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THE ANTERIOR AND POSTERIOR PEDUNCULOPONTINE TEGMENTAL NUCLEUS ARE INVOLVED IN BEHAVIOR AND NEURONAL ACTIVITY OF THE CUNEIFORM AND ENTOPEDUNCULAR NUCLEI

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Abstract—Loss of cholinergic neurons in the mesencephalic locomotor region, comprising the pedunculopontine nucleus (PPN) and the cuneiform nucleus (CnF), is related to gait disturbances in late stage Parkinson's disease (PD). We investigate the effect of anterior or posterior cholinergic lesions of the PPN on gait-related motor behavior, and on neuronal network activity of the PPN area and basal ganglia (BG) motor loop in rats. Anterior PPN lesions, posterior PPN lesions or sham lesions were induced by stereotaxic microinjection of the cholinergic toxin AF64-A or vehicle in male Sprague–Dawley rats. First, locomotor activity (open field), postural disturbances (Rotarod) and gait asymmetry (treadmill test) were assessed. Thereafter, single-unit and oscillatory activities were measured in the non-lesioned area of the PPN, the CnF and the entopeduncular nucleus (EPN), the BG output region, with microelectrodes under urethane anesthesia. Additionally, ECoG was recorded in the motor cortex. Injection of AF64-A into the anterior and posterior PPN decreased cholinergic cell counts as compared to naive controls ($P < 0.001$) but also destroyed non-cholinergic cells. Only anterior PPN lesions decreased the front limb swing time of gait in the treadmill test, while not affecting other gait-related parameters tested. Main electrophysiological findings were that anterior PPN lesions increased the firing activity in the CnF ($P < 0.001$). Further, lesions of either PPN region decreased the coherence of alpha (8–12 Hz) band between CnF and motor cortex (MCx), and increased the beta (12–30 Hz) oscillatory synchronization between EPN and the MCx. Lesions of the PPN in rats had complex effects on oscillatory neuronal activity of the CnF and the BG network, which may contribute to the understanding of the pathophysiology of gait disturbance in PD. **© 2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: gait disturbance, alpha oscillatory activity, cholinergic neurons, pedunculopontine tegmental nucleus, cuneiform nucleus.

INTRODUCTION

The late stages of Parkinson's disease (PD) and progressive supranuclear palsy (PSP) are characterized by postural instability and gait disturbances, which may be refractory to dopaminergic (DA) medication, but also to deep brain stimulation (DBS) of the subthalamic nucleus (STN) or the globus pallidus internus (GPi; Kasashima and Oda, 2003; Schrader et al., 2013; Fasano et al., 2015). Disturbed gait has been related to degeneration of cholinergic neurons in the pedunculopontine nucleus (PPN; Hirsch et al., 1987; Jellinger, 1988; Zweig et al., 1989), which, together with the cuneiform nucleus (CnF), forms the mesencephalic locomotor region (MLR). DBS of the PPN has been tested for treating these symptoms, however, with variable results and with substantial controversy, where exactly the optimal site for stimulation is located (Plaha and Gill, 2005; Ferraye et al., 2010; Moro et al., 2010; Schrader et al., 2013; Nosko et al., 2015).

In primates, the PPN consists of a compact part (PPNc) with a higher density of cholinergic neurons, and a pars dissipata (PPNd), with glutamatergic, GABAergic, and cholinergic neurons (Inglis and Winn, 1995; Ros et al., 2010; Martinez-Gonzalez et al., 2011). The PPN interacts with the basal ganglia (BG) motor loop, but also relays information to the brainstem and spinal cord relevant for postural muscle tone (Alam et al., 2011). Lesions of cholinergic PPN neurons in monkeys induce akinesia, gait and postural changes resistant to DA agents, which closely parallels findings in patients with late stage PD (Kojima et al., 1997; Matsumura and Kojima, 2001; Karachi et al., 2010). In rodents, however, the pedunculopontine tegmental nucleus (PPTg) has primarily been related to non-motor behavior, such as cognition and sensorimotor gating (see Winn, 2006 for review). Bilateral excitotoxic lesions of the complete PPTg did not affect

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Abbreviations: AF64-A, ethylcholine mustard aziridinium ion; ANOVA, analysis of variances; aPPTg, anterior PPTg; BG, basal ganglia; ChAT, choline-acetyltransferase; CnF, cuneiform nucleus; CV, coefficient of variation; DA, dopaminergic; DBS, deep brain stimulation; ECG, electrocardiogram; ECoG, electrocorticogram; EPN, entopeduncular nucleus; FFT, Fast Fourier Transform; FIR, finite impulse response; GPi, globus pallidus internus; ISI, inter-spike interval; LFPs, local field potentials; MCx, motor cortex; MLR, mesencephalic locomotor region; 6-OHDA, 6-Hydroxydopamin; PBS, phosphate-buffered saline; PD, Parkinson's disease; PDF, probability density functions; PFA, paraformaldehyde; PPN, pedunculopontine nucleus; PPNc, pedunculopontine nucleus pars compacta; PPNd, pedunculopontine nucleus pars dissipata; pPPTg, posterior PPTg; PPTg, pedunculopontine tegmental nucleus; PSP, progressive supranuclear palsy; RPM, round per minute; STN, subthalamic nucleus; SU, single unit; Dtx-UII, urotensin II-conjugated diphtheria.

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spontaneous locomotor activity, stability, speed, stride or coordination (Inglis et al., 1994; Olmstead and Franklin, 1994; Winn, 2006). Nevertheless, more recently, Alderson et al. (2008) have observed a reduction in locomotion after lesioning a restricted portion of the anterior but not of the posterior part of the PPTg. These results are consistent with the hypothesis that in rats the anterior PPTg (aPPTg), which is thought to resemble the PPNd, has functions and anatomical connections related to motor processes (Rye et al., 1987; Honda and Semba, 1995), while the posterior PPTg (pPPTg) resembles the PPNc because of a high density of cholinergic neurons and stronger anatomical connections to associative-limbic structures (Olszewski and Baxter, 1982; Manaye et al., 1999; Mena-Segovia et al., 2008). Whether the effects of the aPPTg lesions are achieved through the effects on descending motor projections, or through effects on the BG motor loop, possibly via the CnF as suggested by Alam et al. (2012), has not been investigated.

Ethylcholine mustard aziridinium ion (AF64-A) has been introduced as an irreversible inhibitor of the choline uptake system and choline-related enzymes (Fisher and Hanin, 1980) and described as a potent and selective cholinergic neurotoxin for the PPN that acts in a dose- and site-dependent manner (Kása and Hanin, 1985; Hanin, 1996; Lança et al., 2000). Here we tested the effects of AF64-A-induced cholinergic lesions of either the aPPTg or the pPPTg on rodent gait-related behavior and extracellular neuronal activity of the unlesioned part of the PPTg, as well as on the CnF and the entopeduncular nucleus (EPN), which is regarded the rat equivalent of the human GPi and the output nucleus of the BG motor loop.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats ($N = 20$; weighing 220–230 g; Charles River Laboratories, Germany) were randomly divided into three groups, including naive controls ($N = 8$), aPPTg lesion ($N = 6$) and pPPTg lesion ($N = 6$) animals. Rats were housed in groups of four in standard Macrolon Type IV cages (Techniplast, Hohenpeissenberg, Germany) under a 14-h/10-h light–dark cycle with light on at 07:00 am at a room temperature of 22 ± 2 °C, and with food and water available at all times. All animal procedures were in accordance with the European Council Directive of November 24, 1986 (86/609/EEC) and were approved by the local animal ethics committee. All efforts were made to minimize the number of animals used and their suffering.

PPTg cholinergic lesion

The cholinergic neurotoxin AF64-A solution was prepared from acetylethylcholine mustard HCl (Sigma, Darmstadt, Germany) as described previously by Fisher et al. (1982). In rats, the lesion size after injection of AF64A has been reported to be dose dependent. Therefore, in a preliminary study we tested a dose at the lower end of the dose range reported in the literature (Rodriguez et al., 1998). In our study, injection of 6.75 ng resulted in a loss of cholinergic neurons within the aPPTg or pPPTg areas, as shown by immunohistological staining for cholinergic neurons, without cavitation. The solution was freshly prepared in 1 mg/mL aqueous solution, adjusted with 10 N NaOH to a pH of 11.5–11.7 and kept in room temperature for 30 min with continuous stirring. Thereafter, the pH was adjusted to 7.4 using 6 N HCl and NaOH. The AF64-A solution was further diluted with saline (0.9% NaCl) for a final concentration of 13.5 ng/μL and stored at 4 °C until microinjection within 6 h.

The surgical procedure was adapted according to a protocol for PPTg lesion in rats (Rodriguez et al., 1998). Animals were anesthetized with 3.6% chloral hydrate solution (1 mL/100 g body weight, i.p., Sigma, Germany) and placed in a rodent stereotaxic frame (Stoelting, Wood Dale, Illinois, USA). After making a small incision to expose the scalp, a bone scraper was used to clean the skull above the bregma and lambda and a small craniotomy was made with a dental drill above the PPTg area of each hemisphere. The aPPTg (AP: −7.3 mm posterior to the bregma; ML: ±1.8 mm and V: −7.3 mm; according to the atlas of Paxinos and Watson, 1998) and the pPPTg (AP: −8.3 mm posterior to bregma; ML: ±1.8 mm and V: −7.0 mm) were lesioned bilaterally by microinjection of a total volume of 0.5 μl of AF64-A (6.75 ng) that was injected with a rate of 0.1 μL/min into each PPTg region. Controls received microinjection of vehicle only. The tooth bar was set at −3.3 mm for all coordinates.

Behavior

Three weeks after surgery behavioral testing started. All tests were performed after 30–60-min habituation to the testing room during the day light cycle under artificial light with a fixed intensity and acoustic exposure to a masking noise (playing radio).

Activity box test. To assess spontaneous locomotion, the animals were placed in a black plastic open field (60 × 60 × 30 cm). A video of the animals was recorded for 10 min by a camera installed above the box and the total distance traveled was automatically calculated by a video tracking system using the same settings for all rats (TopScan 1.0, Clever Sys. Inc., Reston, VA, USA).

Rotarod testing. To assess the motor coordination of the animals, an accelerating Rotarod (IITC Life Science, Woodland Hills, CA, USA) was used. The Rotarod consisted of a suspended rod, accelerating for 60 s from 1 round per minute (RPM) to 12 RPM, thereafter continuing at that speed for another 60 s. A trial was stopped when the rat fell off the Rotarod or after 120 s. Three consecutive trials were performed with a rest period of 5 min in between, and the mean duration of rat staying on the Rotarod was calculated. Prior to surgery, the rats were trained for five days to achieve a stable performance.

Automated treadmill gait test. Treadmill gait assessment was performed with the TreadScan system

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