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Deep brain stimulation of the medial septum or nucleus accumbens alleviates psychosis-relevant behavior in ketamine-treated rats

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Abstract

Deep brain stimulation (DBS) has been shown to be effective for relief of Parkinson’s disease, depression and obsessive-compulsive disorder in humans, but the effect of DBS on psychosis is largely unknown. In previous studies, we showed that inactivation of the medial septum or nucleus accumbens normalized the hyperactive and psychosis-related behaviors induced by psychoactive drugs. We hypothesized that DBS of the medial septum or nucleus accumbens normalizes the ketamine-induced abnormal behaviors and brain activity in freely moving rats. Male Long-Evans rats were subcutaneously injected with ketamine (3 mg/kg) alone, or given ketamine and DBS, or injected with saline alone. Subcutaneous injection of ketamine resulted in loss of gating of hippocampal auditory evoked potentials (AEPs), deficit in prepulse inhibition (PPI) and hyperlocomotion, accompanied by increased hippocampal gamma oscillations of 70–100 Hz. Continuous 130-Hz stimulation of the nucleus accumbens, or 100-Hz burst stimulation of the medial septum (1 s on and 5 s off) significantly attenuated ketamine-induced PPI deficit and hyperlocomotion. Medial septal stimulation also prevented the loss of gating of hippocampal AEPs and the increase in hippocampal gamma waves induced by ketamine. Neither septal or accumbens DBS alone without ketamine injection affected spontaneous locomotion or PPI. The results suggest that DBS of the medial septum or nucleus accumbens may be an effective method to alleviate psychiatric symptoms of schizophrenia. The effect of medial septal DBS in suppressing both hippocampal gamma oscillations and abnormal behaviors induced by ketamine suggests that hippocampal gamma oscillations are a correlate of disrupted behaviors.

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1. Introduction

Schizophrenia is a heterogeneous mental disease that includes both positive and negative syndromes [3,27]. Subanesthetic doses of ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist [4], have been shown to induce schizophrenic like symptoms in normal humans [32] and aggravate psychiatric symptoms in schizophrenic patients [33]. Psychiatric symptoms induced by ketamine were accompanied by increased cerebral blood flow in limbic areas such as the anterior cingulate cortex (including medial prefrontal cortex) and insula [33,34]. Ketamine and other NMDA receptor hypofunction models of schizophrenia have contributed to the understanding of the mechanism and treatment of schizophrenia [25,56], extending beyond theories of dopaminergic hyperfunction [19,26,57].

In animals, a single subanesthetic dose of ketamine induces a spectrum of behavioral abnormalities that model the symptoms of schizophrenia in humans. The symptoms include hyperlocomotion...
deficit of prepulse inhibition (PPI) [31,46,69], and loss of gating of hippocampal auditory evoked potentials (AEPs) [48,49]. In addition, a subanesthetic dose of ketamine increased gamma or high-frequency oscillations in many brain areas in animals, including the hippocampus [50], nucleus accumbens [21,22] and auditory, motor and visual cortices [20,31,59]. Schizophrenia in patients is associated with altered gamma-frequency electroencephalogram (EEG), shown as an increase in spontaneous gamma activity [5,23,24], and a decrease in evoked gamma activity or synchronization in other studies [18,67,72]. Altered gamma activity may be a manifestation of the abnormal local inhibitory networks [36,41,76] that underlie schizophrenia [7,10,72].

A neural circuit involving the medial septum, hippocampus and nucleus accumbens is suggested to mediate some of the psychosis-related symptoms induced by an NMDA receptor antagonist in animals [21,42,35,70]. Infusion of muscimol into the medial septum [44,46–48] or selective lesion of medial septal GABAergic neurons [49] normalized the hippocampal gamma waves and the behavioral symptoms induced by an NMDA receptor antagonist, including hyperlocomotion, PPI and AEP deficit. These findings are consistent with the results that the medial septal neurons control the hippocampal EEG [8,74] and hippocampus-mediated behaviors [9]. Inactivation of the nucleus accumbens suppressed locomotor activity induced by low-dose general anesthetics [45].

Recently, deep brain stimulation (DBS) has been used as a therapeutic treatment of several neurological and psychiatric diseases. DBS of a brain area may alleviate the symptoms mediated by the local area, such as tremor in Parkinson’s disease [6,62]. In other cases, DBS may have an effect in normalizing neural circuitry, such as DBS of the nucleus accumbens may be therapeutic for obsessive-compulsive disorder [68] perhaps by suppressing neural activity in the orbitofrontal cortex [51]. The hippocampus and the nucleus accumbens have been proposed as therapeutic targets for DBS in schizophrenic patients [53], in line with the role of a septohippocampal-accumbens circuit in mediating different psychosis-related behaviors. DBS of the ventral hippocampus relieved the deficit in gating of the auditory evoked potentials in an animal model of schizophrenia [12].

Whether DBS is effective in treating psychosis-related symptoms in freely behaving animals has not been experimentally studied. Based on the role of the septohippocampal-accumbens circuit in mediating hyperlocomotion and PPI/AEP deficit, we hypothesized that DBS of the medial septum or the nucleus accumbens will alleviate the behavioral effects induced by ketamine in rats. Since the medial septum also controls hippocampal EEG, we hypothesized that medial septal DBS will also suppress ketamine-induced hippocampal gamma wave increase.

2. Materials and methods

2.1. Surgery

Under pentobarbital anesthesia (60 mg/kg i.p.), male Long-Evans rats weighing between 250 and 300 g were implanted with a pair of Teflon-coated stainless steel stimulating electrodes (127 μm) into the medial septum (anterior–posterior (AP) 0.7, lateral (L) 0 or midline, ventral to skull (V) 6.0 and 6.5, all units in mm, according to the atlas of Paxinos and Watson [58]). In order to avoid the mid sagittal sinus, a drill hole on the skull was made 0.5 mm lateral to midline and the septal electrode was inserted with a 5° slant from the vertical. In rats for septal stimulation, four pairs of electrodes were implanted bilaterally into the hippocampus (AP-3.2, L±1.7; AP-4.6, L±1.2; V 3.3 and 2.3) for recordings of hippocampal AEPs or EEG. For accumbens stimulation, a pair of electrodes was implanted into the right accumbens shell region (AP1.5, L1.2, V7.5 and 7.9), two jeweller’s screws were fixed in the skull over the frontal cortex and cerebellum, to serve as reference and ground for recording hippocampal evoked potentials and EEGs. All electrodes and screws were finally anchored to the skull with dental cement. One week was allowed for the animals to recover from surgery. All experimental procedures were approved by the local Animal Use Committee and conducted according to the guidelines of the Canadian Council for Animal Care. Efforts were taken to minimize the pain and suffering of animals.

2.2. Experimental procedures

Experiments were conducted between 9:00 and 18:00 h. A total of 57 rats were used – 28 for septal DBS with ketamine, 17 for accumbens DBS with ketamine, 12 for testing septal or accumbens DBS alone without administration of ketamine; all rats were implanted with electrodes, including those used for experiments without DBS. Four different responses were recorded in the present study – horizontal locomotion, prepulse inhibition (PPI), gating of auditory evoked potentials and EEG recordings in the hippocampus. For each type of measure, the same rat was given three treatments, in random order with at least one week between treatments: (1) ketamine alone, (2) saline alone or (3) ketamine with DBS of one structure (medial septal or nucleus accumbens). When DBS was applied after ketamine injection, the stimulation started immediately after ketamine injection and continued for the whole duration of any experiment. The one-week separation between treatments was considered adequate since no behavioral and EEG effects of ketamine/saline injection could be detected after one week. In addition, randomization effectively removed effects that may depend on treatment order. Separate rats were used to test the effect of DBS alone, without ketamine, on spontaneous locomotion or PPI. In randomized order, locomotion or PPI tests were done, with or without septal or accumbens DBS on the same rats, without administration of any drugs.

For locomotor activity, horizontal movements of a rat in a Plexiglas chamber (69 × 69 × 49 cm) were measured by the number of interruptions of infrared beams (Columbus Instruments), which were transferred to a microcomputer via an interface. For ketamine injection/control experiments, a rat was habituated for at least 1 h in the chamber, and then the number of infrared beam interruptions was counted every minute, for 5 min during baseline (before injection) and 30 min after ketamine injection with or without DBS, or for 30 min after saline injection alone. For experiments to test the effect of DBS alone versus no stimulation, without ketamine injection, a rat was placed into the chamber and infrared beam interruptions were counted for 30 min, without habituating the rat. No habituation ensured the presence of a certain level of locomotor activity, such that a possible suppressive effect of DBS can be tested.

PPI was measured by SR-LAB (San Diego Instruments, San Diego, CA), using a piezoelectric accelerometer to detect startle amplitude [47]. If DBS was applied after ketamine, it was started immediately after placing a rat into the PPI test chamber. After acclimating to 68-dB white 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 (20-ms broad band noise) of intensity 73, 75, or 80-dB. For each test session, 50 trials were given in randomized order – 10 trials with startle pulse only, 10 trials with no auditory stimulation, and 10 trials with one of the three prepulse intensities followed by a startle pulse. PPI was measured as the difference between the response to the startle pulse alone and the response to the combination of prepulse and 120-dB startle pulse, i.e., PPI (in percent) = 100 × [1 – (mean startle response amplitude after a prepulse/mean amplitude of response to startle alone)]. In this study, the PPI was estimated
using different individual prepulse intensities (73, 75, 80 dB), and the integrated prepulse intensity (mean of all prepulses).

Hippocampal gating of auditory evoked potentials (AEPs) were recorded from a semi-restrained rat placed inside the same chamber used for testing PPI [48]. A loudspeaker was placed above the chamber, in a fixed position of 30° elevated and 24 cm away from the head of the rats. AEPs were recorded following auditory click pairs separated by a conditioning-test (C-T) interval of 500 ms; each click was a white-noise burst at 75 dB of 20 ms pulse duration. Click pairs were given 15 s apart. Single sweeps of the AEP were stored on the computer, and sweeps with movement or electrical artifacts were rejected online and offline. Baseline recording before treatment consisted of 25 sweeps of AEPs, after habituating a rat for 15 min in the restraining chamber. Then, the rat was removed from the recording chamber and received an injection of ketamine (3 mg/kg, subcutaneously injected, sc) or saline (0.1 mL, sc). Ten minutes after injection, the rat was placed back in the chamber for post drug recording with or without DBS. If DBS was applied after ketamine injection, it was started immediately after the rat was placed back into the chamber.

Hippocampal EEG was recorded during awake immobility or walking before injection, which served as baseline recording. Rats were then injected with either ketamine or saline. If DBS was applied after ketamine, it was started immediately after returning the rat to the EEG recording chamber.

2.3. Deep brain stimulation (DBS) and EEG analysis

For DBS, the stimulation was applied across a pair of electrodes at each site (medial septum or nucleus accumbens), with one electrode serving as the cathode and the other as the anode. Preliminary experiment showed similar effects with normal and reversed (opposite) polarity of current flow, since both electrodes were targeted at the same site. The current intensity was determined according to the rat’s response to stimulation during ketamine-induced locomotion. The initial current intensity for each rat was set at 200 μA, and this was increased in 50 μA steps until the stimulation reduced locomotion, or 500 μA was reached. If the initial 200 μA was effective to reduce hyperlocomotion, this current was reduced, in 50 μA steps, so that the lowest current that reduced locomotion was used for DBS. The final DBS parameters for the medial septal site were 100–500 μA (280 ± 51 μA intensity, 0.2 ms pulse duration, and repeating 100 Hz on for 1 s, followed by 5 s off, for 1 min). The 5-s pause between 1-s stimulation trains was meant to avoid the induction of electrographic seizures, since the medial septum has a low seizure threshold [35]. In the nucleus accumbens, which has a higher seizure threshold, DBS was applied for 30 min with continuous 130-Hz stimulus pulses of 250 ± 11 μA (n = 7) and 0.1 ms pulse duration. Preliminary studies indicated that, when compared to ketamine injection alone without DBS, DBS of the medial septum at 10 Hz continuously (200 μA intensity) did not reduce hyperlocomotion at 1–15 min after ketamine, 50 Hz continuous stimulation of the medial septum enhanced behavioral hyperactivity after ketamine (n = 3), while 100 Hz burst (1 s on, 5 s off) septal stimulation significantly reduced ketamine-induced locomotion. 130-Hz frequency was used for DBS of the nucleus accumbens, and this stimulation frequency was shown to be effective in related studies [50,51].

Hippocampal EEG at CA1 stratum radiatum with or without medial septal DBS was analyzed. The EEG was fed into a Grass amplifier filtered from 0.3 Hz to 3 kHz and stored in segments of 2.5 s (2500 points) digitized at 1000 Hz. During DBS, 2048-point segments were selected before the 1-s DBS trains, in order to avoid stimulus artefacts. Artefact-free EEG could not be obtained during continuous 130-Hz DBS of the nucleus accumbens, and thus the effect of accumbens DBS on EEG was not further studied. Each artefact-free EEG segment was tapered at the ends, Fast Fourier Transform, and 5 adjacent frequency bins (0.49 Hz bins) were smoothed by an elliptical function to yield a power spectrum [40]. The average power was calculated for three frequency bands – theta (5–10 Hz), low gamma (30–70 Hz, excluding line power at 58–62 Hz) and high gamma (70–100 Hz). For comparing EEG power changes between treatments, the average power at each time was normalized by the baseline walking EEG power in each experiment.

2.4. Histology

Upon completion of experiment, the rat was deeply anesthetized with urethane (1.3 g/kg i.p.) and transcardially perfused with 0.9% saline followed with 4% formalin. The brain of the rat was removed and cut into 40 μm coronal sections with a microtome. The brain sections were mounted on glass slides and stained with thionin for identifying locations of the electrodes. Histological location of electrodes used for deep brain stimulation in A. the medial septum, B. nucleus accumbens. Upper row, histological plates from the brain atlas of [58] on which electrode locations for effective (solid circles) and ineffective deep brain stimulation (open circles) were indicated. Electrode tip locations from adjacent coronal sections (within 1 mm) were projected onto one plate for each area, with some overlapping solid circles. Lower panel, representative photomicrographs of actual placement, with the electrode tip indicated by a black arrow.

Values were expressed as means ± standard error of the mean. The statistical analysis was performed by Student t test, or...
one two-way analysis of variance (ANOVA). ANOVA with significant main effect or interaction effect was followed by post hoc Newman–Keuls or Fisher LSD test. Significance level was set at $P < 0.05$.

3. Results

3.1. Effect of medial septal stimulation on ketamine-induced hyperlocomotion and PPI deficit

Ketamine increased locomotor activity across the recording cage. DBS of the medial septum reduced locomotor movements, and a rat would remain immobile on one side of the cage, either frozen or with explorative head movements. Occasionally, during adjustment of the stimulating current, high-intensity (>400 µA) stimulation of the medial septum induced wet dog shakes, but without electrophographic seizure activity. In the latter case, the stimulation current was reduced until wet dog shakes disappeared. Septal DBS did not induce any convulsions, or clearly aggressive or defensive behaviors in rats.

As reported previously [46], ketamine injection alone significantly increased locomotor activity compared to either baseline or saline injection at the same time point ($F(2,12) = 18.52, P < 0.001$; two-way repeated measures ANOVA, Fig. 2). Septal stimulation significantly attenuated ketamine-induced hyperlocomotion as compared to no stimulation after ketamine (Fig. 2). Although sporadic differences were observed at certain time points, there was no significant difference between saline injection and “ketamine injection plus septal stimulation” at most time points, as revealed by post hoc Newman–Keuls test.

In separate experiments, spontaneous locomotion of a rat was recorded in sequential experiments with or without septal DBS; no ketamine or saline was injected. Septal DBS, as compared to without DBS, did not significantly affect spontaneous locomotion ($F(1,5) = 0.87, P = 0.87$, two-way repeated measures ANOVA, Supplemental Fig. 1A).

Ketamine injection alone induced a statistically significant PPI decrease, compared to saline injection at the same prepulse intensity (Fig. 3A), as was shown previously [46]. PPI was significantly different among the three treatments that included saline alone injection, ketamine alone injection and “ketamine injection plus septal stimulation”. One-way repeated measures ANOVA revealed that the PPIs for the three treatments were different (Fig. 3A) for a prepulse of 73 dB [$F(2,14) = 4.56, P < 0.05$, Fig. 2A], 75 dB [$F(2,14) = 6.15, P < 0.05$] but not 80 dB. PPIs were also different if all prepulses were included (integrated prepulse) [$F(2,14) = 5.43, P < 0.05$; Fig. 3A]. Post hoc tests indicated that PPI of the “ketamine plus septal stimulation” group was different from that of ketamine injection alone but not from that of saline injection alone. There was no statistically significant difference in the amplitude of startle response among the three treatments (Fig. 3B).

In experiments done without ketamine injection, septal DBS alone, compared to no-DBS condition, did not significantly affect PPI [$F(1,5) = 0.09, P = 0.78$, two-way repeated measures ANOVA, Supplemental Fig. 2A] or startle response ($t = 0.05, P = 0.96$, Supplemental Fig. 2B).

3.2. Effect of nucleus accumbens stimulation on ketamine-induced hyperlocomotion and PPI deficit

Accumbens stimulation reduced movements across the recording cage, but no wet dog shakes or other obvious behaviors were elicited. As shown in Fig. 4, injection of ketamine alone increased horizontal movements as compared to saline alone, but accumbens stimulation after ketamine injection significantly decreased horizontal movements compared to ketamine injection alone [two-way repeated measures ANOVA $F(2,12) = 26.07, P = 0.001$]. Newman–Keuls post hoc test showed that locomotion after ketamine injection alone was significantly different from that after saline injection alone or after “ketamine injection plus

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**Fig. 2.** Effect of medial septal stimulation on ketamine-induced locomotion as a function of time after injection. Ketamine (3 mg/kg sc) injection only (Keta Alone) induced an increase of locomotion detected by the number of interruptions of infrared beams per minute. Medial septal stimulation after ketamine (Keta + Ms-stim) reduced locomotion as compared to Keta Alone; Ms-stim (100 Hz stimulation on for 1 s, and off for 5 s) was applied from 0–30 min. Post hoc Newman–Keuls test following a significant two-way repeated measures ANOVA: *$P < 0.05$, **$P < 0.01$, significantly different from baseline; $P < 0.05$, **$P < 0.01$, significantly different compared to Saline Alone at the same time point.**

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**Fig. 3.** Effect of medial septal stimulation on ketamine-induced deficit of prepulse inhibition (PPI) of an acoustic startle response. A. Ketamine (3 mg/kg sc) injection alone (Keta Alone) induced a decrease in PPI as compared to Saline Alone injection; PPI was measured with prepulses of 73, 75 or 80 dB, or integrated prepulse (average of all prepulses). Medial septal stimulation after ketamine (Keta + Ms-stim) alleviated the ketamine-induced PPI deficit. B. There is no significant difference in the startle amplitude among the 3 groups. *$P < 0.05$, **$P < 0.01$, Newman–Keuls test following one-way repeated measures ANOVA. NS: not significant.
accumbens stimulation”. However, there was no significant difference between locomotion after saline injection alone and that after “ketamine injection plus accumbens stimulation”.

In a separate group of rats given accumbens DBS without the injection of ketamine, accumbens DBS alone, compared to no-DBS condition, did not affect spontaneous locomotion ($F(1,5) = 0.64$, $P = 0.46$, two-way repeated measures ANOVA, Supplemental Fig. 1B).

Accumbens stimulation significantly attenuated ketamine-induced deficit of PPI. One-way repeated measures ANOVA showed an overall significant difference among PPIs of the 3 groups following an integrated prepulse intensity $F(2,12) = 6.05$, $P < 0.05$. As compared to saline-injected rats, a PPI decrease was found after ketamine alone, but not after ketamine with accumbens stimulation ($P < 0.05$, post hoc Newman–Keuls tests, Fig. 5A). The PPI with different prepulse intensities of 73, 75, and 80 dB was also significantly different between ketamine injection alone and saline injection, but not between saline and ketamine injection plus accumbens DBS (Fig. 5A). There was no significant difference in startle amplitude among the 3 groups (Fig. 5B).

The effect of DBS in normalizing PPI deficit after ketamine was not found for stimulating electrodes placed outside of the nucleus accumbens. In 3 rats in which the stimulating electrode missed the nucleus accumbens (Fig. 1B), DBS had no ameliorating effect on ketamine-induced PPI deficit. After ketamine, a PPI (using integrated pulse) of 22 ± 4.2% ($n = 3$) was found during DBS, which was not significantly different from the PPI of 27 ± 5.6% ($n = 3$) without DBS.

In experiments done on separate rats without ketamine injection, there was no significant difference in the PPI recorded during the accumbens DBS condition as compared to the no-DBS condition $F(1,5) = 0.63$, $P = 0.46$, two-way repeated measures ANOVA, Supplemental Fig. 2C) or in the startle amplitude ($t = 0.97$, $P = 0.38$; Supplemental Fig. 2D).

3.3. Effect of medial septal stimulation on ketamine-induced deficit of hippocampal AEPs

Ketamine injection alone significantly reduced gating of the AEPs in the hippocampus, as indicated by an increase in the test to conditioning pulse response (T/C) ratio (Fig. 6A). Since the medial septum is known to modulate electrophysiological responses in the hippocampus, DBS of the medial septum was used to modulate the ketamine-induced alteration in hippocampal electrical activity. Medial septal stimulation after ketamine injection normalized the T/C ratio, such that the ratio was not significantly different from baseline, or different from rats after saline alone injection (Fig. 6A, posthoc Newman–Keuls tests after a significant 2-way ANOVA $F(5,30) = 10.23$, $P < 0.001$). There was no significant difference in the conditioning-pulse response amplitude before and after injections, for the three treatment groups (Fig. 6B).

3.4. Effect of medial septal stimulation on ketamine-induced hippocampal EEG changes

Medial septal stimulation was also used to modulate the hippocampal EEG changes induced by ketamine. Average EEG power at different frequency bands was normalized by the power during baseline walking in each rat. Theta and gamma power decreased from baseline walking to baseline immobility, as seen in the average EEG power in the theta, low (30–70 Hz) gamma and high (70–100 Hz) gamma bands (Fig. 7). As compared to baseline walking, ketamine (3 mg/kg sc) significantly increased high gamma power by 1.5–2 fold at 5–10 min after injection (Fig. 7C). There was no significant increase in the low (30–70 Hz) gamma (Fig. 7B) or theta band power after ketamine (Fig. 7A). Analysis of the frequency and amplitude of theta power peak in individual rats confirmed that there was no significant change in theta power after ketamine, as compared to after saline, or ketamine + DBS.
although there was a trend that ketamine increased peak theta frequency. The average power spectrum (Fig. 7D) showed a robust increase in gamma power at 62–100 Hz at 5 min after ketamine alone, as compared to baseline walking, or 5 min after ketamine with medial septal stimulation. Interestingly, ketamine suppressed the second harmonic of the theta rhythm at ~15 Hz (* in Fig. 7D), similar to the effect of phencyclidine [37]. Two-way block repeated measures (3 treatments × 6 times) ANOVA revealed significant effects on the 70–100 Hz gamma power [treatment F(2,10) = 5.51; P = 0.031; time F(5,25) = 6.95; P = 0.003; and treatment × time interaction F(10,50) = 2.67, P = 0.011]. Post hoc comparisons indicated that the medial septal stimulation significantly reduced the 70–100 Hz gamma power at 5 and 10 min after ketamine injection, as compared injection of ketamine alone (Fig. 7C). The 70–100 Hz gamma power after ketamine + septal DBS was not different from that after saline injection alone. There was no significant treatment or interaction effect on the EEG power at 5–10 Hz theta (Fig. 7A) or 30–70 Hz gamma band (Fig. 7B). The effect of accumbens DBS on EEG was not studied (Methods).

4. Discussion

The present study demonstrated that DBS of the medial septum and the nucleus accumbens alleviated ketamine-induced PPI deficits and hyperlocomotion. Medial septal DBS also suppressed ketamine-induced hippocampal gamma wave increase and hippocampal auditory gating loss. DBS alone did not affect spontaneous PPI or locomotion. As far as we are aware, this is the first report that DBS of limbic areas suppressed ketamine-induced behavioral alterations and hippocampal gamma activity.

4.1. Behaviors and patterns related to deep brain stimulation

Continuous recording of the hippocampal EEG revealed no seizure activity during DBS. High intensity DBS of the medial septum occasionally elicited wet dog shakes, but these were avoided in the present study by reducing septal DBS intensity. Wet dog shakes without electrographic seizures were also induced by d-tubocurarine stimulation [15] and some centrally acting drugs [39]. The absence of seizures indicates that DBS did not alter behaviors by kindling-induced plasticity [60]. Seizures induced by hippocampal and septal stimulations were accompanied by increased locomotion [35,42], as compared to reduced locomotion during DBS.

We showed that stimulation of the medial septum at 100 Hz (with on and off periods) was more effective than stimulation at 10–50 Hz, and confirmed that continuous 130-Hz stimulation of the nucleus accumbens was effective in alleviating the behavioral effects of ketamine. However, the optimal patterns for DBS at each site were not further investigated. During DBS of the medial septum or nucleus accumbens, no aggressive or defensive behaviors were induced in the rats. This contrasted with the hyper-aggressive or aggressive behaviors in rats after large electrolytic lesion [16] or inactivation of the medial septum [43].

4.2. DBS alleviated hyperlocomotion and PPI deficit induced by ketamine

DBS of the two target sites in this study – medial septum and nucleus accumbens – suppressed the hyperlocomotion and reduced the PPI deficit induced by ketamine. We suggest that these data can be explained by convergence of inputs to the nucleus accumbens, which has been implicated in hyperlocomotion and PPI deficits after psychomimetic drugs. The nucleus accumbens receives glutamatergic inputs from the hippocampus [75], as well as dopaminergic inputs from the VTA [73]. In turn, the hippocampus receives cholinergic, GABAergic [1,14], and glutamatergic [11] inputs from the medial septum. Nucleus accumbens, through its ventral pallidum output, has been implicated in mediation of a wide range of abnormal behaviors including hyperlocomotion and PPI deficit [42,55,70].

The neural mechanisms of DBS are not known, and were not specifically studied here. DBS has been suggested to normalize aberrant neural activity [50,51], or induce local inactivation [2,52]. We have previously demonstrated that inactivation of the medial septum by muscimol suppressed the psychosis-related behaviors induced by an NMDA receptor antagonist [43,46,47]. Rats with selective lesion of medial septal GABAergic [49] but not cholinergic neurons [47], showed reduced behavioral symptoms (PPI deficit and hyperlocomotion) induced by an NMDA receptor antagonist. Thus, functional inactivation of septal GABAergic neurons by septal DBS can explain the ameliorating effects of the medial septal DBS on ketamine-induced hyperlocomotion and PPI deficit. On the other hand, septal DBS may normalize ketamine-induced behaviors and hippocampal gamma waves by acting outside of the medial septum, such as through septohippocampal fibers on the hippocampus. In addition, the rats given septal DBS did not show aside from the effects on the hippocampus. In addition, the rats given septal DBS did not show
Ketamine-induced decrease in hippocampal gamma activity is indicative of abnormal inhibitory interneurons [17], disinhibit pyramidal cells, and result in abnormal gamma activity by altering recurrent inhibitory interneurons [36]. The abnormal gamma is indicative of abnormal inhibitory gain [36]. The abnormal gamma is indicative of abnormal inhibitory interneurons [17], disinhibit pyramidal cells, and result in abnormally enhanced gamma activity by altering recurrent inhibitory gain [36]. The abnormal gamma is indicative of abnormal inhibitory processing of the hippocampus and other brain areas. How septal DBS may normalize hippocampal gamma activity is not known. Processing of the hippocampus and other brain areas. How septal DBS may normalize hippocampal gamma activity is not known.

Neither septal or accumbens DBS significantly affected PPI and locomotion in drug-free rats (see supplemental Figs. 1 and 2). This suggests that DBS only affected the altered neural network activity induced by ketamine, but not normal neural activities.

4.3. Medial septal DBS normalized auditory gating deficit and hippocampal gamma induced by ketamine

Ketamine disrupted the gating of auditory evoked potentials in the hippocampus [46,48; Fig. 6]. This was shown as an increase in T/C ratio of the hippocampal AEPs (decrease in auditory gating) after ketamine, without a significant change in the conditioning pulse response amplitude (C). The ketamine-induced increase in T/C ratio was blocked by medial septal DBS (Fig. 6). It has been shown that auditory gating of the hippocampal AEPs depended on inhibition mediated by hippocampal interneurons [54]. Ketamine-induced decrease in hippocampal auditory gating was not observed in rats with selective lesion of GABAergic neurons in the medial septum [49].

In the present study, ketamine (3 mg/kg sc) disrupted behaviors as well as hippocampal gamma activity of 70–100 Hz, but not of 30–70 Hz. Power spectral analysis revealed that the average gamma power increased by 3 mg/kg sc ketamine was of 62–100 Hz frequency (Fig. 7D). Other studies reported that 30–70 Hz hippocampal gamma waves were also increased by ketamine at a higher dose, namely 6 mg/kg sc [46] or 10 mg/kg i.p. [29]. The present study did not record EEG in the nucleus accumbens, where high frequency oscillations of 140–180 Hz were reported after 25 mg/kg i.p. ketamine [21,22]. Thus, whether DBS affects oscillations of gamma or higher frequency in the nucleus accumbens and other areas is not known.

Normal hippocampal gamma activity is generated by interactions between pyramidal cells and local inhibitory interneurons [36,66,76], and ketamine may reduce excitation of inhibitory interneurons [17], disinhibit pyramidal cells, and result in abnormally enhanced gamma activity by altering recurrent inhibitory gain [36]. The abnormal gamma is indicative of abnormal inhibitory processing of the hippocampus and other brain areas. How septal DBS may normalize hippocampal gamma activity is not known. Selective lesion of septal GABAergic neurons reduced the hippocampal gamma induced by NMDA receptor antagonist [49], suggesting that septohippocampal GABAergic neurons, which only innervating inhibitory interneurons [13,30], may play a role in the ketamine-induced hippocampal gamma power increase.

In a mitotoxin-induced developmental model of schizophrenia, it was demonstrated that parvalbumin-containing interneurons were decreased, accompanied by a decrease in conditioned tone-induced gamma waves in the ventral, but not dorsal, hippocampus [41]. Tone-evoked gamma activity in a developmental disruption model is likely the reason why the latter results differed from the spontaneous dorsal hippocampal gamma activity induced by ketamine reported here. In the mitotoxin-induced schizophrenia...
model, DBS of the ventral hippocampus was shown to normalize hippocampal auditory evoked potentials [12].

The result that DBS normalized both psychosis-related behaviors and hippocampal gamma activity suggests that the enhanced hippocampal gamma activity may predispose an animal to psychosis-related behaviors. However, gamma activity is likely correlated with, but not directly causing psychosis-related behaviors. Previous studies indicated that enhanced postictal hippocampal gamma waves, which shared many behavioral correlates with the ketamine-induced gamma waves [47], were present during both immobility and locomotion of freely moving rats [38]. Also, cortical and hippocampal gamma waves were present during urethane anesthesia, when motor and sensory responses were diminished or absent [20].

Schizophrenia is associated with abnormal gamma activities compared to control normal humans. However, both increase and decrease of gamma waves have been reported [18,67,71,72]. Multi-factorial models may account for the variable alteration of gamma waves, such as different positive and negative schizophrenic symptoms [3,27,63] or antipsychotic medication of individual patients [28], and whether gamma activity was spontaneous, evoked or induced. It remains to be shown that DBS is effective to suppress different types of schizophrenia-related gamma activities.

5. General conclusions

The two areas – medial septum, nucleus accumbens – used for DBS in this study have been used for relief of symptoms other than schizophrenia in humans [64,65,68]. The present studies suggest the validity of applying DBS of these areas in therapeutic treatment of schizophrenia.

Conflict of interest

The authors declared that there are no conflict of interests or financial support associated with this work that could have influenced its outcome.

Acknowledgments

This research is supported by grants from Canadian Institutes of Health Research (MOP-15685) and Natural Sciences and Engineering Research Council (1037-2013) to L. Stan Leung.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbvr.2014.03.010.

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