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Research article

Selective cholinergic depletion of pedunculopontine tegmental nucleus aggravates freezing of gait in parkinsonian rats

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HIGHLIGHTS

- Selective PPTg cholinergic lesion via injection of IgG immunotoxin-targeting choline acetyltransferase.
- SNc lesion and combined lesion led to significant changes in many gait parameters.
- Terminal dual stance increased higher in combined lesion rats than SNc lesion rats.
- The combined lesion group showed the most severe freezing among four groups.
- The cholinergic neurons of PPTg play a vital role in the occurrence of gait freezing in PD.

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ABSTRACT

Many patients of advanced Parkinson's disease (PD) suffer from intractable axial symptoms (severe gait and postural impairments), which were recently speculated to be more relevant to cholinergic degeneration in the brainstem than dopaminergic degeneration in the substantia nigra compacta (SNc). To investigate the role of the cholinergic cells of the pedunculopontine tegmental nucleus (PPTg) on motor deficits, especially the axial motor impairments, we measured and analyzed the gait performance of sham lesion rats, SNc dopaminergic lesion rats, PPTg cholinergic lesion rats, and combined lesion rats by using the CatWalk system. Motor performance of PPTg cholinergic lesion rats was also tested on the rotarod. Independent loss of cholinergic neurons in the PPTg did not induce gait disturbance in CatWalk, but PPTg lesion rats showed motor impairments on the rotarod when the demands of the motor task increased. Both SNc lesion rats and combined lesion rats displayed significant changes in many gait parameters, but the terminal dual stance increased much higher in combined lesion group than SNc lesion group. Furthermore, combined lesion rats showed more severe freezing of gait (FOG) than SNc lesion rats during behavioral re-evaluations after lesion. These results suggest that the PPTg cholinergic neurons play a vital role in the occurrence of FOG in PD.

1. Introduction

Parkinson's disease is characterized by dopaminergic neurons degeneration in the substantia nigra pars compacta (SNc), which leads to motor symptoms including resting tremor, rigidity and bradykinesia [1]. Levodopa and deep brain stimulation (DBS) of the subthalamic nucleus (STN) are effective therapies for alleviating those motor symptoms. When PD progresses to advanced stage, however, about half of the patients experience axial symptoms, such as severe gait and postural impairments (more specifically, freezing of gait and falls) that are not ameliorated by levodopa or STN-DBS [2].

In recent years, there is growing evidence that the pedunculopontine nucleus (PPN) plays a vital role in the occurrence of axial symptoms that can be alleviated by PPN-DBS [3–8]. Research on the functions of PPN and the dysfunction of it in PD will contribute to the understanding of the pathological substrates of axial symptoms and the mechanism of PPN-DBS. Furthermore, studies

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Table 1
Summary of the results from previous studies.

Studies	Subjects	Findings
Karachi et al. [9]	Monkeys	Bilateral PPN cholinergic cells lesion induced gait and postural deficits
MacLaren et al. [19]	Rats	Motor impairments only emerged when the demands of the task increased
Kucinski et al. [17]	Rats	Loss of PPTg cholinergic neurons were not necessary to induce falls
Gut NK et al. [18]	Rats	Lesion of PPTg did not affect gait performance of rats
Pienaar et al. [25]	Rats	Activation of PPN cholinergic neurons reversed motor deficits
Jin et al. [14]	Rats	Only anterior PPN lesions decreased the front limb swing time of gait, while not affecting other gait-related parameters
Janickova et al. [15]	Mice	Long-term degeneration of cholinergic neurons from PPTg critically disturbed motor functions

have reported the cholinergic cell loss in PPN of PD patients with refractory gait and postural disorders [9,10]. Consequently, it is reasonable to assume that the cholinergic degeneration may be more relevant to intractable axial symptoms than dopaminergic degeneration. Besides clinical studies that attempted to investigate the relationship between cholinergic dysfunction and axial motor disturbance [11–13], some animal studies also focused on the influence of PPN cell lesion (mainly the cholinergic cells) on motor behavior [9,14–20]. However, the results of these studies were not completely consistent. Although bilateral lesioning of PPN cholinergic cells induced gait and postural deficits in nondopaminergic lesioned monkeys [9], lesion of the pedunculopontine tegmental nucleus (PPTg), the rodent equivalent of the human PPN, did not affect gait performance of rats [18]. And another research group found that motor impairments did not show in rats on execution of individual motor actions but emerged when the demands of the task increased (accelerating rotarod task) after pedunculopontine lesions [19] (Table 1 shows results of previous studies). Therefore, in the present study, we further investigate the role of PPN cholinergic cells on motor deficits, especially the axial motor impairments, by measuring the motor performance of sham lesion rats, SNc dopaminergic lesion rats, PPTg cholinergic lesion rats, and combined lesion rats.

The IgG immunotoxin-targeting choline acetyltransferase (anti-ChAT-SAP) is a tool for eliminating cells that express choline acetyltransferase (ChAT); targeted via the affinity-purified rabbit polyclonal antibody to ChAT, eliminated via saporin [16,21]. We used the anti-ChAT-SAP to create the selective PPTg cholinergic depletion rat model.

2. Materials and methods

2.1. Subjects and study design

Sixty adult male Sprague-Dawley rats, weighing ~300 g at the time of surgery, were used in this study. Animals were housed 2–3 per cage with ad libitum access to water in a standard 12-h light-dark cycle. In order to maintain constant body weight (~300 g) food intake of each rat was limited to ~18 g/day. All procedures were approved and regulated by the Institutional Animal Ethics Committee of Southern Medical University of China.

In CatWalk experiment, 42 rats were randomly assigned to one of four following groups: (1) sham lesion with saline vehicle; (2) unilateral SNc dopaminergic lesion with 6-hydroxydopamine (6-OHDA); (3) unilateral PPTg cholinergic lesion with anti-ChAT-SAP; and (4) unilateral SNc dopaminergic lesion combined with PPTg cholinergic lesion. In rotarod experiment, 18 rats were randomly and evenly assigned to three groups: (1) sham lesion; (2) unilateral PPTg cholinergic lesion; (3) bilateral PPTg cholinergic lesion.

2.2. Surgery and lesions

All rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), placed in a stereotaxic apparatus (Stoelting) and received penicillin (16,000 U, im) before surgery. Unilateral SNc dopaminergic lesion were achieved via injection of 4 μl of 6-OHDA (3 μg/μl free base dissolved in a solution of 0.2 mg/ml L-ascorbicacid in 0.9% w/v saline at a rate of 0.5 μl/min; Sigma) into the right medial forebrain bundle (MFB) at the following coordinates relative to bregma according to the stereotaxic atlas of Paxinos and Watson (2007): −1.8 mm antero-posterior (AP), 2.0 mm medio-lateral (ML), and −8.2 mm dorso-ventral (DV, from the skull surface). 30 min before the 6-OHDA injection, animals received the noradrenergic reuptake inhibitor desipramine (15 mg/kg, ip; Sigma) to minimize damage to noradrenergic neurons. For unilateral PPTg cholinergic lesion, the anti-ChAT-SAP (0.5 μg/μl in phosphate-buffered saline; 250 ng in 0.5 μl per infusion; Advanced Targeting Systems; cat No IT-42) was infused into the right PPTg at the coordinate of −7.8 mm AP, 2.0 mm ML, and −7.2 mm DV. For bilateral PPTg lesion, the same dose of anti-ChAT-SAP was injected into left and right PPTg. Sham lesion was obtained by injecting the same dose of saline.

2.3. Behavioral testing

2.3.1. CatWalk testing

Gait testing was conducted on the CatWalk XT (Noldus information Technology, Wageningen, Netherlands) that was used in our previous studies to analyze gait of unforced moving rats [22,23]. All animals were trained on the CatWalk for one week to ensure an acceptable performance of three consecutive runs. Baseline behavioral evaluations were performed for three days prior to surgery (at least one session per day for a rat; each session contains three consecutive non-interrupted runs). Gait performance was re-evaluated for all rats 21 days after surgery. Table 2 shows the gait parameters and related clinical symptoms.

2.3.2. Freezing of gait (FOG)

During behavioral re-evaluations after lesion, rats showed moderate to severe gait freezing (start hesitation) during testing except the sham lesion group. The time of testing for lesion rats was remarkably longer than baseline evaluations, especially for the combined lesion rats. Some rats suffered from severe start hesitation (rats could not start to walk and stayed still at one end of the walkway) leading to the disability of finishing the required three runs per testing session. The number of unaccomplished sessions (less than three runs) resulted from gait freezing (start hesitation) were counted as the number of FOG, and the percentage of FOG in the total number of testing sessions was calculated for each group.

2.3.3. Rotarod

Rats were trained on a six-lane rotarod (Shanghai Mobiledatum information Technology Co., Ltd, Shanghai, China) at 9 rpm. until they could perform at this speed for 120 s. Individual baseline level of performance was established three days prior to surgery. Rats were placed on the rotarod at 12 rpm, 18 rpm, 24 rpm, 30 rpm and smooth accelerating (0–40 rpm 180 s). Latency to fall was recorded in seconds by an infra-red beam across the rats fall path. If the rat had not fallen by 180 s it was removed from the apparatus. Each rat was tested four times at each speed, with at least 5 min rest