Clinical and Experimental Health Sciences

Cellular, Behavioral, and Locomotor Effects of Oral Nicotine in Male Rats with Bilateral Lesions in the Ventrolateral Striatum Induced with 6-OHDA

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ABSTRACT

Objective: Parkinson's disease is a progressive neurodegenerative disease having a spectrum of non-motor to motor symptoms. Unrelated to motor symptoms of sensory, autonomic, and neuropsychiatric symptoms often appear early in the course of the disease. It is a remarkable observation that patients in the premotor phase can easily quit smoking without help. This study was intended to investigate the interrelation between nicotine and the partial loss of dopaminergic innervation in the ventrolateral striatum induced by 6-OHDA.

Methods: We used an experimental premotor parkinsonism model. The oral nicotine preference of rats was investigated with the two-bottle free choice method. The behaviors related to locomotor activity and emotional state were evaluated with a locomotor activity test, elevated plus maze, and forced swimming test. Histopathological evaluation was performed in the striatum by staining techniques using hematoxylin+eosin (H&E) and immunohistochemistry markers (caspase-3, and MAP-2).

Results: Bilateral 6-OHDA lesions did not lead to a significant alteration in the total locomotor activity or nicotine preference. Nicotine increased horizontal but decreased vertical movements in addition to increasing anxiolytic but also depressive effects in the OHDA lesion group. The number of apoptotic cells was significantly lower in the lesion group receiving nicotine compared to those not receiving nicotine.

Conclusion: Our experimental study points to the role of oral nicotine in male rats with bilateral striatal 6-OHDA lesions in the ventrolateral striatum. Further studies are needed to understand the relationship between loss of dopaminergic innervation in the striatum and nicotine consumption.

Keywords: Nicotine, Parkinson's Disease, 6-OHDA, Ventrolateral Striatum, Experimental.

1. INTRODUCTION

Parkinson's disease (PD), a neurodegenerative disorder, is characterized by motor symptoms resulting from the progressive loss of nigrostriatal dopaminergic neurons. It has been reported that motor symptoms appear with the loss of approximately 30% of dopaminergic neurons in the substantia nigra or 50% of dopaminergic terminals in the striatum (1). In the preclinical phase of PD, nonspecific findings including autonomic dysregulation, sleep disorders, depression, and anxiety are observed (2). Moreover, it has been indicated that smoking cessation seen in the preclinical phase might be an early non-motor sign of PD (3,4). The relationship between nicotine and PD has been known for the last half-century, and the prevalence of PD in smokers is approximately 50% lower than in non-smokers (5). Chuanga (2019) stated that the ease of smoking cessation may occur as a result of the loss of nicotinic responses that occur at the prodromal stage long before the diagnosis of PD (6).

Nicotine has a general protective effect against nigrostriatal degeneration, modulates dopamine release via nAChRs on dopaminergic terminals (7), and decreases L-dopa-induced dyskinesias (8). On the other hand, the possible impact of nicotine on other motor and nonmotor symptoms is still debated. The current study aimed to examine the relationship between striatal dopamine loss and nicotine in rats with the bilateral striatal 6-OHDA lesion based on nicotine consumption, emotional behavior, locomotor activity, and histopathological findings.

2. MATERIAL AND METHODS

2.1. Animals and Experimental Procedure

This study was approved by University Animal Research Ethics Committee (HAYDEK 9/3-2009, Kocaeli University,

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Turkey) and adhered to the principles of the Declaration of Helsinki. The directives of the Animal Research Ethics Regulation in Turkey (2011/28141) and "Europe Communities Council (2010/63/EU)" were abided by throughout the study.

We tested 35 male Wistar, albino rats aged 6-12 months. Each subject was housed individually and had ad-lib access to a standardized ration. They were left in 12 hours light 12 hours dark periods, in 21-24 ^{0}C temperatures, and with a relative humidity of 50-60%.

We divided the animals into three groups; the first group (6 – OHDA lesion + nicotine solution; n=15) (6-OHDA+NW), the second group (Sham lesion + nicotine solution; n=10) (SHAM+NW), and the third group 6-OHDA lesion + tap water; n= 10) (6-OHDA+TW). Subject numbers were preserved in the tests including water consumption, locomotor activity, forced swimming test, and elevated plus maze.

NW and TW were given to the first two groups for three weeks before surgery and for four weeks after the surgery by "two bottles free choice method" (9). During this period, rats in the 6-OHDA+TW group consumed only TW. Water consumption of the subjects was measured after 16:00 once every two days and fresh water bottles were prepared after each measurement. Standardized bottles and droppers were provided to each subject and an equal amount of nicotine solution (NW) and tap water (TW) was filled in each bottle. After 7 weeks of the monitoring period, animals were subjected to locomotor activity, elevated plus maze (EPM), and forced swimming tests (FST). On the first day, locomotor activity and EPM tests were performed, respectively. FST was applied over the next 2 days. During the behavioral tests, nicotine consumption continued in the first two groups. Following behavioral tests, anesthetized animals were perfused transcardially with saline and 4% formaldehyde. Brains were removed and kept in a fixative for histological processing. The chronological order of the experimental procedures is illustrated by the timeline diagram (Figure 1).

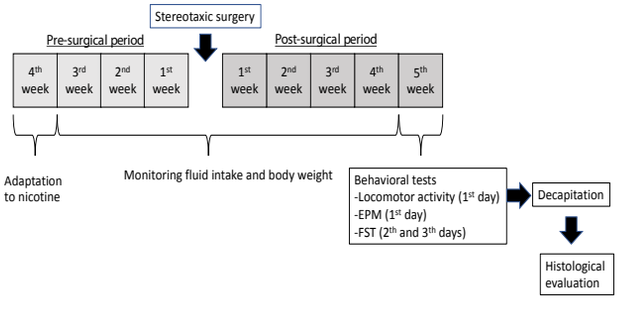

Figure 1. *Illustration of the experimental procedures*

2.2. Preparation of Oral Nicotinic Solution and Oral Nicotine Preference

The subjects in the 6-OHDA+NW and SHAM+NW groups were initially given nicotine (5 micrograms/ml) in water with non-caloric saccharin (4 mg/ml) to suppress the bitter taste of nicotine for a week. After this one-week-long adaptation period, the rats voluntarily consumed fluid from two bottles in their home cages, one with nicotine solution and the other with tap water.

For 3 weeks prior to the stereotaxic surgery, the saccharin dose was reduced to 4 mg/ml, 2 mg/ml, and 1 mg/ml in both NW and TW at one-week intervals, keeping nicotine at a constant dose of 5 microgram/ml. A fixed dose of 1 mg/ml saccharin was kept in both NW and TW from this point onward until the end of the experiment. Rats in the third group were given only tap water with reduced saccharin. Rats were weighted on a weekly basis. The starting and end weights of the bottles with NW and TW were measured separately. The sum of the two was calculated as the total water consumption. The weekly amount of fluid consumption was normalized to 300 g body weight. Nicotine preference was considered as the percentage of NW consumption to total water consumption.

2.3. Neurosurgery

For neurotoxic/sham lesions, stereotaxic surgery was performed in the rats under intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia. To create a bilateral striatal lesion, 2.5 µl of 6-OHDA at the concentration of 4.8 µg/µl dissolved in saline containing 0.1% ascorbic acid was injected into each ventrolateral region of the dorsal striatum (VLS) at the coordinates (AP +1.1, ML ± 3.2, DV 7.2). The internal cannula was introduced according to the rat brain atlas (10). Injections were performed using a 30-gauge cannula attached to the polyethylene tubing connected to a 10 µl Hamilton syringe and controlled by a micro infusion pump set to 0.5 µl/min infusion rate. The cannula was kept inside for two minutes for sufficient diffusion of 6-OHDA, following every infusion. The sham lesion was created using the same procedure as bilateral lesion groups, using an equal volume of the vehicle instead of 6-OHDA.

2.4. Behavioral Tests

Locomotor Activity

The locomotor activity apparatus (May 9803 Activity Monitor, Commat) had a white square plexiglass floor (40cmx40cm) and translucent plexiglass walls with 35cm height surrounding the exterior of this floor. Subjects were acclaimed in the test room for 30 minutes before being placed in the center of the open field. The test lasted for 10 mins. Horizontal, ambulatory, stereotypical, and vertical movements of each subject were recorded. The open field was cleaned with 70% ethanol after each subject and the new subject was placed in the system.

Elevated Plus Maze Test (EPM)

EPM is formed by placing two 10 cm wide platforms made of plexiglass in a "+" shape resulting in four arms perpendicular to each other and a center of 10cmx10cm area. The length of each arm is 50 cm and the maze is placed on 50cm high legs. The long sides of the two open arms are bordered by a 10 cm high white plexiglass and its tip is open. The two closed arms are surrounded on three sides by black plexiglass at a height of 40 cm. At the beginning of testing, each rat was placed on the central platform facing an open arm. The arm that the rat touched with four paws was considered an arm entry. Time spent on open arms (OET), time spent on enclosed arms (CET), open arm entry number (OEN), and enclosed arm entry number (CEN) were recorded. The percentages of the spent time and the number of entrances to the open/closed area were calculated.

Forced Swimming Test

The forced swimming test was performed in a 20 cm diameter, 60 cm height plexiglass cylinder filled with 25 ^{0}C water up to 50 cm height. Each rat was left in the water-filled cylinder twice. They were tested for 15 minutes on the first day, and 5 minutes on the second day. Each rat was kept in a cage covered with a paper towel and heated with a radiant heater for 10 minutes to dry and rest. All procedures were recorded with a video camera. Rats that moved enough to keep their head still above the water and that floated motionlessly were considered immobile (11). All tests were performed before 12:00 a.m. to avoid possible circadian effects on performance.

2.5. Histological Staining

The fixed brain of each rat was embedded in paraffin, and processedfor immunohistochemical and HE staining. Coronal sections of 5 µm were selected at levels between AP 2.28 mm and −0.12 mm area (10). For each animal, three tissue sections were examined. The sections were investigated by caspase-3 (1:1000, CPP32 Ab-4 rabbit polyclonal antibody, biomarkers) and microtubule-associated protein 2 (1:1000, MAP2, mouse monoclonal SMI-52, ab28032, Abcam). primary antibodies using the HRP and ABC staining kits (KP-50AR, Diagnostic Biosystems and sc-2017, Santa Cruz Biotechnology). Sections were visualized by ABC chromogen and mounted for analysis. The coverslips were visualized using the Leica DM2500 microscope with the Leica DFC295 HD camera system at 40x objective. The total number of cells and the caspase-3 labeled cells in the striatum were counted in four randomly chosen square areas (97 x 97 µm) randomly selected from each sample. For this analysis, seven animals were randomly selected by the researcher blinded to the group assignments. For semi-quantitative analysis of MAP-2 staining results, three sections were selected from the striatum of each rat. Staining intensity was graded as follows: 0: no staining, 1: weak; 2: moderate; and 3: intense (12).

2.6. Data Analysis and Statistics

All subjective scorings were performed by a researcher, who was blind to the group assignment of the rats. Results were expressed as mean ± standard error (SEM). Statistical significance levels of data were evaluated with the "GraphPad Prism-8" statistics program. One-way analysis of variance (ANOVA) was used for all behavioral tests and the cell counts. Nicotine preference, NW, TW, and total water consumption were analyzed with two-way ANOVA. Post hoc analysis was performed with Tukey test. Values were considered to be significant at $p<0.05$.

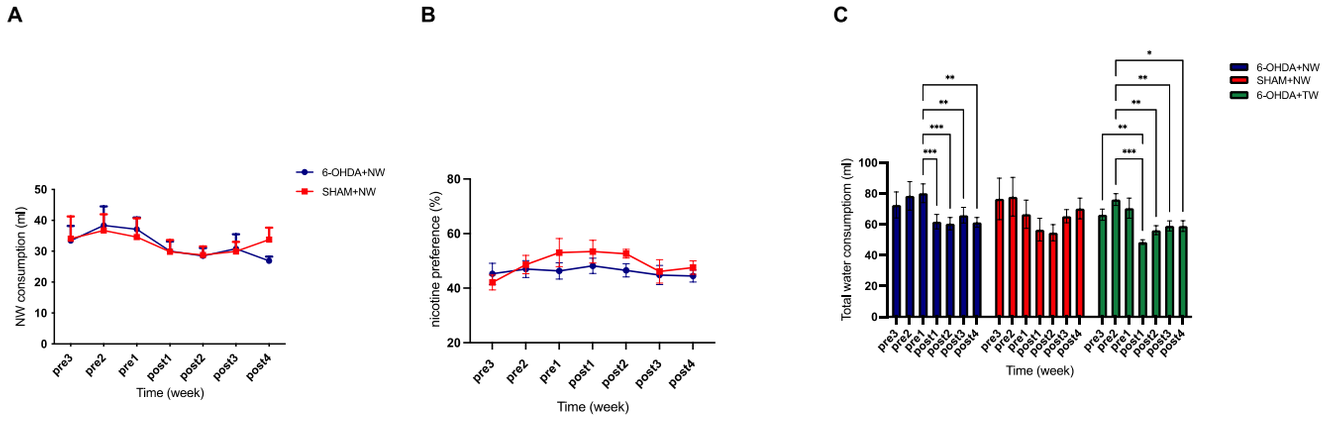

Figure 2. *Nicotine and total water consumption and nicotine preference of the groups*
*NW consumption (A) and preference (B) was not different between the groups and time points. Total water consumption (C) was lower post-lesion than in the pre-lesion period in both groups of 6-OHDA lesions. Total water consumption was lower after lesion in both groups with 6-OHDA lesions. * p <0.05, ** p <0.01, *** p <0.001*

Table 1. *Descriptive statistics. Mean ± SEM for nicotine and total water consumption, and nicotine preference.*

Weeks	pre3	pre2	pre1	post1	post2	post3	post4	Groups
Nicotine water consumption (ml)	33,4 ± 4,8	38,4 ± 6,2	37,1 ± 3,8	30,0 ± 3,1	28,5 ± 2,6	30,8 ± 4,6	26,9 ± 1,4	6-OHDA+NW
	34,1 ± 7,2	36,71 ± 5,2	34,6 ± 6,0	29,8 ± 3,8	28,8 ± 2,8	29,9 ± 3,1	33,8 ± 3,9	SHAM+NW
Nicotine preference (%)	45,3 ± 3,9	47 ± 3,1	46,3 ± 3	48,2 ± 2,8	46,5 ± 2,4	44,8 ± 3,5	44,5 ± 2,2	6-OHDA+NW
	42,1 ± 2,7	48,7 ± 3,4	53 ± 5,2	53,4 ± 4,2	52,6 ± 1,6	46,2 ± 4,3	47,6 ± 2,4	SHAM+NW
Total water consumption (ml)	72,6 ± 8,4	78,5 ± 9,2	80,2 ± 6,1	61,8 ± 4,7	60,5 ± 4,0	66,0 ± 5,1	61,3 ± 3,2	6-OHDA+NW
	76,6 ± 13,4	77,9 ± 12,5	66,6 ± 9,1	56,6 ± 7,3	54,6 ± 5,3	65,3 ± 4,3	70,3 ± 6,8	SHAM+NW
	66,3 ± 3,7	76,1 ± 3,8	70,6 ± 6,5	48,6 ± 1,5	56,2 ± 2,9	59,0 ± 3,3	59,0 ± 3,5	6-OHDA +TW

3. RESULTS

3.1. Temporal Distribution of Nicotine Water and Total Water Consumption

There was no significant effect of "group" and "time" on nicotine preferences (Figures 2A and 2B). All groups exhibited a decrease in consumption of NW and TW in the first week after the operation, which was attributed to the change in post-operative general condition. Two-way analysis of variance showed that water consumption changed significantly over time (F (1.883.60.27) = 12,13; p <0.0001). Total water consumption in the 6-OHDA lesioned rats was overall significantly lower during the post-lesion period than before the lesion irrespective of the experimental group (NW or TW). Tukey analysis showed that in the 6-OHDA+NW group, total water consumption was significantly lower at the 1st, 2nd, 3rd, and 4th weeks after the lesion (p = 0.0002, 0.0001, 0.0023, 0.0093, respectively). In the 6-OHDA+TW group, there was a significant difference between the before and after weeks of the lesion: pre-lesion 3rd week vs. post-lesion1st week (p=0.0066) and pre-lesion 2nd week vs. post-lesion1st, 2nd, 3rd, and 4th weeks (p = 0.0002, 0.0019, 0.0068, 0.0162, respectively) (Figure 2C). NW consumption, TW consumption and nicotine preference results were given in Table 1.

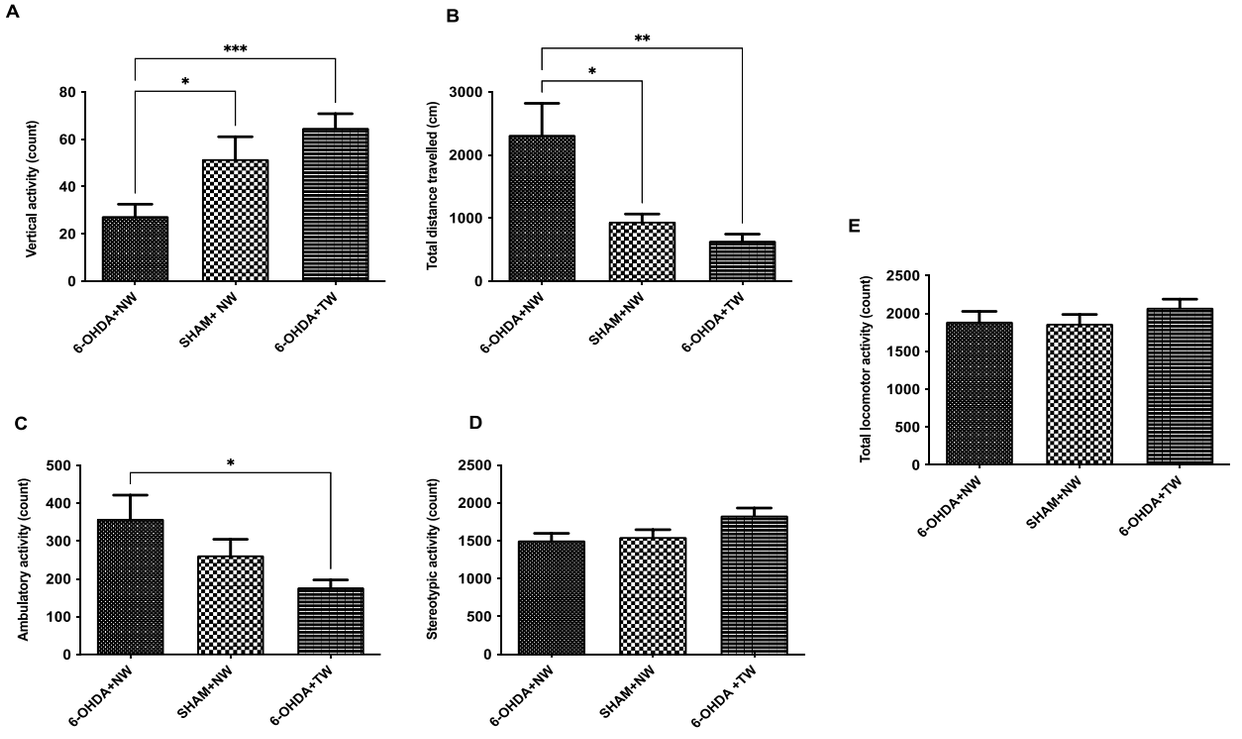

Figure 3. *Subtypes of locomotor activity of the groups*

Vertical movement (A) was lower and total distance traveled (B) was higher in the 6-OHDA+NW group compared to the other groups. Ambulatory action (C) was higher at 6-OHDA + NW compared to 6-OHDA+TW. Stereotypic (D) and total locomotor activity (E) was not different between the groups.

** p <0.05, ** p <0.01, *** p <0.005.*

3.2. Locomotor Activity

A significant overall difference was detected between the three groups in terms of vertical activity ($F(2,32) = 8.66$, $p=0.0010$); and distance traveled ($F(2,32) = 6.04$, $p=0.0060$) (Figure 3A and 3B). There was a trend for a difference between the groups in terms of ambulatory movement ($F(2,32) = 3.171$; $p=0.055$) (Figure 3C). Vertical movements were significantly lower in the 6-OHDA+NW compared to the 6-OHDA+TW ($p=0.0009$) and SHAM+NW ($p=0.0372$) groups (Figure 3A). The total distance traveled was significantly higher in the 6-OHDA+NW group compared to the Sham+NW ($p=0.0376$) and 6-OHDA+TW ($p=0.0094$) groups (Figure 3B). Contrary to the vertical movements, the number of ambulatory movements was significantly higher in the 6-OHDA+NW compared 6-OHDA+TW group ($p=0.0463$) (Figure 3C). These results showed that nicotine had an opposite effect on horizontal compared to vertical movements. There was no significant difference between the groups in terms of stereotypical ($F(2,32) = 3.18$, $p=0.055$), and total locomotor activity ($F(2,32) = 0.6920$, $p=0.5079$) (Figure 3D and 3E). Locomotor activity results (mean ± SEM) were given in Table 2.

Table 2. *Descriptive statistics. Mean ± SEM for the results of locomotor activity and emotional tests*

BEHAVIORAL TESTS	6-OHDA+NW	SHAM+NW	6-OHDA +TW
Ambulatory activity (count)	359.1 ± 62.5	262.4 ± 42.5	177.7 ± 19.5
Vertical activity (count)	27.53 ± 5.0	51.6 ± 9.5	64,9 ± 5.9
Total distance travelled (cm)	2322 ± 497.8	942.5 ± 122.1	641.1 ± 106.0
Stereotypic activity (count)	1506 ± 93.6	1552 ± 94.4	1834 ± 98.7
Total locomotor activity (count)	1892 ± 136.7	1866 ± 121.5	2077 ± 112.5
Time spent of open arm (%)	33.62 ± 7.9	17.7 ± 4.5	10.33 ± 3.9
Duration of immobility (sec)	77.71 ± 10.1	37.8 ± 10.7	14.3 ± 3.2

3.3. Anxiety and Depression-like Behavior

Figure 3 shows the performance of rats in different groups in EPM and FST. The percentage of time spent in open and closed arms also differed significantly between the three groups ($F(2,32) = 3.47$, $p=0.0431$ – Figure 4A). Post-hoc analysis revealed that the 6-OHDA+NW group spent more time in open arms ($p=0.0437$) and less time in closed arms ($p=0.0422$) than rats in the 6-OHDA+TW group. A significant difference in immobility time in FST was detected between the three groups ($F(2,31) = 12.87$, $p<0.0001$). The immobility time in the 6-OHDA+NW group was higher than in the SHAM+NW ($p=0.0109$) and the 6-OHDA+TW ($p<0.0001$) groups. The 6-OHDA+TW and the SHAM+NW groups had similar immobility times ($P=0.2233$) (Figure 4B). Anxiety and depression scores of the groups (mean ± SEM) were given in Table 2.

3.4. Histological and Immunohistochemical Results

The total number of cells was counted in the striatum with the H&E staining. The total number of cells differed between the groups, ($F(2,177) = 34.27$, $p<0.0001$); it was significantly lower in the 6-OHDA+TW group compared to the 6-OHDA+NW ($p=0.0049$) and SHAM+NW ($p<0.0001$) groups, and also in the 6-OHDA+NW group compared to the SHAM+NW group ($p<0.0001$) (Figure 5A). In the H&E staining, the morphologic changes induced in cells of the 6-OHDA+NW group were similar to those observed in cells of the SHAM+NW group. In the 6-OHDA+TW group, pathological changes including apoptotic bodies and cell shrinkage were observed in the cells in the striatum regardless of neurons and glia (Figure 5B).

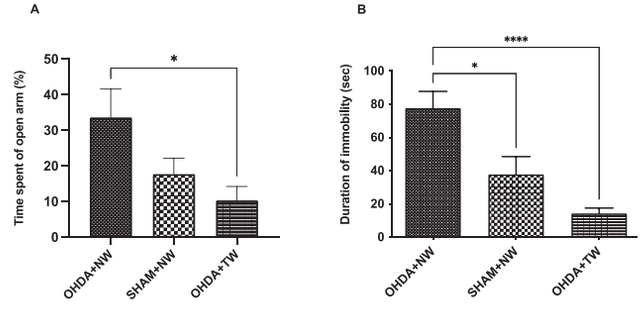

Figure 4. *Anxiety and depression-like behavior scores of groups in the plus maze and forced swimming tests*
*In the 6-OHDA+NW group, the percentage of time spent was higher in open arms (A) compared to the 6-OHDA+TW group. In the forced swimming test, immobility time was higher in the 6-OHDA+NW group than in other groups (B). * p <0.05, **** p <0.0001.*

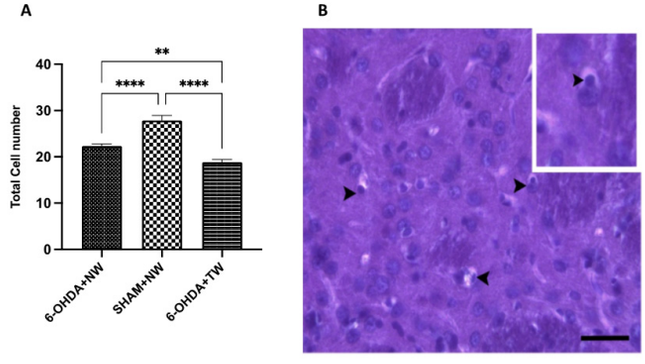

Figure 5. *Total cell numbers and H&E staining in the striatum*
*The figures are showing the number of total cells (A) and morphology of cells (B) in the striatum (6-OHDA+TW group). The majority of the cells exhibited the dark cell degenerative changes (arrowheads). These cells showed the pyknotic nucleus and shrunken cytoplasm. Inset shows dark cell. H&E staining; scale bar 20 µm.** p<0.01 ****p<0.0001*

The number of caspase-3 labeled cells in the striatum was also counted and compared to determine the degree of apoptosis. The total amount of caspase-positive apoptotic cells differed between the groups, ($F(2,177)= 149.0$, $p<0.0001$); it was significantly lower in the 6-OHDA+NW group compared to the 6-OHDA+TW ($p<0.0001$), and in the

SHAM+NW group compared to the 6-OHDA+TW ($p<0.0001$) and 6-OHDA+NW ($p<0.0001$) groups (Figure 6A).

Figure 6 (B, C, D) shows caspase-3 labeled cells in the striatum for all groups; 6-OHDA+NW (B), SHAM+NW (C), and 6-OHDA+TW (D).

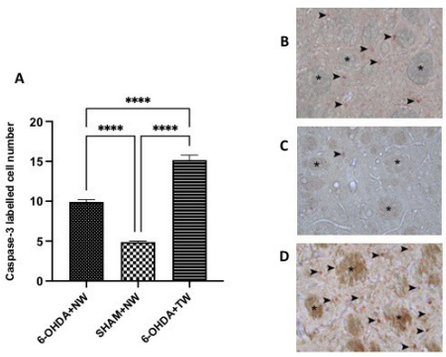

Figure 6. *Apoptotic cell numbers and caspase-3 immunopositive cells in the striatum*
*The figures are showing (A) the number of caspase-3 positive apoptotic cells, and representative images showing the caspase-3 labeled cells in the striatum in the 6-OHDA+NW (B), SHAM+NW (C), and 6-OHDA+TW (D) groups in the striatum. ** p<0.01 ****p<0.0001 Arrowheads, the caspase-3 labeled cells, and asterisks, the fiber bundles*

The 6-OHDA+NW exhibited moderate MAP-2 staining (Figure 7A). The SHAM+NW group showed strong staining while the 6-OHDA+TW group showed weak staining with the MAP-2 (a dendrite-specific marker for the neurons) (Figure 7B-C). The staining of the MAP-2 positive neurites in the striatal neurons was decreased in the 6-OHDA+TW lesion group (Figure 7C).

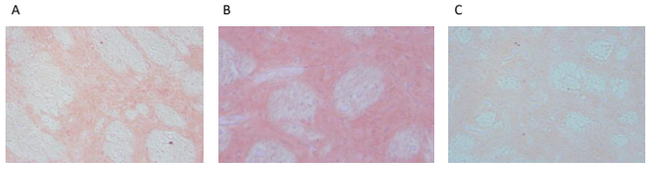

Figure 7. *MAP-2 immunostaining*
Representative images showing the MAP-2 staining in the striatum in the 6-OHDA+NW (A), SHAM+NW (B), and 6-OHDA+TW (C) groups.

4. DISCUSSION

Although nicotine is known to regulate dopamine release in the brain and upregulate specific nAChRs, there is not sufficient data regarding the reduced/stopped nicotine intake in preclinical PD and the potential role of dopaminergic loss in the basal ganglia circuit in this tendency. The current work is the first study that tested the effect of the reduced dopaminergic innervation of VLS on nicotine preference. This study also focuses on the behavioral and emotional effects of nicotine under conditions of denervation of the dopaminergic system in the VLS (13). Our results showed that bilateral striatal lesions did not lead to a significant alteration in total locomotor activity and nicotine consumption/preference. In the 6-OHDA+TW lesion group, nicotine increased the horizontal movement frequency (ambulatory activity and total distance) but decreased the vertical movement frequency. Nicotine also decreased anxiety-like behaviors but increased depression-like behaviors.

More specifically, VLS lesions reduced total water consumption but did not alter nicotine consumption/preference. Previous studies have shown that the VLS contributes to orofacial and forelimb movements (including licking, the facial expression of emotion, and reaching), goal-directed behavior, and reward circuitry (14) with a nonlinear relationship between lesion size and functional impairment (15). The fact that a significant decrease in total water consumption did not generalize to nicotine-water preference in the lesion group may indicate that the lesion does not affect VLS-related motor functions and reward mechanisms in the same way. Longer-term experimental studies using different routes of administration would help clarify the relationship between dopamine depletion in the subfields of the striatum and nicotine preference. Additionally, the wide age range of the animals per group, which is one of the limitations of the study, may have caused higher variability in the data. Future studies should also consider testing older rats to increase the validity of the model.

Total locomotor activity or its subcomponents are affected by motor, sensory and emotional factors (16). In the current study, the total locomotor activity did not differ between the groups, while there were differences in the subcomponent that can be classified as vertical and horizontal (17). In terms of total activity, the absence of a significant difference between the lesion and sham groups can be interpreted as the bilateral lesion of the VLS does not cause evident motor dysfunction. The significant effect of nicotine on horizontal and vertical movements only in the lesion group provides indirect evidence that nicotinic receptors of the cholinergic system are an active contributor to the mentioned motor activities in addition to the mesostriatal dopaminergic system. Previous studies show that nicotine may affect locomotor activity differently depending on the variables such as dose, duration, and internal and external preconditions (18,19). Therefore, the loss of dopaminergic innervation of the striatum might be one of the factors determining the effects of nicotine on locomotor activity patterns, so the VLS seems to be a candidate for one of the functional areas underlying the effects of nicotine. An increase in ambulatory movement induced by nicotine is a common finding, albeit a decrease in vertical movement has been reported at only high doses (20). At this point, it is important to distinguish the nature of the vertical movement. The vertical movement has been considered exploratory (21) as well as stereotypical (22) differing depending on whether the actions are goal-directed or not (23). Thus, these results can be explained in terms of the suppression of complex stereotypical movements as much as the reduction of exploratory behavior by nicotine in rats with VLS lesions, which requires further studies.

The relationship between smoking and neuropsychiatric disorders including depression and anxiety disorder has been known for a long time. Inconsistency of the results of both clinical and preclinical studies has directed the studies to investigate the factors regarding the emotional effects of nicotine. It has been reported that basal conditions arising from age, gender, and genetic structure are among the determining factors. In our study, the use of nicotine in animals with lesions significantly increased the immobility period in FST and duration in the open arm in EPM compared to nicotine use alone or the presence of lesion alone. These results suggest that the VLS might be an area that mediates the emotional effects of nicotine, as also suggested by locomotor activity results. Although depression is often associated with anxiety disorders in clinical practice, these emotional states/behaviors may be independent of each other (24). It should be kept in mind that the period of inactivity, which is typically used as a proxy for hopelessness in FST, may also be affected by factors independent of the presumed hopelessness state. For example, it has been suggested that immobility in FST may also interact with low-level anxiety (24).

The neuroprotective effect of nicotine has been demonstrated in different models generated with the administration of neurotoxic agents, including 6-OHDA (20,25,26,27). One of the limitations of this study is that tyrosine hydroxylase expression per se was not examined and thus striatal dopaminergic innervation could not be evaluated. However, the findings obtained with H&E, caspase-3, and MAP-2 staining have shown that nicotine prevents 6-OHDA-induced striatal cell loss.

In H&E staining, the lowest and highest cell counts were observed in the 6-OHDA+TW and sham+NW groups, respectively. In parallel, caspase-3 immunoreactivity, which reflects apoptotic cell death, is significantly reduced with nicotine use in groups with 6-OHDA lesions. Striatal dopamine denervation results in a significant loss of dendritic spines on medium spiny projection neurons in both animal models of parkinsonism and Parkinson's disease (28,29). Since Parkinson's disease affected the neuronal cytoskeleton, we also studied morphological changes through immunostaining of the dendrite-specific marker MAP-2 in the rat striatum. MAP-2 is found extensively in the somatodendritic areas of neurons, therefore, it is considered a neuron-specific protein (30). The results of the semi-quantitative assessment of MAP-2 immunoreactivity in the striatum (i.e., lower in the 6-OHDA+TW group compared to other groups) suggest that cell loss also includes neurons. Considering that the neuron types in the striatum are GABAergic projection neurons (medium-sized spiny neurons) accounting for 95% of striatal neurons and GABAergic/cholinergic interneurons accounting for 2-3% (31), the probability of loss in these neuron types is quite high. Studies using more specific markers will further elucidate the specific effects at the neuronal level.

5. CONCLUSION

In conclusion, the results of this preliminary study show that the bilateral VLS lesion with 6-OHDA does not affect nicotine preference, nicotine prevents non-dopaminergic neuron loss due to 6-OHDA, and its effects on locomotor activity pattern and emotional behaviors occur only under the lesion condition. The scope of the interpretation of the study results would have increased substantially if there was a full control group (e.g. sham and no nicotine). These results suggested that the behavioral effects of nicotine in animals with lesions, including emotional and motor aspects, may be related to the widespread distribution of nicotinic receptors as well as the predominance of reciprocal connections between anatomically and functionally separated regions in the striatum. The characterization of striatal cell loss caused by 6-OHDA and its prevention by nicotine as well as an explanation of the interaction, if any, between locomotor activity patterns and emotional responses require further studies which include the control group.

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