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Research paper

Hypoactivity of the lateral habenula contributes to negative symptoms and cognitive dysfunction of schizophrenia in rats

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ABSTRACT

Dopaminergic (DAergic) hypofunction in the medial prefrontal cortex (mPFC) has been implicated in the negative and cognitive symptoms of schizophrenia and is regulated by serotonergic (5-HTergic) neurons in the dorsal raphe nucleus (DRN). The lateral habenula (LHb) is a key element in controlling DRN 5-HT neurons. We investigated how the LHb impacts the activity of mPFC neurons and whether it mediates the involvement of DRN on development of symptoms in a pharmacological animal model of schizophrenia. We used immunohisochemistry to assess cytochrome-c oxidase (COX) activity of the LHb in MK-801 model rats and extracellular firing recording to compare firing rates in LHb neurons of acute MK-801-treated rats. The sucrose preference, social interaction, and radial arm maze tests were used to access schizophrenia-like behavior in rats with electrolytically lesioned LHb. Finally, we examined levels of the dopamine D1 receptor (D1R) and tyrosine hydroxylase (TH) in the mPFC, and tryptophan hydroxylase 2 (TPH2) in the DRN of rats with LHb lesions to determine the possible mechanism underlying the schizophrenia-like behavior associated with lesioned LHb. We found that COX levels and LHb neuron firing rates decreased significantly in MK-801-treated animals. The LHb lesions induced negative and cognitive, but not positive symptoms of schizophrenia. The D1R and TH levels decreased in the mPFC while TPH2 expression elevated in the DRN and mPFC of LHb-lesioned rats. These results suggest that LHb hypoactivity may contribute to the negative and cognitive symptoms of schizophrenia by downregulating D1R expression in the mPFC, which might be mediated by DRN 5-HT neurons.

1. Introduction

Schizophrenia is a debilitating chronic psychiatric disorder usually emerging in young adulthood and characterized by positive (hallucinations and delusions), negative (anhedonia and social withdrawal), and cognitive symptoms (memory and attention deficits) (Freedman, 2003; van Os and Kapur, 2009). Although antipsychotic medication can effectively improve the conditions of some schizophrenia patients, especially the positive symptoms, but the quality of life remains poor because of the negative symptoms and cognitive dysfunction (Freedman, 2003).

Multiple factors, including genetics, the environment, monoaminergic system dysfunction are involved in the pathogenesis of schizophrenia (van Os et al., 2010; Guillin et al., 2007; Lewis and Lieberman, 2000). Among these factors, the role of monoaminergic systems, especially the dopamine (DA) system, in the pathogenesis of the disease has received the most attention (Yamazaki et al., 2018; Guillin et al., 2007; Lewis and Lieberman, 2000). Subcortical DA hyperfunction, especially in the dorsal striatum and nucleus accumbens (NAc), is associated with the positive symptoms (Guillin et al., 2007), and medial prefrontal cortex (mPFC) DA hypofunction with the negative symptoms and cognitive disorder (Yamazaki et al., 2018; Lewis and

Abbreviations: DAergic, Dopaminergic; mPFC, medial prefrontal cortex; 5-HTergic, serotonergic; DRN, dorsal raphe nucleus; LHb, lateral habenula; COX, cytochrome-c oxidase; D1R, dopamine D1 receptor; TH, tyrosine hydroxylase; TPH2, tryptophan hydroxylase 2; DA, dopamine; NAc, nucleus accumbens; 5-HT, serotonin; Hb, habenular nucleus; VTA, ventral tegmental area; SN, substantia nigra compact; RID, ratio of the investigation duration; CNS, central nervous system; TPH1, tryptophan hydroxylase 1; D2R, dopamine D2 receptor

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Lieberman, 2000). In recent years, the role of the raphe nucleus serotonin (5-HT) system in the pathogenesis of schizophrenia has attracted great interest because of its strong effect on the neuronal activity in the DAergic system (Di Giovanni et al., 2010). In addition, second-generation antipsychotic drugs reduce cognitive impairment in rats of schizophrenia-like behaviors by inhibiting not only the DA system, but also the 5-HT system (Oyamada et al., 2015; Miyamoto et al., 2005). These studies suggest that the interaction of the two systems may be critically involved in schizophrenia pathogenesis. Thus, it is important to study the role of the upstream structures of the DA and 5-HT systems in the pathogenesis of the disease.

The habenular nucleus (Hb), in particular the lateral habenula (LHb), is a core component located at the dorsal diencephalic conduction system that relays limbic forebrain inputs to midbrain structures (Wang and Aghajanian, 1977; Herkenham and Nauta, 1977, 1979). The LHb is one of the few brain regions that can simultaneously regulate the activities of the ventral tegmental area (VTA), substantia nigra compact (SN) DA system, and dorsal raphe nucleus (DRN) 5-HT system (Jhou et al., 2009; Sego et al., 2014). LHb excitation inhibits the activities of DAergic and 5-HTergic neurons in the VTA/SN and DRN (Ji and Shepard, 2007; Stern et al., 1979). This inhibition is thought to be exerted indirectly, by activating GABAergic neurons in the VTA/SN and DRN, or in the rostromedial tegmental nucleus, which projects to DAergic and 5-HTergic neurons in the VTA/SN and DRN, respectively (Sego et al., 2014). The LHb has shown to play key roles in regulating multiple behavioral and physiologic functions, including cognition, reward, pain sensitivity, and sleep and wake (Hikosaka, 2010; Li et al., 2016). This plurality is attributed to the several roles of the LHb in regulating the activities of monoaminergic systems (Hikosaka, 2010; Zhao et al., 2015; Li et al., 2016). The disruption of these behavioral functions is associated with the development of psychiatric disorders (Lecourtier and Kelly, 2007). Recently, the LHb has attracted more attention for its role in the pathogenesis of depression (Li et al., 2011; Lecca et al., 2014, 2016; Cui et al., 2018). However, the impact of the LHb on schizophrenia is less clear. Previous observations found that compare to normal individuals, patients with schizophrenia exhibit a lower capillary density in the bilateral Hb (Bernstein et al., 2016), when administrated of antipsychotics, glucose metabolism of the LHb was increased (Dedeurwaerdere et al., 2011). The LHb was reported to show a higher incidence of calcification in patients with schizophrenia (Sandyk, 1992). In addition, continuous administration of amphetamine and cocaine, which induce schizophrenia-like symptoms, cause severe degeneration of the fasciculi retroflexus (efferent fibers of the LHb) (Ellison, 2002). In a recent imaging study, the bilateral Hb was found to have a significantly decreased absolute volume and increased functional connectivity, mainly with the mPFC, in schizophrenic patients, and the enhanced Hb-mPFC connectivity was positively correlated with the patients' Brief Psychiatric Rating Scale scores (Zhang et al., 2017). These findings suggest that abnormal Hb function, particularly its effects on the mPFC, may contribute to the pathogenesis of schizophrenia. DA hypofunction in the mPFC also contributes to the negative symptoms and cognitive dysfunction in schizophrenia (Yamazaki et al., 2018; Lewis and Lieberman, 2000), and the DA D1 receptor (D1R) levels decreased in the mPFC of schizophrenic patients (Okubo et al., 1997). The mPFC receives inputs not only from DAergic VTA neurons, but also from 5-HTergic DRN neurons (Seamans and Yang, 2004; Puig and Gulledge, 2011). Indeed, oral administration of the 5-HT antagonist ASP5736 results in the activation of the mPFC DAergic system (Yamazaki et al., 2018). In addition, the LHb controls the activities of DRN 5-HT neurons (Stern et al., 1979; Sego et al., 2014).

This study aimed to investigate the mechanisms underlying the role of the LHb in schizophrenia, focusing on how the LHb impacts the function of mPFC and whether this mediates the development of schizophrenia symptoms through affecting the DRN 5-HTergic system. We examined the activity of cytochrome *c* oxidase (COX) in the LHb of MK801-induced schizophrenia rats as an indicator of LHb function. We also studied the effects of the symptom-inducing agent on the firing rates of LHb neurons. Electrolytic LHb lesion was performed to elucidate the relationship among LHb function, schizophrenia symptoms, and the activities of the DA and 5-HT systems in the mPFC and dorsal striatum.

2. Methods and materials

2.1. Animals

Adult male Wistar rats (220–260 g) were used in all animal experiments. The study protocol was approved by the Jilin University Animal Care and Use Committee, and was in compliance with the Chinese Law for the care and use of laboratory animals. The rats were housed under normal laboratory conditions (room temperature 22 ± 2 °C, 12-h light-dark cycle, lights on at 7 a.m), with free access to food and water.

2.2. MK-801 schizophrenia model preparation

Dizocilpine (MK-801; M107, Sigma, USA) was dissolved in saline (0.9% NaCl, 0.5 mg/ml) and administered intraperitoneally (i.p., 0.5 mg/kg/day) into rats (n = 8) at 6 p.m. for 6 days to establish the schizophrenia model (Kondziella et al., 2006). The rats in control group (n = 4) were administered intraperitoneally with saline (1 ml/kg) under the same conditions.

2.3. Single-cell recordings of LHb neuronal activity

Rats were anesthetized (urethane, 1.2 g/kg i.p.) and positioned on an S-R stereotaxic apparatus (Narishige Corporation, Japan). Glass microelectrodes (impedance 8–15 MΩ, 1–2 μm tip) filled with 0.5 M NaCl and 2% Pontamine Sky Blue were implanted into the LHb (3.0–4.2 mm posterior to the bregma, 0.6–1.0 mm lateral to the midline, and 4.2–4.6 mm ventral to the dura) by a hydraulic drive and stepping motor (PC-5 N, Narishige, Tokyo, Japan) to record LHb neuronal electrical activity. Briefly, single-unit extracellular recordings were amplified and filtered (0.3–30 kHz bandpass) by a microelectrode amplifier (ME2-8301; Nihon Kohden, Tokyo, Japan) and monitored continuously with a dual-beam storage oscilloscope (VC-10; Nihon Kohden, Tokyo, Japan). LHb neuronal potentials were digitized using a data acquisition system (ML-112; ADI, Sydney, Australia). When a discriminated single neuron was detected and maintained stable firing for 5–10 min, MK-801 (0.5 mg/kg, i.p., n = 47) or saline (0.9%, 1 ml/kg, i.p., n = 11) was injected. Each rat was only recorded once to avoid interference from prior recordings. The analysis of neuronal pattern were according to a previous study (Yang et al., 2018), burst-type neurons were defined as clusters of spikes beginning with a maximal inter-spike interval of 20 ms and ending with a maximal inter-spike interval of 100 ms. The minimum intra-burst interval was set at 100 ms and the minimum number of spikes in a burst was set at 2. Recording sites were confirmed by pontamine sky blue staining after the electrophysiological study (Fig. 1e), and the rats of which the recording sites were outside LHb were removed (MK-801, n = 7; saline, n = 2).

2.4. LHb electrolytic lesion

Rats were anesthetized with chloral hydrate (350 mg/kg, i.p.) and placed on an S-R stereotaxic instrument. The electrode was implanted into the LHb (3.0–4.2 mm posterior to the bregma, 0.6–1.0 mm lateral to the midline, and 4.2–4.6 mm ventral to the dura), and a 0.35 mA DC current was applied for 40 s by an electronic stimulator (SEN-7130; Nihon Kohden, Tokyo, Japan) in lesion group (n = 45). For sham group (n = 30), no current was applied to the electrodes (Supplementary Fig. 2). The rats of which the lesion sites were outside LHb were

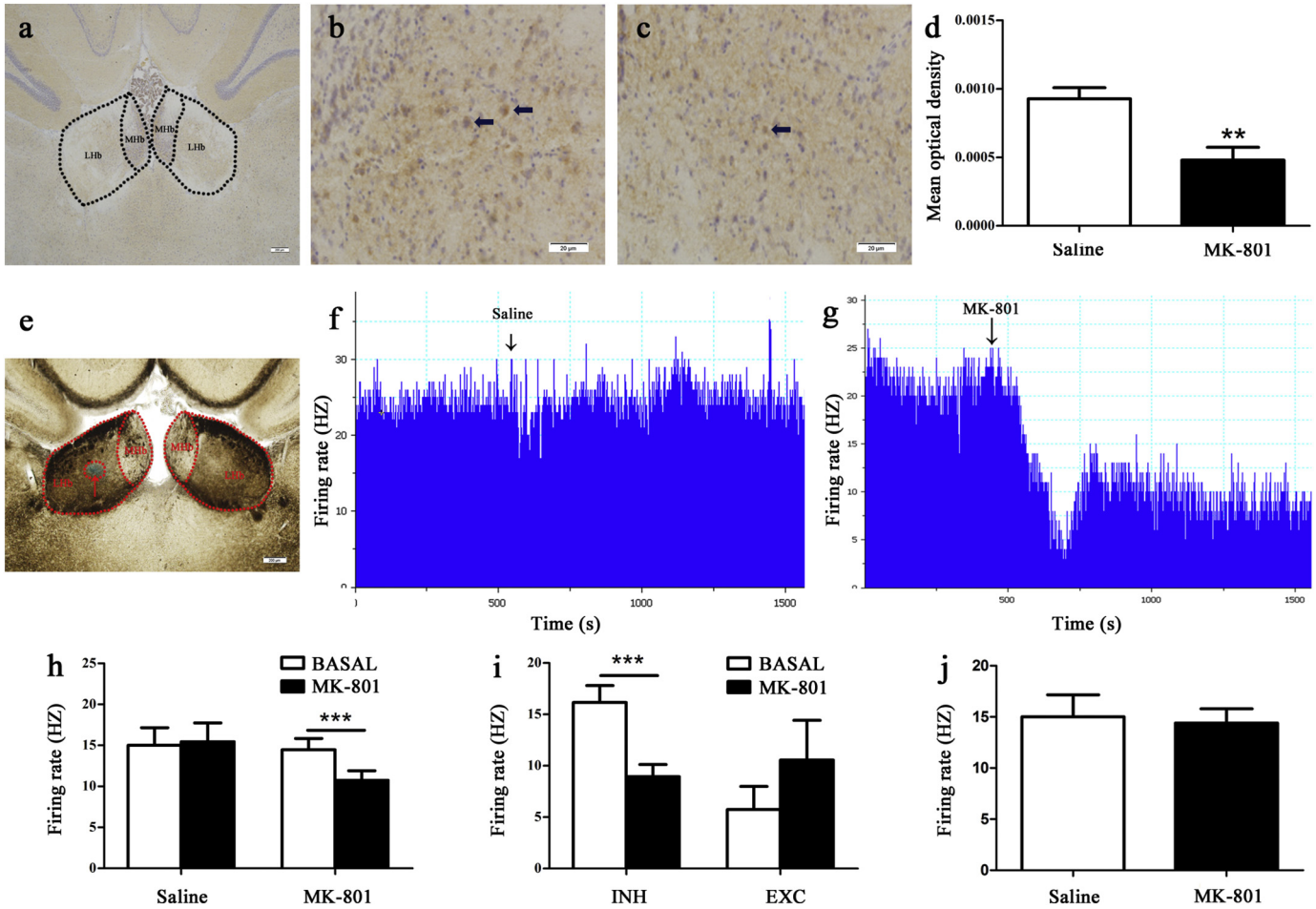

Fig. 1. Effect of MK801 on activity of the LHb neurons. (a, b, c) The representative immunohistochemical stainings (scale bar: a = 200 μm; b, c = 20 μm) for the LHb COX in (b) saline and (c) MK801-induced schizophrenia groups, respectively. (d) The statistical summary for COX activity the LHb of the two groups. (e) The histological identification of recording site in the LHb. The electrophysiological activity of LHb neurons from rats receiving (f) saline and (g) MK-801, respectively. The statistical summary for (h) firing rates of LHb neurons treated with saline and MK-801, (i) firing rates of neurons excited and inhibited by MK-801, (j) the baseline firing rate between saline and MK-801 groups. INH, neurons inhibited by MK801 (n = 21); EXC, neurons excited by MK801 (n = 5), neurons unaffected by MK801 (n = 14) are not shown. Data are expressed as the means ± SEM. ** $P < .01$, *** $P < .001$ compared with saline and baseline before MK801.

excluded (Lesion, n = 9; sham, n = 0).

2.5. Sucrose preference test

At the beginning of the test, each rat was presented with 2 bottles of 1% (w/v) sucrose for 3 days, followed by 2 bottles of water for 1 day. For the next 24 h, the rat was given the free choice between 2 bottles containing 1% sucrose solution and water. The bottles were swapped in the middle of the test to avoid side preference. Sucrose preference was calculated as the percentage of the sucrose solution consumed relative to the total liquid intake.

2.6. Social interaction test

The social interaction test was conducted as previously described (File et al., 2004). Rats were housed individually for 24 h before the test. A same-strain juvenile male rat was introduced into the cage of the test rat for 5 min. After a retention interval of 30 min, the test rat was exposed to the same juvenile rat for another 5 min. The total duration of social investigation was calculated as the total time spent by the test rat sniffing, grooming, or closely following the juvenile rat. The ratio of the investigation duration (RID) of the second to the first encounter was calculated. An RID of 1 indicates the lack of recognition.

2.7. Radial arm maze test

The test procedure for an 8-arm radial maze has been described in detail previously (Mizuno et al., 2000). The rats were food-restricted for 1 week until their body weights dropped by 15% before experiment. During first 2 days conditioning, scattered food (60 mg) were respective placed at the center of the maze device and at the front, middle and end of the eight arms of the maze, then rats are allowed to explore freely for 5 min in the maze to acclimate the experimental environment. On subsequent days, food was placed only on the arms, and finally only in the food cups at the arm ends (training). During the test proper, 4 arms were baited with 1 food placed in each food well. The baited arms were the same for a given rat but varied among rats. The rat was placed in the center of the maze and allowed to explore it until all 4 pellets were retrieved or 5 min had elapsed. Working memory errors were defined as reentries into baited arms, and reference memory errors as entries into unbaited arms. The time elapsed between the beginning of the test session and the rats obtaining all available food was recorded. The rats were tested once daily for 8 consecutive days.

2.8. Open-field test

Rats were carried to the test room 2 h before the test. The animals were placed in the center of the open field and allowed to explore the

apparatus for 5 min. Rat's behavior was videotaped and subsequently analyzed. The open field was cleaned with 70% ethanol between tests.

2.9. Stereotyped behavior analysis

Stereotyped behavior consists of motor responses that are repetitive, invariant, and seemingly without purpose or goal. During the test, the animals were placed into the test apparatus and videotaped for 30 min, and the stereotyped behavior were scored for 1 min every 5 min from the 30 min video, according to a scale described previously (Kelley and Delfs, 1994). The behaviors like staying still, grooming, moving head up or down, sniffing, bobbing, licking, biting, gnawing, and moving the mouth were included for scoring. A value of 1 was given once any of specific aforementioned behavior occurring during the 1 min of rating.

2.10. Quantitative COX histochemistry

COX activity was quantified as previously described (Shumake et al., 2003). Briefly, following anesthesia, the brains were removed, frozen, and cut into 30-μm-thick coronal sections using a freezing microtome (CM1950; Leica, Nussloch, Germany). Before staining, sections were air-dried at room temperature (22 ± 2 °C) for 5 min and fixed in phosphate buffer containing 0.5% glutaraldehyde and 10% sucrose. After washing with phosphate buffer three times for 5 min, the slides were incubated in phosphate buffer containing 0.05% diaminobenzidine, 0.01% cytochrome *c*, and 5% sucrose in darkness at room temperature for 10–15 min. The sections were dehydrated with ethanol, cleared with xylene, and coverslipped. All sections were stained in the same batch in order to rule out interbatch variability. Prior to measuring optical density by software Image Pro Plus 6.0, background was subtracted for all images first. After this, images were inverted to negative in order to obtain optical density values.

2.11. Western blotting

The proteins were electrophoretically separated on a 12% SDS-PAGE gel and transferred onto PVDF membranes. The primary antibodies used were against D1 (D1R 1:1000; ab20066, Abcam, UK), D2 receptor (D2R; 1:1000; ab21218, Abcam, UK), tyrosine hydroxylase (TH; 1:500; bs-0016R, Bioss, China), tryptophan hydroxylase 1 (TPH1; 1:500; bs-1215R, Bioss, China), TPH2 (1:500; bs-8729R Bioss, China), and β-actin (1:1000; bs0061R, Bioss, China). The primary antibodies were detected with a secondary horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (1:5000; bs-0295-HRP, Bioss, China). High-sensitivity ECL reagents was used to visualize the specific bands.

2.12. Data analysis

SPSS v16.0 and GraphPad Prism 5.0 were used for statistical analyses and graph generation. Changes in the firing rate of LHb neurons from baseline to the post-MK-801-injection levels were assessed by Student's paired *t*-test. Drug response was defined as a 20% change in the firing rate from baseline as previously described (Shen et al., 2012). For the radial arm maze data, repeated-measures ANOVA was used to assess the reference and working memory errors, total arm entries, and total time elapsed. Social interaction test data were analyzed by two-way ANOVA. Student's unpaired *t*-test was used in the other analyses. All data are expressed as means ± S.E.M. Significance was defined as $P < .05$.

3. Results

3.1. Changes of the LHb COX activity in MK-801-induced model of schizophrenia

Intraperitoneal administration of MK-801 for 6 days induces schizophrenia-like behaviors in rats (Kondziella et al., 2006). In the present study, this treatment was found to result in 48.2% decrease in LHb COX activity compared to control mice (0.0004798 ± 0.00009389 vs. 0.0009265 ± 0.00008127, t [10] = 3.345, $P < .01$; Fig. 1a-d). This result indicates that decreased LHb activity may be associated with the onset of schizophrenia.

3.2. Effects of MK-801 on LHb neuronal activity

Recording sites were confirmed by pontamine sky blue staining after the electrophysiological study (Fig. 1e). Eventually, a total of 40 LHb neurons were recorded from 40 rats. MK-801 evoked three types of responses: 52.5% (21/40) of the neuronal firing rates were decreased, 12.5% (5/40) increased, and the rest showed no significant change (Table S1). We found that MK-801 decreased the average firing rates of LHb neurons from 14. 47 ± 1.371 Hz to 10.74 ± 1.168 Hz (t [39] = 4.814, $P < .0001$; Fig. 1 g, h), injection of an equal amount of saline did not affect the firing rates of LHb neurons in normal rats (15.00 ± 2.156 Hz to 15. 45 ± 2.277 Hz; t[8] = 0.6342, $P = .5437$; Fig. 1 f, h). Meanwhile, MK-801 reduced the firing rates of LHb neurons inhibited by MK801 from 16.15 ± 1.660 Hz to 8.934 ± 1.197 Hz (t [20] = 8.907, $P < .001$; Fig. 1i), and the average inhibition was 46.63 ± 4.09% (Table S1). The latency to onset of suppression was 441.7 ± 124.2 s, and the mean duration of suppression was 2134 ± 467.9 s (Table S1). There was no difference of the baseline firing rate between saline and MK-801 groups (15.00 ± 2.156 Hz to14.47 ± 1.371 Hz; t[47] = 0.1952, $P = .8461$; Fig. 1j). We then analyzed the firing pattern of neurons in the LHb and found no alteration in the percent of burst- and tonic-type neurons in the LHb of normal rats before and after MK-801 injection (Supplementary Fig. 1), suggesting that a single MK-801 injection may not change the firing pattern of the neurons in LHb.

3.3. Negative and cognitive symptoms in LHb-lesioned rats

Quantitative behavioral tests were used to assess the symptoms of schizophrenia 4 weeks after the LHb lesion. According to the previous publications of schizophrenia, social interaction and sucrose preference tests were respectively utilized to assess the social withdrawal and anhedonia, acting as the negative symptoms of schizophrenia (Bitanihirwe et al., 2010; Neill et al. 2010). And the cognitive deficit of schizophrenia was evaluated by radial arm maze, a test for detecting the spatial learning and memory in rodents (Prades et al. 2017; Koh et al., 2018). In the LHb-lesioned rats, the mean sucrose preference was 49.20 ± 9.62%, while in the sham group it was 80.86 ± 5.15%. The LHb-lesion-induced decrease in sucrose preference was statistically significant (t[15] = 2.56, $P < .05$; Fig. 2a). No significant change was observed in total fluid intake (t[15] = 0.52, $P = .61$; Fig. 2b). These results indicate that LHb lesions can induce an anhedonia-like state in rats.

To investigate the effects of LHb lesions on social behaviors, we subjected LHb-lesioned and sham groups to a social interaction test (Fig. 2c). We found a significant difference between the groups (F[1, 38] = 4.18, $P < .05$) and a significant interaction of group and trial block (F[1, 38] = 5.29, $P < .05$).The main effect of trial block was not significant (F[1, 38] = 3.01, $P = .09$). The RIDs of the second to the first encounter were 0.50 ± 0.09 and 1.39 ± 0.31 in the sham and LHb lesion groups, respectively. The difference between the groups was significant (t[19] = 2.35, $P < .05$, Fig. 2d), suggesting that LHb lesions can induce social dysfunction.

We used repeated-measures ANOVA to assess reference memory errors, working memory errors, total time elapsed, and total arm entries in LHb-lesioned and sham-treated rats. For the interaction, a significant difference in the reference memory error rate was found between the two groups (F[1, 136] = 5.32, $P < .05$, Fig. 2e), while no difference was observed in working memory errors (F[1, 136] = 1.85, $P = .18$,

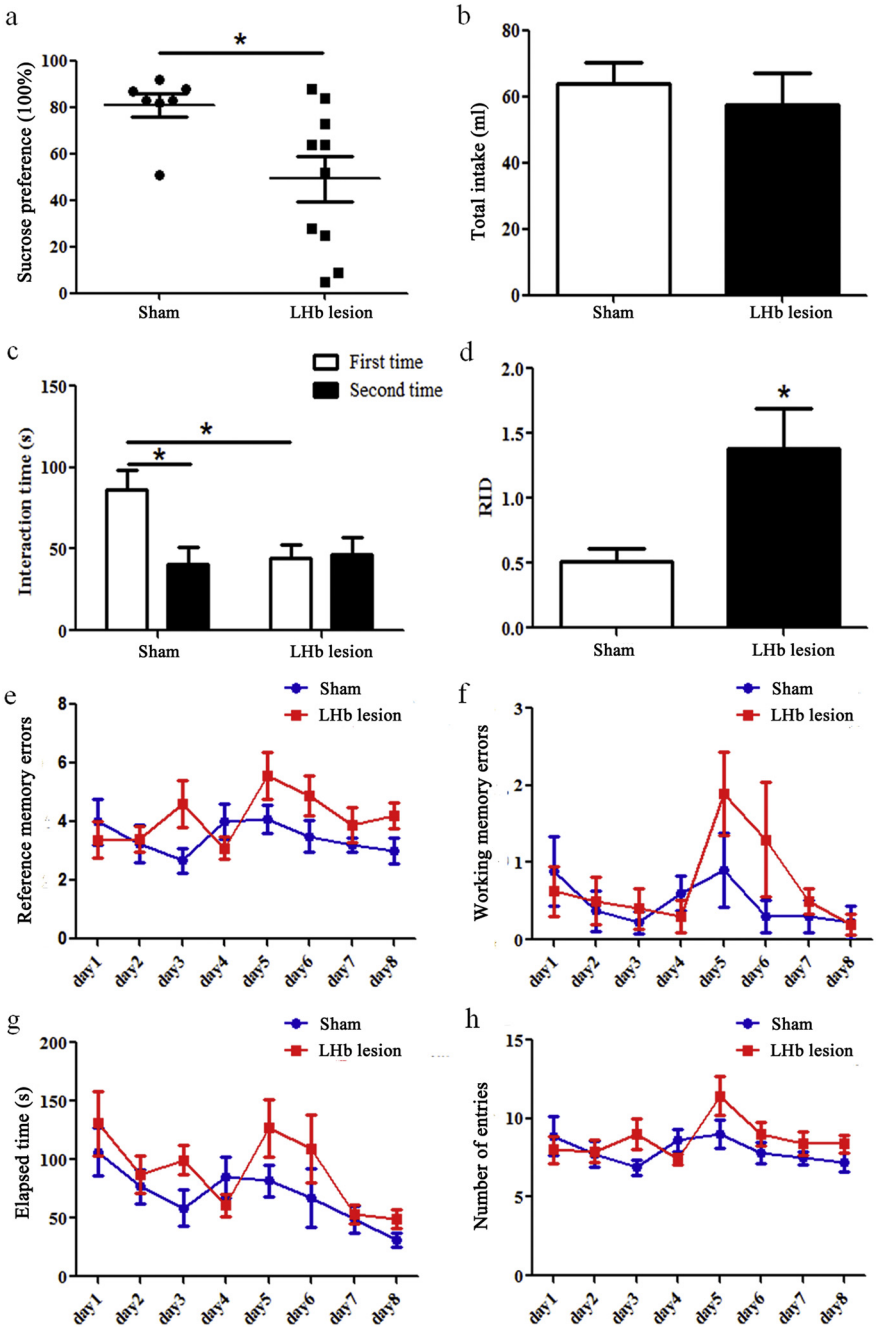

Fig. 2. Negative and cognitive symptoms in LHb lesioned rats. The following parameters are compared between the LHb lesioned group and the sham control group: (a) sucrose preference, (b) sucrose and water total intake, (c) interaction time in the first and (d) second recognition time and the ratio of the investigation duration (RID), (e) the performance in radial arm maze, including the reference memory errors, (f) the working memory errors, (g) the elapsed time and (h) the total number entries for the whole task. Data are expressed as the means ± SEM. * P < .05, compared with their respective control group.

Fig. 2f) or total number of entries ($F[1, 136] = 3.51$, $P = .06$, Fig. 2g). Total time elapsed also showed a significant difference between the groups ($F[1, 136] = 5.14$, $P < .05$, Fig. 2h). Taken together, these results suggest that LHb lesions can produce behavioral symptoms of cognitive impairment.

Stereotyped behavior and hyperlocomotion which reflect the positive symptoms of schizophrenia in rats were respectively examined by stereotyped behavior and open-field test (Sams-Dodd. 1998; Ernst et al., 2012). The difference in total distance traveled between the sham (2094 ± 198.5 cm) and LHb-lesioned (1972 ± 172.0 cm) groups was not significant ($t[20] = 0.3822$, $P = .71$; Fig. 3a). There was also no significant difference in stereotypy scores between the groups (5.7 ± 0.5 vs. 6.1 ± 0.6, $t[14] = 0.4932$, $P = .63$; Fig. 3b). These results show that LHb lesions are not sufficient to cause positive symptoms of schizophrenia. Therefore, the LHb may play a pivotal role in mediating the negative and cognitive, but not positive symptoms of schizophrenia.

3.4. Effects of LHb lesion on mPFC COX activity

In the sham group, the mean COX optical density was 0.001787 ± 0.0001124, while that in the LHb-lesioned group was 0.001458 ± 0.00006281. The decrease in COX activity in the lesion group compared with sham group was significant ($t[10] = 2.55$,

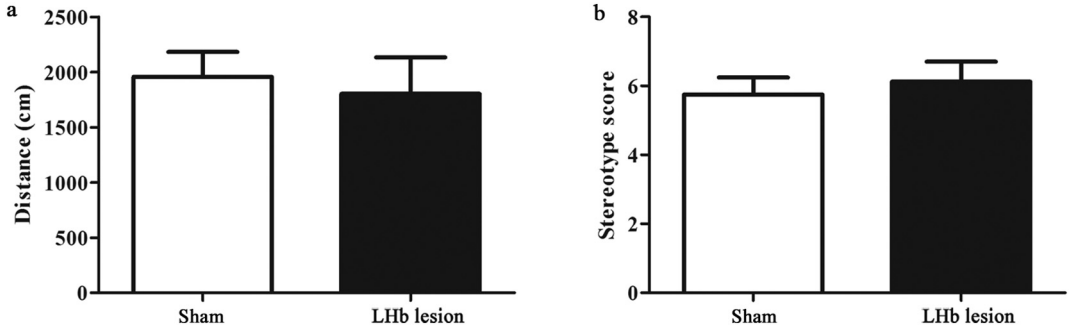

Fig. 3. The positive related symptoms between the sham group and lateral habenula lesion group. Statistical analysis shows that there was no difference in the (a) distance moved in the open field and (b) stereotype score between the two groups. Data are expressed as the means ± SEM.

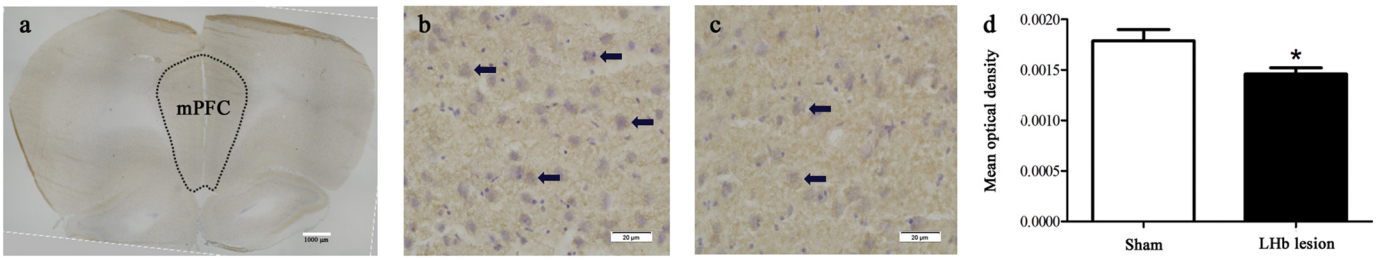

Fig. 4. Effects of LHb lesion on the COX activity in the mPFC neurons. (a, b, c) The representative images (scale bar: a = 1000 μm; b, c = 20 μm) of COX activity of the mPFC in (b) sham group and (c) LHb lesion group, respectively. (d) The statistical analysis of COX activity of the mPFC. Data are expressed as the means ± SEM. * $P < .05$ compared with the sham group.

$P < .05$, Fig. 4a-d), indicating that mPFC activity was decreased after LHb lesions.

3.5. Expression of TH, D1R, and TPH2 in the mPFC after LHb lesion

TH, D1R, and TPH2 expression levels in the mPFC were examined to clarify the underlying mechanisms of the negative and cognitive symptoms caused by the LHb lesions (Fig. 5a). The D1R level in the LHb-lesioned group was substantially reduced compared with sham group (t[2] = 8.482, P < .05, Fig. 5c). In addition, the levels of TH, the rate-limiting enzyme in DA synthesis, decreased in the mPFC after LHb lesion (t[2] = 6.503, P < .05, Fig. 5c). These data suggest that the mPFC DA system was hypofunctional after LHb lesion. 5-HT is known to regulate DAergic function in the mPFC. Therefore, we further investigated the expression of TPH2, the rate-limiting enzyme in 5-HT synthesis mainly expressed in the central nervous system (CNS), and found that the TPH2 level was significantly increased in the LHb-lesioned group (t[2] = 7.732, $P < .05$, Fig. 5c). In contrast, the expression levels of TPH1 were similar between the groups (t[2] = 2.009, $P = .18$; Fig. 5c).

3.6. Expression of TH, D1R, and TPH2 in the dorsal striatum after LHb lesion

The positive symptoms are associated with DA hyperfunction in subcortical regions, such as the striatum (Guillin et al., 2007). However, we failed to observe positive-like symptoms 4 weeks after LHb lesion; hence, we investigated the expression of TH, D1R, D2R, TPH1, and TPH2 in the dorsal striatum, thought to have increased DAergic activity in schizophrenia. No significant differences were found in the expression levels of D1R (t[2] = 0.09327, $P = .93$; Fig. 5b, d) or TH (t[2] = 1.213, $P = .35$; Fig. 5b, d) in the dorsal striatum after the LHb lesions. In contrast, the D2R level was significantly increased (t[2] = 4.393, $P < .05$; Fig. 5b, d). The TPH2 level was also elevated in the LHb-lesioned group (t[2] = 5.236, P < .05, Fig. 5b, d) compared with that in the sham group; however, the expression of TPH1 in the dorsal striatum was not affected (t[2] = 1.739, $P = .22$, Fig. 5b, d).

3.7. Expression of TPH1 and TPH2 in the DRN after LHb lesions

Considering the effect of DRN 5-HT on DAergic function in the mPFC and dorsal striatum, we examined TPH2 expression in the DRN. The TPH2 levels were significantly increased in the LHb-lesioned group (t[2] = 8.291, P < .05; Fig. 6a, b), while there was no difference in TPH1 expression between the two groups (t[2] = 1.917, $P = .20$; Fig. 6a, b).

4. Discussion

Previous studies have demonstrated that the administration of noncompetitive NMDAR antagonists, such as phencyclidine, ketamine, and MK-801, can induce schizophrenia-like psychopathologic symptoms in nonpsychotic individuals that are almost indistinguishable from those of schizophrenic patients (Malhotra et al., 1997). In the present study, MK-801 was used to produce an animal schizophrenia model showing the prominent alterations of behavior characteristic of the human disease, including positive symptoms such as hyperactivity and stereotypical behavior, negative symptoms such as social withdrawal and anhedonia, and cognitive dysfunction manifesting as learning and memory impairment (Jentsch and Roth, 1999). Cavelier et al. showed that COX activity was significantly decreased in the caudate nuclei and cortices of schizophrenic patients, indicating that large-scale hypofunction was present in the schizophrenic brain (Cavelier et al., 1995). In the present study, we investigated the change in COX activity of LHb neurons in MK-801-induced chronic schizophrenia model and found significantly decreased COX activity in LHb of this model. This result suggest that LHb may be hypofunctional in MK801-induced schizophrenia rats, consistent with the previous results showing higher levels of calcification in the thalamus of patients with schizophrenia than healthy controls (Sandyk, 1992). In addition, extracellular firing rate recordings demonstrated that MK-801 affected 65% of LHb neuronal electrical activity with a predominantly inhibiting effect (81% of MK-

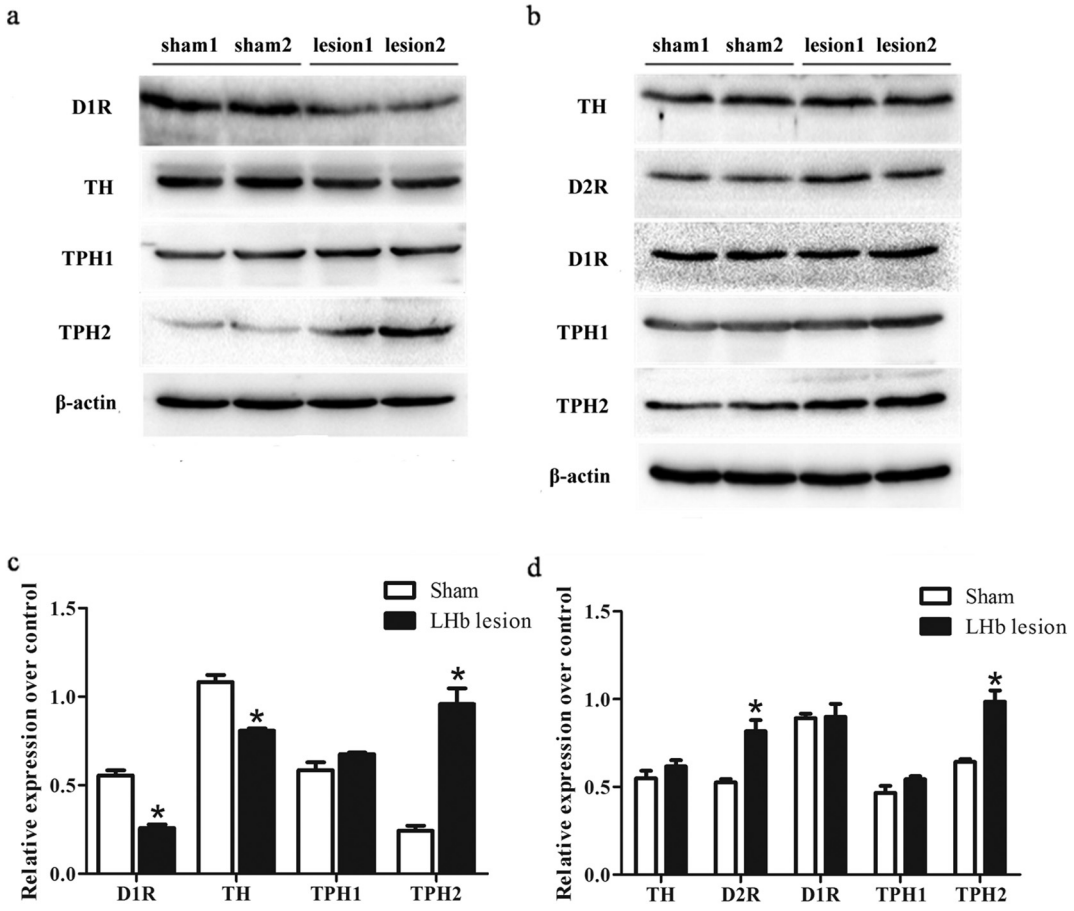

Fig. 5. The expression of TH, D1R and TPH2 in the mPFC and dorsal striatum after LHb lesion. (a) The representative gel pictures of the western blot analysis and (c) the statistical summary for the level of D1R, TH, TPH1 and TPH2 expression in the mPFC. (b) The representative gel pictures of the western blot analysis and (d) the statistical summary for the level of TH, D2R, D1R, TPH1 and TPH2 in the dorsal striatum. Data are expressed as the means ± SEM. * $P < .05$, compared with the sham group.

801-induced-responsive neurons showed inhibitory effects), suggesting that MK-801 induced schizophrenia may act through inhibiting the activity of mostly neurons in LHb. A recent imaging study provided further evidence of Hb hypofunction in schizophrenia by showing the bilateral Hb volume was significantly reduced in schizophrenic patients (Zhang et al., 2017). However, whether LHb hypofunction was involved in the etiology of schizophrenia remained unclear.

Electrolytic lesion of the Hb was a drastic but still valid approach to reduce activity of the area that employed in many studies (Tian and Uchida, 2015; Lecourtier et al., 2004). We investigated schizophrenia-like behaviors in LHb-lesioned rats with social interaction and sucrose preference tests, which are widely used to assess social withdrawal and anhedonia as negative symptoms of schizophrenia. LHb-lesioned rats showed deficits in both social interaction and sucrose preference. However, Lecourtier et al. (2004) failed to observe social withdrawal in whole-Hb-lesioned rats. This discrepancy may be attributable to the different test method used to assess social withdrawal. In the present study, we calculated the combined time spent in social investigation, including sniffing, grooming, and closely following the juvenile rat, rather than only the time spent sniffing, as Lecourtier reported. In addition, only the LHb, rather than the entire Hb, was damaged in our study. Because the medial Hb and LHb have distinct anatomical and physiological characteristics (Kim and Chang, 2005), this difference may also have contributed to the discrepancy.

Working memory deficits represent a core neuropsychological dysfunction in schizophrenia (Silver et al., 2003). In the radial arm maze test, deficits were observed in the LHb-lesioned group. These results are consistent with those of Lecourtier et al. (2004), who found that Hb-lesioned rats exhibited significant deficits in the latency to find the hidden platform, and in the distance swum before finding it, in the Morris water maze test. Therefore, our findings strongly indicate that LHb hypofunction is critical for the cognitive disorder in schizophrenia.

In a recent imaging study, the bilateral Hb showed significantly increased functional connectivity, mainly with the mPFC, in schizophrenia patients (Zhang et al., 2017), suggesting that the LHb may contribute to the pathogenesis of schizophrenia by affecting mPFC function. Our experiments further showed that there was a significant decrease in COX activity in the mPFC of LHb-lesioned rats. In addition, TH and D1R expression was significantly down-regulated, indicating that DA function was reduced in the mPFCs of LHb-lesioned rats. In support of this view, a postmortem study showed that the number of TH-labeled axons in entorhinal cortex (EC) layers III and VI, as well as PFC layer VI, was decreased in schizophrenics (Akil et al., 1999, 2000). These results suggest that schizophrenia might be associated with deficits of DA transmission in the EC and PFC. A positron emission tomography study showed that prefrontal D1R availability decreased in schizophrenics, and this reduction was correlated with the patients' poor performance in the Wisconsin Card Sort Task (Okubo et al., 1997), consistent with our results. Therefore, the negative symptoms and cognitive disorders induced by LHb lesion in rats may be related to mPFC DA hypofunction.

DA hypofunction in the mPFC has been reported to be important in the development of the negative symptoms and cognitive disorders in schizophrenics (Yamazaki et al., 2018; Lewis and Lieberman, 2000), which is supported by clinical observations. Second-generation antipsychotic drugs improve both the positive and negative symptoms, as

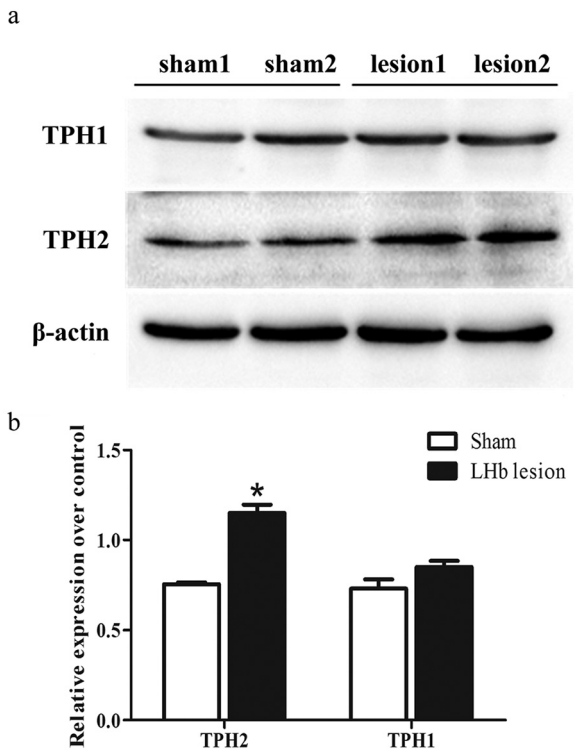

Fig. 6. The expression of TPH1 and TPH2 in the DRN after lateral habenula lesions. (a) The representative gel pictures of the western blot analysis and (b) the statistical summary for the levels of TPH 1 and TPH 2 in the DRN. Data are expressed as the means ± SEM. * P < .05, compared with the sham group.

well as the cognitive dysfunction. Their mechanism of action is thought to involve the inhibition of the 5-HT type 2A receptor in the mPFC, which facilitates cortical DAergic transmission (Miyamoto et al., 2005; Jakab and Goldman-Rakic, 1998; Willins et al., 1997). These observations suggest a link between 5-HT hyperfunction and the pathogenesis of the negative symptoms and cognitive disorders in schizophrenia.

In this study, LHb lesions up-regulated TPH2 expression in both the mPFC and DRN, suggesting a concomitant increase in 5-HT function in both regions. The TPH2 expression stimulation may be attributable to the reduced inhibitory effect of the lesioned LHb on DRN neurons. DRN 5-HT neurons have been shown to project to the mPFC and inhibit mPFC pyramidal neurons. In addition, electrical stimulation of the dorsal and median raphe nuclei inhibit the firing rates of 66% of recorded neurons in the mPFC, and this inhibition was blocked by 5-HT type 1A receptor antagonists (Puig et al., 2005). The DRN also inhibits mPFC neurons indirectly, by suppressing midbrain DA neuronal activity. These studies suggest that the mPFC DA hypofunction caused by LHb lesion may be achieved by increasing DRN 5-HT neuronal activity.

Stereotyped behavior and psychotic agitation are typical positive symptoms of schizophrenia (Porsolt et al., 2010), which are associated with subcortical DA hyperfunction, especially in the dorsal striatum and NAc (Guillin et al., 2007). LHb stimulation can transiently suppress the activity of 97% of DA neurons in the VTA and SN (Ji and Shepard, 2007). This observation indicates that LHb lesion can stimulate DA release in the NAc and striatum, which receive projections from DA neurons in the VTA and SN. However, in the present study, LHb-lesioned rats showed no significant increase in open-field locomotion or stereotyped behavior. Moreover, no significant differences were found in D1R or TH expression in the dorsal striatum after LHb lesions, although the D2R and TPH2 levels were increased. One possible explanation for these findings is that the LHb-lesion-induced DRN hyperactivity results in the suppression of midbrain DA function. Further research is needed to test this possibility.

In summary, we found that LHb neuronal activity was decreased significantly in MK801-induced schizophrenia rats. LHb lesions induced the negative and cognitive, but not positive symptoms of schizophrenia. The D1R and TH levels were decreased in mPFC, while TPH2 expression was elevated in the DRN and mPFC of LHb-lesioned rats. These results suggested that LHb hypoactivity may contribute to the negative and cognitive symptoms of schizophrenia by down-regulating mPFC D1R.

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Disclosures

The authors declare that there are no conflict financial interests with the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.expneurol.2019.05.005.

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