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Septohippocampal GABAergic neurons mediate the altered behaviors induced by n-methyl-D-aspartate receptor antagonists.

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Septohippocampal GABAergic Neurons Mediate the Altered Behaviors Induced by *N*-methyl-D-Aspartate Receptor Antagonists

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ABSTRACT: **We hypothesize that selective lesion of the septohippocampal GABAergic neurons suppresses the altered behaviors induced by an *N*-methyl-D-aspartate (NMDA) receptor antagonist, ketamine or MK-801. In addition, we hypothesize that septohippocampal GABAergic neurons generate an atropine-resistant theta rhythm that coexists with an atropine-sensitive theta rhythm in the hippocampus. Infusion of orexin-saporin (ore-SAP) into the medial septal area decreased parvalbumin-immunoreactive (GABAergic) neurons by ~80%, without significantly affecting choline-acetyltransferase-immunoreactive (cholinergic) neurons. The theta rhythm during walking, or the immobility-associated theta induced by pilocarpine, was not different between ore-SAP and sham-lesion rats. Walking theta was, however, more disrupted by atropine sulfate in ore-SAP than in sham-lesion rats. MK-801 (0.5 mg/kg i.p.) induced hyperlocomotion associated with an increase in frequency, but not power, of the hippocampal theta in both ore-SAP and sham-lesion rats. However, MK-801 induced an increase in 71–100 Hz gamma waves in sham-lesion but not ore-SAP lesion rats. In sham-lesion rats, MK-801 induced an increase in locomotion and an impairment of prepulse inhibition (PPI), and ketamine (3 mg/kg s.c.) induced a loss of gating of hippocampal auditory evoked potentials. MK-801-induced behavioral hyperlocomotion and PPI impairment, and ketamine-induced auditory gating deficit were reduced in ore-SAP rats as compared to sham-lesion rats. During baseline without drugs, locomotion and auditory gating were not different between ore-SAP and sham-lesion rats, and PPI was slightly but significantly increased in ore-SAP as compared with sham lesion rats. It is concluded that septohippocampal GABAergic neurons are important for the expression of hyperactive and psychotic symptoms an enhanced hippocampal gamma activity induced by ketamine and MK-801, and for generating an atropine-resistant theta. Selective suppression of septohippocampal GABAergic activity is suggested to be an effective treatment of some symptoms of schizophrenia.** © **2012 Wiley Periodicals, Inc.**

KEY WORDS: **hippocampus; theta rhythm; gamma rhythm; auditory gating; behavioral hyperactivity; prepulse inhibition; schizophrenia; medial septum; NMDA receptor antagonist**

INTRODUCTION

The hippocampal theta rhythm correlates with the moment-to-moment behavior of an animal (Vanderwolf, 1969). Theta rhythm may participate in spatial navigation, sensorimotor processing and sensorimotor gating (O'Keefe and Nadel, 1978; Bland and Oddie, 2001; Buzsaki, 2002). The integrity of the medial septum is essential for the hippocampal theta rhythm (Stewart and Fox, 1990), which may depend on three types of septohippocampal neurons—cholinergic neurons that contact both hippocampal principal cells and interneurons (Frotscher and Leranth, 1985), GABAergic fibers that only contact hippocampal GABAergic inter neurons (Freund and Antal 1988; Toth et al. 1997; Takacs et al., 2008), and glutamatergic neurons (Sotty et al., 2003; Colom et al., 2005) that excite pyramidal cells (Huh et al., 2010).

Both atropine-sensitive and atropine-resistant inputs are involved in generating the hippocampal theta rhythm during voluntary behavior (Vanderwolf, 1988; Bland and Colom, 1993; Leung, 1998). Atropine-sensitive theta involves muscarinic cholinergic receptors and possibly cholinergic septohippocampal afferents. The atropine-resistant theta is characterized by its lack of sensitivity to muscarinic antagonists (Vanderwolf, 1975, 1988) or to abolition of the muscarinic cholinergic signaling pathway (Shin et al., 2005). The neurotransmitter underlying atropine-resistant theta has been suggested to be serotonergic (Vanderwolf, 1988) or glutamatergic and involving N-methyl-D-aspartate receptors (Vanderwolf and Leung, 1983; Leung and Desborough, 1988; Buzsaki, 2002; Leung and Shen, 2004). In addition, atropine-resistant theta may involve septohippocampal glutamatergic neurons (Bland et al., 2007; Huh et al., 2010) and septohippocampal GABAergic neurons (Stewart and Fox, 1989; Lawson and Bland, 1993; Lee et al., 1994; Manseau et al., 2008). Septohippocampal GABAergic neurons are suggested to be critical for pacing hippocampal theta (Simon et al., 2006; Hangya et al., 2009) by firing at distinct phases of the theta rhythm (Borhegyi et al. 2004). One aim of the present study is to study the participation of septohippocampal GABAergic neurons in theta generation.

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Another aim of this study was to examine the participation of septohippocampal GABAergic neurons in the psychotic behaviors induced by NMDA receptor antagonists, ketamine and MK-801. A low dose of ketamine induced positive and negative symptoms of schizophrenia in humans (Krystal et al., 1994; Lahti et al., 2001), including a disruption of prepulse inhibition (PPI) and auditory gating (Braff et al., 2001; Boeijinga et al., 2007). Systemic injection of ketamine and other NMDA receptor antagonists, such as phencyclidine and MK-801, also induced schizophrenia-like symptoms in animals that include increased locomotor activity, loss of PPI (Swerdlow et al., 1998; Ma and Leung, 2000; Ma et al., 2004) and loss of gating of hippocampal auditory evoked potentials (Miller and Freedman, 1995; Ma et al., 2009). NMDA receptor antagonist was inferred to block atropine-resistant theta in the hippocampus (Vanderwolf and Leung, 1983; Leung, 1985; Buzaki, 2002), but the relation of theta suppression to schizophrenia is unclear. Instead, GABAergic circuit dysfunction following NMDA receptor blockade has been proposed as mechanisms for schizophrenia (Benes and Berretta, 2001; Gonzalez-Burgos et al., 2011). Schizophrenic symptoms in behaving animals were accompanied by an increase in hippocampal gamma waves of 71-100 Hz (Leung, 1985; Whittington et al., 2000; Ma and Leung, 2000, 2007; Ma et al., 2004, 2009), indicating disruption of a recurrent inhibitory network (Leung, 1982, 1998; Mann et al., 2005). In past studies, we showed that infusion of a $GABA_A$ receptor agonist, muscimol, into the medial septum alleviated the NMDA antagonist-induced changes in hippocampal gamma waves, sensorimotor gating, behavioral hyperlocomotion and hippocampal auditory evoked potentials (Ma and Leung, 2007; Ma et al., 2004, 2009), possibly by septal modulation of hippocampal interneurons (Takacs et al., 2008). Here, we hypothesize specifically that the septohippocampal GABAergic neurons mediate the behavioral deficits following a systemic NMDA receptor antagonist. Our previous study indicated that the schizophrenic behaviors induced by NMDA receptor antagonist were not alleviated by selective lesion of septal cholinergic inputs to the hippocampus by 192-IgG-saporin (Ma et al., 2004).

It has been recently demonstrated that a low dose of the ribosome toxin saporin (SAP) conjugated to an orexin-2 receptor, or ore-SAP, selectively lesioned the septal GABAergic neurons leaving the cholinergic neurons almost intact (Smith and Pang, 2005). By selective lesion of the septal GABAergic neurons with ore-SAP, this study examined the involvement of septohippocampal GABAergic neurons in atropine-resistant hippocampal theta rhythm and the psychotic symptoms induced by an NMDA receptor antagonist.

MATERIALS AND METHODS

Male Long-Evans hooded rats (Charles River Canada, St. Constance, Quebec, Canada) were housed in pairs in Plexiglas cages and kept on a 12/12 h light/dark cycle at a temperature of 22 ± 1°C, with ad libitum food and water. All experimental procedures were approved by the local Animal Use Committee.

Under sodium pentobarbital (60 mg/kg i.p.) anesthesia, the rats were implanted with recording electrodes (125 μm Teflon-insulated stainless steel wires) in stratum radiatum and stratum oriens of the hippocampal CA1 region on both sides (AP -3.5, L + 2.7), and ventral (V) from skull surface 2.3–3.3; units in mm), according to the stereotaxic atlas of Paxinos and Watson (2007). Rats were rested 7 days after surgery.

Lesion of GABAergic neurons in the medial septum was performed using orexin-saporin (ore-SAP; Chemicon, Temecula, CA). The injection protocol below was modified from that of Smith and Pang (2005), who reported that 140 ng ore-SAP selectively lesioned septal GABAergic and not cholinergic neurons. Ore-SAP (100 ng/μl in sterile saline) was infused bilaterally into the medial septal area (AP 0.7, L ± 0.5) at 5.7 mm (0.3 μl) and 7.8 mm (0.4 μl) below the skull surface, giving a total of 140 ng ore-SAP bilaterally. At each site, a constant infusion rate of 0.05 μl/ min was maintained by a pump (Harvard Apparatus, South Natick, MA) pushing the solution through a 30-gauge Hamilton syringe (Ma et al., 2004); the injection needle remained in place for 10 min before retraction to allow for diffusion. Sham lesion rats were infused with equal volumes of 0.9% saline. Rats were recorded 2–5 weeks after infusion.

Immunocytochemistry of choline acetyltransferase (ChAT) and parvalbumin (Parv) was performed at the end of experiments, using procedures published elsewhere (Ma et al., 2004, 2009). Parv immunopositive cells in the medial septum are shown to project to the hippocampus and are almost exclusively GABAergic, as shown by colabeling with GABA or gamma aminobytric acid decarboxylase (GAD) immunoreactivity (Freund and Antal1989; Gritti et al., 2003). In contrast, <10% of calbindin-immunopositive or calretinin-immunopositive cells in the basal forebrain were GAD immunopositive, and few calretinin-immunopositive cells project to the hippocampus (Gritti et al., 2003). Sections of 40-μm thick were cut from ore-SAP and sham lesion rats and the number of cells immunopositive to Parv or ChAT was quantified in three representative coronal sections (40 μm) at anterior (∼AP 0.7), middle (∼AP 0.4) and posterior (∼AP 0.2) levels of the medial septum-diagonal band of Broca region. A digital image of a selected section was captured by camera at 100 × magnification from a Nikon microscope, and cells were counted from the digital image. Cell counts were confirmed by a second person who was not aware of the lesion procedures. The sites of the electrode placements were verified histologically in 40-μm frozen sections of the brain stained with thionin (Fig. 1A).

Psychotic behaviors in rats were induced by injection of an NMDA receptor antagonist, ketamine (3 mg/kg, subcutaneously s.c.) or MK-801 (0.5 mg/kg intraperitoneally injected i.p.). Since MK-801 showed more long-lasting effects than ketamine, it was used for the study of prepulse inhibition and locomotion, while ketamine was used for studying auditory gating. The dose of ketamine was chosen in accordance with our previous study showing that ketamine at 3 mg/kg s.c. gave a

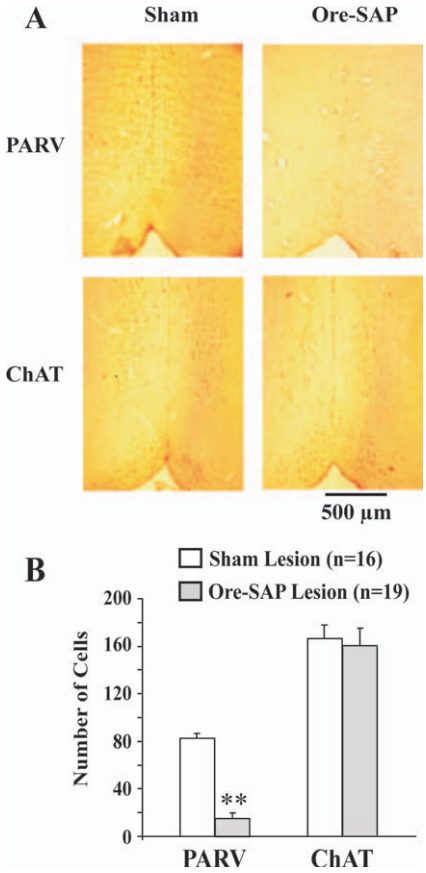

FIGURE 1. Parvalbumin (PARV) and choline acetyltransferase (ChAT) immunopositive neurons after medial septal infusion of orexin-saporin (Ore-SAP-lesion) or saline (Sham-lesion). A: Representative sections of ore-SAP and sham lesion rat, showing near complete loss of GABAergic neurons labeled by PARV immunoreactivity but preservation of cholinergic neurons labeled by ChAT immunoreactivity. B: Cell counts of ChAT and PARV immunopositive neurons in the medial septum and diagonal band of Broca at ~A 0.4, Paxions and Watson's atlas (2007). **$P < 0.0003$ (Bonferroni adjusted *t*-test) difference between ore-SAP-lesion and sham-lesion groups. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

maximal effect on hippocampal auditory gating deficit without affecting the response amplitude of the conditioning pulse (Ma et al., 2009). In preliminary experiments, we demonstrated, in control rats without septal infusion, the effect of MK-801 (0.5 mg/kg i.p.) by itself or in combination with 50 mg/kg i.p. atropine sulfate (injected 15 min before MK-801) on hippocampal theta rhythm and locomotion. MK-801 at dose of 0.5 mg/kg i.p. was shown to be optimal for inducing coordinated locomotor activity without ataxia (Ma and Leung, 2007).

For recording of auditory evoked potentials, a rat was semirestrained in a Plexiglas chamber (21 cm × 10 cm × 10 cm) that did not allow the rat to turn around. A loudspeaker was placed above the chamber, in a fixed position of 30° elevated and 24 cm away from the rat's head. Semirestraint reduced movement artifacts and allowed sound clicks to be delivered at a relatively constant intensity to both ears of the rat. The same chamber was used for testing prepulse inhibition (Ma et al., 2004; Ma and Leung, 2007). The implanted electrodes were connected to a flexible cable that led through an opening of the restraining chamber. Auditory evoked potentials were acquired at the stratum radiatum electrode following auditory click pairs separated by a conditioning-test (C-T) interval of 500 ms; each click was a white-noise burst of 20 ms pulse duration and at 75 dB. Click pairs were given 15-s apart. Additional C-T intervals (300 and 1,000 ms) were also used in selected experiments. Single sweeps of the auditory evoked potential were stored on the computer and those sweeps with clear movement or electrical artifacts were rejected online, and additional sweeps could also be rejected offline.

Baseline recording before drug (25 sweeps) was performed after habituating a rat for 15 min in the restraining chamber. Then, the rat was removed from chamber and injected with ketamine (3 mg/kg s.c.) or saline (0.1 ml s.c.). Ten minute after injection, the rat was placed back in the chamber for post drug recording. Response to the C or T pulse was measured by the maximal negative deviation from the baseline of the average evoked potential (15–25 sweeps). The ratio of the T response to the C response, or the T/C ratio (normally < 1), was used to measure auditory gating. A low T/C ratio indicates high gating, while a high T/C ratio (near 1) indicates low gating.

Prepulse inhibition (PPI) was assessed in a Plexiglas startle chamber (SR-LAB, San Diego Instruments, San Diego, CA), using a piezoelectric accelerometer to detect startle amplitude, as described elsewhere (Ma et al., 2004). After acclimating to 68 dB noise, the rat was given different sound stimuli—a startle pulse only (120 dB 40-ms broad band burst), or a startle pulse preceded 100 ms by a prepulse intensity of 73, 75, or 80-dB (20-ms broad band noise). For each test session, 50 trials were given in randomized order—10 trials with startle pulse only, 10 trials with no stimulation, and 10 trials of each one of the three prepulse intensities followed by a startle pulse. The intertrial interval was 15 s. PPI was measured as the difference between the response to the startle pulse alone and the response to prepulse-startle, or PPI (in percent) = 100 * [1 - (mean startle response amplitude after a prepulse /mean amplitude of response to startle alone)]. In this study, mean values of the three prepulse intensities of 73, 75, 80 dB (integrated prepulse intensity) as well as individual prepulse intensities were used to calculate the PPI.

Horizontal movements (locomotion) of a rat were measured by the number of interruptions of infrared beams in a Plexiglas chamber (69 × 69 × 49 cm^3). Four independent infrared sources, at 23 cm intervals, were located on a horizontal plane 5 cm above the floor, with photodiode detectors on the other side. Interruptions of the beams were counted and transferred to a microcomputer via an interface (Columbus Instruments). For spontaneous activity in a novel environment, recording of beam interruption started immediately after a rat was transferred from its home cage into the chamber, and 10 min counts were made for 1 h. For experiments with MK-801 injection, a rat was habituated for at least 1 h in the chamber before injection. The number of infrared beam interruptions

was counted every 10 min, for 30 min during baseline (before injection), and for 2 h after MK-801 (Ma and Leung, 2007).

EEG Recordings and Analysis

Hippocampal EEGs were recorded before and after drug treatments. Baseline recordings were collected in all rats during walking and immobile conditions. Two gross behavioral states were distinguished: (1) walking, broadly defined to include horizontal movements, rearing and turning, and (2) awake-immobility, defined as staying motionless on the floor of the recording cage with head held up against gravity. The EEG signals were filtered between 0.3 and 100 Hz, recorded on a polygraph (Grass 7D) and sampled at 200 Hz by a microcomputer. At least 30 s of EEG was manually selected from each minute of EEG recording and subjected to power spectral analysis, using segments of 5.12 s (1,024 points sampled at 200 Hz; Leung et al., 1982). The power spectra were plotted in logarithmic units, with calibration of 6.15 log units = 1.0 mV peak-to-peak sine wave. The peak theta rhythm was measured as the rise in theta power, in logarithmic units, from a minimum at 3–6 Hz to the peak at 4–10 Hz. If no peak was found at 4–10 Hz, theta power was considered to be zero. The rise of theta reflects accurately the magnitude of theta oscillations at 4–10 Hz; measurement of absolute theta does not distinguish between oscillatory and nonoscillatory responses (Leung et al., 1982).

Gamma power was measured by the mean integrated power in frequency bands of 30–70 Hz and 71–100 Hz. Mean integrated power is defined as the sum of power within a frequency band divided by the number of frequency bins within the bandwidth. The power of 58–62 Hz was not used in the sum, to avoid the 60-Hz line frequency. For analysis of the time effect of a drug on the integrated EEG power, each power was normalized by the baseline (100%).

The effect of atropine sulfate (50 mg/kg i.p.) and pilocarpine (25 mg/kg i.p.) on hippocampal EEG during immobility and walking (Leung, 1985) was studied in sham and ore-SAP lesion rats. EEG was recorded during baseline (before drug) and at 15–45 min after atropine sulfate or 15–30 min after pilocarpine. Statistical analyses were performed using repeated measure analysis of variance (ANOVA), followed by Newman-Keuls's post hoc test, or by Student's *t* test. *P*-values of < 0.05 were considered to be statistically significant.

RESULTS

Orexin-Saporin Infused in the Medial Septal Area Selectively Lesioned GABAergic Neurons

Orexin-saporin was infused into the medial septum to selectively lesion GABAergic septal neurons. As compared to sham lesion rats, ore-SAP lesion rats showed a large decrease in the number of Parv-immunopositive, putatively GABAergic neurons (Fig. 1A). The number of Parv-immunopositive neurons in ore-SAP lesion rats was significantly decreased to ~20% of that in sham lesion rats, and the decrease was significant at each of the three anteroposterior levels of the septal area (Bonferroni adjusted *t*-test, $P < 0.0003$; Fig. 1B). However, there was no difference in the number of ChAT-immunopositive, putatively cholinergic neurons as seen in representative sections (Fig. 1A). Quantitative cell counts confirmed that there was no significant difference in the ChAT-immunopositive neuronal counts between sham and ore-SAP lesion rats (*t*-test, $P > 0.13$ at each of three sections).

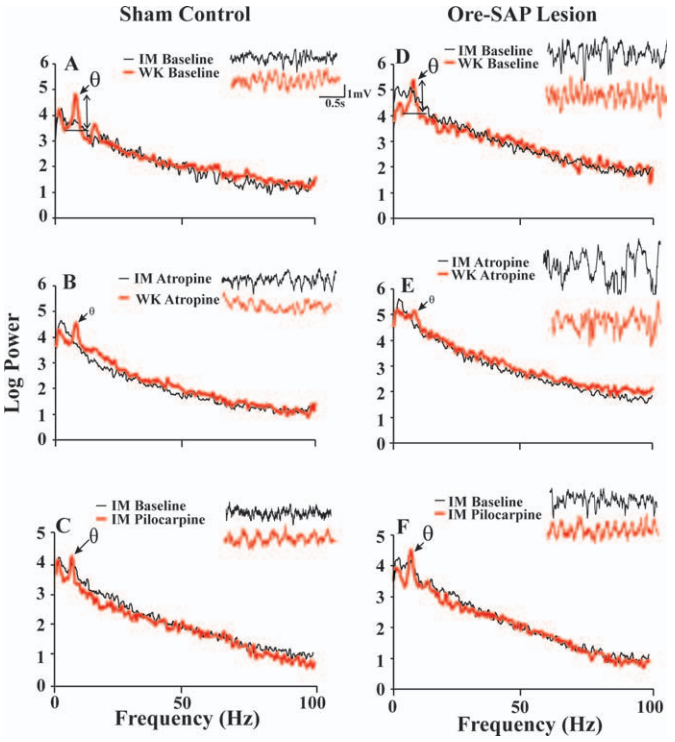

FIGURE 2. Power spectra of hippocampal EEG in representative sham (left column) and septal orexin-saporin (Ore-SAP, right column) lesion rats. Recordings were made before (A and D, baseline) and after injections of 50 mg/kg i.p. atropine sulfate (B and E) or 25 mg/kg i.p. pilocapine hydrochloride (C and F), and recorded during walking (WK) or awake-immobile (IM) conditions. Representative EEG tracings at surface or deep hippocampal electrodes are shown in inset of each part of the figure. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Hippocampal EEG After Ore-SAP Lesion in the Septum

In the ore-SAP lesion rats as well as sham lesion rats, theta power was higher during walking as compared with awake-immobility (Figs. 2A,D). During walking, the rise of the peak theta power of the ore-SAP lesion rats was not different from that in sham lesion rats (Fig. 3A). However, atropine disrupted theta in ore-SAP lesion rats more than sham lesion rats. After injection of atropine sulfate (50 mg/kg i.p.), theta power dur-

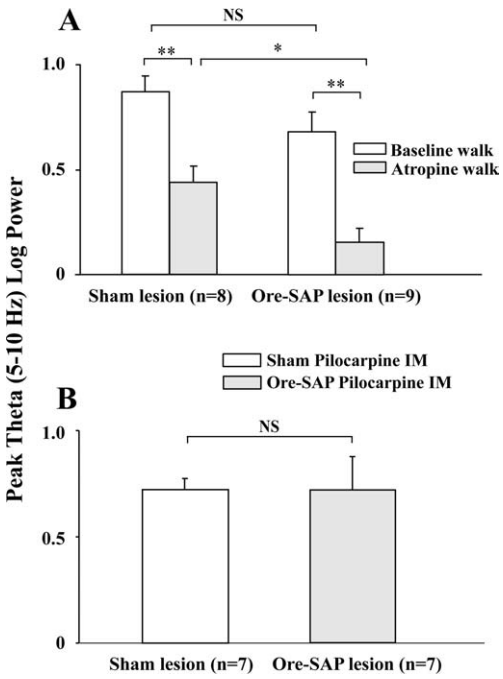

FIGURE 3. Effects of systemic injection of 50 mg/kg i.p. atropine sulfate (A) or 25 mg/kg i.p. pilocarpine hydrochloride (B) on hippocampal theta power in medial septal orexin-saporin lesion (Ore-SAP lesion) or sham lesion rats. EEGs were recorded under walking (walk) conditions for atropine-treated rats and under immobile (IM) conditions for pilocarpine treated rats. *$P < 0.05$; **$P < 0.01$ (t test) difference between Ore-SAP lesion and sham lesion groups. NS: not significant. Hippocampal theta power was assessed by the rise of the power peak above minimum (Methods).

ing walking decreased by 0.53 + 0.12 log units in ore-SAP lesion rats, significantly more than the decrease of 0.43 + 0.06 log units in sham lesion rats ($P < 0.05$; Figs. 2B,E and 3A). Gamma power (30–70 Hz and 71–100 Hz) during walking was not different between ore-SAP and sham lesion rats, with or without atropine sulfate injection (data not shown). An immobility-associated theta rhythm was induced by injection of 25 mg/kg i.p. pilocarpine in both ore-SAP and sham lesion rats (Figs. 2C,F), with theta power not significantly different between ore-SAP and sham lesion rats (Fig. 3B).

MK-801-Induced Changes in Hippocampal EEGs and Effect of Septal Ore-SAP Lesion

NMDA receptor antagonists are known to reduce hippocampal theta power (Leung and Desborough, 1988; Leung and Shen, 2004), and the combination of an NMDA receptor antagonist and atropine/scopolamine was shown to strongly suppress hippocampal theta during walking (Vanderwolf and Leung, 1983; Leung, 1985; Horvath et al., 1988). Since the effects of MK-801 had not been reported, we studied hippocampal EEG with MK-801 (0.5 mg/kg i.p.) alone, with or without pretreatment of atropine sulfate (50 mg/kg i.p.). In confirmation of previous studies on other NMDA receptor antagonists, MK-801 alone did not affect theta power but the combination of MK-801 and atropine sulfate strongly suppressed theta, and spontaneous locomotion, in control intact rats without septal infusion (Supporting Information Fig. 1).

Hippocampal EEGs in sham and ore-SAP lesion rats were analyzed before and after injection of MK-801. MK-801 (0.5 mg/kg i.p.) injection did not affect the peak hippocampal theta power in sham lesion rats [$F(8,48) = 1.66$, $P = 0.13$] or ore-SAP lesion rats [$F(8,48) = 0.85$, $P = 0.56$; Fig. 4E]. Both sham and ore-SAP lesion rats showed an increase in theta peak frequency after MK-801 injection (Fig. 4F). Repeated measures two-way ANOVA showed a trend of a higher theta peak frequency in sham as compared with ore-SAP lesion rats [Fig. 4F; group effect $F(1,8) = 3.70$, $P = 0.06$], with a significant time effect [$F(1,8) = 4.14$, $P < 0.001$], although Newman-Keuls post hoc test did not indicate statistical significance at a specific

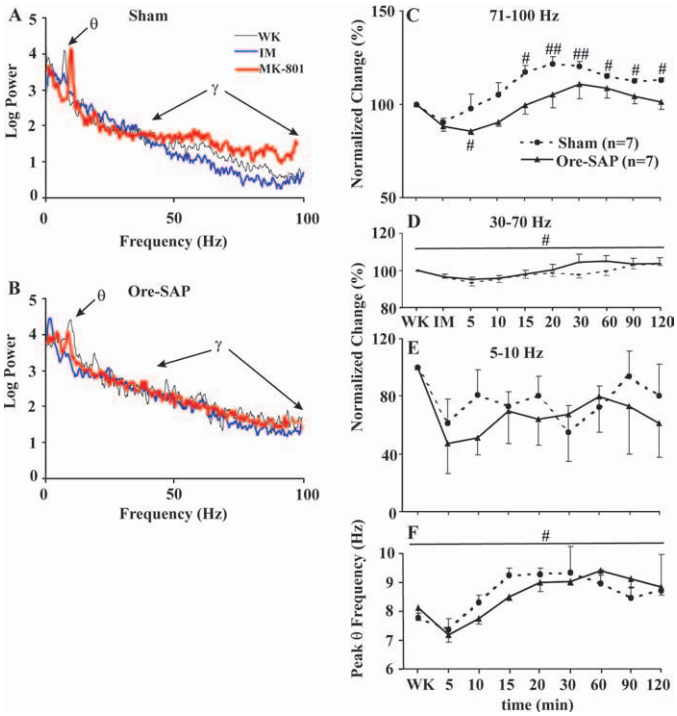

FIGURE 4. Hippocampal EEG changes after injection of MK-801 (0.5 mg/kg i.p.). A,B: Representative hippocampal EEG spectra 20 min after MK-801 as compared with baseline walk (WK) and awake-immobility (IM) before drug for orexin-saporin lesion rats (Ore-SAP, B) and sham lesion rats (Sham, A). An increase in gamma waves was observed in sham but not in Ore-SAP rat after MK-801. C–E: Normalized change of integrated hippocampal EEG power (baseline walk = 100%) of group data (mean ± standard error of the mean) of ore-SAP and sham rats, for 71–100 Hz gamma (C), 30-70 Hz gamma (D) and 5-10 Hz theta rhythm (E). F: Peak theta frequency in the hippocampus increased in both sham and ore-SAP lesion rats after MK-801 injection. # $P<0.05$, ## $P<0.01$, difference with baseline walking after one-way repeated ANOVA followed by Newman-Keuls post hoc test. # above line indicates significant time effect in ANOVA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

time point. In sham lesion rats, average peak theta frequency was 7.77 + 0.16 Hz during baseline and 9.34 + 0.89 Hz at 30 min after MK-801 administration. In ore-SAP lesion rats, the baseline peak theta frequency was 8.12 + 0.44 Hz and 9.04 + 0.13 Hz at 30 min after MK-801 injection.

An increase in gamma activity was observed after MK-801 in sham (Fig. 4A) but not in ore-SAP lesion rats (Fig. 4B). Group data confirmed that the normalized 71–100 Hz gamma power after MK-801 was significantly higher in sham lesion rats as compared with ore-SAP lesion rats [Fig. 4C; two-way (group × time) ANOVA group effect $F(1,9) = 28.7$, $P < 0.0001$, with a nonsignificant ($P > 0.4$) group × time interaction effect $F(9,120) = 0.99$]. Only the sham lesion group shows a significant increase in 71–100 Hz gamma power with time after MK-801 [Fig.4C; $F(9,54) = 9.47$, $P < 0.0001$, one-way ANOVA]. The ore-SAP group shows a significant decrease in 71–100 Hz gamma power at 5 min after injection (Fig. 4C; one-way ANOVA time effect $F(9,54) = 6.35$, $P < 0.0001$). During baseline walking (before drug), the magnitude of the integrated 71–100 Hz gamma power was not different between sham (1.80 + 0.16 log units) and ore-SAP lesion (1.76 + 0.05 log units) rats ($t = 0.27$; $P = 0.82$, t test).

MK-801 injection did not result in a significant difference in the normalized gamma power of 30–70 Hz between sham and ore-SAP lesion rats (Fig. 4D) in either group effect $F(1,9) = 3.30$, $P = 0.07$ or group × time effect $F(1,9) = 0.65$, $P = 0.75$ (two-way ANOVA). However, there was a significant time effect [$F(1,9) = 4.53$, $P < 0.0001$] although Newman-Keuls post-hoc test did not reveal statistical significance at a specific time point. There was no difference in the magnitude of integrated 30–70 Hz gamma power during baseline walking between sham and ore-SAP lesion rats.

Septal Ore-SAP Lesion Reduced MK-801-Induced Locomotion

Spontaneous horizontal locomotion, without drug injection, was assessed in a novel environment. Both sham and ore-SAP lesion groups showed an initially high level of movements (beam interruptions) that decreased with time, but the number of movements per time was not different between groups at any time [Fig. 5A; two-way repeated measures ANOVA group effect $F(1,72) = 0.0008$, $P = 0.99$, time effect: $F(5,72) = 15.15$, $P < 0.0001$; group × time interaction: $F(5,72) = 1.83$, $P = 0.12$].

In a separate experiment, locomotor activity induced by MK-801 (0.5 mg/kg i.p.) injection was assessed. During baseline before injection of MK-801, there was no difference in the habituated baseline locomotion between the two groups of rats (Fig. 5B). After MK-801 injection, locomotor activity increased greatly in sham lesion rats, significantly more than in ore-SAP lesion rats (Fig. 5B), as confirmed by a two-way repeated measures ANOVA [main group effect $F(1,12) = 10.31$, $P < 0.01$, time effect $F(12,144) = 18.620$, $P < 0.0001$, and group × time interaction $F(12,144) = 3.141$, $P < 0.001$].

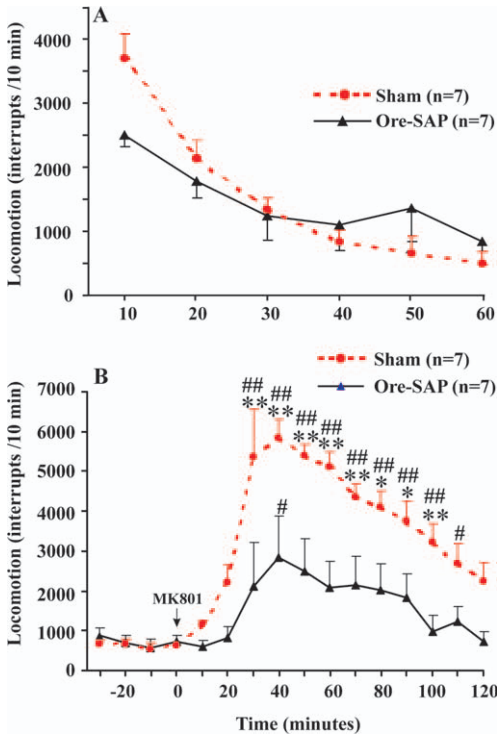

FIGURE 5. Locomotion in open field and after injection of MK-801. A: Locomotor activity in a novel open field, as indicated by the number of interruptions of infrared beams, was not significantly different between orexin-saporin lesion rats (Ore-SAP) as compared to sham lesion rats (Sham). B: Horizontal locomotion before and after 0.5 mg/kg i.p.MK-801, rats were habituated in the environment before measurements started 30 min before MK-801. Ore-SAP rats as compared to sham rats showed decreased number of beam interruptions after MK-801 injection. *$P < 0.05$, **$P < 0.01$ difference between groups; #$P < 0.05$, ##$P < 0.01$, difference with baseline of a particular group, Newman-Keuls post-hoc test after two-way ANOVA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Septal Ore-SAP Lesion Reduced MK-801-Induced Impairment of PPI

PPI was assessed in ore-SAP lesion rats as compared to sham lesion rats. Without drug injection, there was a significant difference in the PPI between ore-SAP and sham lesion rats when all the individual prepulse intensities (73, 75, 80 dB) were included [two-way (group × intensity) ANOVA main effect $F(1,30) = 8.33$, $P < 0.01$; Fig. 6A). Amplitude of the startle response, without prepulse, was not different between the two groups ($t = 1.79$. $P = 0.18$, Fig. 6B). In the rats given MK-801 (15 min before the start of PPI testing), as compared with the respective group without MK-801 (compare Fig. 6C with Fig. 6A), sham lesion rats showed a marked decrease in PPI while ore-SAP lesion rats did not show a significant change in PPI. After MK-801 injection, PPI was significantly different between ore-SAP-lesion and sham-lesion groups [two-way ANOVA group effect, $F(1,45) = 26.85$, $P < 0.0001$], with significant post hoc differences for the individual prepulse intensities and the integrated intensity (Fig. 6C). There was no sig-

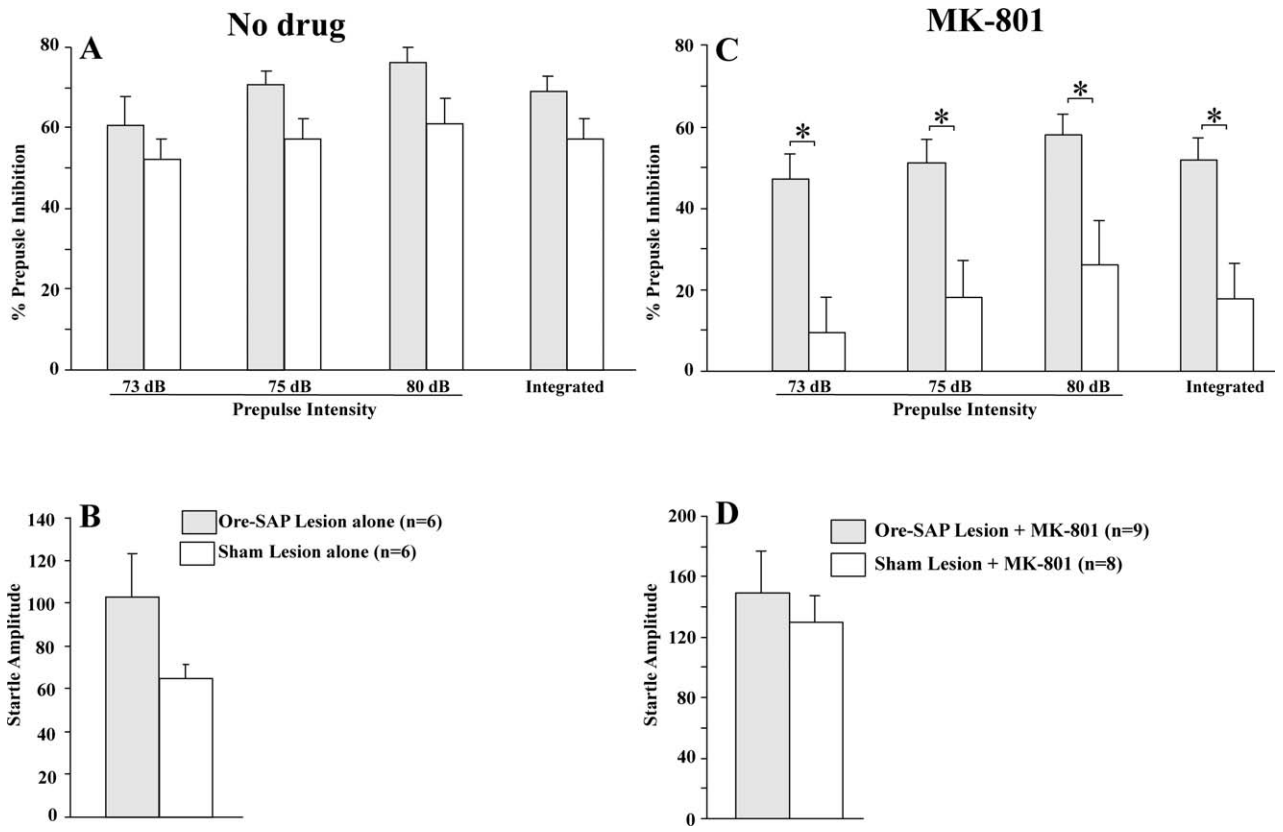

FIGURE 6. Septal orexin-saporin (Ore-SAP) lesion rats, without drugs, showed an increased prepulse inhibition (PPI) across three individual prepulse intensities (2-way ANOVA), but not for the integrated prepulse intensity (paired *t*-test, A). Ore-SAP lesion alone did not affect startle amplitude (arbitrary units, B) compared to sham lesion rats. PPI decreased after MK-801 (0.5 mg/kg i.p.) injection in sham-lesion rats as compared to ore-SAP lesion rats at each prepulse intensity and the integrated intensity (C). D: Startle amplitude was not different between ore-SAP and sham lesion rats after MK-801 injection. *$P < 0.05$, difference between sham and ore-SAP lesion group after two-way ANOVA (A), and followed by Newman-Keuls post-hoc test (C).

nificant difference in the startle amplitudes between ore-SAP and sham lesion groups ($t = 0.58$, $P = 0.59$, Fig. 6D).

Ore-SAP Lesion Reduced Ketamine-Induced Impairment of Hippocampal Auditory Evoked Potential

During baseline before drug, both ore-SAP lesion and sham lesion rats showed auditory gating, or suppression of the test response (amplitude T) as compared with the conditioning response (amplitude C) in the average hippocampal auditory evoked potential (Fig. 7A). Baseline T/C ratio in sham lesion rats was 0.26 ± 0.07, not significantly different from that of 0.33 ± 0.06 in ore-SAP lesion rats ($t = 0.79$, $P = 0.45$; t test). After injection of ketamine (3 mg/kg s.c.), sham but not ore-SAP lesion rats showed an increase in T/C ratio (Fig. 7A). Ketamine increased the T/C ratio from baseline to 0.84 ± 0.12 in sham lesion rats ($t = 4.41$, $P < 0.01$, t test). However, ketamine did not significantly change the T/C ratio of ore-SAP lesion rats, which was 0.40 ± 0.06 after ketamine, similar to that before injection ($t = 0.87$; $P = 0.41$, t test). There was no significant difference in C amplitude before and after ketamine in sham lesion rats (before: 0.73 + 0.10 mV, after: 0.58 + 0.08 mV, $t = 1.08$, $P = 0.32$) or in ore-SAP lesion rats (before: 0.90 + 0.16 mV, after: 1.05 + 0.17 mV, $t = 0.84$, $P = 0.42$, Fig. 7B).

DISCUSSION

This study showed that selective lesion of the septohippocampal GABAergic neurons by infusing ore-SAP into the medial septum suppressed behavioral hyperactivity, auditory gating and PPI deficits induced by an NMDA-receptor antagonist, with minimal disruption of baseline (no drug) locomotion and hippocampal auditory gating. The results suggest that septohippocampal GABAergic neurons participate in an atropine-resistant hippocampal theta rhythm during normal walking, and in generating the enhanced hippocampal gamma rhythm of 71–100 Hz induced by an NMDA receptor antagonist.

The two effects of ore-SAP lesion of septohippocampal GABAergic neurons in this study that of attenuating atropine-resistant theta and reducing the schizophrenic symptoms

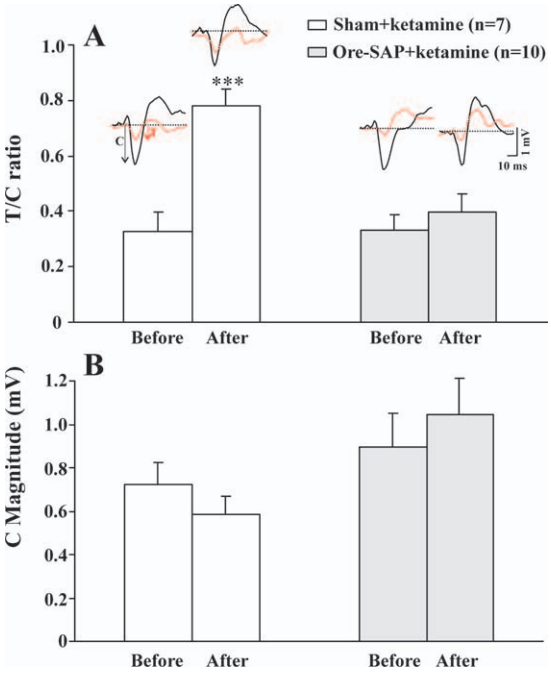

FIGURE 7. Auditory gating was disrupted by ketamine (3 mg/kg s.c.) in sham lesion rats but not in orexin-saporin (Ore-SAP) lesion rats. Average evoked potential was recorded in CA1 stratum radiatum of the hippocampus, and the second-click response (red dotted trace) and the first-click response (black solid trace) were overlaid, with indication of the measurement of the peak response to the 1st conditioning click (*C*) and 2nd test click (*T*). The *T/C* ratio was increased significantly after ketamine in sham lesion rats, but not in ore-SAP lesion rats (A). There was no difference in the magnitude of conditioning pulse responses between sham lesion and septal ore-SAP lesion rats, or before and after ketamine in the same group (B). ***$P < 0.01$ (*t* test). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

induced by an NMDA receptor antagonist, are considered as independent effects. While septal GABAergic neuronal lesion reduced both atropine-resistant theta and schizophrenic symptoms, there is no evidence that the reduction of atropine-resistant theta after lesion relieves the schizophrenic symptoms, because little atropine-resistant theta could be demonstrated after NMDA receptor blockade. Independent of theta generation, an NMDA antagonist induced disinhibiton in the hippocampus, prefrontal cortex and other brain areas (Grunze et al., 1996; Li et al., 2002), consistent with the hypothesis that GABAergic neuronal dysfunction may induce schizophrenic symptoms (Benes and Berretta, 2001; Gonzalez-Burgos et al., 2011).

In this study, lesion of septal Parv immunopositive (GABAergic) but not ChAT immunopositive (cholinergic) neurons resulted from 140 ng of ore-SAP infused into the medial septal area, confirming the recent results of Lecourtier et al. (2011). Not all GABAergic neurons in the septum are labeled by Parv, since Parv immunopositive neurons constitute only a fraction of GAD immunopositive neurons in the medial septum (Gritti et al., 2003; Zaborszky et al., 2005; Pang et al., 2011). However, the Parv immunopositive neurons likely constitute a high proportion of GABAergic neurons that project to the hippocampus (Freund and Antal, 1989; Gritti et al., 2003). Smith and Pang (2005) reported that 140 ng of ore-SAP selectively lesioned Parv immunopositive neurons, while 280 ng resulted in loss of both Parv immunopositive and cholinergic neurons, and abolished the hippocampal theta rhythm (Gerashchenko et al., 2001). The reason of selective loss of GABAergic as compared to cholinergic neurons at a low ore-SAP dose is not clear, since both GABAergic and cholinergic neurons have orexin-2 receptors (Wu et al., 2002, 2004; Stanley and Fadel, 2011). This study did not assess the damage to septohippocampal glutamatergic neurons, and the involvement of these neurons in theta generation or NMDA antagonist-induced behaviors in freely moving animals is not known.

Participation of Septal GABAergic Neurons in Normal and Schizophrenia-Like Behaviors

The study shows that GABAergic septohippocampal neurons are involved in schizophrenia-like behaviors induced by an NMDA receptor antagonist. The latter behaviors were also suppressed by infusion of a low dose (0.25 μg) of muscimol into the medial septum (Ma and Leung, 2007, Ma et al., 2004; 2009) while selective lesion of the cholinergic neurons in the medial septum by 192-IgG saporin did not affect the PPI deficit and hyperlocomotion induced by phencyclidine (Ma et al., 2004).

Auditory gating in the hippocampus is likely mediated by hippocampal inhibitory interneurons (Miller and Freedman, 1995), and blockade of hippocampal $GABA_B$ receptor inhibition induced a hippocampal auditory gating loss (Ma and Leung, 2011). We infer that the loss of hippocampal auditory gating may reflect hippocampal disinhibition induced by ketamine, an NMDA receptor antagonist (Grunze et al., 1996), and hippocampal disinhibition is mediated by septohippocampal GABAergic neurons inhibiting hippocampal GABAergic interneurons (Freud and Antal, 1988; Toth et al., 1997). In intact animals, hippocampal disinhibition mediated by both NMDA receptor antagonist and septohippocampal GABAergic neuronal activity may induce an increase in dopamine release in the nucleus accumbens (Mogensen et al., 1993; Pennartz et al., 1994), which results in an increase in locomotion and PPI. Thus, lesion of septohippocampal GABAergic neurons may reduce hippocampal disinhibition and normalize locomotor activity and PPI. However, septohippocampal GABAergic neurons acting on structures outside of the hippocampus, such as subcortical areas (Semba, 2000), may also contribute to the behaviors induced by an NMDA receptor antagonist.

Selective lesion of the septohippocampal GABAergic neurons only marginally affected baseline behaviors in this study. Locomotion in a novel open field and baseline auditory gating were not significantly affected by ore-SAP lesion of the medial septum. Baseline PPI was, however, increased slightly but significantly in ore-SAP lesion as compared to sham lesion rats. In other studies, selective lesion of septohippocampal GABAergic

neurons by GAT1-saporin (Pang et al., 2011) did not affect general locomotion in an open field but disrupted working spatial memory without affecting reference memory in the water maze (Pang et al., 2011) or radial arm maze (Dwyer et al., 2007). However, ore-SAP (140 ng in the medial septum) disrupted spatial reference memory retention in the water maze for several days (Smith and Pang, 2005; Lecourtier et al., 2011).

Septohippocampal GABAergic Neurons Participate in Atropine-Resistant Hippocampal Theta

In this study, selective lesion of the septohippocampal GABAergic neurons did not significantly affect theta power during baseline walking, but decreased theta power during walking after atropine. Hippocampal theta power is suggested to result from driving of septohippocampal GABAergic neurons on hippocampal neurons since both septohippocampal cholinergic neuronal firing and muscarinc cholinergic synaptic activity may be too slow for theta-frequency driving (Stewart and Fox, 1990; Simon et al., 2006). However, in the present study, a ~80% decrease in septohippocampal GABAergic neurons did not significantly affect theta power during no-drug walking, suggesting that septohippocampal GABAergic neurons are not critically important in theta power or frequency during baseline walking. Alternatively, the loss of septohippocampal GABAergic neurons may result in compensatory mechanisms, which may include increase in cholinergic activity in the septum, and/or an increase in theta-rhythmic driving by septohippocampal glutamatergic neurons. The latter compensatory mechanisms are speculative since cholinergic and glutamatergic neuronal activities have not been measured. On the other hand, theta power during walking after atropine was significantly reduced in ore-SAP lesion than sham lesion rats, suggesting that septohippocampal GABAergic neurons contribute to the atropine-resistant theta in behaving rats. Disruption of atropine-resistant theta during walking was also reported in rats with septal ibotenic acid lesion, but loss of septal GABAergic neurons was not measured (Leung et al., 1994).

Pilocarpine-induced atropine-sensitive theta in behaving rats did not differ in power between ore-SAP and sham lesion rats. This pilocarpine-induced theta activity was atropine-sensitive, and our result did not support the participation of septohippocampal GABAergic neurons in generating a muscarinic receptor activated theta (Alreja et al., 2000; Wu et al., 2000). However, a general decrease in amplitude of the hippocampal theta rhythm in urethane-anesthetized rats and behaving rats was reported after kainic acid lesion of septohippocampal GABAergic neurons (Yoder and Pang, 2005).

Relation of Hippocampal Gamma Waves to Schizophrenic Behaviors

After administration of MK-801, hippocampal gamma waves of 71–100 Hz were significantly increased in sham lesion rats but not in ore-SAP lesion rats. This suggests that the MK-801 induced hippocampal gamma (71–100 Hz) increase requires the integrity of the septal GABAergic neurons. Septohippocampal GABAergic input may enhance gamma oscillations through modulation of a local hippocampal recurrent inhibition network (Leung, 1982, 1998; Mann et al., 2005). The mechanism of this enhancement is not clear, but disinhibition of pyramidal cells may increase the operating bias and gain of the pyramidal cell population (Leung, 1982).

Clinical studies suggested a link between schizophrenia and aberrant gamma oscillations recorded in the scalp EEG (Clementz et al., 1997; Baldeweg et al., 1998; Barr et al., 2010). We have shown here an important role of the GABAergic septohippocampal neurons in mediating the various symptoms of schizophrenia in animals, perhaps in association with an enhanced gamma rhythm in the hippocampus. We suggest that selective inactivation of septohippocampal GABAergic neurons may control the behavioral symptoms of schizophrenia.

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