, external capsule; FA, fractional anisotropy; FDR, false discovery rate; FOV, field of view; FWHM, full width at half maximum; GM, gray matter; HE, hematoxylin and eosin; M1, primary motor cortex; MAM, methylazoxymethanol acetate; MBP, myelin basic protein; MD, mean diffusivity; MRI, magnetic resonance imaging; NeuN, neuronal nuclei; NMDAR, N-methyl-D-aspartate receptor; PBS, phosphate-buffered saline; PCP, phencyclidine; PNSS, positive and negative syndrome scale; PV, parvalbumin; RARE, rapid acquisition with relaxation enhancement; ROIs, regions-of-interest; RSD, retrosplenial cortex; TR, repetition time; VBA, voxel-based analysis; VBM, voxel-based morphometry; vStr, ventral striatum; WM, white matter.

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dysfunctions, resembling those found in first episode schizophrenia (Sams-Dodd, 1999; Eyjolfsson et al., 2006; Barnes et al., 2015). Compared to acute treatment, repeated administration of NMDAR antagonists appears to be better in modeling schizophrenia-like behaviors and histopathology (Jentsch and Roth, 1999; Castane et al., 2015). Repeated or neonatal exposure to NMDAR antagonists can induce prolonged cognition impairments in rodents (Latysheva and Rayevsky, 2003; Egerton et al., 2008). The histopathological changes induced by chronic NMDAR antagonist administration, such as hippocampal and cortical neuron apoptosis (Olney et al., 1989; Genius et al., 2012; Gomes et al., 2015) and myelin breakdown in the frontal cortex and corpus callosum (cc) (Xu and Li, 2011), are also akin to those found in schizophrenia patients.

Magnetic resonance imaging (MRI) studies have consistently shown that first-episode schizophrenia patients are characterized by structural deficits in the brain involving both gray matter (GM) and white matter (WM). Widespread cortical thinning was observed in fronto-temporal, parietal and occipital regions (Sprooten et al., 2013), and morphometric changes in subcortical regions, including the hippocampus and basal ganglia, were also frequently reported (Kuhn et al., 2012; Stegmayer et al., 2014; Okada et al., 2016). Atrophy (Walterfang et al., 2009; Bora et al., 2011) and impaired microstructural integrity (Kong et al., 2011; Lee et al., 2013; Holleran et al., 2014) in the cc and external capsule (ec) are the most consistently observed schizophrenia-related WM abnormalities. Imaging-derived brain structural changes in schizophrenia patients not only correlate with clinical symptoms (Whitford et al., 2009; Kuhn et al., 2012), but also show response to antipsychotic treatment (van Haren et al., 2007; Ozcelik-Eroglu et al., 2014).

Less is known whether the animal models of schizophrenia are associated with brain structural changes similar to those found in clinical patients. A 10-day ketamine treatment induced hippocampal atrophy in C57 mice (Schobel et al., 2013). Sub-chronic PCP administration in rats was recently shown to result in GM density loss in the hippocampus, anterior cingulate cortex, ventral striatum (vStr), and amygdala (Barnes et al., 2015). In a rat model of prenatal exposure to methylazoxymethanol acetate (MAM), the schizophrenia-like animals had enlargement of lateral and third ventricles, reduced hippocampal volumes and reduced fractional anisotropy (FA) in the cc and cingulum (Chin et al., 2011). In addition, arterial spin labeling perfusion imaging and manganese-enhanced MRI studies demonstrated that neurodevelopmental and pharmacological models of schizophrenia are associated functional abnormalities in the brain, involving both the cortex and sub-cortical nuclei (Risterucci et al., 2005; Malkova et al., 2014).

In this study, we used MRI to investigate structural changes of the brain in rats subjected to a 14-day treatment of a selective NMDAR antagonist MK801, with a daily dose of 0.2 mg/kg body weight. MK801 treatment in this regimen was previously shown to be able to induce persisting schizophrenia-like space working cognition impairment in rats (Li et al., 2011). Unlike ketamine and PCP, which may produce a non-selective multi-system neurochemical perturbation via direct and/or indirect effects, MK801 is a strict selective NMDAR antagonist (Wong et al., 1986), and thus better for modeling NMDAR hypofunction in schizophrenia research (Kapur and Seeman, 2002). Voxel-based morphometry (VBM) was used to measure regional GM and WM volumes. Diffusion tensor imaging (DTI) was used to assess microstructural integrity of WM fibers. MRI observations were verified with histopathological assessments, including hematoxylin and eosin (HE) staining and neuronal nuclei (NeuN) staining for neuronal loss, Golgi-Cox staining for dendritic arborization, parvalbumin (PV) immunohistology for interneuron deficits and myelin basic protein (MBP) immunohistology for demyelination. The main goal of the study is to evaluate whether the model of repeated MK801 administration exhibits imaging intermediate phenotypes and neuropathological manifestations resembling those found in clinical schizophrenia.

EXPERIMENTAL PROCEDURES

Animal and treatment

All animal protocols were approved by the Institutional Animal Care and Use Committee. Forty-three male Sprague–Dawley rats, about 2 months old and weighing 303 ± 20 g, were used. The animals were housed in standard individually ventilated cages connected to a ventilation rack in a temperature-controlled (23 ± 1 °C) environment with 12-h light/12-h dark cycle (i.e., lights on at 08:00 h AM). Standard pallet food (Wanhejiaxing, Wuhan, China) and tap water were available *ad libitum*. The rats were randomly assigned into either the Saline group (n = 21) or the MK801 (n = 22) group. MK801 (Sigma, St. Louis, MO) was dissolved in physiological saline, and diluted to 5 mg/ml solution. The animals in the MK801 and Saline groups received daily intraperitoneal injection of MK801 solution (0.2 mg/kg body weight) and the same amount of saline, respectively, in the morning for 14 consecutive days. A subset of the animals (n = 11 for the Saline group; n = 12 for the MK801 group) were first imaged 7 days later after the last injection (i.e., the wash-out period). These animals, along with the rest that did not undergo MRI scans, were then decapitated for histopathological assessments.

MRI acquisition

All MRI experiments were performed on a 7.0 T/20-cm Bruker Biospec scanner. A 72-mm-diameter volume coil was used for radiofrequency pulse transmission, and a quadrature surface coil for signal detection. T_2-weighted anatomical images were acquired from 36 contiguous coronal slices with a rapid acquisition with relaxation enhancement (RARE) sequence, repetition time (TR) 6450 ms, effective echo time (TE_{eff}) 40 ms, matrix size 256 × 256, field of view (FOV) 30 mm × 30 mm, slice thickness 0.5 mm, RARE factor 4 and 4 averages. DTI was performed with a 4-shot spin-echo echo planar imaging sequence, an encoding scheme of 60 gradient