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Research paper

Activation of calcium-impermeable GluR2-containing AMPA receptors in the lateral habenula produces antidepressant-like effects in a rodent model of Parkinson's disease

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ABSTRACT

Evidence indicates that depression is closely related to hyperactivity of the lateral habenula (LHb). However, it is not clear how activation and blockade of AMPA receptors (AMPARs) in the LHb affect depressive-like behaviors, particularly in Parkinson's disease-related depression. In this study, unilateral 6-hydroxydopamine lesions of the substantia nigra pars compacta (SNc) in rats induced depressive-like behaviors and led to hyperactivity of LHb neurons compared to SNc sham-lesioned rats. Interestingly, intra-LHb injection of AMPAR agonist (*S*)-AMPA produced antidepressant-like effects in the two groups of rats and antagonist NBQX induced depressive-like behaviors, although (*S*)-AMPA excited LHb neurons and NBQX inhibited these neurons. We further found that intra-LHb injection of (*S*)-AMPA excited dopaminergic neurons in the anterior ventral tegmental area (aVTA) and serotonergic neurons in the dorsal raphe nucleus (DRN), which increased release of DA and 5-HT in the medial prefrontal cortex (mPFC), while NBQX induced the opposite effects. Further, lesioning the GABAergic rostromedial tegmental nucleus did not alter the proportions of the responses of these neurons to AMPAR stimulation. Additionally, lesions of the SNc reduced the level of p-GluR2-S880 in the LHb, which can increase the surface expression of calcium-impermeable GluR2-containing AMPARs (CI-AMPARs). This change in SNc-lesioned rats enhanced effects of (*S*)-AMPA and NBQX on the behaviors, LHb neuronal firing and release of DA and 5-HT. Collectively, antidepressant-like effects produced by (*S*)-AMPA attribute to activation of LHb neurons expressing CI-AMPAR, which excites aVTA dopaminergic neurons and DRN serotonergic neurons via the direct projection, thereby increasing release of mPFC DA and 5-HT.

1. Introduction

Most neurons in the lateral habenula (LHb) are glutamatergic (Brinschwitz et al., 2010; Li et al., 2011; Aizawa et al., 2012), and their axons innervate to the monoaminergic cell groups in the midbrain via the direct projection (Omelchenko et al., 2009; Brinschwitz et al., 2010; Bernard and Veh, 2012; Goncalves et al., 2012; Sego et al., 2014; Petzel et al., 2017) or a GABAergic relay in the rostromedial tegmental nucleus (RMTg; Jhou et al., 2009; Kaufling et al., 2009; Goncalves et al., 2012; Sego et al., 2014; Petzel et al., 2017). Based on its connections with the monoaminergic transmitter systems, it is thought to play a crucial role in the regulation of depression. Studies have found that the LHb is hyperactive during depressive-like states (Morris et al., 1999; Shumake et al., 2003; Li et al., 2011, 2013; Lawson et al., 2017; Yang et al., 2018), and that the reduction of LHb hyperactivity by pharmacological inhibition and deep brain stimulation or lesioning the LHb ameliorates depressive symptoms (Yang et al., 2008; Sartorius et al., 2010; Li et al., 2011; Meng et al., 2011; Winter et al., 2011). Further, hyperactivity of LHb neurons is involved in increased presynaptic glutamate release and up-regulation of β-calcium/calmodulin-dependent protein kinase type II, which enhances function of postsynaptic AMPARs (Li et al., 2011, 2013).

Although Parkinson's disease (PD) is well characterized by motor symptoms including bradykinesia, rigidity and rest tremor, a range of non-motor symptoms, such as depression, anxiety and cognitive deficits, are increasingly recognized (Lohle et al., 2009). Depression is a

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frequently encountered neuropsychiatric co-morbidity in PD; however, the neurobiological mechanism of PD-related depression is complex. Preclinical studies have also found that 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra pars compacta (SNc) in rats induce depressive-like behaviors (Winter et al., 2007; Santiago et al., 2010, Santiago et al., 2014; Sourani et al., 2012; Han et al., 2015, 2016; Wang et al., 2017) and increase the firing activity of LHb neurons (Han et al., 2015; Wang et al., 2017), and that electrolytic lesions of the LHb and pharmacological inhibition by local administration of $GABA_A$ receptor agonist muscimol produce antidepressant-like effects in the lesioned rats (Luo et al., 2015; Wang et al., 2017). In addition, lesions of the SNc in rats increase release of glutamate in the LHb, which leads to hyperactivity of LHb neurons (Wang et al., 2017). These studies suggest that LHb hyperactivity may play an important role in PD-related depression.

The LHb primarily receives glutamatergic afferents from the medial globus pallidus, cortex and lateral hypothalamus (Herkenham and Nauta, 1977; Kim and Lee, 2012; Yetnikoff et al., 2015) and expresses AMPA receptors, which are involved in depression (AMPARs; Alt et al., 2006; Li et al., 2011; Maroteaux and Mameli, 2012; Meye et al., 2013). AMPARs are composed of four subunits (GluR1–GluR4; Traynelis et al., 2010) and can be divided into calcium-impermeable GluR2-containing and calcium-permeable GluR2-lacking AMPARs (CI- and CP-AMPARs, respectively; Keinanen et al., 1990; Seeburg et al., 1998). Thus, glutamate released from the presynaptic terminals in the LHb mediates its fast excitatory action through these receptors. In addition, a recent study from our laboratory has found that CP-AMPARs in the LHb are involved in antidepressant-like effects in both SNc sham-lesioned and SNc-lesioned rats (Zhang et al., 2019). Despite the LHb projects to the dopaminergic ventral tegmental area (VTA) and serotonergic raphe nuclei including dorsal and median raphe nuclei (DRN and MRN, respectively) via the direct and indirect pathways (Metzger et al., 2017; Petzel et al., 2017) and there are major functional differences between the anterior and posterior part of the VTA (aVTA and pVTA, respectively; Sanchez-Catalan et al., 2014), effects of activation and blockade of LHb AMPARs on depressive-like behaviors remain unclear, particularly in PD-related depression, and not much is known about how AMPARs in the LHb affect the firing activity of dopaminergic and seriotonergic neurons in different midbrain structures and release of dopamine (DA) and serotonin (5-HT) in the medial prefrontal cortex (mPFC), which are involved in the regulation of depression (Steketee, 2003; Matsumoto and Hikosaka, 2007; Metzger et al., 2017). Therefore, in this study, we designed a series of experiments to address these issues. In addition, change in protein expression of AMPAR GluR2 subunit was also observed after DA depletion.

2. Materials and methods

2.1. Animals and drugs

Male Sprague-Dawley rats (Experimental Animal Center of Xi'an Jiaotong University, Xi'an, China) that weighed 280–330 g were used. The rats were kept at constant room temperature (21 ± 1 °C) under a regular light/dark schedule (light, 8:00–20:00 h). Food and water were supplied *ad libidum*. The experiments were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH publication, 8th edition, 2011), and with the authorization of the Animal Care Committee of Xi'an Jiaotong University. All efforts were made to minimize the number of animals used and their suffering. In this study, rats were randomly assigned to four groups: SNc sham-lesioned (SNc sham), SNc-lesioned (SNc lesion), SNc sham- and RMTg-lesioned (SNc sham + RMTg lesion), and SNc- and RMTg-lesioned rats (SNc + RMTg lesion).

The following drugs were used: desipramine hydrochloride, 6-OHDA hydrochloride, apomorphine hydrochloride and ibotenic acid (Sigma-Aldrich, St. Louis, MO, USA), and (*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [(*S*)-AMPA, selective AMPAR agonist] and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide disodium salt (NBQX, potent AMPAR antagonist; Tocris, Bristol, UK). Desipramine, (*S*)-AMPA and NBQX were dissolved in saline; 6-OHDA and apomorphine were prepared in saline containing 0.02% ascorbic acid, and ibotenic acid was dissolved in 0.1 M phosphate-buffered saline (pH 7.4). These drugs were prepared on the day of the experiment.

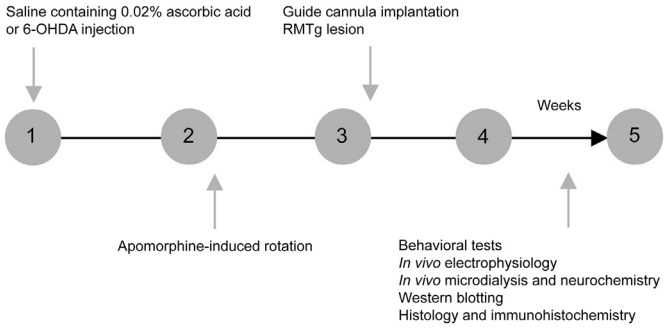

Fig. 1. Schematic representation of time line and summary of the different experiments.

2.2. Experimental procedures

All experiments were performed during the fourth week after saline containing 0.02% ascorbic acid or 6-OHDA injection into the SNc, and each rat was used only once in each experiment. Animals were subjected to a sequence of the experiments as summarized in Fig. 1. In this study, the stereotaxic coordinates (in mm) were taken from the atlas of Paxinos and Watson (2004) using bregma and dura mater as references.

2.3. 6-OHDA lesion surgeries

6-OHDA lesions of the left SNc in rats were carried out as previously described (Wang et al., 2009). For details, see Supplementary materials and methods.

2.4. Excitotoxic lesions of the RMTg

Lesions of the RMTg were performed in the SNc sham and SNc lesion groups, respectively. Surgical procedures were performed, as above, and then ibotenic acid (2 μg/0.2 μl) was injected into the left RMTg (AP – 7.1, ML 0.7, DV 7.4; Paxinos and Watson, 2004). After surgery, the animals were allowed to recover for at least one week before starting electrophysiological recordings.

2.5. Guide cannula implantation and intra-LHb injections

Guide cannula implantation and intra-LHb injections were performed as described in Supplementary materials and methods. After surgery, the rats underwent a recovery period of one week before behavioral tests.

2.6. Behavioral tests

The open field test, sucrose preference test and forced swim test (FST) were performed in the SNc sham and SNc lesion groups, respectively. The rats were tested in an isolated room between 8:00 and 11:00 am. All behavioral tests were recorded with a digital video camera (HR-550E; Sony, Tokyo, Japan) and analyzed by two observers blinded to the group. To assess effects of 6-OHDA lesion and intra-LHb injection of (*S*)-AMPA or NBQX on spontaneous locomotor activity and depressive-like behaviors, the following groups were formed: saline/

saline, saline/(*S*)-AMPA (0.01875, 0.0375 or 0.075 μg/rat) and NBQX/(*S*)-AMPA (1.0 μg/rat and 0.075 μg/rat), and saline/NBQX (0.25, 0.5 or 1.0 μg/rat).

2.6.1. Open field test

The open-field test was performed to measure effects of 6-OHDA lesion and the drugs on spontaneous locomotor activity as previously described (Tadaiesky et al., 2008). The details are provided in Supplementary materials and methods.

2.6.2. Sucrose preference test

The sucrose preference test was conducted as previously reported to examine anhedonia (Sclafani and Ackroff, 2003). The details of the test are shown in Supplementary materials and methods. Decreased sucrose consumption is indicative of anhedonia, which is one of the core symptoms of depression (Sclafani and Ackroff, 2003).

2.6.3. FST

The FST was used to examine depressive-like behavior as previously described (Porsolt et al., 1978). The detailed procedures are described in Supplementary materials and methods. Long immobility time is regarded as depressive-like behavior (Porsolt et al., 1978).

2.7. In vivo electrophysiological recordings

Single-unit extracellular recordings were performed in all groups of rats as previously described (Han et al., 2015).

2.7.1. Single-unit recordings of LHb neurons

The microelectrodes were lowed into the left LHb (AP – 3.6–4.2, ML 0.4–1.0, DV 4.2–4.8; Paxinos and Watson, 2004), and the firing activity of the neurons was recorded in the SNc sham and SNc lesion groups, respectively. In order to assess effects of activation and blockade of LHb AMPARs on the firing activity of the neurons in the two groups of rats, intra-LHb injection of (*S*)-AMPA (2.5 ng/40 nl) or NBQX (66.67 ng/40 nl) was performed with a glass micropipette connected via polyethylene tubing to a 1-μl microsyringe, and then the firing activity of the neurons was observed as previously described (Han et al., 2015).

2.7.2. Single-unit recordings of aVTA and pVTA dopaminergic neurons

For observing the firing activity of aVTA and pVTA dopaminergic neurons and effects of activation and blockade of LHb AMPARs on the firing activity of these neurons in the SNc sham and SNc lesion groups, the microelectrodes were directed to the left aVTA (AP – 4.5–5.0, ML 0.6–1.0, DV 7.3–8.4) or pVTA (AP – 5.5–6.0, ML 0.6–1.0, DV 7.3–8.4; Paxinos and Watson, 2004; Petzel et al., 2017), and then the firing activity of the neurons was recorded in the two groups of rats, respectively. Dopaminergic neurons were identified according to previously described electrophysiological characteristics (Grace and Bunney, 1983), including a biphasic or triphasic action potential, a characteristic long duration (> 2.5 ms) often with an inflection on the initial rising phase and a slow spontaneous firing rate (0.5–8 Hz) occurring sometimes in bursts. Further, intra-LHb injection of (*S*)-AMPA (0.0375 μg/0.3 μl) or NBQX (0.5 μg/0.3 μl) was performed with a glass micropipette implanted in the left LHb (AP – 3.7, ML 0.4, DV 4.6; Paxinos and Watson, 2004) at a 16° angle, which was attached to a 1-μl microsyringe by a polyethylene tubing, and then the firing activity of the dopaminergic neurons was observed in the two groups of rats. In addition, we examined effect of RMTg lesion on the firing activity of aVTA and pVTA dopaminergic neurons in the SNc sham + RMTg lesion and SNc + RMTg lesion groups. The firing activity of the neurons was recorded in the two groups of rats, respectively, and changes in the firing activity of the neurons after intra-LHb injection of (*S*)-AMPA or NBQX were observed, as above.

2.7.3. Single-unit recordings of DRN and MRN serotonergic neurons

In order to examine the firing activity of DRN and MRN serotonergic neurons and effects of activation and blockade of LHb AMPARs on the firing activity of the neurons, the microelectrodes were placed to the left DRN (AP – 7.4–7.6, ML 1.7–2.1, DV 5.0–6.5) or MRN (AP – 7.8–8.0, ML 1.6–2.1, DV 6.2–7.2; Paxinos and Watson, 2004) at a 16° angle, and then the firing activity of the neurons was recorded in the SNc sham and SNc lesion groups, respectively. Serotonergic neurons were identified by their characteristic biphasic or triphasic action potential waveforms of > 2.0 ms duration and basal firing rates of 0.1–4 Hz (Aghajanian et al., 1978). In some experiments, intra-LHb injection of (*S*)-AMPA (0.0375 μg/0.3 μl) or NBQX (0.5 μg/0.3 μl) was performed, and then the firing activity of the serotonergic neurons was observed in the two groups of rats. In another experiment, we examined effect of RMTg lesion on the firing activity of DRN and MRN serotonergic neurons in the SNc sham + RMTg lesion and SNc + RMTg lesion groups. The firing activity of the neurons was recorded in the two groups of rats, respectively, and changes in the firing activity of the neurons after intra-LHb injection of (*S*)-AMPA or NBQX were observed, as above.

2.8. In vivo microdialysis and neurochemistry

Published procedures were used (Wang et al., 2017). All microdialysis experiments were performed in unanesthetized and freely moving rats. In the SNc sham and SNc lesion groups, changes in the levels of extracellular DA and 5-HT in the left mPFC after intra-LHb injection of (*S*)-AMPA (0.0375 μg/0.3 μl) or NBQX (0.5 μg/0.3 μl) were observed using high-performance liquid chromatography with electrochemical detector. In addition, tissue content of DA in the left striatum of rats used in Western blotting was measured (Han et al., 2015). For details, see Supplementary materials and methods.

2.9. Western blotting

In the SNc sham and SNc lesion groups, Western blotting was performed to examine protein expression of total GluR2 subunit (t-GluR2) and phosphorylated GluR2 subunit at serine 880 site (p-GluR2-S880) in the LHb. The detailed procedures and all antibodies used in this study are described in Supplementary materials and methods.

2.10. Histology and immunohistochemistry

After the experiments, rats were administered an overdose of urethane and transcardially perfused with saline, followed by 4% paraformaldehyde. The brains were quickly removed and fixed for 4 h in 4% paraformaldehyde, and then were cytoprotected in 30% sucrose solution until sunken. Brain slices of 40 μm were obtained with a microtome. The sections were processed for cresyl violet staining to verify anatomical placement of the the cannula, recording site and microdialysis probe. To determine the extent of degeneration of dopaminergic neurons in the SNc and VTA, tyrosine hydroxylase (TH) immunohistochemical staining was performed in the four groups of rats as previously described (Wang et al., 2009). In addition, the extent of ibotenic acid lesions of the RMTg in the SNc sham + RMTg lesion and SNc + RMTg lesion groups was identified on sections stained with neuronal nuclei (NeuN) immunohistochemistry (Furlong and Carrive, 2007).

2.11. Data analysis and statistics

In the present study, behavioral, electrophysiological and microdialysis data were only analyzed from rats with almost total loss of TH immunoreactive (TH-ir) neurons in the left SNc, and verified anatomical placement of the cannula, recording site and microdialysis probe. In addition, only rats with loss of NeuN-immunoreactive neurons in the left RMTg by > 70% and depletion of DA tissue content in the left

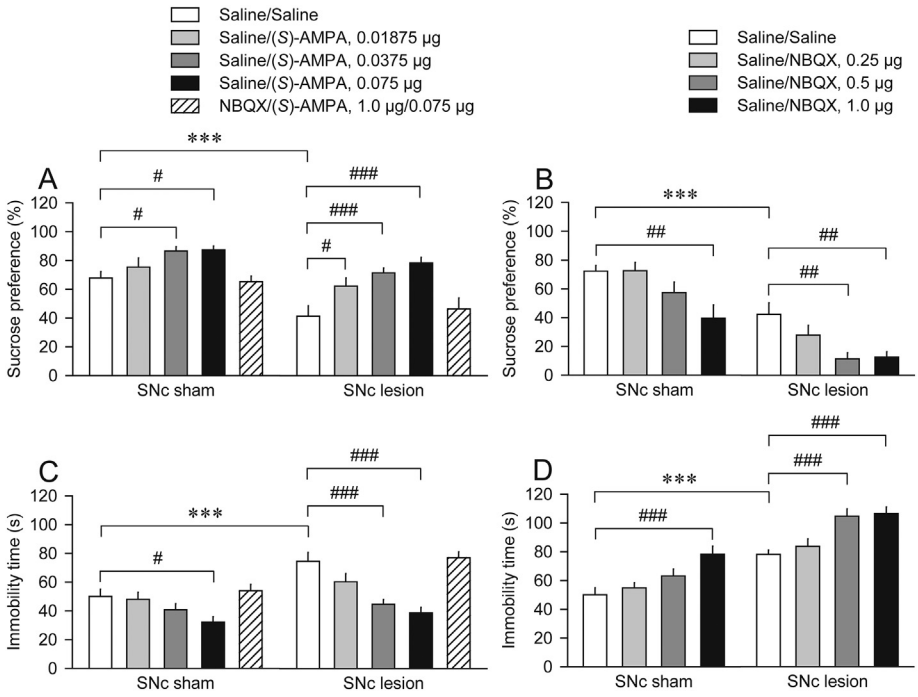

Fig. 2. Effects of 6-OHDA lesion, intra-LHb injection of AMPAR agonist (*S*)-AMPA and antagonist NBQX on depressive-like behaviors measured by the sucrose preference test and FST. Unilaterally lesioning the SNc in rats decreased sucrose preference (A, B) and increased immobility time (C, D) compared to SNc sham-lesioned rats. In the two groups of rats, intra-LHb injection of (*S*)-AMPA increased sucrose preference (A) and decreased immobility time (C) compared to saline injection into the LHb in the same group, while NBQX decreased sucrose preference (B) and increased immobility time (D). The dose producing these behavioral effects in SNc-lesioned rats was lower than those in SNc sham-lesioned rats (A–D). $^{***}P < .001$ vs. SNc sham-lesioned rats; $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$ vs. saline injection into the LHb in the same group; two-way ANOVA followed by Bonferroni's test. Data are presented as means ± SEM; n = 10–12 rats/group.

striatum by > 90% were considered for the analysis of the firing rate of the dopaminergic and serotonergic neurons and the results of Western blotting, respectively.

For electrophysiological data, basal firing activity of LHb neurons was recorded for 5–10 min before intra-LHb injection of (*S*)-AMPA or NBQX, and the following parameters were calculated: (i) the mean firing rate, and (ii) the mean coefficient of variation (COV; the ratio between standard deviation of the interspike interval and mean interspike interval, reflecting the degree of regularity of neuronal firing; Fedrowitz et al., 2003). Changes in the firing rate of LHb neurons were analyzed per 5 min epoch before and after intra-LHb injection of (*S*)-AMPA or NBQX. The COV of the neurons was compared in a period of 5 min preceding and after injection of the drugs. In addition, basal firing activity of the dopaminergic and serotonergic neurons was recorded for 3–5 min before intra-LHb injection of (*S*)-AMPA or NBQX, respectively; and then the mean firing rate of the neurons was calculated. Changes in the firing rate of the dopaminergic and serotonergic neurons were compared 1 min before and after injection of the drugs. In this study, a change of > 20% of basal firing rate was considered a significant alteration for an individual neuron (Fan et al., 2011). For microdialysis data, the average of initial three consecutive dialysates was defined as 100% of basal transmitter release before intra-LHb injection of (*S*)-AMPA or NBQX. For immunohistochemical data, the count of TH-ir neurons in the SNc and VTA (around 5.0 mm posterior to bregma) and NeuN immunoreactive neurons in the RMTg (around 7.1 mm posterior to bregma) was performed on three consecutive sections per rat as previously described (Furlong and Carrive, 2007; Wang et al., 2009).

All the data are presented as the mean ± S.E.M. Statistical analyses were performed with the SigmaStat (Systat, San Jose, CA, USA). Student's *t*-test, two-way ANOVA followed by Bonferroni's test, Manne Whitney *U* test, Friedman repeated measures analysis of variance on ranks followed by Dunn's multiple tests, Wilcoxon Signed Rank test, χ^2 test or two-way ANOVA with repeated measures followed by Bonferroni's test was used, when appropriate. The differences were considered significant at $P < .05$.

3. Results

3.1. Dopaminergic neuron counting and tissue content of DA

Unilateral 6-OHDA injections into the SNc in rats induced differential loss of tyrosine hydroxylase immunoreactive neurons in the ipsilateral SNc (− 93%; $P < .001$) and VTA (− 34%; $P < .001$, unpaired Student's *t*-test; Supplementary Fig. 1A-C) compared to rats in the SNc sham group. The lesions also significantly decreased tissue content of DA in the ipsilateral striatum (− 96%; $P < .001$, unpaired Student's *t*-test; Supplementary Fig. 1D).

3.2. Effects of dopaminergic lesion and activation and blockade of LHb AMPARs on locomotor activity

In the SNc sham and SNc lesion groups, we tested effects of unilaterally lesioning the SNc and intra-LHb injection of AMPAR agonist (*S*)-AMPA or antagonist NBQX on horizontal and vertical activities in the open field test. A two-way ANOVA (lesion × drug) showed a significant effect on locomotor activity for the lesion (horizontal activity: $F_{1,\ 105} = 134.4$, $P < .001$, Supplementary Fig. 2A; $F_{1,\ 76} = 62.4$, $P < .001$, Supplementary Fig. 2B; vertical activity: $F_{1,\ 105} = 20.11$, $P < .001$, Supplementary Fig. 2C; $F_{1,\ 76} = 10.81$, $P < .01$, Supplementary Fig. 2D), but not for the drugs (horizontal activity: $F_{4,\ 105} = 0.23$, $F_{3,\ 76} = 1.2$, Supplementary Fig. 2A, B; vertical activity: $F_{4,\ 105} = 0.6$, $F_{3,\ 76} = 0.09$, Supplementary Fig. 2C, D) and their interaction (horizontal activity: $F_{4,\ 105} = 0.09$, $F_{3,\ 76} = 0.07$, Supplementary Fig. 2A, B; vertical activity: $F_{4,\ 105} = 0.37$, $F_{3,\ 76} = 1.34$, Supplementary Fig. 2C, D). Post hoc analysis revealed that intra-LHb injection of the drugs did not affect locomotor activity compared to saline injection into the LHb in the same group (Supplementary Fig. 2A-D).

3.3. Effects of dopaminergic lesion and activation and blockade of LHb AMPARs on depressive-like behaviors

As shown in Fig. 2A and B, unilateral 6-OHDA lesioning and intra-LHb injection of (*S*)-AMPA or NBQX affected sucrose consumption in the sucrose preference test. A two-way ANOVA (lesion × drug) showed a significant difference on sucrose consumption for the lesion ($F_{1,}$

$_{105}$ = 26.18, P < .001, Fig. 2A; $F_{1,\ 72}$ = 66.19, P < .001, Fig. 2B) and for the drugs [$F_{4,\ 105}$ = 12.92, P < .001 for (*S*)-AMPA, Fig. 2A; $F_{3,\ 72}$ = 10.03, P < .001 for NBQX, Fig. 2B], but not for their interaction [$F_{4,\ 105}$ = 0.83 for (*S*)-AMPA, $F_{3,\ 72}$ = 1.21 for NBQX, Fig. 2A, B]. In both the SNc sham and SNc lesion groups, post hoc analysis showed that treatment with (*S*)-AMPA significantly increased sucrose consumption compared to rats treated with saline in the same group, and the dose producing statistical significance in the SNc lesion group were lower than those in the SNc sham group (SNc sham vs. SNc lesion: 0.0375 vs. 0.01875 μg; Fig. 2A). (*S*)-AMPA increased sucrose preference in the two groups of rats, indicating an antidepressant-like response. In contrast to effect of (*S*)-AMPA, treatment with NBQX significantly decreased sucrose consumption in the two groups of rats. Likewise, the dose reaching statistical significance in the SNc lesion group was lower than that of the SNc sham group (SNc sham vs. SNc lesion: 1.0 vs. 0.5 μg; Fig. 2B). These results indicate that NBQX induces a depressive-like response. Pretreatment with NBQX blocked effect of (*S*)-AMPA in both groups (Fig. 2A).

As illustrated in Fig. 2C and D, unilateral 6-OHDA lesioning and intra-LHb injection of (*S*)-AMPA or NBQX also affected immobility time in the FST. A two-way ANOVA (lesion × drug) showed a significant difference on immobility time for the lesion ($F_{1,\ 100}$ = 23.37, P < .001, Fig. 2C; $F_{1,\ 76}$ = 89.46, P < .001, Fig. 2D) and the drugs [$F_{4,\ 100}$ = 15.27, P < .001 for (*S*)-AMPA, Fig. 2C; $F_{3,\ 76}$ = 15.15, P < .001 for NBQX, Fig. 2D], but not for their interaction [$F_{4,\ 100}$ = 2.1 for (*S*)-AMPA, $F_{3,\ 76}$ = 0.96 for NBQX, Fig. 2C, D]. In both the SNc sham and SNc lesion groups, post hoc analysis showed that treatment with (*S*)-AMPA significantly decreased immobility time compared to rats treated with saline in the same group, and the dose producing statistical significance in the SNc lesion group was lower than those in the SNc sham group (SNc sham vs. SNc lesion: 0.075 vs. 0.0375 μg; Fig. 2C). (*S*)-AMPA decreased immobility time in the two groups of rats, indicating an antidepressant-like response. In contrast to effect of (*S*)-AMPA, treatment with NBQX showed an increase in immobility time in the two groups of rats. Likewise, the dose reaching statistical significance in the SNc lesion group was lower than that of the SNc sham group (SNc sham vs. SNc lesion: 1.0 vs. 0.5 μg; Fig. 2D). These results indicate that NBQX induces a depressive-like response. Prior injection of NBQX blocked effect of (*S*)-AMPA in both groups (Fig. 2C). These behavioral results also suggest that depletion of DA enhances the response of LHb neurons to AMPAR stimulation.

3.4. Effects of dopaminergic lesion and activation and blockade of LHb AMPARs on the firing activity of LHb neurons

The LHb is divided into the medial and lateral division (LHbM and LHbL, respectively; Aizawa et al., 2012; Petzel et al., 2017); therefore, we observed whether there are differences in the mean firing rate and the mean COV of the neurons between the LHbM and LHbL. In the SNc sham and SNc lesion groups, the mean firing rate of the neurons between the LHbM and LHbL was no significant, respectively (Fig. 3A-E). Compared to LHbM neurons, the mean COV of LHbL neurons didn't reach significance level in the two groups of rats (Fig. 3F, G). In the SNc lesion group, the mean firing rate of LHb neurons was significantly higher than that of the SNc sham group (P < .01, unpaired Student's *t*-test; Fig. 3H), and the mean COV of these neurons was also significantly increased (P < .01, Manne Whitney *U* test; Fig. 3I), indicating that depletion of DA leads to hyperactivity of LHb neurons.

In the SNc sham and SNc lesion groups, intra-LHb injection of (*S*)-AMPA significantly increased the mean firing rate of the neurons compared to before injection, respectively (both P < .001, Friedman repeated measures analysis of variance on ranks), and the duration of significant excitatory effect in the SNc lesion group was longer than that of the SNc sham group (SNc sham vs. SNc lesion: 10 vs. 15 min; Fig. 3J-L). In contrast to excitatory effect of (*S*)-AMPA, intra-LHb injection of NBQX significantly decreased the mean firing rate of the neurons in the two groups of rats (both P < .001, Friedman repeated measures analysis of variance on ranks; Fig. 3M-O). Likewise, the duration of significant inhibitory effect in the SNc lesion group was longer than those in the SNc sham group (SNc sham vs. SNc lesion: 10 vs. 20 min; Fig. 3M-O). As regards the COV, neither (*S*)-AMPA nor NBQX induced a change in both groups (Fig. 3P-S). These data also suggest that lesions of the SNc enhance the response of LHb neurons to AMPAR stimulation.

3.5. Effects of activation and blockade of LHb AMPARs on the firing activity of dopamiergic and serotonergic neurons in the midbrain

We further examined changes in the firing rate of aVTA and pVTA dopaminergic neurons and DRN and MRN serotonergic neurons after intra-LHb injection of (*S*)-AMPA or NBQX in the SNc sham and SNc lesion groups, because glutamatergic efferents of the LHb mainly project to midbrain monoaminergic nuclei (Metzger et al., 2017; Petzel et al., 2017).

3.5.1. Changes in the firing activity of the dopaminergic neurons

Compared to rats in the SNc sham group, lesions of the SNc in rats significantly increased the mean firing rate of aVTA and pVTA dopaminergic neurons, respectively (both P < .05, unpaired Student's *t*-test; Fig. 4A-D), indicating that the depletion of DA leads to hyperactivity of dopaminergic neurons in both the aVTA and pVTA.

In the SNc sham and SNc lesion groups, intra-LHb injection of (*S*)-AMPA significantly increased the mean firing rate of aVTA dopaminergic neurons compared to before injection, respectively (SNc sham: P < .05; SNc lesion: P < .01; paired Student's *t*-test; Fig. 5A, B); however, (*S*)-AMPA, at the same dose, significantly decreased the mean firing rate of pVTA dopaminergic neurons (SNc sham: P < .01; SNc lesion: P < .001; paired Student's *t*-test; Fig. 5C). In the two groups of rats, (*S*)-AMPA excited the majority of aVTA dopaminergic neurons (both groups: 67%; Fig. 6A) and inhibited the majority of pVTA dopaminergic neurons (Fig. 6B).

Conversely, intra-LHb injection of NBQX significantly decreased the mean firing rate of aVTA dopaminergic neurons compared to before injection in the two groups of rats (both P < .001, paired Student's *t*-test; Fig. 5F, G); however, NBQX, at the same dose, significantly increased the mean firing rate of pVTA dopaminergic neurons (SNc sham: P < .001; SNc lesion: P < .01; paired Student's *t*-test; Fig. 5H). In the SNc sham and SNc lesion groups, NBQX inhibited the majority of aVTA dopaminergic neurons (Fig. 6E) and excited the majority of pVTA dopaminergic neurons (Fig. 6F).

3.5.2. Changes in the firing activity of the serotonergic neurons

Lesions of the SNc in rats significantly increased the mean firing rate of DRN and MRN serotonergic neurons compared to rats in the SNc sham group, respectively (DRN: P < .001; MRN: P < .01; unpaired Student's *t*-test; Fig. 4E-H), indicating that SNc lesions cause hyperactivity of serotonergic neurons in both the DRN and MRN.

In the two groups of rats, intra-LHb injection of (*S*)-AMPA significantly increased the mean firing rate of DRN serotonergic neurons compared to before injection, respectively (both P < .01, paired Student's *t*-test; Fig. 5D); however, (*S*)-AMPA, at the same dose, significantly decreased the mean firing rate of MRN serotonergic neurons (both P < .001, paired Student's *t*-test; Fig. 5E). In the SNc sham and SNc lesion groups, (*S*)-AMPA excited the majority of DRN serotonergic neurons (Fig. 6C) and inhibited the majority of MRN serotonergic neurons (Fig. 6D).

Further, intra-LHb injection of NBQX significantly decreased the mean firing rate of DRN serotonergic neurons compared to before injection in the SNc sham and SNc lesion groups (both P < .01, paired Student's *t*-test; Fig. 5I); however, NBQX, at the same dose, significantly increased the mean firing rate of MRN serotonergic neurons (both P < .001; Fig. 5J). In the two groups of rats, NBQX inhibited the majority of DRN serotonergic neurons (Fig. 6G) and excited the majority of

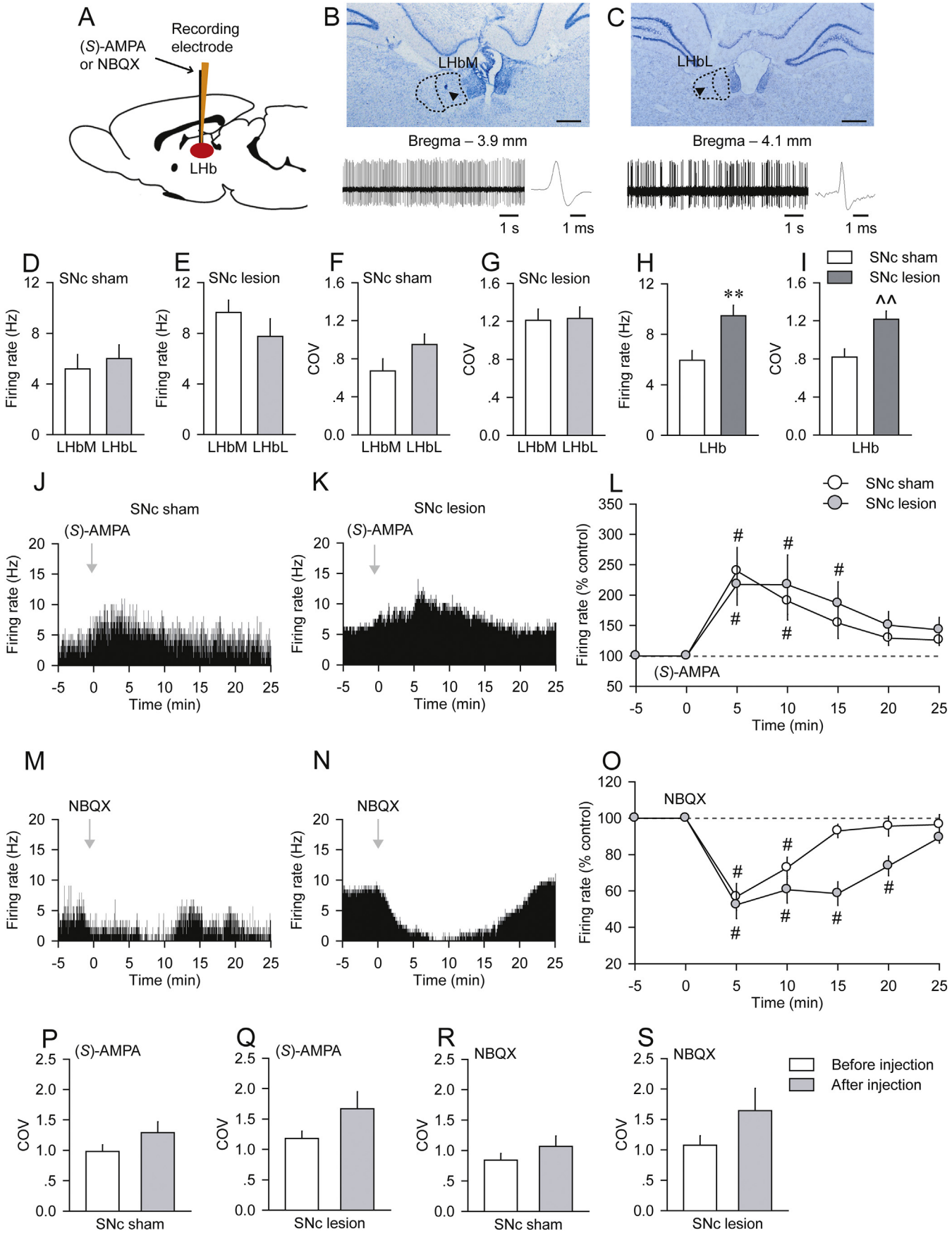

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Fig. 3. Changes in the firing activity of LHb neurons after 6-OHDA lesion, intra-LHb injection of AMPAR receptor agonist (*S*)-AMPA and antagonist NBQX. (*S*)-AMPA or NBQX injected into the LHb and single-unit recordings (A). Photomicrographs of cresyl violet staining showing the recording sites in the LHbM and LHbL marked with iontophoretically injected pontamine sky blue (arrowheads) and extracellular recordings of representative LHbM and LHbL neurons showing the spontaneous firing activity and action potential waveform in SNc sham-lesioned rats (B, C). In SNc sham-lesioned and SNc-lesioned rats, there were no significant differences in the mean firing rate (D, E) and the mean COV (F, G) of the neurons between the LHbM and LHbL, respectively (SNc sham: LHbM, $n = 15$ neurons from 10 rats, LHbL, $n = 17$ neurons from 11 rats; SNc lesion: LHbM, $n = 21$ neurons from 14 rats, LHbL, $n = 14$ neurons from 8 rats). Unilateral lesions of the SNc in rats increased the mean firing rate of LHb neurons compared to SNc sham-lesioned rats (H; SNc sham: $n = 32$ neurons from 21 rats; SNc lesion: $n = 35$ neurons from 22 rats), and also increased mean COV of the neurons (I). The representative firing rate histograms showing intra-LHb injection of (*S*)-AMPA (2.5 ng/40 nl) increased the firing rate of the neurons in both sham-lesioned and SNc-lesioned rats (J, K), while NBQX (66.67 ng/40 nl) decreased the firing rate of the neurons (M, N). (*S*)-AMPA (L; $n = 14$–16 neurons/group) and NBQX (O; $n = 15$–16 neurons/group; each neuron from a rat) induced excitation and inhibition in the two groups of rats, respectively; however, the duration of the excitatory and inhibitory effects in SNc-lesioned rats was longer than that of SNc sham-lesioned rats (J–O). The injection of (*S*)-AMPA or NBQX did not alter mean COV of the neurons compared to before injection in the two groups of rats (P-S). $^{**}P < .01$ vs. SNc sham-lesioned rats, unpaired Student's *t*-test; $^{\wedge\wedge}P < .01$ vs. SNc sham-lesioned rats, Manne Whitney *U* test; $^{\#}P < .05$ vs. baseline, Friedman repeated measures analysis of variance on ranks followed by Dunn's multiple tests. Data are presented as means ± SEM. Scale bars: B, C = 500 μm.

MRN serotonergic neurons (Fig. 6H).

3.6. Effects of activation and blockade of LHb AMPARs on the firing activity of dopamiergic and serotonergic neurons in the midbrain after lesioning of the RMTg

Evidence has suggested the existence of two separate pathways from the LHb to the dopaminergic and serotonergic midbrain nuclei, a direct

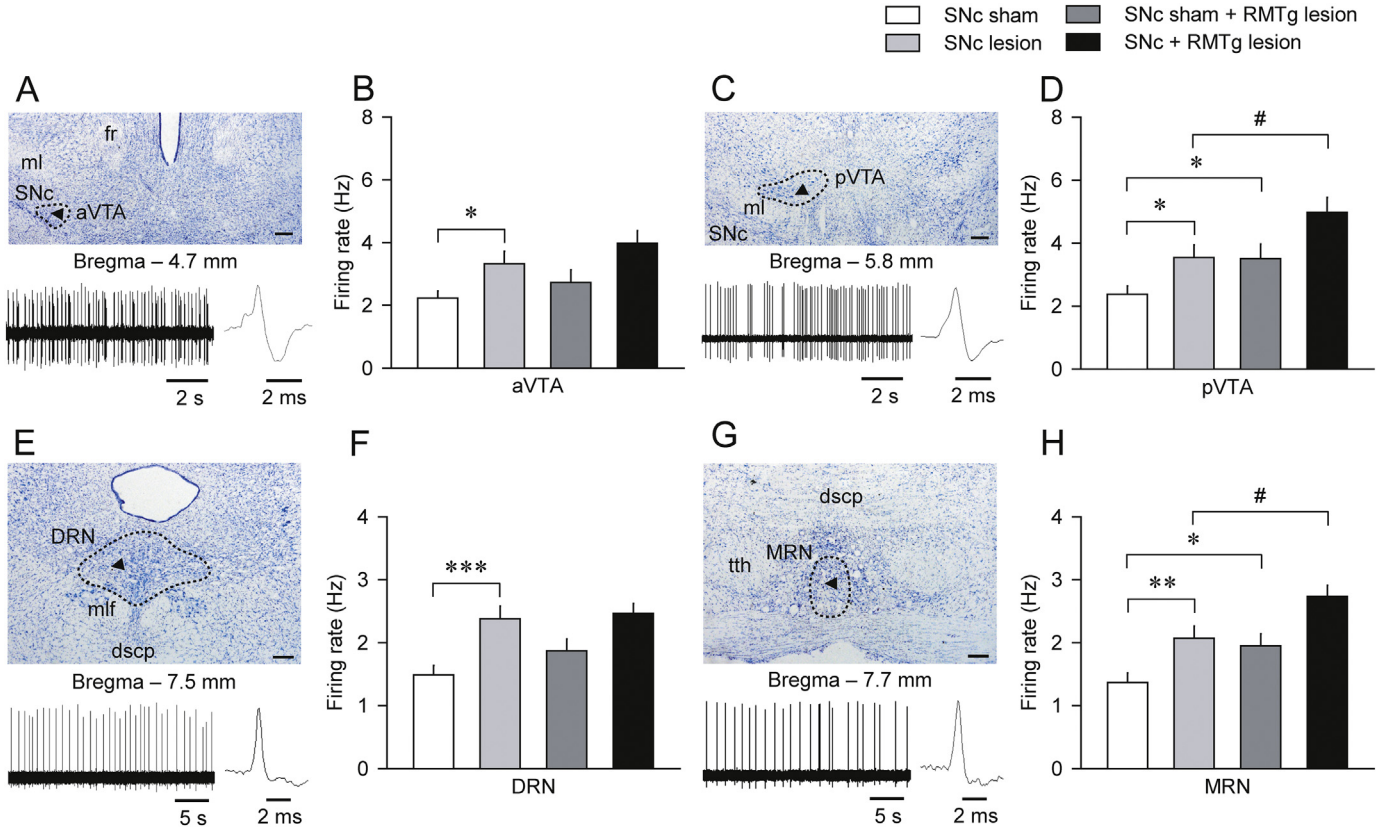

Fig. 4. Changes in the firing rate of dopaminergic neurons in the aVTA and pVTA and serotonergic neurons in the DRN and MRN after unilaterally lesioning the SNc and RMTg. Photomicrographs of cresyl violet staining showing the recording sites (arrowheads) in the aVTA (A), pVTA (C), DRN (E) and MRN (G) marked with iontophoretically injected pontamine sky blue in SNc sham-lesioned rats, respectively. Extracellular recordings of representative aVTA and pVTA dopaminergic neurons (A, C) and DRN and MRN serotonergic neurons (E, G) showing the spontaneous firing activity and action potential waveform in SNc sham-lesioned rats. Unilateral lesions of the SNc in rats increased the mean firing rate of dopaminergic neurons in the aVTA and pVTA (B, D) and serotonergic neurons in the DRN and MRN (F, H) compared to rats in the SNc sham group, respectively (SNc sham: aVTA, $n = 26$ neurons from 20 rats, pVTA, $n = 26$ neurons from 18 rats, DRN, $n = 28$ neurons from 28 rats, MRN, $n = 27$ neurons from 19 rats; SNc lesion: aVTA, $n = 24$ neurons from 20 rats, pVTA, $n = 26$ neurons from 18 rats, DRN, $n = 30$ neurons from 28 rats, MRN, $n = 29$ neurons from 20 rats). In the SNc sham + RMTg lesion group, lesions of the RMTg increased the mean firing rate of dopaminergic neurons in the pVTA and serotonergic neurons in the MRN compared to the SNc sham group, respectively, but not in the aVTA and DRN (B, D, F, H; SNc sham + RMTg lesion: aVTA, $n = 22$ neurons from 20 rats, pVTA, $n = 22$ neurons from 17 rats, DRN, $n = 24$ neurons from 18 rats, MRN, $n = 23$ neurons from 20 rats). In the SNc + RMTg lesion group, lesioning the RMTg also increased the mean firing rate of dopaminergic neurons in the pVTA and serotonergic neurons in the MRN compared to the SNc lesion group, without affecting the mean firing rate of dopaminergic neurons in the aVTA and serotonergic neurons in the DRN (B, D, F, H; SNc + RMTg lesion: aVTA, $n = 21$ neurons from 17 rats, pVTA, $n = 22$ neurons from 20 rats; DRN, $n = 23$ neurons from 16 rats, MRN, $n = 25$ neurons from 20 rats). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs. SNc sham group; $^{\#}P < .05$ vs. SNc lesion group; unpaired Student's *t*-test. Data are presented as means ± SEM. dscp, decussation of the superior cerebellar peduncle; fr, fasciculus retroflexus; ml, medial lemniscus; mlf, medial longitudinal fasciculus; tth, trigeminothalamic tract. Scale bars, A, C, E, G = 200 μm.

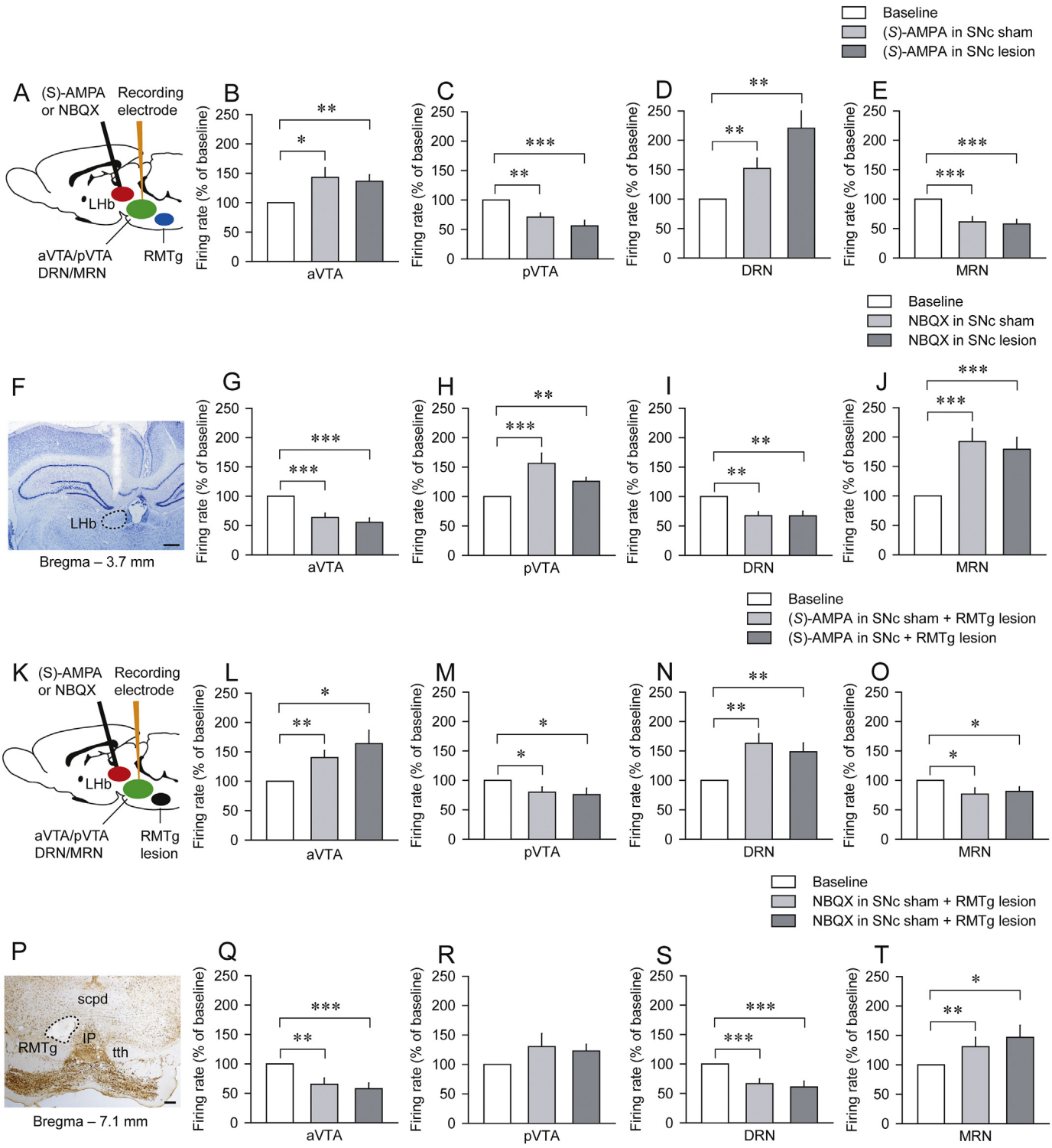

Fig. 5. Changes in the mean firing rate of dopaminergic neurons in the aVTA and pVTA and serotonergic neurons in the DRN and MRN after intra-LHb injection of AMPAR agonist (*S*)-AMPA and antagonist NBQX. (*S*)-AMPA (0.0375 μg/0.3 μl) or NBQX (0.5 μg/0.3 μl) injected into the LHb and single-unit recordings (A, K). Photomicrographs of cresyl violet staining showing the tip of the injection track aimed at the LHb of SNc sham-lesioned rat (F) and NeuN immunostaining showing the extent of ibotenic acid lesion in the RMTg of SNc-lesioned rat (P). (*S*)-AMPA increased the mean firing rate of aVTA dopaminergic neurons and DRN serotonergic neurons compared to baseline in the SNc sham and SNc lesion groups, respectively (B, D; n = 12–15 neurons/group), whereas it decreased the mean firing rate of pVTA dopaminergic neurons and MRN serotonergic neurons (C, E; n = 12–15 neurons/group). NBQX induced the opposite effects on the mean firing rate of these neurons compared to (*S*)-AMPA in the two groups of rats (G–J; n = 12–15 neurons/group). In the SNc sham + RMTg lesion and SNc + RMTg lesion groups, (*S*)-AMPA also excited aVTA dopaminergic neurons and DRN serotonergic neurons compared to baseline, respectively (L, N; n = 11–12 neurons/group) and inhibited pVTA dopaminergic neurons and MRN serotonergic neurons (M, O; n = 11–13 neurons/group). Likewise, NBQX induced the opposite effects on the mean firing rate of these neurons compared to (*S*)-AMPA in the two groups of rats (Q–T; n = 10–12 neurons/group). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs. baseline; paired Student's *t*-test. Data are presented as means ± SEM. Only one neuron was tested per rat. IP, interpeduncular nucleus; scpd, superior cerebellar peduncle, descending limb; tth, trigeminothalamic tract. Scale bars, F = 500 μm, *P* = 200 μm.

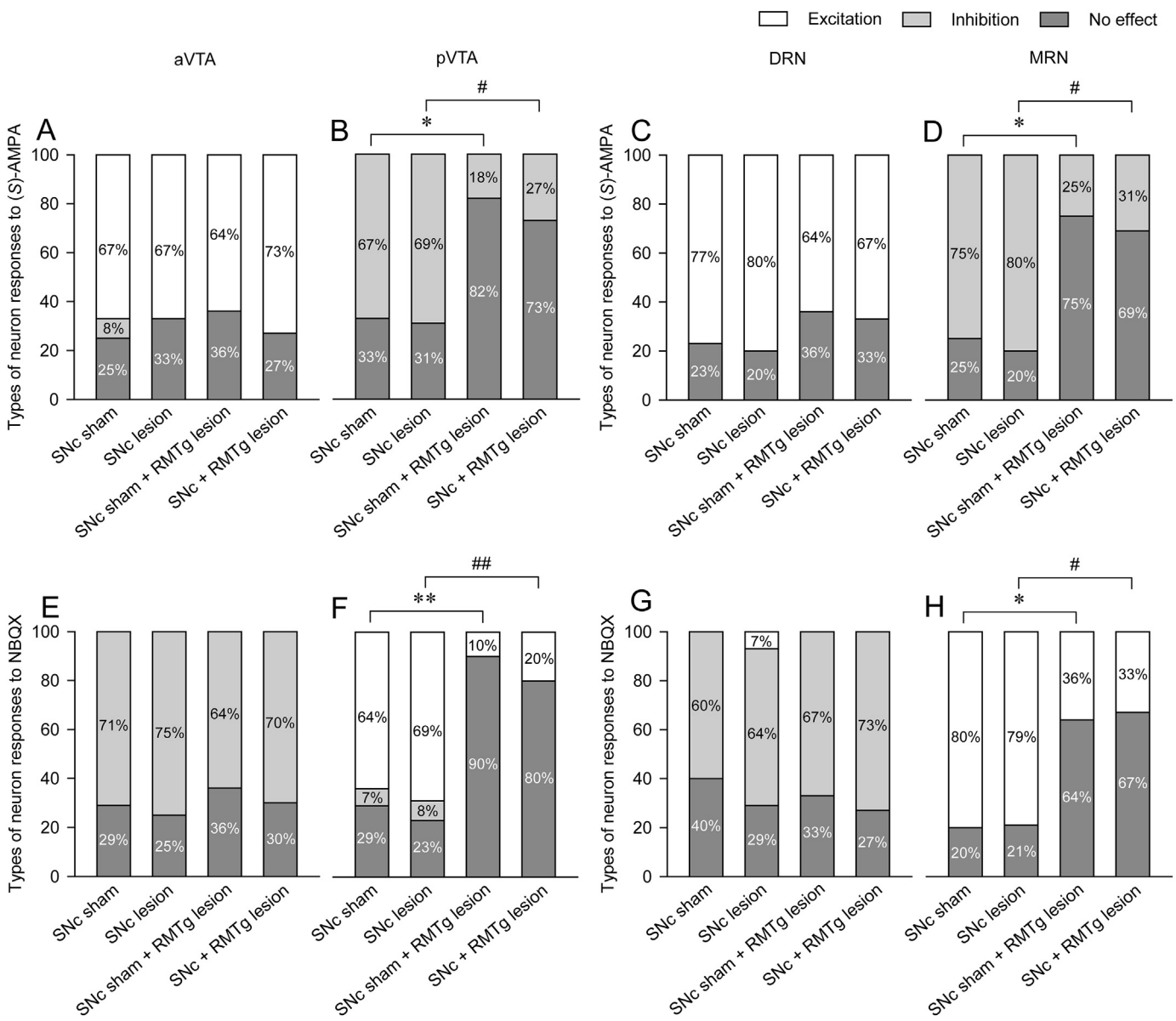

Fig. 6. Effects of SNc and RMTg lesions on the responses of dopaminergic and serotonergic neurons in different midbrain structures to intra-LHb injection of AMPAR agonist (*S*)-AMPA and antagonist NBQX. In the four groups of rats, (*S*)-AMPA excited most aVTA dopaminergic neurons (A; n = 11–12 neurons/group) and DRN serotonergic neurons (C; n = 12–15 neurons/group). Compared to the SNc sham group, lesions of the SNc in rats did not alter the percentages of excited aVTA dopaminergic and DRN serotonergic neurons, respectively (A, C). Likewise, there were no differences in the percentages of excited aVTA dopaminergic and DRN serotonergic neurons between the SNc sham + RMTg lesion and SNc + RMTg lesion groups (A, C). NBQX induced the opposite effects in most aVTA dopaminergic neurons (E; n = 10–14 neurons/group) and DRN serotonergic neurons (G; n = 11–15 neurons/group) compared to (*S*)-AMPA in the four groups of rats. In the SNc sham and SNc lesion groups, (*S*)-AMPA inhibited most pVTA dopaminergic neurons (B; n = 12–13 neurons/group) and MRN serotonergic neurons (D; n = 12–15 neurons/group). Compared to the SNc sham and SNc lesion groups, RMTg lesions decreased the percentages of inhibited pVTA dopaminergic neurons (B; n = 11 neurons/group) and MRN serotonergic neurons (D; n = 12–13 neurons/group) in the SNc sham + RMTg lesion and SNc + RMTg lesion groups, respectively. NBQX excited most pVTA dopaminergic neurons (F; n = 13–14 neurons/group) and MRN serotonergic neurons (H; n = 14–15 neurons/group) in the SNc sham and SNc lesion groups; however, RMTg lesions decreased the percentages of excited pVTA dopaminergic neurons (F; n = 10 neurons/group) and MRN serotonergic neurons (H; n = 11–12 neurons/group) in the SNc sham + RMTg lesion and SNc + RMTg lesion groups compared to the SNc sham and SNc lesion groups, respectively. $^{*}P < .05$, $^{**}P < .01$ vs. SNc sham; $^{\#}P < .05$, $^{\#\#}P < .01$ vs. SNc lesion; χ^2 test. Data are presented as means ± SEM. Only one neuron was tested per rat.

and an indirect via the GABAergic RMTg (Metzger et al., 2017; Petzel et al., 2017). In the SNc sham and SNc lesion groups, we therefore lesioned the RMTg, and then observed changes in the firing activity of the dopaminergic and serotonergic neurons after intra-LHb injection of (*S*)-AMPA or NBQX.

3.6.1. Changes in the firing activity of the dopaminergic neurons

In the SNc sham + RMTg lesion and SNc + RMTg lesion groups, lesions of the RMTg did not alter the mean firing rate of aVTA dopaminergic neurons compared to the SNc sham and SNc lesion groups, respectively (Fig. 4B); however, the lesions significantly increased the mean firing rate of pVTA dopaminergic neurons in the two groups of rats (both $P < .05$, unpaired Student's t-test; Fig. 4D), indicating that GABAergic outputs of the RMTg inhibit pVTA dopaminergic neurons without affecting aVTA dopaminergic neurons.

In the two groups of rats, intra-LHb injection of (*S*)-AMPA significantly increased the mean firing rate of aVTA dopaminergic neurons compared to before injection, respectively (SNc sham + RMTg lesion:

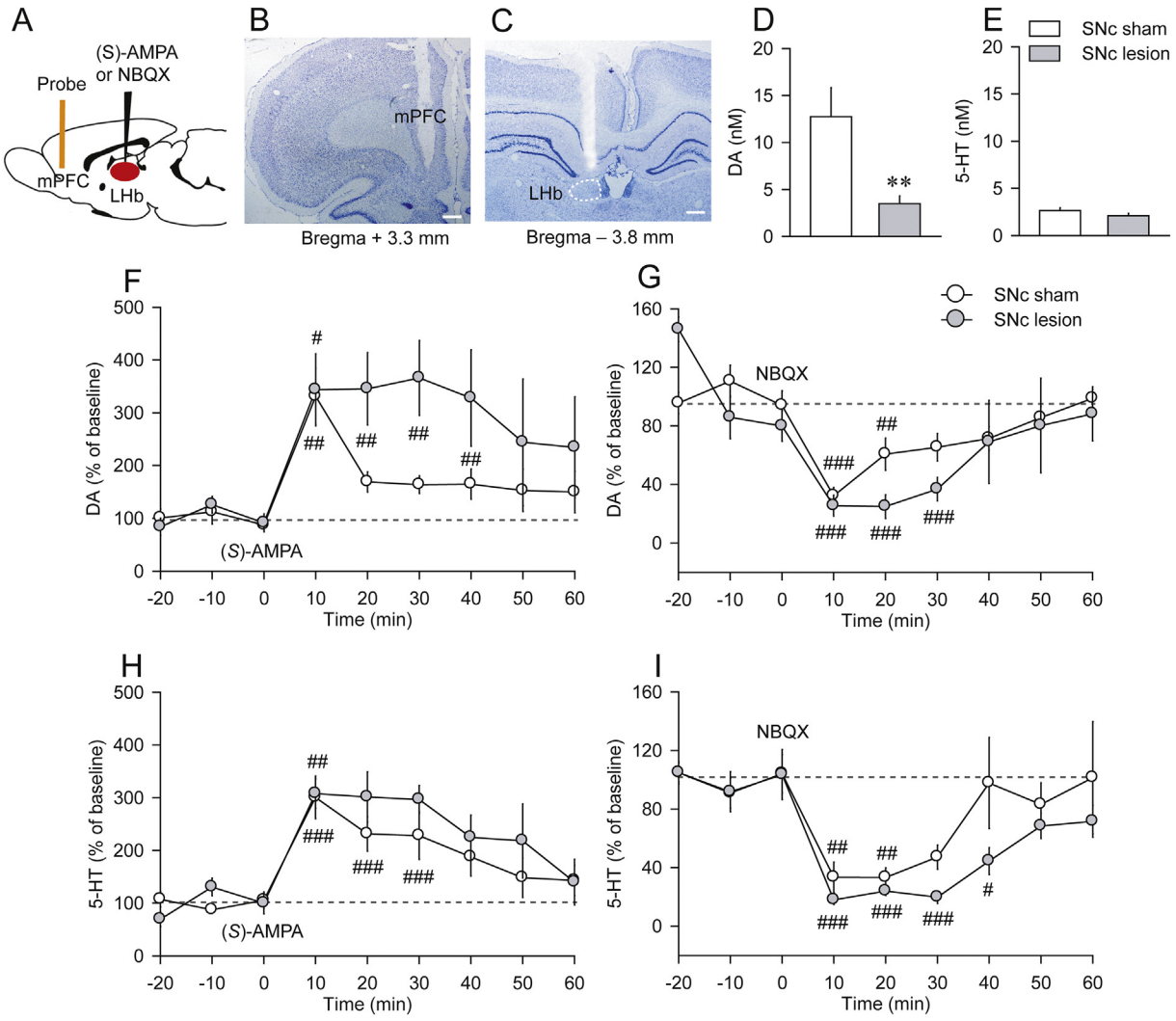

Fig. 7. Changes in the levels of extracellular DA and 5-HT in the mPFC after 6-OHDA lesion, intra-LHb injection of AMPAR agonist (*S*)-AMPA and antagonist NBQX. The experimental protocol (A). Photomicrographs of cresyl violet staining showing the site of microdialysis probe in the mPFC (B) and the tip of the injection track aimed at the LHb (C) of SNc-lesioned rat. Unilateral lesions of the SNc in rats decreased the level of DA in the ipsilateral mPFC compared to SNc sham-lesioned rats (D), and did not alter the level of 5-HT (E; $n = 12$ rats/group). Intra-LHb injection of (*S*)-AMPA (0.0375 μg/0.3 μl) increased the levels of DA and 5-HT in the mPFC of SNc sham-lesioned and SNc-lesioned rats (F, H; $n = 8$–9 rats/group), while NBQX (0.5 μg/0.3 μl) decreased the levels of DA and 5-HT in the mPFC (G, I; $n = 8$–9 rats/group). Although (*S*)-AMPA and NBQX increased or decreased DA and 5-HT release in the two groups of rats, the duration of significant changes in SNc-lesioned rats was longer than that of SNc sham-lesioned rats. $^{**}P < .01$ vs. SNc sham-lesioned rats, unpaired Student's *t*-test; $^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$ vs. baseline, two-way ANOVA with repeated measures followed by Bonferroni's test. Data are presented as means ± SEM. Scale bars, B, C = 500 μm.

$P < .01$; SNc + RMTg lesion: $P < .05$; paired Student's *t*-test; Fig. 5K, L); however, (*S*)-AMPA, at the same dose, significantly decreased the mean firing rate of pVTA dopaminergic neurons (both $P < .05$, paired Student's *t*-test; Fig. 5M). In the SNc sham + RMTg lesion and SNc + RMTg lesion groups, (*S*)-AMPA excited the majority of aVTA dopaminergic neurons (Fig. 6A), but the majority of pVTA dopaminergic neurons were unaffected (Fig. 6B).

Conversely, intra-LHb injection of NBQX significantly decreased the mean firing rate of aVTA dopaminergic neurons compared to before injection in the two groups of rats, respectively (SNc sham + RMTg lesion: $P < .01$; SNc + RMTg lesion: $P < .001$; paired Student's *t*-test; Fig. 5P, Q). NBQX, at the same dose, increased the mean firing rate of pVTA dopaminergic neurons in both the SNc sham + RMTg lesion and SNc + RMTg lesion groups, but did not reach a significant level (Fig. 5R). In the two groups of rats, NBQX inhibited the majority of aVTA dopaminergic neurons (Fig. 6E); however, the majority of pVTA dopaminergic neurons were unaffected (Fig. 6F).

Compared to the SNc sham and SNc lesion groups, intra-LHb injection of (*S*)-AMPA or NBQX did not significantly alter the percentages of different types of aVTA dopaminergic neuron responses in the SNc sham + RMTg lesion and SNc + RMTg lesion groups, respectively (Fig. 6A, E); however, (*S*)-AMPA significantly decreased the percentage of inhibited pVTA dopaminergic neurons and increased the percentage of unaffected neurons in the two groups of rats (SNc sham + RMTg lesion: $\chi^2 = 5.49$, $df = 1$, $P < .05$; SNc + RMTg lesion: $\chi^2 = 5.92$, $df = 1$, $P < .05$; χ^2 test; Fig. 6B), and NBQX significantly decreased the percentage of excited pVTA dopaminergic neurons and increased the percentage of unaffected neurons (SNc sham + RMTg lesion: $\chi^2 = 8.9$, $df = 1$, $P < .01$; SNc + RMTg lesion: $\chi^2 = 7.46$, $df = 1$, $P < .01$; χ^2 test; Fig. 6F). These results suggest that neurons expressing AMPAR in the LHb directly regulate the activity of aVTA dopaminergic neurons and indirectly influence pVTA dopaminergic neurons via the RMTg.

3.6.2. Changes in the firing activity of the serotonergic neurons

Lesions of the RMTg in the SNc sham + RMTg lesion and SNc + RMTg lesion groups did not alter the firing rate of DRN serotonergic neurons compared to the SNc sham and SNc lesion groups, respectively (Fig. 4F); however, the lesions significantly increased the

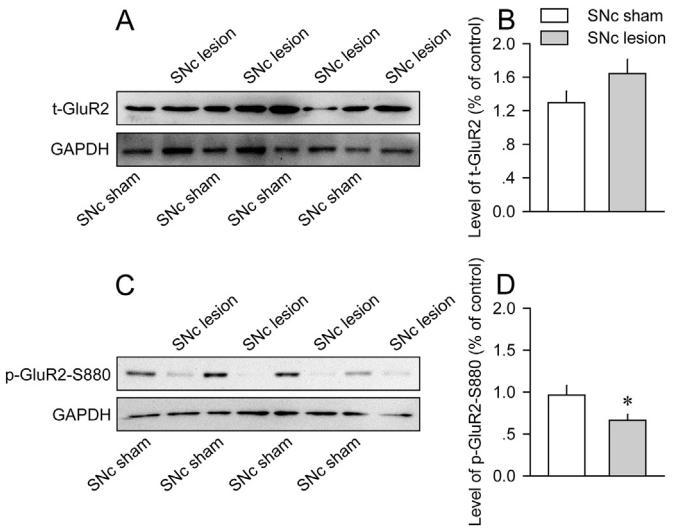

Fig. 8. Protein expression of t-GluR2 and p-GluR2-S880 subunits in the LHb. Representative bands for t-GluR2 (A) and p-GluR2-S880 (C) subunits in the LHb of SNc sham-lesioned and SNc-lesioned rats. Unilateral lesions of the SNc in rats did not alter level of t-GluR2 subunit in the ipsilateral LHb compared to SNc sham-lesioned rats (B; $n = 15$ rats/group); however, the lesions decreased level of p-GluR2-S880 in the LHb (D; $n = 16$ rats/group). *$P < .05$ vs. SNc sham-lesioned rats; unpaired Student's *t*-test. Data are presented as means ± SEM.

firing rate of MRN serotonergic neurons in the two groups of rats (both $P < .05$, unpaired Student's *t*-test; Fig. 4H), indicating that GABAergic outputs of the RMTg inhibit MRN serotonergic neurons without affecting DRN serotonergic neurons.

In the SNc sham + RMTg lesion and SNc + RMTg lesion groups, intra-LHb injection of (*S*)-AMPA significantly increased the mean firing rate of DRN serotonergic neurons compared to before injection, respectively (both $P < .01$, paired Student's *t*-test; Fig. 5N); however, (*S*)-AMPA, at the same dose, significantly decreased the mean firing rate of MRN serotonergic neurons (both $P < .05$, paired Student's *t*-test; Fig. 5O). In the two groups of rats, (*S*)-AMPA excited the majority of DRN serotonergic neurons (Fig. 6C), but the majority of MRN serotonergic neurons were unaffected (Fig. 6D).

Further, intra-LHb injection of NBQX significantly decreased the mean firing rate of DRN serotonergic neurons compared to before injection in the two groups of rats, respectively (both $P < .001$, paired Student's *t*-test; Fig. 5S); however, NBQX, at the same dose, significantly increased the mean firing rate of MRN serotonergic neurons (SNc sham + RMTg lesion: $P < .01$; SNc + RMTg lesion: $P < .05$, paired Student's *t*-test; Fig. 5T). In the two groups of rats, NBQX inhibited the majority of DRN serotonergic neurons (Fig. 6G), but the majority of MRN serotonergic neurons were unaffected (Fig. 6H).

Compared to the SNc sham and SNc lesion groups, intra-LHb injection of (*S*)-AMPA or NBQX did not significantly alter the percentages of different types of DRN serotonergic neuron responses in the SNc sham + RMTg lesion and SNc + RMTg lesion groups, respectively (Fig. 6C, G); however, (*S*)-AMPA significantly decreased the percentage of inhibited MRN serotonergic neurons and increased the percentage of unaffected neurons in the two groups of rats (SNc sham + RMTg lesion: $\chi^2 = 6.0$, $df = 1$, $P < .05$; SNc + RMTg lesion: $\chi^2 = 6.89$, $df = 1$, $P < .05$; χ^2 test; Fig. 6D), and NBQX significantly decreased the percentage of excited MRN serotonergic neurons and increased the percentage of unaffected neurons (SNc sham + RMTg lesion: $\chi^2 = 5.11$, $df = 1$, $P < .05$; SNc + RMTg lesion: $\chi^2 = 5.42$, $df = 1$, $P < .05$; χ^2 test; Fig. 6H). These results suggest that neurons expressing AMPAR in the LHb directly regulate the activity of DRN serotonergic neurons and indirectly influence MRN serotonergic neurons via the RMTg.

3.7. The levels of extracellular DA and 5-HT in the mPFC after dopaminergic lesion and activation and blockade of LHb AMPARs

Lesions of the SNc in rats significantly decreased the level of extracellular DA in the mPFC compared to rats in the SNc sham group (−73%; $P < .01$, unpaired Student's *t*-test; Fig. 7A-D); however, the lesions did not alter the level of extracellular 5-HT in the mPFC (Fig. 7E), indicating that lesions of the SNc decrease DA release in the mPFC.

Intra-LHb injection of (*S*)-AMPA increased the level of extracellular DA in the mPFC compared to the baseline in the SNc sham and SNc lesion groups, respectively (Fig. 7F). A two-way ANOVA with repeated measures showed a significant effect of time ($F_{6, 42} = 5.42$, $P < .001$), but no effect of group ($F_{1, 42} = 3.68$) or time × group interaction ($F_{6, 42} = 1$). (*S*)-AMPA also increased the level of extracellular 5-HT in the mPFC compared to the baseline in the two groups of rats (Fig. 7H). The statistical analysis showed a significant effect of time ($F_{6, 42} = 8.52$, $P < .001$), but no effect of group ($F_{1, 42} = 2.11$) or time × group interaction ($F_{6, 42} = 0.42$). However, the duration of significant effects produced by (*S*)-AMPA on DA and 5-HT release in the SNc lesion group was longer than that of the SNc sham group (SNc sham vs. SNc lesion: DA, 10 vs. 40 min; 5-HT, 10 vs. 30 min; Fig. 7F, H). Conversely, intra-LHb injection of NBQX decreased the level of extracellular DA in the mPFC compared to the baseline in the SNc sham and SNc lesion groups, respectively (Fig. 7G). A two-way ANOVA with repeated measures showed a significant effect of time ($F_{6, 42} = 11.34$, $P < .001$), but no effect of group ($F_{1, 42} = 0.52$) or time × group interaction ($F_{6, 42} = 0.87$). NBQX also decreased the level of extracellular 5-HT in the mPFC compared to the baseline in the two groups of rats (Fig. 7I). The statistical analysis showed a significant effect of time ($F_{6, 42} = 11.29$, $P < .001$), but no effect of group ($F_{1, 42} = 2.72$) or time × group interaction ($F_{6, 42} = 0.95$). Likewise, the duration of significant effects produced by NBQX on DA and 5-HT release in the SNc lesion group was longer than those in the SNc sham group (SNc sham vs. SNc lesion: DA, 20 vs. 30 min; 5-HT, 20 vs. 40 min; Fig. 7G, I). These results indicate that activation and blockade of LHb AMPARs regulate DA and 5-HT release in the mPFC of the two groups of rats, and DA depletion enhances the response of LHb neurons to AMPAR stimulation.

3.8. Protein expression of AMPAR GluR2 subunit in the LHb after dopaminergic lesion

Compared to rats in the SNc sham group, unilateral lesions of the SNc in rats did not alter expression of total GluR2 subunit in the ipsilateral LHb (t-GluR2; Fig. 8A, B); however, the lesions significantly reduced the level of phosphorylated GluR2 subunit at serine 880 site (p-GluR2-S880; − 35%; $P < .05$, unpaired Student's *t*-test; Fig. 8C, D), indicating that lesions of the SNc reduce phosphorylation of AMPAR GluR2 subunit serine 880 in the LHb.

4. Discussion

4.1. Activation of LHb AMPARs increases the levels of extracellular DA and 5-HT in the mPFC, which produce antidepressant-like effects

The behavioral data showed that lesions of the SNc in rats decreased sucrose preference and increased immobility time compared to rats in the SNc sham group, indicating the induction of depressive-like responses. The results are in good agreement with all previous studies (Winter et al., 2007; Santiago et al., 2010, Santiago et al., 2014; Sourani et al., 2012; Han et al., 2015, 2016; Wang et al., 2017). These findings suggest involvement of the dopaminergic transmitter system in the onset of PD-related depression.

Accumulating data indicate that the LHb plays an important role in depression, because it is implicated in the regulation of dopaminergic activity in the VTA and serotonergic activity in the raphe nuclei (Lecca et al., 2014; Proulx et al., 2014). Studies have found that the LHb

exhibits increased firing and metabolic activity during depressive-like states including depressed patients and rodent models of depression (Morris et al., 1999; Shumake et al., 2003; Li et al., 2011, 2013; Lawson et al., 2017; Yang et al., 2018), and that inhibition of hyperactivity of the LHb by the local administration of $GABA_A$ receptor agonist muscimol or deep-brain stimulation of the LHb imporves depressive-like behaviors (Yang et al., 2008; Sartorius et al., 2010; Li et al., 2011; Meng et al., 2011; Winter et al., 2011). Likewise, our studies have also shown that activation of LHb $5\text{-}HT_{2C}$ receptors increases the firing activity of LHb neurons and the expression of depressive-like behaviors in rats with unilateral lesions of the SNc, whereas intra-LHb injection of muscimol inhibits the neurons and produces antidepressant-like effects, which attribute to increased release of 5-HT in the mPFC (Han et al., 2015; Wang et al., 2017). Based on these previous studies, we consider that hyperactivity of LHb neurons is associated with depressive-like behaviors, and the hyperactivity may be one of the pathophysiological mechanisms of depression caused by various reasons.

The LHb receives glutamatergic projections from various brain regions (Herkenham and Nauta, 1977; Kim and Lee, 2012; Yetnikoff et al., 2015), and expresses AMPARs, which are the main glutamate receptor in this region (Li et al., 2011; Maroteaux and Mameli, 2012; Meye et al., 2013). A study has found that withdrawal from chronic voluntary ethanol drinking induces depressive-like symptoms and increases LHb AMPAR and Ca^{2+}/calmodulin-dependent protein II activity, and their inhibitions decrease depressive-like behaviors and alcohol consumption in rats (Li et al., 2017). Further, our recent study has shown that blockade of LHb CP-AMPARs produces antidepressant-like effects in both SNc sham-lesioned and SNc-lesioned rats, which involve in reduced firing rate of LHb neurons and increased DA and 5-HT release in the mPFC (Zhang et al., 2019). However, effects of activation and blockade of LHb AMPARs on depressive-like behaviors remain unclear, particularly in PD-related depression. Interestingly, the behavioral and electrophysiological results in this study showed that intra-LHb injection of selective AMPAR agonist (*S*)-AMPA produced antidepressant-like effects in both the SNc sham and SNc lesion groups, while injection of potent AMPAR antagonist NBQX induced or increased the expression of depressive-like behaviors in the two groups of rats, respectively, although (*S*)-AMPA increased the firing activity of LHb neurons and NBQX inhibited the neurons. These results indicate that the changes between the behaviors and neuronal activity induced by (*S*)-AMPA or NBQX are inconsistent compared to previous reports. The discrepancy may be explained by release of DA and 5-HT in the mPFC after intra-LHb injection of (*S*)-AMPA or NBQX, because the LHb strongly controls the output of midbrain monoaminergic nuclei that provide DA and 5-HT to the forebrain, and the two monoamines and mPFC are involved in the regulation of depression (Steketee, 2003; Matsumoto and Hikosaka, 2007; Metzger et al., 2017). Further, the monoamine hypothesis, which suggests the deficiency of monoamines in depression, has been widely used to explain the pathophysiology of depression and mechanisms of action of antidepressants; the reason is that the antidepressant treatments increase levels of monoamines in the brain (Nestler et al., 2002; Berton and Nestler, 2006). In the present study, in vivo microdialysis results showed that lesions of the SNc in rats decreased in the level of extracellular DA in the mPFC compared to rats in the SNc sham group, and the level of extracellular 5-HT was not altered, which are consistent with our recent study (Wang et al., 2017). The result also suggests that decreased release of DA in the mPFC may be involved in the onset of PD-related depression. Further, intra-LHb injection of (*S*)-AMPA increased the levels of extracellular DA and 5-HT in the mPFC of both SNc sham-lesioned and SNc-lesioned rats; conversely, injection of NBQX decreased release of DA and 5-HT in the mPFC. From these findings, increased and decreased DA and 5-HT release in the mPFC support that the behavioral results showing that activation of LHb AMPARs produced antidepressant-like effects and blockade of the AMPARs induced depressive-like behaviors.

In the present study, the dose of (*S*)-AMPAR and NBQX producing significant behavioral effects in the SNc lesion group was lower than those in the SNc sham group, and the duration of (*S*)-AMPAR and NBQX action on the firing rate of LHb neurons and release of mPFC DA and 5-HT in SNc-lesioned rats was prolonged compared to SNc sham-lesioned rats, suggesting the abnormal expression of LHb AMPARs after depletion of DA, which enhances the effects of (*S*)-AMPA and NBQX. Further, glutamatergic synapses in the LHb express CI- and CP-AMPARs, and they mediate fast excitatory transmission onto neurons (Li et al., 2011; Maroteaux and Mameli, 2012; Meye et al., 2013). In CI-AMPARs, enhanced phosphorylation of GluR2 subunit at S880 site accelerates endocytosis of GluR2 subunit and reduces the abundance of surface-expressed CI-AMPARs (Wang et al., 2014). The Western blotting data showed that lesions of the SNc in rats did not alter the level of t-GluR2 subunit in the LHb compared to rats in the SNc sham group; however, the lesions significantly reduced the level of p-GluR2-S880, indicating that degeneration of the nigrostriatal pathway reduces phosphorylation of GluR2 subunit at S880 site, and then increases the surface expression of GluR2-containing CI-AMPARs, which is responsible for enhanced effects of (*S*)-AMPA and NBQX on the behaviors, firing activity of LHb neurons and release of mPFC DA and 5-HT in rats with lesions of the SNc.

4.2. Activation of LHb AMPARs increases the firing activity of aVTA dopaminergic neurons and DRN serotonergic neurons, which lead to increased release of DA and 5-HT in the mPFC

In addition to the direct projection from the LHb to the VTA and raphe nuclei, the LHb also projects to these structures indirectly via the GABAergic RMTg (Metzger et al., 2017; Petzel et al., 2017). Moreover, evidence has indicated the presence of functional differences between the aVTA and pVTA (Sanchez-Catalan et al., 2014). Although the electrophysiological identification of VTA dopaminergic neurons is related to multiple factors, such as recording methods and recording durations (Margolis et al., 2010), the electrophysiological criteria in extracellular recording in vivo are reliable and widely adopted. In this study, we found that lesions of the SNc in rats significantly increased the mean firing rate of both aVTA and pVTA dopaminergic neurons compared to rats in the SNc sham group. Likewise, the lesions also significantly increased the mean firing rate of DRN and MRN serotonergic neurons, respectively. These results are confirmed by previous reports (Wang et al., 2009; Zhang et al., 2019).

The GABAergic projections from the RMTg make synapses onto dopaminergic neurons in the VTA (Balcita-Pedicino et al., 2011), and inhibition of the RMTg increases dopaminergic neuron activity in the midbrain, while RMTg stimulation inhibits their firing (Hong et al., 2011; Lecca et al., 2011, 2012; Bourdy et al., 2014). These studies indicate that the RMTg is an important inhibitory control center for dopaminergic neurons in the midbrain. In addition, RMTg GABAergic neurons synapse onto putative glutamatergic neurons in the DRN (Gras et al., 2002; Sego et al., 2014). In the present study, the results showed that lesions of the RMTg did not alter the mean firing rate of aVTA dopaminergic neurons and DRN serotonergic neurons compared to the SNc sham and SNc lesion groups, respectively; however, the lesions significantly increased the mean firing rate of pVTA dopaminergic neurons and MRN serotonergic neurons, suggesting that the GABAergic outputs of the RMTg inhibit pVTA dopaminergic neurons and MRN serotonergic neurons, but not aVTA dopaminergic neurons and DRN serotonergic neurons, which are consistent with our recent study (Zhang et al., 2019).

Despite a study has found that most LHb neurons expressing CP-AMPAR control the activity of pVTA dopamnergic neurons and MRN serotonergic neurons indirectly via the GABAergic RMTg (Zhang et al., 2019), changes in the firing activity of different dopaminergic and serotonergic cell groups after activation and blockade of LHb AMPARs are unknown. In this study, intra-LHb injection of (*S*)-AMPA significantly increased the mean firing rate of aVTA dopamniergic neurons

and DRN serotonergic neurons compared to before injection in the SNc sham and SNc lesion groups, respectively; however, (*S*)-AMPA significantly decreased the mean firing rate of pVTA dopamniergic neurons and MRN serotonergic neurons. In the SNc sham + RMTg lesion and SNc + RMTg lesion groups, (*S*)-AMPA also significantly increased the mean firing rate of aVTA dopamniergic neurons and DRN serotonergic neurons and decreased the mean firing rate of pVTA dopamniergic neurons and MRN serotonergic neurons. In the four groups of rats, intra-LHb injection of NBQX produced the opposite effects compared to (*S*)-AMPA, respectively. These results suggest that activation or blockade of LHb AMPARs differentially regulates the firing activity of dopaminergic and serotonergic neurons in different midbrain structures. Because reduction of phosphorylation of GluR2-S880 in the LHb can increase the surface expression of the GluR2-containing CI-AMPARs in SNc-lesioned rats (Wang et al., 2014), we consider that (*S*)-AMPA increases the firing activity of LHb neurons expressing CI-AMPAR and then enhances glutamatergic outputs of the LHb, which lead to a increase in the firing activity of aVTA dopaminergic neurons and DRN serotonergic neurons, suggesting that LHb neurons expressing CI-AMPAR directly target dopaminergic neurons in the aVTA and serotonergic neurons in the DRN. This hypothesis is supported by previous studies showing that the LHb directly projects to the aVTA, DRN and MRN (Bernard and Veh, 2012; Goncalves et al., 2012; Sego et al., 2014; Petzel et al., 2017), and LHb axons form synapses with dopaminergic and GABAergic neurons of the VTA (Omelchenko et al., 2009). In addition, NBQX inhibited aVTA dopaminergic neurons and DRN serotonergic neurons, which further confirms that glutamatergic outputs from the LHb directly regulate the firing activity of these neurons.

For decreased the mean firing rate of pVTA dopaminergic neurons and MRN serotonergic neurons after intra-LHb injection of (*S*)-AMPA, it can be postulated that (*S*)-AMPA excites LHb neurons expressing CI-AMPAR that project to the RMTg, which increase the firing activity of GABAergic neurons in the RMTg and then produces inhibition of pVTA dopaminergic neurons and MRN serotonergic neurons, suggesting that LHb neurons expressing CI-AMPAR indirectly regulate the activity of dopaminergic neurons in the pVTA and serotonergic neurons in the MRN via the RMTg. Likewise, this notion is also supported by the observations that the LHb projects to the RMTg, which sends GABAergic projections to the VTA and synapses onto VTA dopaminergic neurons (Jhou et al., 2009; Balcita-Pedicino et al., 2011; Petzel et al., 2017). Further, NBQX increased the mean firing rate of pVTA dopaminergic neurons and MRN serotonergic neurons, we consider that inhibition of LHb neurons induced by NBQX decreases the activity of GABAergic neurons in the RMTg, which causes the disinhibition of pVTA dopaminergic neurons and MRN serotonergic neurons.

Although intra-LHb injection of (*S*)-AMPA and NBQX increased or decreased the mean firing rate of dopaminergic and serotonergic neurons in different midbrain structures, the response of each dopaminergic and serotonergic neuron to activation or blockade of AMPARs in the LHb was different. In the present study, we found that (*S*)-AMPA excited most aVTA dopaminergic neurons and DRN serotonergic neurons in the four groups of rats, and there was no difference in the proportion of excited neurons between the SNc sham and SNc sham + RMTg lesion groups, as well as between the SNc lesion and SNc + RMTg lesion groups. Combined with the Western blotting results, these findings suggest that most LHb neurons expressing CI-AMPAR directly target dopamnergic neurons in the aVTA and serotonergic neurons in the DRN. Further, (*S*)-AMPA inhibited most pVTA dopaminergic neurons and MRN serotonergic neurons in the SNc sham and SNc lesion groups, respectively; however, it did not alter the firing activity of most pVTA dopaminergic neurons and MRN serotonergic neurons in the SNc sham + RMTg lesion and SNc + RMTg lesion groups. Compared to the SNc sham and SNc lesion groups, the proportion of (*S*)-AMPA-induced inhibition was significantly decreased in the SNc sham + RMTg lesion and SNc + RMTg lesion groups, respectively, suggesting that most LHb neurons expressing CI-AMPAR target pVTA dopamnergic neurons and MRN serotonergic neurons indirectly via the GABAergic RMTg. In the four groups of rats, NBQX inhibited most aVTA dopaminergic neurons and DRN serotonergic neurons. In addition, NBQX excited most pVTA dopaminergic neurons and MRN serotonergic neurons in the SNc sham and SNc lesion groups, but did not alter the activity of most pVTA dopaminergic neurons and MRN serotonergic neurons in the SNc sham + RMTg lesion and SNc + RMTg lesion groups. These findings further suggest that glutamatergic outputs from the LHb differentially control the firing activity of different dopaminergic and serotonergic cell groups in the midbrain.

In conclusion, the present study indicates that activation of LHb AMPARs by (*S*)-AMPA produces antidepressant-like effects in both SNc sham-lesioned and SNc-lesioned rats, although (*S*)-AMPA increases the firing activity of LHb neurons. In additon, blockade of the AMPARs by NBQX induces the opposite effects on the behaviors and neuronal firing. Changes in the behaviors are associated with the fact that neurons expressing AMPARs in the LHb mainly target aVTA dopaminergic neurons and DRN serotonergic neurons via the direct pathway and then influence the firing activity of the dopaminergic and serotonergic neurons and release of DA and 5-HT in the mPFC, which are involved in the regulation of depression. Further, the reduced phosphorylation level of GluR2 subunit at the S880 in the LHb increases surface expression of GluR2-containing CI-AMPARs, which enhances effects of (*S*)-AMPA and NBQX on the behaviors, firing activity of LHb neurons and release of mPFC DA and 5-HT in SNc-lesioned rats. Therefore, these results further suggest that most neurons expressing CI-AMPAR in the LHb directly control the firing activity of aVTA dopaminergic neurons and DRN serotonergic neurons. Considering the complexity of disorders involving the VTA, raphe nuclei and LHb dysfunction, the present study provides insight into involvement of the brain regions and LHb CI-AMPARs in the disorders, particularly in PD-related depression.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.expneurol.2019.113058.

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