

Original Article

Dopamine level in the striatum exhibits circadian rhythms in the rat model of Tourette syndrome

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Abstract: Dopamine dysfunction is implicated in the pathogenesis of tic disorders. To explore whether TD symptoms might be related to the circadian variations of dopamine in the striatum, in this study we established rat models of tic disorder (TD) induced by iminodipropionitrile (IDPN). 120 Sprague-Dawley male rats were divided into two groups: control group and IDPN treatment group. The rats in treatment group were injected 150 mg/kg IDPN for 7 consecutive days, while rats in control group were injected saline. The rats in each group were subjected to open field test at daily time points: 1:00, 5:00, 9:00, 13:00, 17:00, 21:00, continuously for 7 days. Then the rats were killed at the 6 different time points during the 24 hours period. The rat striatums were quickly removed and the levels of dopamine and 5-HT were measured by high-performance liquid chromatography. The results showed that dopamine level and the excitability of the rats in treatment group exhibited rhythmic features. The peak of DA secretion was at 5 am, when the rats exhibited the highest excitability. Our results suggest that 5 am is an important time point for drug intervention to alleviate the condition of TS.

Keywords: Tic disorder, iminodipropionitrile, rat, dopamine, rhythm

Introduction

A number of evidences suggest a central dopamine dysfunction in the pathogenesis of tic disorders, and the regulation of dopamine level is effective for improving the diseased condition [1-3]. Moreover, functional brain imaging techniques have shown that dopamine release from the striatum is abnormal in patients with Tourette syndrome [4]. Several studies have shown that the dopamine release and decomposition in the striatum of normal rats were different at different time periods. Within 24 hours, the circadian variation of the dopamine shows the rhythm [5]. Interestingly, the onset of tic symptoms tends to have a rhythm within one day [6, 7]. Therefore, we speculate that there is a link between dopamine level in the striatum and the onset of tic symptoms.

To explore whether TD symptoms might be related to the circadian variations of dopamine in the striatum, in this study we established rat models of tic disorder (TD) induced by iminodi-

propionitrile (IDPN) [8]. Next we examined the state of excitability of the rats at the different time points during a day, detected the content of dopamine in the striatum and evaluated their behaviors.

Materials and methods

Animals

Total 120 male Sprague-Dawley (SD) rats (6-week old) were obtained from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China) and acclimatized for a week before the experiments. Animal welfare and experimental procedures were performed strictly in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996) and approved by Institutional Animal Care and Use Committee (IACUC) of Shanghai Jiaotong University. These animals were housed in cages (4 animals/cage) with free access to food and water, and maintained at a controlled tempera-

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ture 19-25°C and humidity environment with 12 h light/12 h dark cycle (LD 12:12).

Grouping of animals

The 120 rats were randomly divided into treatment group (n=60) and control group (n=60). Among treatment and control groups, the rats were further randomly divided into 6 subgroups by the time points examined, respectively, as follows: 1:00 group, 5:00 group, 9:00 group, 13:00 group, 17:00 group, 21:00 group (n=10).

Treatment of animals

The rats in treatment group were intraperitoneally injected 150 mg/kg IDPN. Equal volume of saline was injected into the rats of control group, and then IDPN was given daily for consecutive 7 days at a dosing volume of 1 mL/100 g in treatment group. At the end of treatment, the rats were subjected to behavior and biochemical tests.

Behavior test

Rat behaviors were observed by the investigators who were familiar with the stereotyped behavior, but not familiar with the experimental design. 5 min after administration, the investigator observed the behavior of rats for every 5 min for a total of 6 observation periods and scored them. The stereotypy actions included bites (teeth touching the cage, wood chips, vacuous chewing), taffy pulling (raises of the forepaw), self-gnawing, licking not associated with grooming, head shaking, paw buffeting, quick aversion, episodic utterances. The behaviors were scored as follows: 0 Asleep, resting in place or normal activity in place; 1. Increased sniffing and head raising; 2. Discontinuous increased sniffing with body raising; 3. Discontinuous increased sniffing, licking with head and body raising primarily in one place, with occasional rapid burst of locomotor activity (2-5 steps); 4. Continuous sniffing, biting, head bobbing and repetitive body raising/wall climbing in place; 5. Continuous sniffing, biting, licking, head bobbing, and continuous body raising/wall climbing wherein forepaws do not touch cage floor [9].

Anxious behavior and locomotion were evaluated by open field test. The open field box was a square box (60 cm × 60 cm × 40 cm), made

out of wood with the bottom divided into nine squares of 20 cm × 20 cm. Before the start of the experiment, each animal was placed in the box for 15 min for adaptation. Then the ambulating and rearing movements confined to the area near the sides of the open-field were recorded by a video camera within 15 min in the light condition. The rats in each group were recorded in the daily time points: 1:00, 5:00, 9:00, 13:00, 17:00, 21:00, continuously for 7 days. After all behavioral tests in rats, the imaging data was observed by two in order to minimize subjective errors.

Measurement of dopamine and 5-hydroxytryptamine levels

Two weeks later, the rats (8-week old) including 60 from control group and 60 from treatment group were killed at six time points: 1:00 am, 5:00 am, 9:00 am, 13:00 pm, 17:00 pm, 21:00 pm. The striatums were quickly removed and the blood was rapidly washed away with icy saline, with filter paper drying, and then immediately frozen on dry ice and stored at -80°C. The striatal tissues were thawed at room temperature and immersed in a small amount of cold 0.4 mol/L HClO₄ solution. After ultrasonic treatment, the homogenates of striatums were subjected to high-performance liquid chromatography (HPLC) analysis to measure the levels of dopamine and 5-hydroxytryptamine. The HPLC system comprised a reverse-phase column (MA-5 ODS, 15064.6-mm ID, Eicom), a model L-6000 pump (Hitachi), and an electrochemical detector (ECD-100, Eiercom). The mobile phase consisted of 85 mmol/L citric acid-100 mmol/L sodium acetate, 0.2 mmol/L disodium EDTA, 1.2 mmol/L sodium octane sulfonate, and 5% methanol in deionized water. The flow rate was 1 mL/min, and the pH was adjusted to 3.5. The levels of dopamine and 5-hydroxytryptamine were expressed as ng/mg wet weight of brain tissue.

Statistical analysis

All data were expressed as the mean ± SD and analyzed using the SPSS version 12 software (SPSS Inc., Chicago, IL, USA). The multi-group comparisons were carried out by one-way analysis of variance and Student's t-test was used to determine the difference between the two groups. *P*<0.05 was accepted as statistically significant.

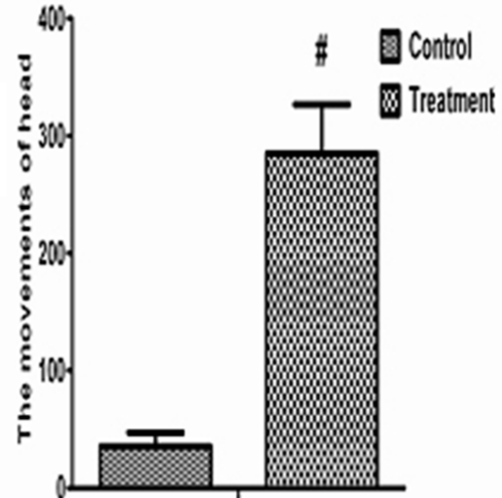
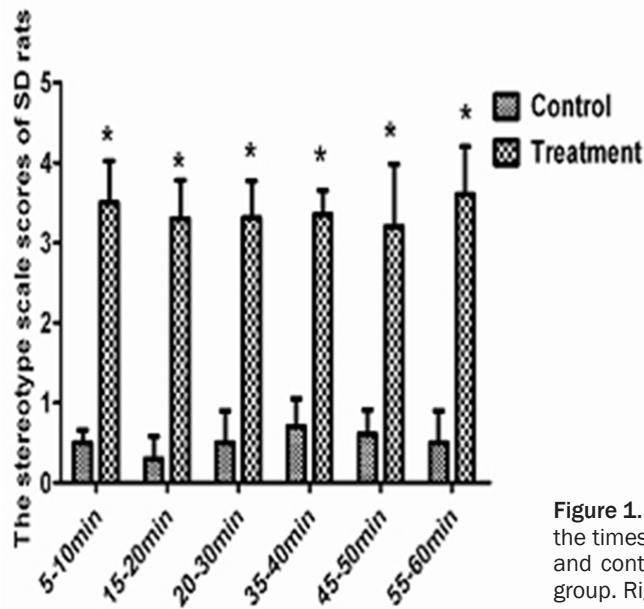


Figure 1. Comparison of the stereotypy scale scores and the times of head movement in the rats of model group and control group. Left: * $P < 0.05$ compared to control group. Right: # $P < 0.05$ compared to control group.

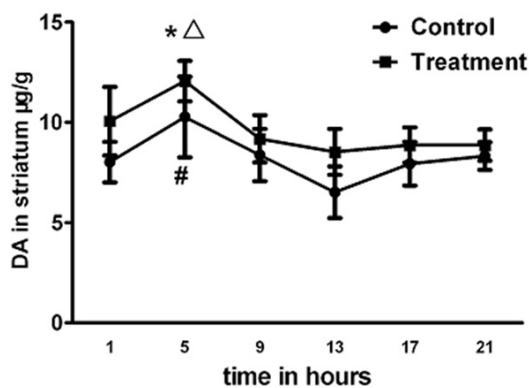


Figure 2. DA level in the striatum of rats of model group and control group. DA level was measure by HPLC. * $P < 0.05$ treatment group at 5 am vs. control group at 5 am; # $P < 0.05$ control group at 5 am vs. control group at other time points; [△] $P < 0.05$ treatment group at 5 am vs. treatment group at other time points.

Results

Establishment of a rat model with tic disorders

The rats of treatment group were intraperitoneally injected 150 mg/kg IDPN. Equal volume of saline was injected into the rats of control group. The stereotypy scale scores were observed and recorded at 5-10 min, 15-20 min, 25-30 min, 35-40 min, 45-50 min and 55-60 min after the treatment. The behavioral changes of the rats exposed to IDPN treatment were

obvious. The stereotypy scale scores in treatment group were significantly higher than in control group ($P < 0.05$), and the times of head movement in model group were also higher than that in control group ($P < 0.05$) (Figure 1). These data demonstrate that we successfully established rat model with tic disorders by injection of IDPN.

DA and 5-HT levels in the striatum of rats in two groups

In control group, striatum level of dopamine level was the highest at 5:00 am and the lowest at 13:00 pm (Figure 2), while striatum level of 5-HT was the highest at 13:00 pm and the lowest at 17:00 pm (Figure 3).

In treatment group, striatum level of dopamine level was the highest at 5:00 am and the lowest at 13:00 pm (Figure 2), while striatum level of 5-HT was the lowest at 17:00 pm (Figure 3).

Comparison of striatum DA and 5-HT levels in two groups at different time points

DA level in the striatum of treatment group was higher than that in control group, especially at 5:00 am when the difference was significant ($P < 0.05$, Figure 2). 5-HT level in the striatum of treatment group was lower than that in control group, especially at 17:00 pm when the difference was significant ($P < 0.05$, Figure 3).

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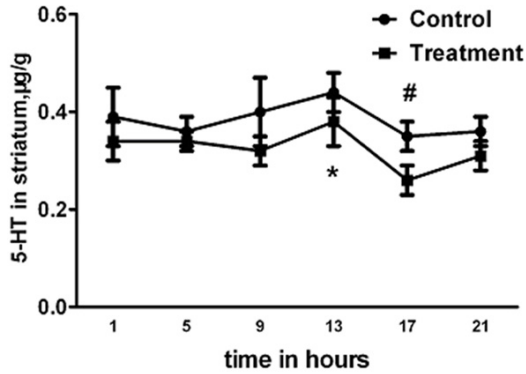


Figure 3. 5-HT level in the striatum of rats of model group and control group. 5-HT level was measure by HPLC. # $P<0.05$ treatment group at 17 pm vs. control group at 17 pm; * $P<0.05$ treatment group at 13 pm vs. treatment group at other time points.

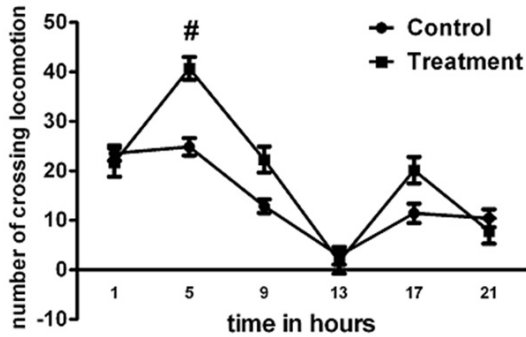


Figure 4. Comparison of the number of crossing locomotion of rats of model group and control group. # $P<0.05$ treatment group at 5 am vs. treatment group at other time points.

Although DA level in the striatum of treatment group was higher than that in control group, IDPN did not affect the circadian rhythm of DA release (Figure 2). Similarly, although 5-HT level in the striatum of treatment group was lower than that in control group, IDPN did not affect the circadian rhythm of 5-HT release (Figure 3).

Comparison of the behaviors of rats in two groups

The rats in treatment group exhibited circadian changes in the number of crossing locomotion, grooming and rearing over 24 hours (Figures 4-6). In treatment group, the number of crossing locomotion at 5 am was significantly higher than at other time points ($P<0.05$, Figure 4). Two peaks for the number of crossing locomo-

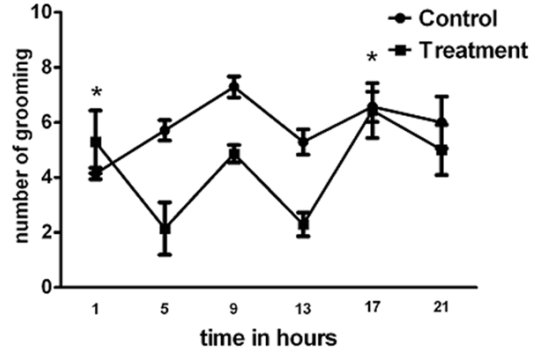


Figure 5. Comparison of the number of grooming of rats of model group and control group. * $P<0.05$ treatment group at 17 pm vs. treatment group at other time points.

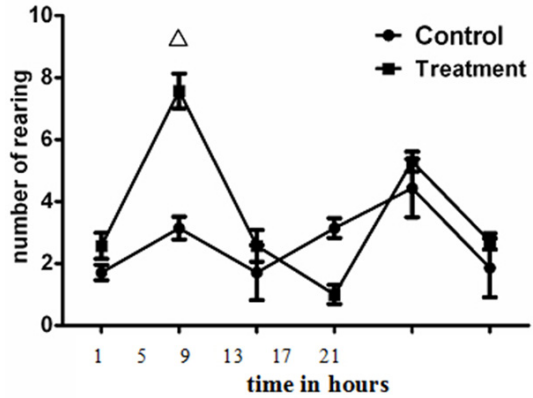


Figure 6. Comparison of the number of rearing of rats of model group and control group. Δ $P<0.05$ treatment group at 5 am vs. treatment group at other time points.

tion were observed at 5 am and 17 pm in treatment group, but not so obvious in control group (Figure 4). Similarly, the number of grooming at 5 am was significantly higher than at other time points ($P<0.05$, Figure 6). Two peaks for the number of rearing were observed at 5 am and 17 pm in treatment group, but not so obvious in control group (Figure 6).

Discussion

Abnormality in the neurotransmitters dopamine (DA) and serotonin (5-HT) plays an important role in the pathophysiology of TS [9-11]. Furthermore, a previous study showed an increased DA content in 46 subjects with TS compared to 40 age-matched normal controls [12]. Dopamine system consists of three parts: the

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dopaminergic nigrostriatal system, the mesencephalic limbic DA system and tuberoinfundibular system. Among them, the dopaminergic nigrostriatal system and mesencephalic limbic DA system exhibit different daily rhythm [13].

It has been reported that DA level in the striatum of normal rats and mice showed rhythmic changes [14, 15]. In this study we found that 8-week old normal SD rats exhibited the highest DA concentration at 5 am and the lowest DA concentration at 17 pm. There was a peak for dopamine at 5 am and a valley at 13 pm in TD model group. The dopamine level in these two groups both exhibited circadian rhythms and the peak was at 5 am. In our study, there was a peak for 5-HT at 13 pm and a valley at 17 pm in model group. The rats in control group showed the highest 5-HT concentration at 13 pm time and the lowest 5-HT concentration at 17 pm. IDPN did not affect the rhythmic features and peak or valley values of 5-HT level in the striatum. 5-HT in the striatum is released from the raphe nucleus nerve endings. It has been shown that 5-hydroxy indole acetic acid (5-HTAA) and tryptophan levels in the basal ganglia area were decreased in TS patients [16]. In addition, 5-HT agonist ziprasidone could promote the dopamine transporter and increased frequency of blinking [17]. Therefore, we speculate that 5-HT is likely to be involved in TD indirectly through regulating DA level.

The excitatory changes at different time points in control and TD model rats were measured by the open field test. Open field test is widely applied in animal behavior studies to evaluate the spontaneous activity when the animals are exposed to unfamiliar environment [18]. Evidence has shown positive correlation between the number of grooming and the ability to adapt to the surrounding environment. When rats begin to adapt to a strange environment, grooming will increase. Our results showed that the excitability of rats had a circadian rhythm and varied with double peak curves, and the peaks occurred at 5:00 and 17:00, respectively.

In summary, this study showed that dopamine level in the striatum exhibits circadian rhythms in the rat model of Tourette syndrome. The peak of DA secretion was at 5 am, when the rats exhibited the highest excitability. Therefore,

5 am is an important time point for drug intervention to alleviate the condition of TS. Further studies are needed to confirm our findings.

Acknowledgements

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Disclosure of conflict of interest

None.

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