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Impaired hippocampal-dependent memory and reduced parvalbumin-positive interneurons in a ketamine mouse model of schizophrenia

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ABSTRACT

The hippocampus of patients with schizophrenia displays aberrant excess neuronal activity which affects cognitive function. Animal models of the illness have recapitulated the overactivity in the hippocampus, with a corresponding regionally localized reduction of inhibitory interneurons, consistent with that observed in patients. To better understand whether cognitive function is similarly affected in these models of hippocampal overactivity, we tested a ketamine mouse model of schizophrenia for cognitive performance in hippocampal- and medial prefrontal cortex (mPFC)-dependent tasks. We found that adult mice exposed to ketamine during adolescence were impaired on a trace fear conditioning protocol that relies on the integrity of the hippocampus. Conversely, the performance of the mice was normal on a delayed response task that is sensitive to mPFC damage. We confirmed that ketamine-exposed mice had reduced parvalbumin-positive interneurons in the hippocampus, specifically in the CA1, but not in the mPFC in keeping with the behavioral findings. These results strengthened the utility of the ketamine model for preclinical investigations of hippocampal overactivity in schizophrenia.

1. Introduction

Neurocognitive deficits are a key clinical feature of schizophrenia that predate the onset of psychosis and predict long-term disability in patients (Corigliano et al., 2014; Eastvold et al., 2007; Green, 1996; Green et al., 2004; Jahshan et al., 2010; Nuechterlein et al., 2014). Existing antipsychotics and nootropics however have limited efficacy in improving cognition and functional outcomes in schizophrenia. Preclinical animal models developed to recapitulate many of the features of the illness have been used to investigate neurocognitive deficits and their treatments (Dudchenko et al., 2013; Lipska and Weinberger, 2000; Moore et al., 2013; Young et al., 2012), and these models commonly pointed to deficits in hippocampal- and prefrontal-mediated functional domains, consistent with their known importance in learning and memory.

Recent evidence from animal models and human neuroimaging studies has indicated altered brain excitability in the medial temporal lobe as a potential driver of pathology in schizophrenia. Specifically, the evidence suggests that hippocampal dysfunction in schizophrenia patients, and also observed in well-established animal models of the schizophrenia, may be due to aberrant hippocampal overexcitability (e.g., Lodge and Grace, 2007; Medoff et al., 2001; Sanderson et al., 2012; Schobel et al., 2009, 2013; Tregellas et al., 2014; Zierhut et al., 2010). In one such study, hippocampal overactivity was observed early during a prodromal stage and predicted clinical progression to overt psychosis within 2 years (Schobel et al., 2009). The level of overactivity has also been found to correlate with worse cognitive performance in schizophrenia patients (Tregellas et al., 2014). Consistent with those findings, aberrant excitability in the hippocampus has been observed in neurodevelopmental animal models of schizophrenia that used the NMDA receptor antagonist ketamine (Schobel et al., 2013) or the antimitotic compound methylazoxymethanol acetate (e.g., Lodge and Grace, 2007). As adults, animals exposed to those agents prenatally or during adolescence have significantly higher hippocampal metabolic basal activity (Schobel et al., 2013) and in vivo hippocampal firing rates (Gill et al., 2011; Lodge and Grace, 2007) compared to vehicle-treated controls. In vitro slice recordings from the treated animals have also provided evidence for neuronal hyperexcitability of principal neurons in the hippocampus that could be partially normalized with diazepam (Sanderson et al., 2012). Thus, both preclinical and clinical data suggest that overactivity is a condition contributing to hippocampal dysfunction in this illness.

One important correlate of underlying circuit perturbation that is relevant to the pathogenesis of overactive hippocampus is the loss of GABAergic inhibitory interneuron populations. Individuals with schizophrenia have normal total number of neurons in the hippocampus (Heckers et al., 1991; Konradi et al., 2011; Schmitt et al., 2009; Walker et al., 2002), but significantly reduced parvalbumin- and somatostatin-

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expressing interneurons both in patients (Konradi et al., 2011; Torrey et al., 2005; Zhang and Reynolds, 2002) and in animal models (e.g., Gilani et al., 2014; Gill and Grace, 2014; Lodge et al., 2009; Schobel et al., 2013). The loss of inhibitory network integrity in schizophrenia suggests that boosting inhibitory control in the hippocampus may be beneficial. Indeed, treatments with a $GABA_A$ α5 positive allosteric modulator in an animal model were shown to be effective in reducing evoked excitatory response in the hippocampus and normalizing dopamine dysfunction (Gill et al., 2011). An alternate strategy involving tightly regulating glutamate transmission was shown to be effective too, at least in reducing hypermetabolism and ameliorating loss of parvalbumin in the mouse hippocampus (Schobel et al., 2013). Whether these treatments, currently still in preclinical testing, are beneficial for enhancing cognition has not been demonstrated yet.

Towards that goal, we set out to determine if the preclinical models used to recapitulate hippocampal hyperactivity in schizophrenia produce cognitive dysfunction. Specifically, we examined a ketamine mouse model that displays hippocampal hyperactivity akin to that seen in schizophrenia patients (Schobel et al., 2013) for functional impairment in hippocampal- and prefrontal-mediated memory tasks. While the ketamine model is well established and produces schizophrenia-like symptoms including impaired cognition in other exposure regimens (e.g., Olney et al., 1999; Neill et al., 2010), it is not known whether the specific induction protocol of ketamine exposure that produced hippocampal overactivity leads to cognitive dysfunction. Differences in induction regimens in this area have been noted to impede consistency in findings and translational integrity (Gilmour et al., 2012). We first established that mice exposed to this ketamine regimen during a month in adolescence show hyper-responsiveness as adults to a dopamine agonist, a commonly used behavioral assay to validate dopaminergic perturbation that is central to the disease. We then showed that these mice displayed impaired hippocampal-dependent trace fear memory but intact medial prefrontal cortex (mPFC)-mediated working memory. The expression of inhibitory interneurons in those brain areas was in line with the behavioral findings; that is, parvalbumin-positive interneurons were decreased in the hippocampus but not in the mPFC.

2. Materials and methods

2.1. Subjects

Male C57/BL6 mice were obtained at 4 weeks old from The Jackson Laboratory (Bar Harbor, Maine). The mice were housed in cohorts of 4 per cage at 25 °C and maintained on a 12-h light/dark cycle. Once ketamine exposure was initiated, the mice were housed individually. All cages were lined with corncob bedding and a nestlet for nest building. Food (Purina autoclave laboratory rodent diet) and water were provided ad libitum. All procedures in the current investigations were approved by the Institutional Animal Care and Committee in accordance with the National Institutes of Health directive.

2.2. Ketamine exposure

Ketamine (VedCo; 100 mg/ml concentration) was diluted to 1.6 mg/ml, and injected at a volume of 10 ml/kg of body weight (Schobel et al., 2013). Mice were injected subcutaneously three times a week (Monday, Wednesday, and Friday) with saline or ketamine (16 mg/kg) for a month starting at 1-mo of age. Following the month treatment, the mice were left undisturbed until adulthood at 4–6 months old for behavioral testing.

2.3. Amphetamine-induced locomotor activity

Each mouse was placed in an open field chamber (42 cm × 42 cm × 30.5 cm) in which locomotion was tracked with the VersaMax animal activity monitoring system (AccuScan Instruments, Columbus, OH). After 30 min of baseline activity, the mouse was taken out of the chamber and injected intraperitoneally with a small dose of amphetamine (0.5 mg/kg in a volume of 10 ml/kg; Sigma). The mouse was then returned to the chamber for another 60 min of activity monitoring. Total distance traveled and movement time were the dependent measures.

2.4. Trace fear conditioning

The trace fear conditioning apparatus and procedures were identical to those described in Smith et al. (2007). Briefly, each conditioning chamber was either square (17.78 cm wide × 17.78 cm deep × 30.48 high; model #H10-35 M-04; Coulbourn Instruments, Whitehall, PA) or octagonal (radius 21.59 cm and 30.48 high; model #H10-35 M-08; Coulbourn Instruments), with a grid floor through which a footshock could be delivered. Half of the square and half of octagonal chambers were scented with two drops of vanilla extract in the drop pan. During testing, the grid floor was replaced with a solid wooden floor coated with a white colored sealant. A speaker was mounted on the back wall for the delivery of a tone. The tone and shock were created via a peripheral Coulbourn tone generator (model #A69-20) and animal shocker (model #H13-16). A computer that interfaced with Coulbourn Graphic State software controlled all stimuli onset and duration.

On the first day (acclimatization day), mice were pre-exposed to the training chambers for 12 min; no tone or shock was delivered. On the second day (training day), the mice were placed in the same chambers as on the first day for 4 min to acclimatize. The mice were then given six conditioning trials involving 70-dB tone (conditioned stimulus) and a 2-s, 0.5-mA shock (unconditioned stimulus). Each trial consisted of a 20-s baseline interval, a 20-s tone presentation, an 18-s trace interval, a 2-s shock, and a 40-s post-shock interval. On the third day (testing day), the mice were placed in a novel test chamber for 3 min (i.e., mouse trained in a square chamber was tested in a octagonal chamber, and vice versa), and then given four 100-s testing trials. Each trial consisted of 20-s interval baseline interval, a 20-s tone (70 dB) presentation, and a 60-s post-tone interval (see Fig. 2A for schematic representations of the training and testing trials). Scoring was done from a video at 1-s interval increments for freezing during the 40-s post-shock interval on training day and the 60-s post-tone interval on testing day.

2.5. Delayed response task

Mice were tested on a delayed response task in modified operant chambers. The chambers were made of aluminum front and back walls, clear polycarbonate sides and ceiling, and stainless-steel rods floor (Med Associates, St Albans, VT). Each chamber had a programmable food cup for the delivery of liquid reward, and was connected to a vacuum for the removal of liquid. Infrared photocells around the food cup monitored the time spent and number of entries into the cup. Each chamber had two retractable mouse levers to the left and right sides of the food cup. A house light provided ambient light inside the sound-attenuating shell that housed the chambers.

Prior to training, mice were food deprived and pre-exposed to a 10% sucrose solution in their home cages, which served as a reward in the task. The food-deprived mice were water restricted subsequently during training (1 h water access each day in the home cage) to increase motivation and were maintained at approximately 85% free-feeding body weight throughout the study. At the start of the training phase, the mice were habituated to the chambers and were given food cup magazine training. Each mouse was given 60 trials of sucrose presentation at a pseudo-random interval each day for two days. The sucrose (50 μl of 20% *w*/*v*) was presented in the food cup for 5 s before removal by vacuum. Next, the mice were shaped to lever press for sucrose delivery under a fixed ratio schedule (FR-1) for 12 sessions over six days;

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