ORIGINAL ARTICLE

Sleep Science

Olfactory impairment is related to REM sleep deprivation in rotenone model of Parkinson's disease

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ABSTRACT

Introduction: Olfactory dysfunction affects about 85-90% of Parkinson's disease (PD) patients with severe deterioration in the ability of discriminate several types of odors. In addition, studies reported declines in olfactory performances during a short period of sleep deprivation. Besides, PD is also known to strongly affect the occurrence and maintenance of rapid eye movement (REM) sleep. **Methods:** Therefore, we investigated the mechanisms involved on discrimination of a social odor (dependent on the vomeronasal system) and a non-social odor (related to the main olfactory pathway) in the rotenone model of PD. Also, a concomitant impairment in REM sleep was inflicted with the introduction of two periods (24 or 48 h) of REM sleep deprivation (REMSD). Rotenone promoted a remarkable olfactory impairment in both social and non-social odors, with a notable modulation induced by 24 h of REMSD for the non-social odor. **Results:** Our findings demonstrated the occurrence of a strong association between the density of nigral TH-ir neurons and the olfactory discrimination capacity for both odorant stimuli. Specifically, the rotenone-induced decrease of these neurons tends to elicit reductions in the olfactory discrimination ability. **Conclusions:** These results are consistent with the participation of the nigrostriatal dopaminergic system mainly in the olfactory discrimination of a non-social odor, probably through the main olfactory pathway. Such involvement may have produce relevant impact in the preclinical abnormalities found in PD patients.

Keywords: Olfactory discrimination; Dopamine; Rotenone; REM sleep deprivation; Parkinson disease.

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease that affects a growing segment of the population with the progression of age[1,2]. Currently, diagnosis occurs only in the presence of motor symptoms, although, several non-motor signs have been recognized as important features of this disease, and precedes motor symptoms. Two of these well-documented prodromal signs are the sleep and olfactory disturbances. In fact, studies demonstrated the occurrence of rapid eye movement (REM) sleep suppression inflicted by the nigrostriatal lesion[3,4], pharmacological dopaminergic blockade[5] or dopamine (DA) transporter knockout (DAT-KO)[6]. In addition, olfactory dysfunction affects about 85-90% of PD patients[7,8] with remarkable deterioration of detection, discrimination and odor identification[9-11].

Such impairment in PD, originally described by Ansari and Johnson[12], is supported by neuropathological findings of Lewy bodies presence in the olfactory bulb, olfactory tract and anterior olfactory nucleus in preclinical Braak stages prior to significant nigral degeneration[13]. Moreover, it has been reported worsening of olfactory function in the smelling of certain odors in detriment of others, using the odor identification test[14]. Regarding experimental studies in rodents, two distinct odors may be categorized: social and non-social. Accordingly, social odors present a more intricate processing, requiring the participation of the main olfactory pathway and mostly the vomeronasal system[15,16]. Whereas, non-social odors are exclusively processed by the main olfactory pathway[16].

Interestingly, sleep deprivation adversely affected the olfactory performance in rats[17,18]. Disturbances in olfactory function of people with REM sleep disturbances are also found[17,19-22]. In this sense, various studies have observed and discussed the involvement of the dopaminergic system in olfaction, since DA seems to operate as a key player in the modulation of the glomerular activity generated from the sensory afferents to mitral/tuffed cells[8,23-26]. Conjointly, it has been found an enormous increase in the number of tyrosine hydroxylase immunoreactive (TH-ir) interneurons compared to controls in the glomerular layer of the olfactory bulb from PD patients[24,27]. It is discussed that this increment could be responsible for the inhibition of glomerular activity, promoting the hyposmia[28,29]. Such mechanism is attributed to the inhibitory effect of DA[24,27], mediated by D2 receptors activity[30], causing the suppression of the olfactory information. A very similar deficit has been recently described after the intranigral administration of rotenone in rats, reinforcing such process[31]. In fact, the mechanisms linking these findings remain unclear. However, a recent study reported a direct axonal dopaminergic projection from the substantia nigra pars compacta (SNpc) to the olfactory bulb of rats[32]. Therefore, it is suggested that the degeneration of the nigro-olfactory dopaminergic fibers contribute to the occurrence of hyposmia in PD[32]. However, the neurobiological basis of olfactory deficits produced by REMSD alone or associated to a nigrostriatal lesion remains to be clarified.

In view of that, in the present study we employed the intranigral rotenone model of PD leading to a massive mitochondrial inhibition and selective degeneration of dopaminergic neurons in the SNpc[31,33-35]. It is therefore feasible that the well-known dopaminergic supersensitivity effect induced by REMSD[5,36], and also by rotenone nigral lesion[35], could affect the activity of dopaminergic system, generating an increase in their inhibitory effect, therefore promoting a more pronounced disruption in olfactory function. Accordingly, the main goal of this study was to test if such pattern of olfactory dysfunction may vary for social (dependent on the vomeronasal system) and non-social (dependent on the main olfactory pathway) odors.

Experimental Procedures

Animals

Male Wistar rats weighing 280–320 g at the beginning of the protocols were used. They were housed in groups of five in polypropylene cages with wood shavings as bedding and maintained in a temperature-controlled room (22±2ºC) on a 12-h light-dark cycle (lights on at 7:00 AM) with food and water provided *ad libitum*. All experiments were conducted in accordance with guidelines of Brazilian Guide for Care and Use of Laboratory Animals (COBEA) and the protocol complies with the recommendations of Federal University of Paraná and was approved by the Institutional Ethics Committee (approval ID # 651).

Experimental design

First experiment - Determination of non-social odor preference

Non-social olfactory preference test in a radial maze

In this experiment, we aimed to determine a pattern of olfactory preference for a non-social odor. To execute that we exposed 10 rats to different non-social odors (mint, musk, vanilla, lemon and water as a control) in a radial maze with five arms. The central zone was a pentagon (20 cm x 20 cm x 25 cm height), while the arms had a square shape (17 cm x 17cm x 25 cm height). The animals were free to enter and explore all the arms of the radial maze during the sessions. At the end of the arms the odor was presented to the animals in plastic containers (50 mL falcon tube) with several small holes (about 1 mm of diameter each). Inside the containers there was a filter paper (3 cm x 1 cm) soaked with 100 µL of the odor essence (Essências Curitiba, Brazil) or water (control). Each rat was tested in 6 different sessions of 3 minutes. All the odors (including the water - control) were presented simultaneously (1 odor/arm) during the sessions, however, the sequence of the odors within the arms was changed for each session in order to avoid a spatial learning bias. Clean sawdust was included in all of the arms to dilute odorants and work as a consistent background odor for all non-social odor containers.

All the sessions were video-recorded for subsequent analysis of the following parameters: frequency of arms exploration and time of arms exploration. Results are presented as mean of the 6 sessions (Figure 2) and indicated that the

lemon odor was preferred as an indicator of non-social odor preference; hence, we selected this odor as a non-social olfactory stimulus for the second experiment.

Second experiment - Possible olfactory impairment generated by SNpc lesion associated with REMSD

Before the stereotaxic surgeries, the rats were randomly distributed in two groups: sham (n=10) and rotenone (n=10). Seven days after the rotenone nigral infusion the animals were subjected to the olfactory discrimination task (ODT), for both social and non-social odors, in three different time-points: 24 h before REMSD (Baseline); immediately after 24 or 48 h of REMSD (REMSD) and 24 or 48 h after that (Rebound) (Figure 1). We executed this protocol twice, each time for a different period of REMSD tested: 24 h (sham n=10; rotenone n=10) (Figure 1A) and 48 h (sham n=10; rotenone n=10) (Figure 1B). Immediately, after the last time-point tested, the rats had their brains perfused and fixed for subsequent immunohistochemical analysis of SNpc TH-ir neurons.

Stereotaxic surgery

Rats were sedated with intraperitoneal xylazine (10 mg/kg; Syntec do Brasil Ltda, Brazil) and anesthetized with intraperitoneal ketamine (90 mg/kg; Syntec do Brasil Ltda, Brazil). The following coordinates were used to the bilateral injury, bregma as a reference: SNpc (AP)=−5,0 mm, (ML)=± 2,1 mm e (DV)=−8,0 mm (Paxinos and Watson, 2005). Needles were guided to the region of interest for a bilateral infusion of 1 μL of rotenone (12 μg/μL), or of dimethylsulfoxide - DMSO (Sigma-Aldrich®, United States) for the sham group. Using an electronic infusion pump (Insight Instruments, Ribeirão Preto, Brazil) at a rate of 0.33 μL/min[31,35,37,38].

REMSD and Rebound procedures

REMSD was performed as previously described by Tufik et al.[36], using the single platform method. Rats were individually placed on a circular platform (6.5 cm in diameter) in a cage (23×23×30 cm) filled with water up to 1 cm below the platform level. At the onset of each REM sleep episode, the animal experiences a loss of muscle tonus and falls into the water, thus being awakened. When platforms of this size are used, REM sleep is completely eliminated[39]. Throughout the study, the experimental room was maintained at controlled conditions (22 ± 2ºC, 12:12 h light/dark cycle, lights on 7:00 a.m.). Food and water were provided *ad libitum* by placing chow pellets in a dispenser positioned inside the cage and water bottles on a grid located on top of the tank. The duration of REMSD periods was equivalent to the duration of the respective rebounds.

Social and non-social odor discrimination task (ODT)

This test was previously described by Soffié and Lamberty and subsequently modified by Prediger et al.[40-42] and recently used by Rodrigues et al.[31]. A rectangular arena (60 x 40 x 50 cm) bisected by a dividing, with door allowing free passage between the two compartments was used. There was a period of adaptation in the apparatus for 5 minutes, during which the animals were free to explore both compartments with fresh sawdust. For the social discrimination, during the test, one compartment presented sawdust loaded with the familiar odor of the animal (obtained from its exposure to this sawdust during the preceding 48 h).

The other compartment presented new clean sawdust, designated as a non-familiar odor. For the non-social discrimination, during the test, both compartments presented clean sawdust, however, in one compartment there was lemon essence (100 μL in a filter paper inside of the pierced 50 mL falcon tube) and in the opposite compartment water as a control (equally presented).

The test started by placing the animal in the middle of the discrimination box, and the exploratory behavior in the compartments was recorded during 5 minutes. It is expected that the animal with olfactory impairment tends to explore both compartments equally, indicating absence of discrimination. The opposite is also expected if the olfactory function is intact, i.e., when animals prefer to explore a particular compartment[43].

As a parameter of discrimination, the "discrimination index (DI)" was calculated by dividing the difference in exploration time between the two compartments (non-familiar compartment - familiar compartment) by the total amount of exploration for both compartments (non-familiar compartment + familiar compartment). DI was then multiplied by 100 to express it as a percentage[31,35]. DI equals to zero corresponds

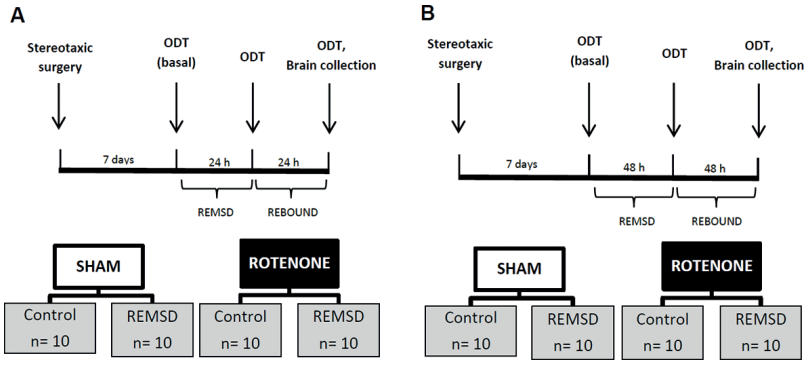

Figure 1. Schematic representation of the second experiment. A. 24 h of REMSD. B. 48 h of REMSD. Olfactory discrimination task (ODT), REM sleep deprivation (REMSD).

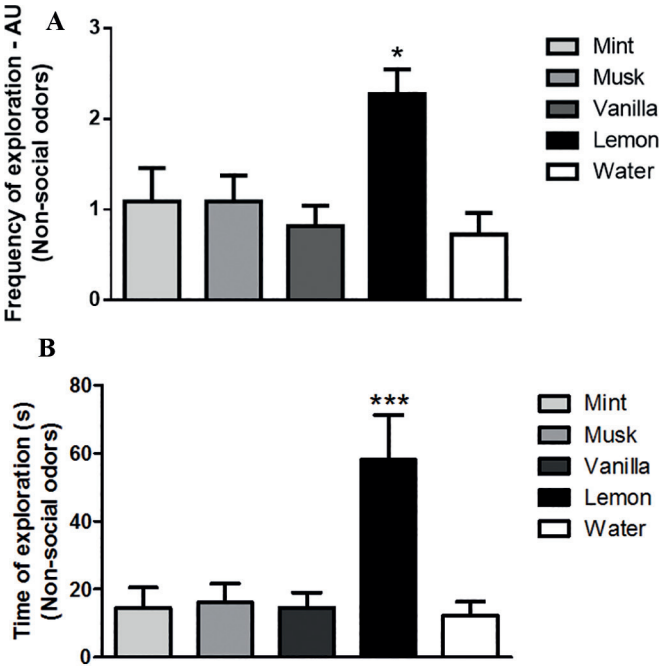

Figure 2. Determination of non-social odor preference. A. Frequency of exploration (arbitrary units - AU) spent in each odorant stimuli. B. Time (s) of exploration spent in each odorant stimuli. The bars represent the mean ± standard error of the mean, n=10 per group, *P≤0.05, ***P≤0.001. One-way ANOVA followed by the Newman-Keuls test.

to a full preference towards non-familiar odor. Negative scores correspond to a preference towards familiar odor.

TH-ir immunohistochemistry

Density of TH-ir neurons was estimated within the SNpc. Animals were deeply anesthetized, with ketamine, immediately after the behaviors tests, and were transcardially perfused with saline first, then with 4% of the fixative solution formaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed from the skulls and were immersed for 48 h in that fixative solution at 4ºC. Subsequently, the brains were placed in 30% sucrose solution for 3 days and were frozen at -80ºC before sectioning. Series of 40 µm thick sections were cut on a cryostat (-20°C) in the frontal plane, and collected at the -4.92 mm to -5.52 mm from the bregma[44].

Tissue sections were incubated with primary mouse anti-TH antibody, diluted in phosphate-buffered saline containing 0.3% Triton X-100 (1:500; Chemicon, CA, USA) overnight at 4ºC. Biotin-conjugated secondary antibody incubation (1:200 anti-mouse # Vector Laboratories, USA), was performed for 2 h at room temperature. After several washes in phosphate-buffered saline, antibody complex was localized using the ABC system (Vectastain ABC Elite kit, Vector Laboratories, USA) followed by 3,3-diaminobenzidine reaction with nickel enhancement.

The sections were then mounted onto gelatin-coated slides and coverslipped after dehydration in ascending concentrations of ethanol-xylene solutions. Cell density counts were conducted making use of the software ImageJ (https://imagej.nih.gov/ij/). Counts were done on twelve sections (corresponding to the 480 µm interval), and an average density per section (and consequently for each animal) was obtained. For each group a mean value was calculated and converted to a percentage relative to the sham group, and compared with rotenone group. The mean density of TH-ir neurons in each hemisphere was considered representative of the SNpc neuronal cells in each animal. The images were obtained using a motorized Axio Imager Z2 microscope (Carl Zeiss, Jena, DE), equipped with an automated scanning VSlide (Metasystems, Altlussheim, DE).

Statistical Analysis

Differences between groups in the ODT were analyzed by two-way analysis of variance (ANOVA) with lesion as the between-subjects factor, REMSD as the within-subjects factor and interaction between these factors as the interaction factor - followed by the Bonferroni post hoc test. The pattern of olfactory preference for a non-social odor was analyzed by one-way ANOVA followed by the Newman-Keuls multiple comparison test. TH-immunohistochemistry was analyzed by unpaired two-tailed t Test. Pearson's correlation coefficients (r) were calculated to establish relationships between the percentage of TH-ir neurons density and the DI obtained from social and non-social odors. Values were expressed as mean ± standard error of mean (SEM). The level of significance was set at $p \leq 0.05$.

RESULTS

First experiment - Determination of non-social odor preference

As can be seen in Figure 2, the animals were exposed to a number of different non-social odors. According to the frequency of exploration parameter (Figure 2A) the rats exhibited a significant preference for the lemon odor ($p<0.05$) in comparison to the others tested [$F_{(4,54)}=4.84$; $p=0.002$]. In addition, considering the time of exploration (Fig. 2B), the animals showed an equal increment ($p<0.001$) of this parameter for the lemon odor compared to the others [$F_{(4,54)}=6.88$; $p=0.0002$].

Second experiment - Possible olfactory impairment generated by SNpc lesion associated with REMSD

Figure 3 shows the DI obtained from the ODT of social (Figure 3A) and non-social odors (Figure 3B). Accordingly, the rotenone group demonstrated a significant increase ($p<0.01$) in the DI compared to the sham group in the baseline. Likewise, the rotenone group remained exhibiting an increased DI after 24 h of REMSD ($p<0.001$) compared to the sham baseline group as indicated by the lesion [$F_{(2,30)}=1.74$; $p=0.19$], time [$F_{(2,28)}=0.84$; $p=0.44$] and interaction [$F_{(1,30)}=9.21$; $p\leq0.01$] factors.

Regarding the non-social ODT (Figure 3B), it was observed a significant increase in the DI of the rotenone group ($p<0.05$) compared to the sham group in the baseline. In addition, both REMSD groups showed an increment ($p<0.05$) in the DI when compared to the sham baseline group as demonstrated by

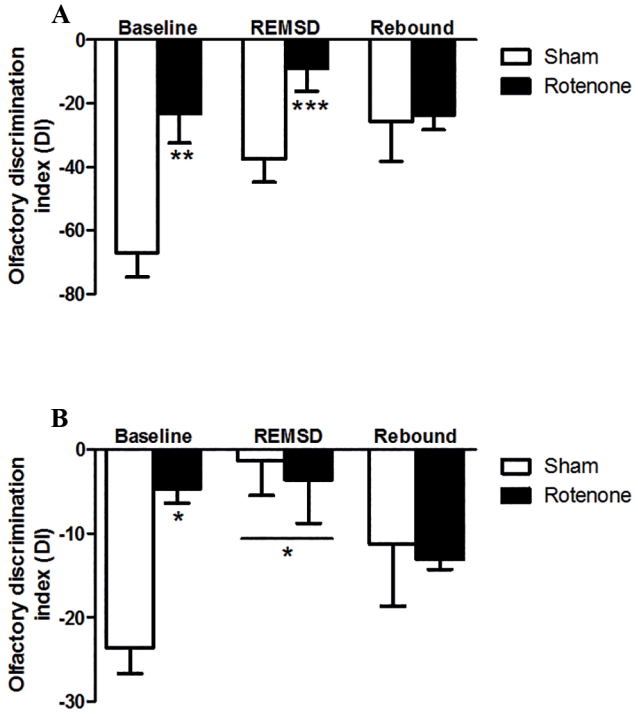

Figure 3. Olfactory discrimination index (DI) obtained from 24 h of REMSD and 24 h of rebound. A. Social odor. B. Non-social odor (lemon). The bars represent the mean ± standard error of the mean, n=10 per group, *P≤0.05, **P≤0.01, ***P≤0.001 compared to the sham baseline. Two-way ANOVA followed by the Bonferroni test.

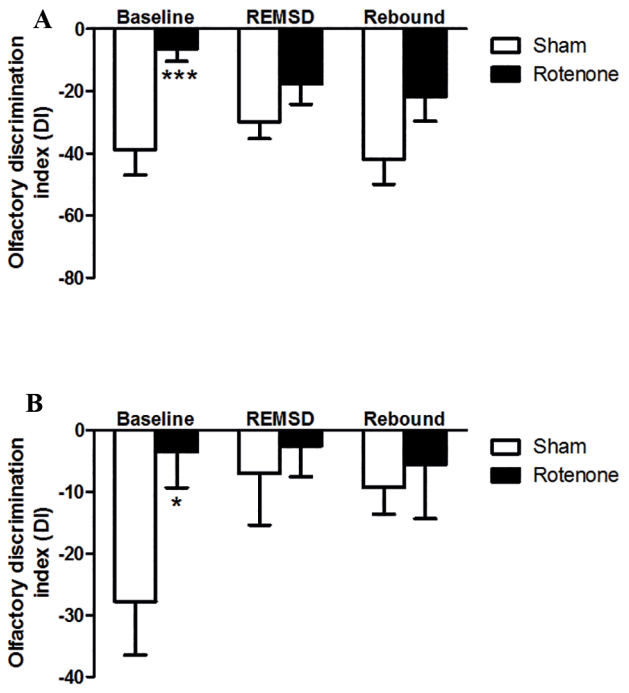

Figure 4. Olfactory discrimination index (DI) obtained from 48 h of REMSD and 48 h of rebound. A. Social odor. B. Non-social odor (lemon). The bars represent the mean ± standard error of the mean, n=10 per group, *P≤0.05, **P≤0.01, ***P≤0.001 compared to the sham baseline. Two-way ANOVA followed by the Bonferroni test.

the lesion [$F_{(2,20)}$=2.09; p=0.18], REMSD [$F_{(2,20)}$=1.29; p=0.30] and interaction [$F_{(1,30)}$=1.2; p=0.32] factors.

Regarding the 48 h of REMSD exposure (Figure 4) the rotenone group exhibited a significant increase (p<0.001) in the DI, compared to the sham group in the baseline. However, we did not detect significant differences between the groups tested concerning the discrimination of a social odor following 48 h of REMSD and its respective rebound period (Figure 4A), as revealed by the lesion [$F_{(2,52)}$=9.44; p=0.003], REMSD [$F_{(2,52)}$=0.22; p=0.8] and interaction [$F_{(1,52)}$=1.03; p=0.36] factors. The analysis of the ODT for the non-social odor (Figure 4B) showed the occurrence of a significant increment (p<0.05) in the DI for the rotenone in comparison to the sham control group in the baseline. Analogously, it was not observed significant differences between the groups after the 48 h period of REMSD and its respective rebound according to the lesion [$F_{(2,52)}$=3.56; p=0.05], REMSD [$F_{(2,52)}$=1.32; p=0.27] and interaction [$F_{(1,52)}$=1.42; p=0.25] factors.

To access the extension of the neuronal lesion inflicted by rotenone we determined the density of TH-ir neurons within the SNpc, since this region is highly populated by such neurons. In fact, we observed a significant reduction of about 40% (p<0.0001; t=7.92 df=25) in the TH-ir neurons density in comparison to the sham group (Figure 5).

Pearson's correlation coefficients (Table 1) revealed significant moderate negative correlations (r=-0.7; p=0.006) and (r=-0.7; p=0.003) between the percentage of TH-ir neurons density and the DI obtained for a social odor at the baseline periods of analysis. However, this level of correlation was only observed after 24 h of REMSD (r=-0.6; p=0.02) and not at 48 h of REMSD (r=-0.2; p=0.43) or their respective rebound periods (r=-0.07; p=0.8), (r=0.04; p=0.9).

Significant correlations were also detected for the non-social odor exposure at the baseline periods (r=-0.9; p=0.0004) and (r=-0.8; p=0.001). Conversely, this outcome was not observed after 24 (r=-0.2; p=0.64) or 48 h (r=0.007; p=0.9) of REMSD and their respective rebounds (r=0.1; p=0.8) and (r=0.3; p=0.24).

DISCUSSION

Neurotoxic effects of rotenone are typically related to nigrostriatal dopaminergic neurotransmission mimicking PD[35,38,45]. In the current study, we observed that the occurrence of TH-ir neuronal loss in the SNpc is able to inflict an olfactory impairment for both, social and non-social odors. Moreover, REMSD most likely generated a similar, however, more predominantly deficit in the discrimination of a non-social odor. Of note, this effect was related to a shorter period of REMSD (24 h). In fact, this is the first study, according to our knowledge, that compares the variations of the olfactory performances using different olfactory stimuli, social and non-social odor (lemon), after a rotenone exposure in different periods of REMSD.

Furthermore, the relationship between dopaminergic neurotransmission and REM sleep is a recent theme in the literature, and growing evidence suggests a significant impact of REM sleep disturbances in PD[46].

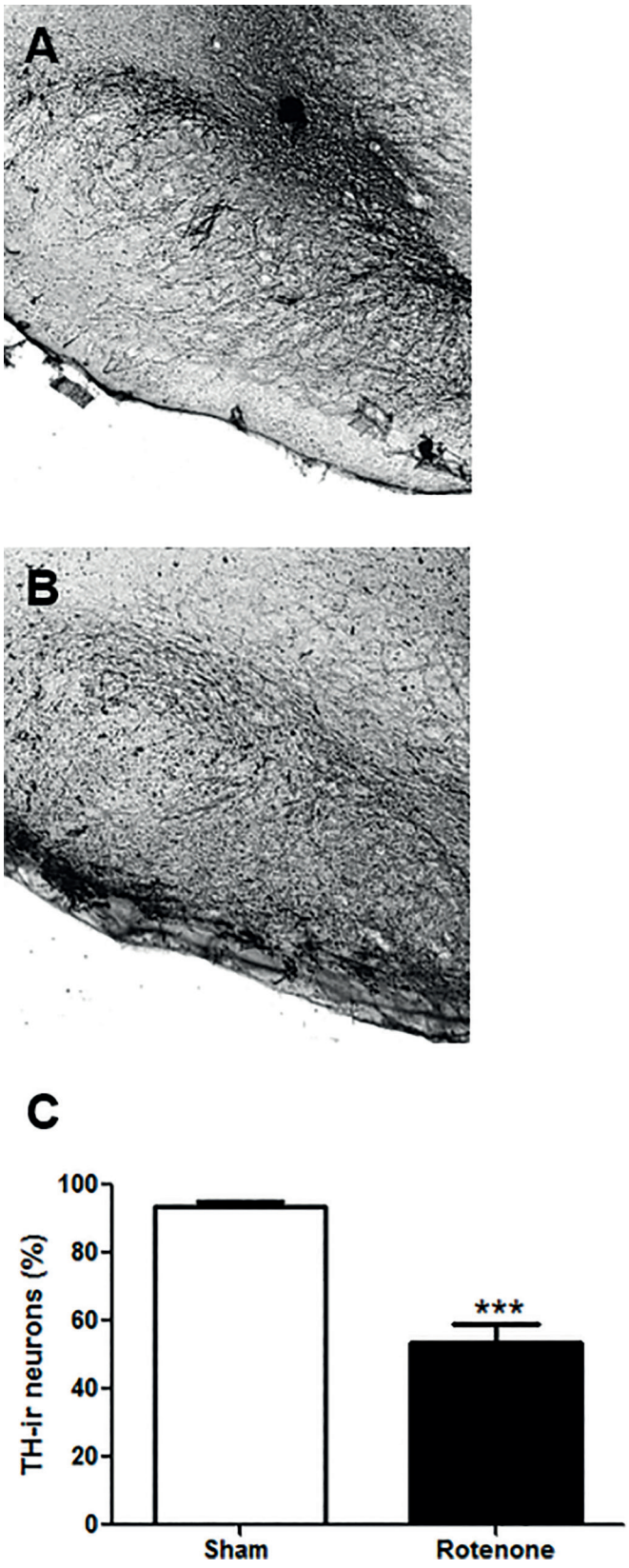

Figure 5. Representative immunohistochemistry labeling of SNpc TH-ir neurons. A. Sham group. B. Rotenone group. C. Percentage of TH-ir neurons density in relation to sham group. The bars represent the mean ± standard error of the mean, n=5 per group. ***P≤0.001. Two-tailed t Test.

Table 1. Pearson's correlations between the percentage of TH-ir neurons density within the SNpc and DI obtained from social and non-social odors.

Correlations	Periods		
	Baseline	REMSD	Rebound
Social odor			
TH-ir neurons x DI 24 h	r=-0.7 ; P=0.006*	r=-0.6 ; P=0.02*	r=-0.07 ; P=0.8
TH-ir neurons x DI 48 h	r=-0.7; P=0.003 *	r=-0.2 ; P=0.43	r=0.04 ; P=0.9
Non-social odor			
TH-ir neurons x DI 24 h	r=-0.9 ; P=0.0004*	r=-0.2 ; P=0.64	r=0.1 ; P=0.8
TH-ir neurons x DI 48h	r=-0.8; P=0.001*	r=0.007; P=0.9	r=0.3; P=0.24

*Significant correlations are indicated.

Besides, electrophysiological data indicated that the absence of half of the SNpc TH-ir neurons, in rats, provoked a major impairment in the sleep-wake parameters, predominantly in REM sleep[3]. Several sleep deprivation protocols show that REM sleep is related to dopaminergic neurotransmission, through the emergence of a robust dopaminergic D2 supersensitivity[36,47,48]. Further, in DAT-KO mice the selective activation of D2 receptors promoted recovery of REM sleep, suggesting that this receptor is related to REM sleep regulation[6]. Here we showed that REMSD associated with rotenone nigral lesion did not enhance this inhibitory effect generated by D2 receptors, therefore, not promoting a synergic olfactory impairment. This suggests the occurrence of different intensities of dopaminergic activation engaged by both manipulations.

Corroborating other studies, which also evaluated the participation of the dopaminergic system in the olfactory function[24,31,49,50], rotenone alone, appears to consistently reduce the DI in both social and non-social odor (lemon). Even more recently, it has been demonstrated, the existence of a direct dopaminergic projection, from the SNpc to the olfactory bulb, probably influencing olfactory performance, particularly in PD[32].

We observed that 24 h of REMSD affected the discrimination of lemon scent, independently of the rotenone lesion. Besides, a significant negative correlation (r=-0.7, p=0.006) is observed at the baseline period, between the percentage of SNpc TH-ir neurons density and DI, for the social odor. This finding indicates that decreased density in the SNpc neurons is associated to decreased olfactory discrimination performance.

A similar correlation (r=-0.6, p=0.02) is also detected after 24 h of REMSD, but not at the respective rebound period (r=-0.07, p=0.8) indicating that the olfactory impairment, for this condition (social odor), may be occurring although limited to a shorter period of REMSD. In addition, strong correlations obtained at the baseline for the non-social odor (lemon) (r=-0.9, p=0.0004 and r=-0.8, p=0.01) suggest a more prominent impairment in the discrimination of this modality of odorants compared to social odors.

Studies have shown that the preference for certain odors can be an important motivational factor[51,52]. In fact, social odors allow the transmission of some level of information between individuals. This characteristic can be exemplified by the presence of compounds with this type of odorant[15], such as (methylthio)methanethiol, present in the mice urine[53]. Indeed, a highly complex mixture of volatile and non-volatile molecules[15] forms the mixed composition of this type of social odor.

Thus, this processing seems to be more intricate, requiring the participation of both the main olfactory pathway and the vomeronasal system[15,16], which interact functionally and anatomically, to perform this function[54]. Whereas, odors destitute of social components are processed exclusively by the main olfactory pathway[16].

At this point a note of caution should be added. Despite the differences in olfaction between humans (microsmatic) and rats (macrosmatic), several studies observed declines in olfactory performances during a short period of sleep deprivation in both humans[20,21,55] and rats[17,18]. Therefore, more studies are needed to determine if these findings, obtained from animal models, could be extrapolated for a human condition.

Interestingly, we detected a strong association between the density of nigral TH-ir neurons and the olfactory discrimination capacity for both odorant stimuli, reinforcing the role of the nigro-olfactory projections for odors processing mechanisms. In addition, REMSD and nigrostriatal lesion can induce dopaminergic D2 supersensitivity[3,36,48,56]. The activation of this class of dopamine receptors modulates also the gamma-aminobutyric acid receptors (GABAA) of the mitral/tufted cells, facilitating GABAergic neurotransmission from TH-ir periglomerular neurons[57]. This fact should lead to an increase in the inhibition of the mitral/tufted cells[57]. Furthermore, when the pre-synaptic D2 receptors is selectively blocked, the release of glutamate is increased, thus producing an increase in DA and GABA levels through higher activation of dopaminergic juxtaglomerular neurons[7,58].

CONCLUSIONS

In conclusion, the present data provide novel evidence concerning the participation of the dopaminergic system mainly in the olfactory discrimination of a non-social stimulus, that is, the main olfactory pathway. Rotenone promoted a remarkable olfactory impairment in both types of odors with a notable modulation induced by 24 h of REMSD of the later.

The statistical correlations strongly suggest the occurrence of an association between the density of nigral TH-ir neurons and the olfactory discrimination capacity for both odorant stimuli. Specifically, the occurrences of manipulations that decrease these neurons tend to elicit reductions in the olfactory discrimination ability.

The DA modulation may have important roles in synaptic plasticity in the bulb, since there is a direct projection from the SNpc to the olfactory bulb[32] and also DA levels are increased during the odor learning process[59]. This phenomenon also affects other areas such as the striatum and SNpc. Therefore, these changes may have potential impact in the preclinical abnormalities found in PD patients.

Conflict of interests

The authors have declared that no conflict of interests exists.

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