Neurotoxicology 73 (2019) 175–182

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Nicotine improved the olfactory impairment in MPTP-induced mouse model of Parkinson's disease

Jing Yang[a,b,1], Dong-Jun Lv[a,b,1], Ling-Xi Li[a,b,1], Ya-Li Wang[c], Di Qi[b], Jing Chen[a], Cheng-Jie Mao[a], Fen Wang[b], Yi Liu[c], Li-Fang Hu[b], Chun-Feng Liu[a,b,c,*]

[a] Department of Neurology and Suzhou Clinical Research Center of Neurological Disease, The Second Affiliated Hospital of Soochow University, Suzhou, 215004, China
[b] Institute of Neuroscience, Soochow University, Suzhou, 215123, China
[c] Department of Neurology, Suzhou Municipal Hospital of Nanjing Medical University, Suzhou, 215008, China

ARTICLE INFO

Keywords:
Parkinson's disease
Olfactory dysfunction
Nicotine
cHolinergic system

ABSTRACT

Olfactory impairment is an early feature of patients with Parkinson's disease (PD). Retrospective epidemiological studies reported lower scores on the University of Pennsylvania Smell Identification Test (UPSIT) in non-smokers than smokers with PD and showed an inverse correlation between susceptibility to PD and a person's history of smoking. But the mechanisms by which cigarettes affect olfaction in PD are not fully understood. So we investigated the effect of nicotine on the olfactory function in 1-methyl-4-phenyl-1, 2, 3, 6 tetrahydropyridine (MPTP)-treated mice. We observed that nicotine improved locomotor activity and protection against dopaminergic neuron loss in the midbrain in MPTP-treated mice. Compared to controls, MPTP-treated mice showed a deficit of odor discrimination and odor detection, which were alleviated by nicotine treatment. But no significant changes were found in olfactory memory in MPTP-treated mice. Moreover, we detected a marked decrease of Choline acetyltransferase (ChAT) expression in the olfactory bulb (OB) in MPTP-treated mice, which was also attenuated by nicotine administration. In addition, nicotine ameliorated the loss of cholinergic neurons and dopaminergic innervation in the horizontal limb of the diagonal band (HDB), which is the primary origin of cholinergic input to the OB. Our results suggested that nicotine could improve the olfactory impairment by protecting cholinergic systems in the OB of MPTP-treated mice. And nicotine protection of cholinergic systems in the OB is relevant to attenuating dopaminergic neuron loss in the midbrain and HDB.

1. Background

Parkinson's disease (PD) is a movement disorder characterized by rigidity, bradykinesia, posture instability and resting tremor that was first described by James Parkinson in 1817. But it is recently recognized that non-motor features such as depression, blepharospasm, orthostatic hypotension, sleep disorders, constipation and dysosmia also occur in PD patients (Gao and Wu, 2016). Moreover, non-motor symptoms often appear earlier than the motor dysfunction, and the smell impairment is the most salient non-motor feature of PD(Kang et al., 2016). Previous studies reported that about 75%–90% of PD patients suffer from smell dysfunction (Cao et al., 2016).

As we know that the olfactory bulb (OB) represents a convergence point for inputting odor signals and includes the synapse transferring odor information between the olfactory receptor neuron and higher cortical regions. The vomeronasal is a second olfactory sense organ in the mouse. It is the first stage of the accessory olfactory system and has important consequence for the sensing of structurally and functionally distinct chemical stimuli. The OB mainly receives cholinergic inputs, which originate from the horizontal limb of the diagonal band (HDB) (Devore and Linster, 2012). The loss of cholinergic neurons in the HDB and deficits in cholinergic interneurons in OB are frequently observed in PD, and they might be the cause of olfactory dysfunction (Krosnowski et al., 2012). The olfactory function is controlled by HDB cholinergic neurons which release acetylcholine (Ach) acting on both nicotine and muscarinic receptor (nAChR and mAChR). Cigarette smokers have a lower risk of developing PD and many studies have showed an inverse correlation between olfactory dysfunction and

* Corresponding author at: Department of Neurology, The Second Affiliated Hospital of Soochow University, Suzhou, 215004, China.
E-mail addresses: 743066590@qq.com (J. Yang), 1040529522@qq.com (D.-J. Lv), 356176440@qq.com (L.-X. Li), 435108964@qq.com (Y.-L. Wang), 810728704@qq.com (D. Qi), jing_ch.china@hotmail.com (J. Chen), drchengjiemao@163.com (C.-J. Mao), wangfen_1982@126.com (F. Wang), liuyizr@163.com (Y. Liu), hulifang@suda.edu.cn (L.-F. Hu), liuchunfeng@suda.edu.cn (C.-F. Liu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.neuro.2019.02.008
Received 18 October 2018; Received in revised form 3 February 2019; Accepted 11 February 2019
Available online 10 April 2019
0161-813X/

smoking in PD (Sharer et al., 2015). However, the underlying mechanism is poorly understood. Nicotine is the main psychoactive component of cigarettes. Interestingly, numerous work showed the neuroprotection of nicotine on dopaminergic neurons against 1-methyl-4-phenyl-1, 2, 3, 6 tetrahydropyridine (MPTP) toxicity (Singh et al., 2009). Recent studies showed the loss of cholinergic neurons and dopaminergic innervation in the HDB in MPTP parkinsonian monkeys (Mundinano et al., 2013). The previous report also demonstrated the dopaminergic input to the HDB was provided primarily by axons of neurons whose cell bodies resided in the ventral tegmental area (VTA) (Senatorov and Renaud, 1999). So it is curious to define whether nicotine may improve the olfactory impairment by regulating cholinergic systems in the OB of MPTP-treated mice.

In this study, we examined the effect of nicotine on locomotor activity and olfactory function, along with the level of choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH) expression in OB and HDB, in mice treated with or without MPTP.

2. Materials and methods

2.1. Reagents and antibodies

MPTP (Sigma, M0896, USA) and nicotine (Sigma Chemical Company).

We listed all primary antibodies used in this study as follows: anti-TH (Sigma, T1299, USA), anti-ChAT (Abcam, ab18736, UK), anti-ChAT (Millipore, AB143, USA) and anti-β-actin (Sigma, A3854, USA). All the secondary antibodies used for western blot were from Jackson lab (USA).

2.2. Animals

Eight-week-old male C57BL/6 mice were obtained from Shanghai Laboratory Animal Center (SLAC, Shanghai, China), and housed at a 12/12 h light/dark cycle at 24 °C temperature, with access to food and water ad libitum. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Soochow University.

2.3. Treatments

The animals were divided into four groups: control, nicotine, MPTP, and nicotine + MPTP group. To test the effect of nicotine on the olfaction of PD model, the mice were received intraperitoneally (i.p.) with nicotine at 1 mg/kg or normal saline (0.9% NaCl) daily for 3 weeks, and treated with MPTP − HCl (14 mg/kg, i.p.) or normal saline at 2-h intervals over an 8-h period on the 8th day.

2.4. Rotarod test

The rotarod test was applied to evaluate the motor coordination of the mice on the 21st day after nicotine injection for the first time (Gu et al., 2017). The mice were trained twice daily at a rotation speed of 25 rpm for two successive days before testing. On the 3rd day, the speed was kept to 25 rpm in a test session. The mouse was placed on the rotating lane of the rod and the timer was started. Once the mouse fell off the rod, the time latency to fall was recorded. Each mouse was tested for three trials at 5-min intervals, with a maximum trial length at 300 s per trial. The average time latency on the rod of three test trials for each mouse was calculated.

2.5. Open field test

To assess the general locomotor activity in mice, the open field test was used in our study on the 21st day after nicotine treatment (Goes et al., 2014). The mice were placed in the center of the testing chamber (40 cm × 40 cm × 35 cm) to move freely, with a video tape recorder above the chamber. Each mouse was placed in the testing chamber with white floors for 10 min adaptation, followed by a 5 min recording period using a computerized automatic analysis system (ANY-maze, Stoelting, USA). The apparatus was cleaned with cotton pad wetted with 75% ethanol each time before next session. During the test video recording was carried out and scoring was done by a blind observer. The locomotor speed was calculated and presented as data for analysis. (The locomotor speed = The total locomotor distance / 300 s).

2.6. Buried pellet test

To measure the odor detection in the mice, we performed a buried pellet test, as previously reported (Lehmkuhl et al., 2014). Individually housed mice were food-restricted on a diet to 90% of body weight from 2 days prior to the test and during the experimental period. Before testing and during food restriction, give each mouse 1–2 pieces of the pellets to be used during the test. This step is important because the mice need to be familiar with the pellet odor. A piece of the pellet was buried 0.5 cm below the bedding that was approximately 5 cm deep in the test cage (45 cm × 24 cm × 20 cm). In each trial, the mouse was placed at the center of the cage at the beginning. When the pellet was detected and eaten by mice, the timer stopped and recorded. If the mouse did not find the pellet within 5 min, the test stopped and a score of 300 s was noted for it. The bedding in the test chamber was cleaned for each trial. The buried pellet test was performed for 3 consecutive days and each mouse received one trial per day. The pellet was buried in a different spot in the cage between trials.

2.7. Olfactory memory test

To measure odor memory in the mouse, we carried out a test of detecting the chocolate. The testing box consisted of a square arena (80 cm × 80 cm) and wall (60 cm high). We made four cases (5 cm × 5 cm × 5 cm) that filled up with sands of different odors (water, alcohol, chilies, paprika). And we put the four cases on the four corners of the testing box. Then we buried the chocolate in a case of random odor sand. The mouse was placed in the center of the box at the beginning. When the chocolate was detected and eaten, the timer stopped and recorded. If the mouse did not find the chocolate within 5 min, the test stopped and a score of 300 s was noted. Each mouse was tested twice at a two-day interval. The location of the odor sand which bury the test chocolate was changed at random for each trial, but the chocolate was buried the same odor sand. The relative ratio of noting the time of trial-2 to trial-1 (trial 2/trial 1) was calculated and analyzed.

2.8. Block test

To assess the mouse ability to discriminate the social scent, we performed a block test according to a previous study (Tillerson et al., 2006). To take odor of mice respectively, each wooden block (2 cm × 2 cm × 2 cm) was sealed in a plastic bag with 8 g of animal beddings from the home cages of tested mice for 24 h. At least 1 h before testing, habituate the mice in their home cage without the water bottle, feeder bin. In each trial, test mice were moved to the testing area that put two kinds of wooden blocks of different odor (one block with its own smell, another one with unfamiliar another mouse' odor). Then the test mouse could smell the two kinds of wooden blocks of different odor. Change gloves between each trial so that the scents are not exchanged between animals/blocks. The timer was used to record the time per mouse sniffed each block in a 120 s trail. The relative ratio of sniffing time of novel scent to own scent (novel scent/own scent) was calculated and analyzed.

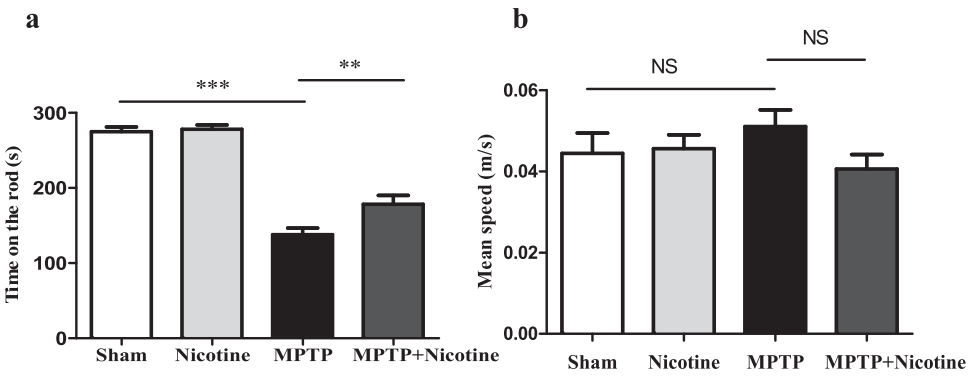

Fig. 1. Effect of nicotine on Locomotor activity in MPTP-treated mice. (a) Rotarod performance in different experimental groups. N = 5–7 per group. (b) The mean speed in the open field test showed no difference among different groups. N = 5–7 per group. $^{**}P < 0.01$ and $^{***}P < 0.001$. One-way analysis of variance and Tukey's post-hoc test.

2.9. Western blot

Brain tissues of striatum, substantia nigra (SN), HDB or OB were homogenized in lysis buffer containing 25 mM Tris, 150 mM NaCl, 5 mM EDTA, 1% NP-40, pH 7.5, with protease inhibitor cocktail tablets (Roche Diagnostics, Penzberg, Germany) and heated at 95 °C for 5 min. Protein lysates were separated on 8% SDS-PAGE gels and transferred onto polyvinylidene fluoride membranes. Next, blots were blocked with 5% dry milk powder in Tris-buffered saline/Tween20 buffer (TBST: 10 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 8) at 37 °C for 1 h, and then incubated with appropriate primary antibodies at 4 °C. After that, blots were briefly washed and probed with secondary antibodies for another 1 h. Blots were finally visualized using a chemiluminescence kit (Bio-Rad, 170–5061) and analyzed using ImageJ software (National Institute of Health, Bethesda, MD, USA). β-actin was used as the loading control for different protein analyses.

2.10. Immunostaining

Animals were anesthetized with pentobarbital sodium, and perfused with phosphate buffer solution (0.1 M PBS, pH 7.2), followed by 4% (w/v) paraformaldehyde fixative. Then the brains were harvested, post fixed overnight, dehydrated, and embedded in paraffin wax. Paraffin coronal slices were cut in 4 μm thick using a sliding microtome.

We used immunohistochemistry to observe TH neurons distribution in SNpc and VTA. Paraffin sections were blocked with 3% hydrogen peroxide for 20 min and then boiled in 10 mM citrate buffer (pH 6.0) for 10 min for epitope-retrieval. Next, the sections were treated with 0.5% Triton X-100 for 10 min and blocked with 5% BSA in TBST for 1 h. After that, the sections were incubated with mouse anti-TH antibody overnight at 4 °C and stained using the DAB kit (Gene Tech, GK500705, China). The pictures were taken under a microscope (Carl Zeiss, Germany). The number of dopaminergic neurons in SNpc were bilateral counted in one out of every neighboring twenty sections from AP-3.16 to AP-3.80 mm by two researchers blind to the treatment condition.

Immunofluorescence was applied to view ChAT neurons and TH nerve fibers distribution in HDB. The sections were treated in the same way as above and were incubated with rabbit anti-ChAT (1:100, Millipore, AB143) or mouse anti-TH antibody (1:500, Sigma, T1299) overnight at 4 °C. After that, sections were washed in 0.01-M phosphate-buffered saline 3 times and incubated with donkey anti-rabbit IgG (HtL) Alexa Fluor 488 or donkey anti-mouse IgG (HtL) Alexa Fluor 488 conjugate secondary antibody at 37 °C for 1 h. After washing 3 times, the nucleus was stained by DAPI (Vector Laboratories, Burlingame, CA, USA). Subsequently, the sections were visualized by a microscope (Carl Zeiss, Germany). The counting of cholinergic neurons in HDB is similar to that of TH neurons in SNpc. The percentage of TH nerve fibers in HDB was calculated as previously described (Mundinano et al., 2013). It was analyzed using ImageJ software and the average values of 3 consecutive sections per animal were calculated. Images were converted to 8 bit-RGB stacked (green channel), and a background subtraction procedure was performed. Ten randomly selected areas of each layer were outlined and the same threshold limits were defined for each image to select TH structures. TH-positive area fraction is calculated by ImageJ software automatically.

2.11. Statistical analysis

Data are presented as mean ± SEM. The GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) was applied for the statistical analyses. Behavioral results were statistically compared with a one-way analysis of variance (ANOVA), followed by Newman-Keuls post hoc test, for comparison between experimental groups. For neurochemical analyses and immunohistochemistry experiments, results were statistically analyzed by one-way ANOVA followed by Tukey's post hoc test (Baranyi et al., 2016), and P values less than 0.05 were regarded as statistical significance.

3. Results

3.1. Nicotine treatment ameliorated the locomotor deficit in MPTP-induced mice

In order to assess the effect of nicotine, several behavioral tests were performed at 3 weeks after nicotine treatment in saline- or MPTP-treated mice. The rotarod test revealed that the time on the rotarod was obviously reduced in MPTP-treated mice ($P < 0.001$) compared with control group (Fig. 1a). This was ameliorated by nicotine treatment at 1 mg/kg once daily. But no significant difference was observed in the mean of locomotion speed among all tested groups (Fig. 1b). Nicotine treatment alone had no obvious effect on motor coordination.

3.2. Nicotine treatment ameliorated the Dopamine neuron loss in MPTP-induced mice

We employed Western blot and immunostaining to observe the effect of nicotine treatment on MPTP-induced mice in Dopamine neurons. Western blot analyses showed a marked decrease of TH expression in both the striatum (Fig. 2a) and SN (Fig. 2b) in MPTP-treated mice ($P < 0.001$), which was attenuated by nicotine treatment. Consistent with this, the immunostaining revealed a similar change of TH expression levels in MPTP- and nicotine-treated mice. Dopaminergic innervation in the striatum was obviously reduced in MPTP-treated mice. Nicotine treatment resulted in higher levels of TH expression as compared to MPTP group (Fig. 2c). We also counted the number of TH positive neurons in SNpc and found a reduction ($P < 0.001$) of TH-immunoreactivity in the SNpc of MPTP-treated group. And nicotine-treated mice showed an increase ($P < 0.05$) of TH-immunoreactivity compared with MPTP-treated animals (Fig. 2d, f). Apart from SNpc, MPTP also led to an obvious loss of TH positive neurons in VTA ($P < 0.01$). But this dopaminergic neuron loss was also prevented by nicotine treatment (Fig. 2d, e). Nicotine treatment alone did not affect

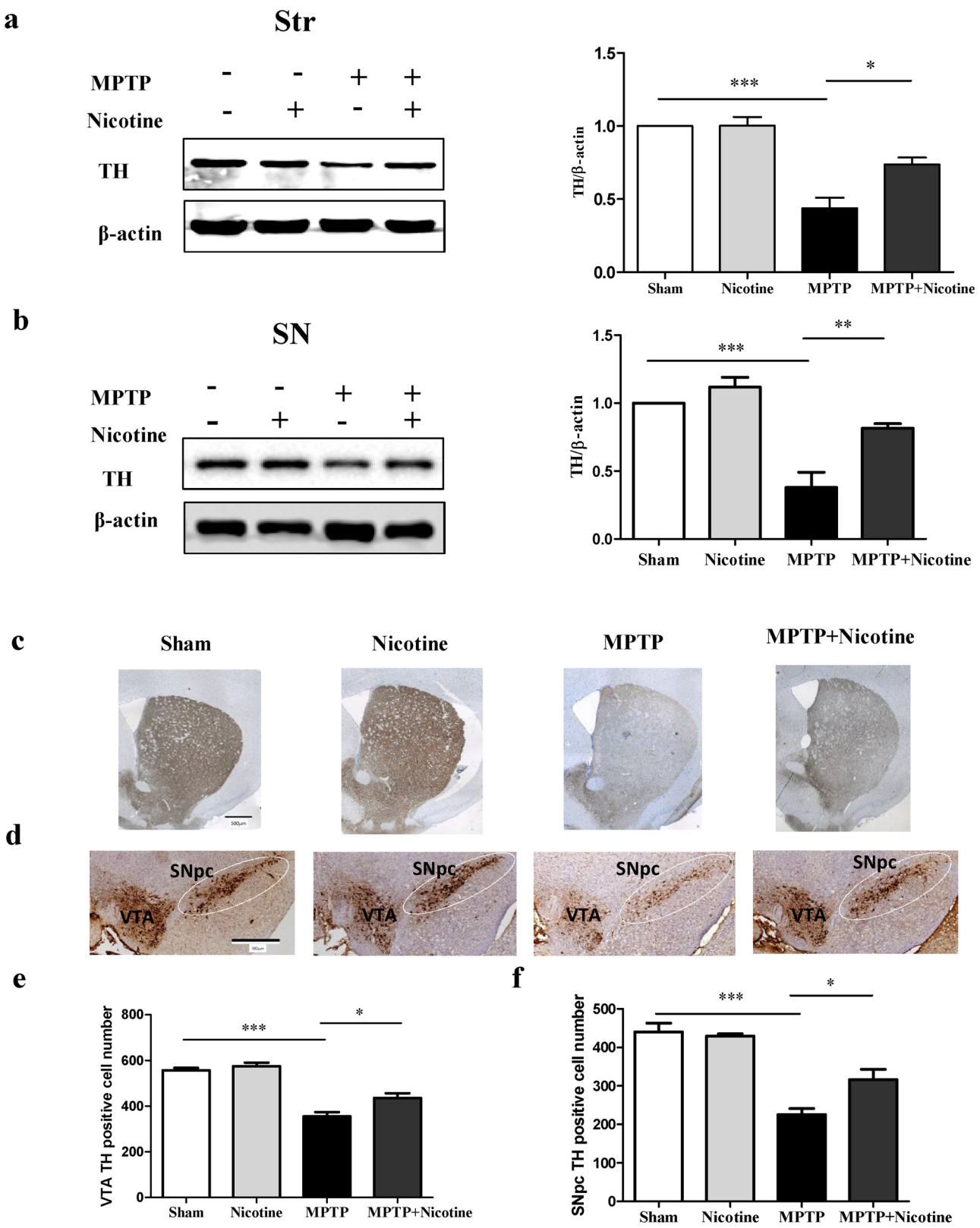

Fig. 2. Effect of nicotine on the expression of TH protein in the nigrostriatal pathway in MPTP-treated mice.(a, b) The TH expression in the striatum (a) and SN (b) as determined by western blot. N = 3 per group. (c) Representative images of the dopaminergic innervation in the striatum, as visualized by TH immunostaining. N = 4–5 per group. Scale bar at 500μm. (d) Representative pictures for TH-immunoreactivity in the SNpc and VTA. N = 4–5 per group. Scale bar at 500μm. (e, f).The quantitative data for the number of TH positive neurons in the SNpc (e) and VTA (f). The data was collected from coronal sections of the SNpc and VTA mice for each group. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. One-way analysis of variance and Tukey's post-hoc test.

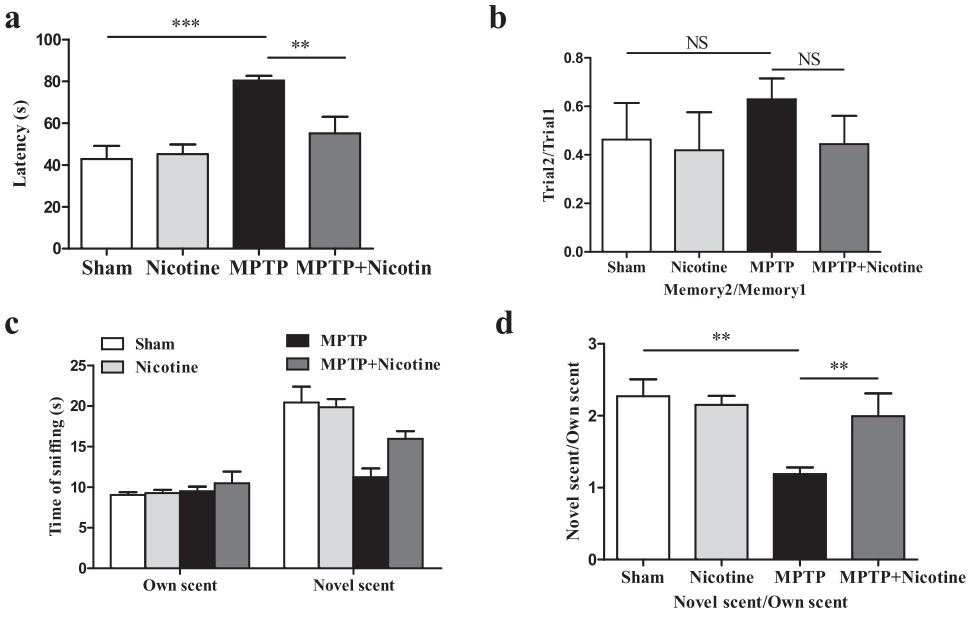

Fig. 3. Effect of nicotine on the olfactory behavior in MPTP-treated mice. (a)The buried pellet test showed that MPTP-treated mice had a longer latency to find the pellet than controls. And nicotine-treated mice got shorter time when they grew up as compared with MPTP-treated mice. N = 6–8 per group. (b) The ratios of trial 2/trial 1 were recognized the performance of capacity of the olfactory memory in mice. The ratios of MPTP-treated mice slightly higher than control, and slightly lower than nicotine-treated mice, but the statistical analysis showed no significant difference. N = 4–7 per group. (c) This histogram showed that mice sniff the time on novel scent vs own scent for each group. (d) The ratios of novel scent/own scent used to show the capacity of social scent discrimination in mice. The ratio of MPTP-treated mice obvious decrease as compared with sham group. And the ratio of nicotine-treated mice showed a reduction compared with MPTP-treated mice, N = 5–9 per group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. One-way analysis of variance and Tukey's post-hoc test.

TH expression in these related regions.

3.3. Nicotine prevented the olfactory impairment in MPTP-treated mice

We also evaluated the effect of nicotine on olfactory function using different assays. In the buried pellet test, MPTP-treated mice showed a longer latency to find the pellet compared to control group ($P < 0.001$). But this was shortened in the presence of nicotine treatment ($P < 0.05$) (Fig. 3a). In the odor memory test, we use the ratio of trial-2 to trial-1 (trial 2/trial 1) to evaluate odor memory. But the difference among various groups was not significant (Fig. 3b). In the block task, the ratio of the time to sniff the novel scent over its own scent (novel/own scent) was used to assess the odor discrimination. We observed that this ratio in MPTP-treated mice was obviously less than control mice ($P < 0.01$), although the time of sniffing its own scent did not alter significantly. This indicates MPTP caused a damage to odor discrimination in mice. And this impairment was partially rescued by nicotine administration, as indicated by the increased ($P < 0.05$) ratio of novel scent/own scent compared with MPTP group (Fig. 3c, d). These results imply that nicotine could improve the odor detection and discrimination but not odor memory in MPTP-induced mice.

3.4. Nicotine attenuated the decreases of ChAT expression in both the OB and HDB induced by MPTP

To examine the mechanism by which nicotine affected the olfactory function, we studied the protein level of ChAT in both OB and HDB because cholinergic system acts as the major regulator of olfactory function. A significant decrease of ChAT expression was found in the OB of MPTP-treated mice ($P < 0.001$), which was prevented by nicotine treatment ($P < 0.05$) (Fig. 4a). Similarly, treatment with nicotine attenuated the decline of ChAT expression in the HDB in MPTP-treated mice ($P < 0.05$) (Fig. 4b). Meanwhile, we observed the changes of cholinergic neurons through immunofluorescence, which were consistent with western blot (Fig. 4c). The number of ChAT neurons in the HDB of MPTP-treated mice decreased ($P < 0.001$), and nicotine treatment prevented the loss of ChAT neurons in MPTP-treated mice ($P < 0.01$) (Fig. 4d).

3.5. Nicotine protected the dopaminergic innervation in HDB

Compared with control group, MPTP-treated mice showed a reduction in TH protein level in the HDB ($P < 0.01$). This was prevented by nicotine treatment ($P < 0.05$) (Fig. 5a, b). Immunofluorescence images in the HDB also exhibited similar decreased changes of dopaminergic innervation in MPTP-treated mice ($P < 0.001$), what's more, nicotine treatment restored this damage ($P < 0.01$) (Fig. 5c). We also calculated the percentage of TH nerve fibers in HDB, and the results were consistent with changes in TH protein level of the four groups (Fig. 5d).

4. Discussion

In this study, we demonstrated that MPTP-treated mice had obvious olfactory dysfunction, along with motor deficit and nigral dopaminergic neuron loss. Chronic treatment with nicotine not only exhibited protection against dopaminergic neuron loss in the midbrain but also improved the olfactory function in MPTP-treated mice. Moreover, our results showed nicotine alleviated MPTP-induced decrease of ChAT expression in the HDB and OB, which is critical for olfaction modulation (D'Souza and Vijayaraghavan, 2014).

Olfactory dysfunction in the identification, discrimination, and odor memory is associated with PD (Xiao et al., 2014). Different from humans, the olfactory system in the rodent is particularly complex. It has two separate olfactory pathways: main olfactory system and accessory olfactory system. Although both systems were involved in odor detection, the main olfactory system is dominant in a common molecular analyzer that is capable of detecting different subtle (Rodriguez et al., 2002). Herein we make the block test to evaluate the odor discrimination of social scent in mice. We found all except MPTP-treated mice spent more time on novel scent in relative to own scent.

Olfactory threshold test was used to be an odor detection assay. As early as in 1976 the alterations of olfactory threshold were demonstrated in PD patients (Ansari and Johnson, 1975). The olfactory threshold is often measured using smell identification tests such as UPSIT in the clinic. Studies found a strong inverse association between smoking and PD (Thacker et al., 2007). Lucassen et al found higher scores on the UPSIT in 22 smokers with PD than in 54 PD patients who had never smoked (Lucassen et al., 2014). Here we adopted a kind of common and simple method to examine the olfaction in mice. Our results showed that MPTP-treated mice spent much more time in finding the hidden pellet than controls. And nicotine treatment could save time in seeking the pellet. This indicates that MPTP enhanced the olfactory threshold in mice, which was attenuated by nicotine.

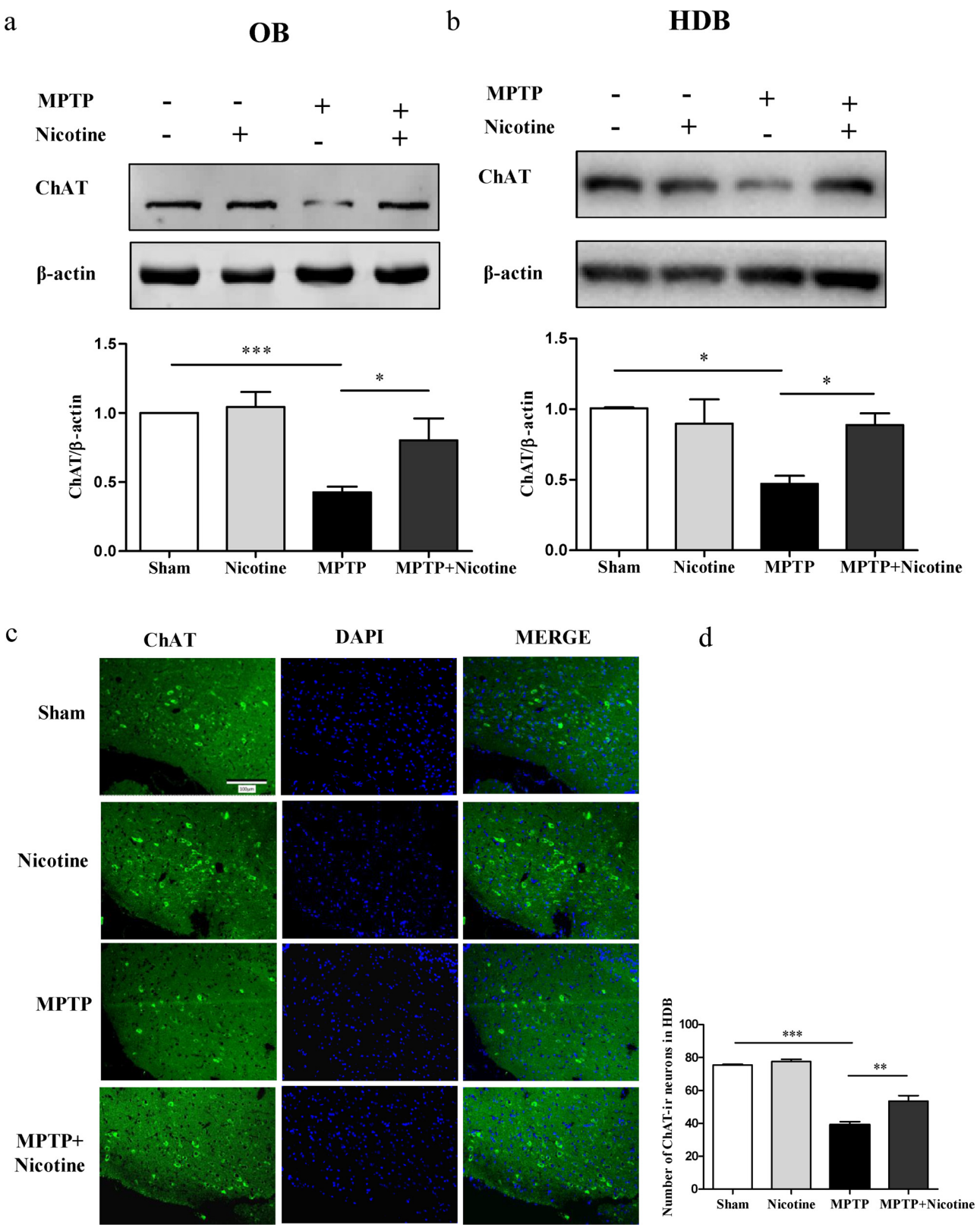

Fig. 4. Nicotine attenuated ChAT expression in the MPTP-induced OB and HDB. (a, b) The expression of ChAT in OB(a) and HDB(b) was determined by western blot. N = 3 per group. (c) Changes of ChAT neurons distribution in HDB, as revealed by immunofluorescence. (d) The number of the positive neurons was collected from coronal sections of the HDB mice for each group. Scale bar at 100μm. N = 3 per group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. One-way analysis of variance and Tukey's post-hoc test.

The olfactory memory is another standard to evaluate dysosmia and can be divided into two distinct ways: long-term memory and short-term memory. The short-term memory seems to demand more extensive encephalic regions including olfactory bulb, piriform and entorhinal cortices and the hippocampus, with the entorhinal cortex as a key region (Sanchez-Andrade et al., 2005). In this study, we tested the

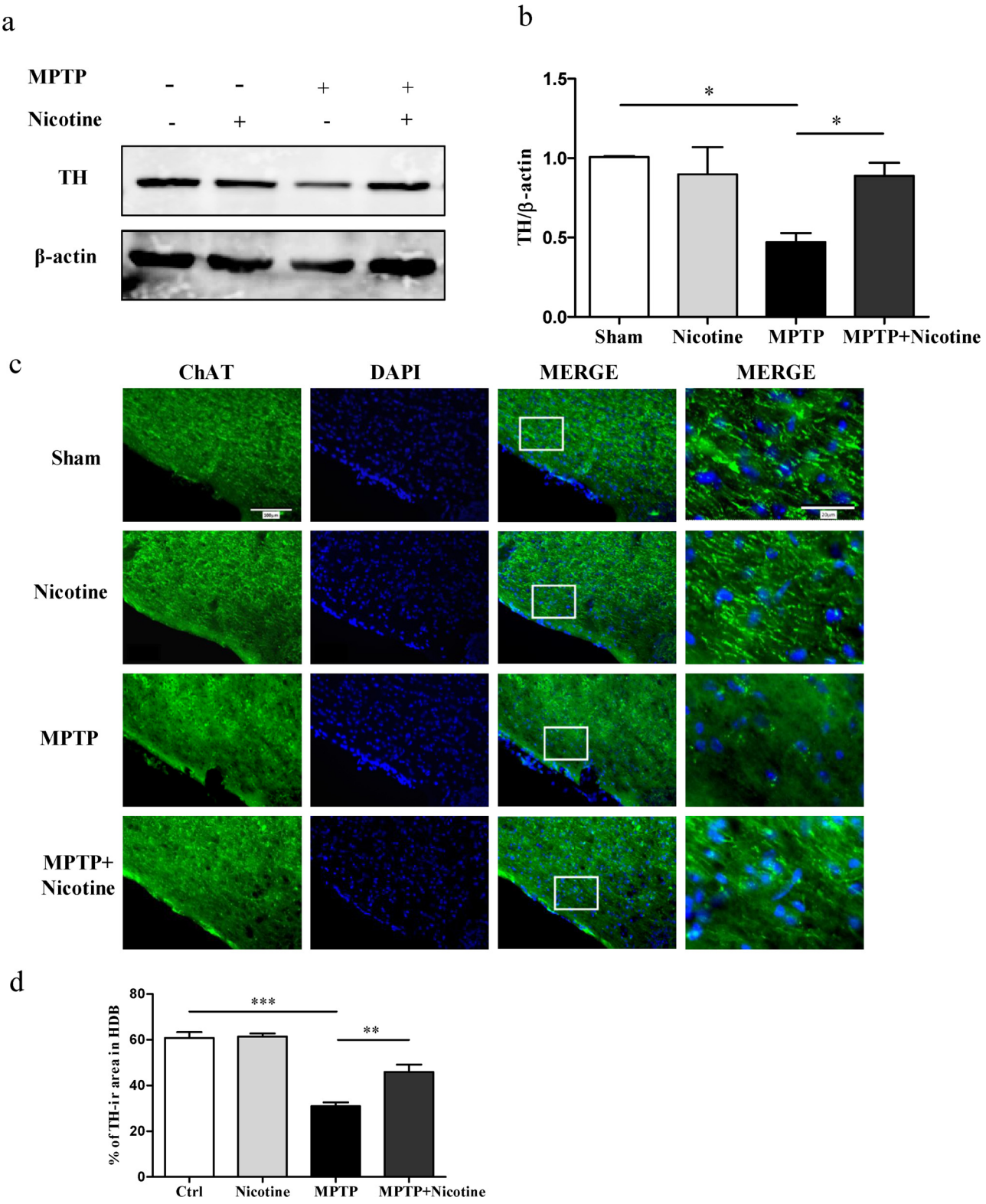

Fig. 5. Nicotine attenuated TH expression in HDB. (a, b) TH expression in HDB as determined by western blot. N = 3 per group. (c) The distribution of TH nerve fibers in the HDB, as demonstrated by immunofluorescence. (d) The percentage of TH-ir area in the HDB was quantified through coronal sections of the HDB mice for each group. Scale bar at 100μm and 20μm. N = 3 per group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. One-way analysis of variance and Tukey's post-hoc test.

short-term memory which requires a distributed neural system in mice. Our study showed that there was no effect of MPTP on olfactory memory, consistent with the previous report that no significant changes were found in the olfactory memory of αSyn^{A53T} mice(Zhang et al., 2015), which is a commonly used animal model of PD. What's more, nicotine did not affect it.

Odor dysfunction is an early 'pre-clinical' sign of PD, but its mechanisms remain unclear. Several factors such as the neuron loss in OB, Lewy body formation in the olfactory system (Duda, 2010), and microglial activation within the olfactory bulb (Lalancette-Hebert et al.,

2009), have been proposed for the hyposmia in PD. Nevertheless, it is incontrovertible that OB plays a critical role in the olfactory system. The structures of OB are sophisticated. OB consists of various neuronic systems including cholinergic, serotonergic, dopaminergic and noradrenergic systems. The cholinergic system acts as the major regulator of olfactory function (Cui et al., 2006). Cholinergic dysregulation in OB may contribute to the olfactory dysfunction in PD. The cholinergic interneuron in the OB mainly receives the projections from HDB in the basal forebrain, which adjust cortical activity by a relative autocephalous method. Inputting fibers from HDB is complete at postnatal day 12. Then they could distribute into different layers of OB (Salcedo et al., 2011). Our studies showed that the cholinergic interneurons in OB and HDB may be damaged in MPTP-treated mice as ChAT expression was downregulated in these two regions. The mechanisms for this cholinergic neuron loss need further study. But our results are consistent with a previous study that the cholinergic neurons of the vertical limb in the diagonal band were reduced in MPTP-treated mice (Szego et al., 2011). Interestingly, our findings showed that nicotine could rescue this reduction of ChAT in OB and HDB in MPTP-injected mice.

The previous study reported a loss of cholinergic neurons and a decrease of dopaminergic innervation in the HDB of MPTP-treated monkey (Mundinano et al., 2013). Our results found the reduction of cholinergic neuron and depletion of dopaminergic innervation of HDB in MPTP-treated mice. Although previous studies show selective toxicity of MPTP to dopaminergic neurons, our and other studies suggested that dopamine decrease in itself might reduce the cholinergic neurons in HDB (Heise et al., 2005). Moreover, we found nicotine alleviated the loss of cholinergic neuron and dopaminergic innervation of HDB in MPTP-treated mice. Besides, treatment with nicotine protected against the loss of dopaminergic neurons in the VTA and SNpc of MPTP-treated mice, consistent with previous reports (Li et al., 2000). As we know VTA is the origin of the central dopamine input to the HDB. Nicotine could activate different nicotine receptors such as α4β2 or α7nAChRs. The α7nAChR is extensively expressed in the VTA and SNpc. A previous study showed that nicotine activated PI3K/Akt/Bcl-2 signal pathway via the activation of α7nAChRs and thus protected dopaminergic neurons (Akaike, 2009).

In sum, our findings demonstrate that nicotine could improve hyposmia, as seen in MPTP-treated mice, by increasing the cholinergic tone in basal forebrain through its neuroprotection against dopaminergic neuron loss in the midbrain.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (91649114);Jiangsu Provincial Key R&D Program(BE2018658); Jiangsu Provincial Medical Key Discipline Project (ZDXKB2016022); Suzhou Clinical Research Center of Neurological Disease (Szzx201503) and also partly supported by Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References

Akaike, A., 2009. Functional changes induced by long-term stimulation of nicotinic acetylcholine receptors. Foreword. Biol Pharm. Bull. 32 (3), 317.
Ansari, K.A., Johnson, A., 1975. Olfactory function in patients with Parkinson's disease. J. Chronic Dis. 28 (9), 493–497.
Baranyi, M., et al., 2016. Novel (Hetero)arylalkenyl propargylamine compounds are protective in toxin-induced models of Parkinson's disease. Mol. Neurodegener. 11, 6.
Cao, M., et al., 2016. Olfactory dysfunction in parkinson's disease patients with the LRRK2 G2385R variant. Neurosci. Bull. 32 (6), 572–576.
Cui, Q.L., Fogle, E., Almazan, G., 2006. Muscarinic acetylcholine receptors mediate oligodendrocyte progenitor survival through Src-like tyrosine kinases and PI3K/Akt pathways. Neurochem. Int. 48 (5), 383–393.
D'Souza, R.D., Vijayaraghavan, S., 2014. Paying attention to smell: cholinergic signaling in the olfactory bulb. Front. Synaptic Neurosci. 6, 21.
Devore, S., Linster, C., 2012. Noradrenergic and cholinergic modulation of olfactory bulb sensory processing. Front. Behav. Neurosci. 6, 52.
Duda, J.E., 2010. Olfactory system pathology as a model of Lewy neurodegenerative disease. J. Neurol. Sci. 289 (1-2), 49–54.
Gao, L.L., Wu, T., 2016. The study of brain functional connectivity in Parkinson's disease. Transl. Neurodegener. 5, 18.
Goes, A.T., et al., 2014. Neuroprotective effects of swimming training in a mouse model of Parkinson's disease induced by 6-hydroxydopamine. Neuroscience 256, 61–71.
Gu, P.S., et al., 2017. Mulberry fruit ameliorates Parkinson's-disease-related pathology by reducing alpha-synuclein and ubiquitin levels in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid model. J. Nutr. Biochem. 39, 15–21.
Heise, C.E., et al., 2005. Cell survival patterns in the pedunculopontine tegmental nucleus of methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys and 6OHDA-lesioned rats: evidence for differences to idiopathic Parkinson disease patients? Anat. Embryol. (Berl.) 210 (4), 287–302.
Kang, W., et al., 2016. The predictive value of SS-16 in clinically diagnosed Parkinson's disease patients: comparison with (99m)Tc-TRODAT-1 SPECT scans. Transl. Neurodegener. 5, 15.
Krosnowski, K., et al., 2012. Diverse populations of intrinsic cholinergic interneurons in the mouse olfactory bulb. Neuroscience 213, 161–178.
Lalancette-Hebert, M., et al., 2009. Live imaging of Toll-like receptor 2 response in cerebral ischaemia reveals a role of olfactory bulb microglia as modulators of inflammation. Brain 132 (Pt 4), 940–954.
Lehmkuhl, A.M., Dirr, E.R., Fleming, S.M., 2014. Olfactory assays for mouse models of neurodegenerative disease. J. Vis. Exp. 90, e51804.
Li, Y., King, M.A., Meyer, E.M., 2000. alpha7 nicotinic receptor-mediated protection against ethanol-induced oxidative stress and cytotoxicity in PC12 cells. Brain Res. 861 (1), 165–167.
Lucassen, E.B., et al., 2014. History of smoking and olfaction in Parkinson's disease. Mov. Disord. 29 (8), 1069–1074.
Mundinano, I.C., et al., 2013. Reduced cholinergic olfactory centrifugal inputs in patients with neurodegenerative disorders and MPTP-treated monkeys. Acta Neuropathol. 126 (3), 411–425.
Rodriguez, I., et al., 2002. Multiple new and isolated families within the mouse superfamily of V1r vomeronasal receptors. Nat. Neurosci. 5 (2), 134–140.
Salcedo, E., et al., 2011. Activity-dependent changes in cholinergic innervation of the mouse olfactory bulb. PLoS One 6 (10), e25441.
Sanchez-Andrade, G., James, B.M., Kendrick, K.M., 2005. Neural encoding of olfactory recognition memory. J. Reprod. Dev. 51 (5), 547–558.
Senatorov, V.V., Renaud, L.P., 1999. Projections of medullary and pontine noradrenergic neurons to the horizontal limb of the nucleus of diagonal band in the rat. Neuroscience 88 (3), 939–947.
Sharer, J.D., et al., 2015. Olfactory dysfunction in Parkinson's disease: positive effect of cigarette smoking. Mov. Disord. 30 (6), 859–862.
Singh, S., et al., 2009. The expression of CYP2D22, an ortholog of human CYP2D6, in mouse striatum and its modulation in 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease phenotype and nicotine-mediated neuroprotection. Rejuvenation Res. 12 (3), 185–197.
Szego, E.M., et al., 2011. Dopamine-depletion and increased alpha-synuclein load induce degeneration of cortical cholinergic fibers in mice. J. Neurol. Sci. 310 (1-2), 90–95.
Thacker, E.L., et al., 2007. Temporal relationship between cigarette smoking and risk of Parkinson disease. Neurology 68 (10), 764–768.
Tillerson, J.L., et al., 2006. Olfactory discrimination deficits in mice lacking the dopamine transporter or the D2 dopamine receptor. Behav. Brain Res. 172 (1), 97–105.
Xiao, Q., Chen, S., Le, W., 2014. Hyposmia: a possible biomarker of Parkinson's disease. Neurosci. Bull. 30 (1), 134–140.
Zhang, S., Xiao, Q., Le, W., 2015. Olfactory dysfunction and neurotransmitter disturbance in olfactory bulb of transgenic mice expressing human A53T mutant alpha-synuclein. PLoS One 10 (3), e0119928.