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Mild dopaminergic lesions are accompanied by robust changes in subthalamic nucleus activity

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A B S T R A C T

The subthalamic nucleus (STN) is a major player in the input and output of the basal ganglia motor circuitry. The neuronal regular firing pattern of the STN changes into a pathological bursting mode in both advanced Parkinson's disease (PD) and in PD animals models with severe dopamine depletion. One of the current hypothesis, based on clinical and experimental evidence, is that this typical burst activity is responsible for some of the principal motor symptoms. In the current study we tested whether mild DA depletion, mimicking early stages of PD, induced deficits in motor behaviour and changes in STN neuronal activity. The present study demonstrated that rats with a mild lesion (20–40% loss of DA neurons) and a slowed motor response, but without gross motor abnormalities already have an increased number of bursty STN neurons under urethane anaesthesia. These findings indicate that the early increase in STN burst activity is a compensatory mechanism to maintain the dopamine homeostasis in the basal ganglia.
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1. Introduction

The subthalamic nucleus (STN), is a major player in the input and output of the basal ganglia motor circuitry [2,33]. Firstly, the STN forms together with the striatum the basal ganglia entry sites for cortical input [1,25]. Secondly, the STN strongly modulates the basal ganglia output nuclei by its glutamatergic efferents and is also an output nucleus itself by its direct cortical projections [13,30].

Animal studies indicate that STN neurons normally fire in a regular manner [26,28,42]. In both PD patients and severe animal models of PD, the STN's neuronal regular firing pattern changes into a pathological bursting mode [6,8]. One of the current hypothesis is that this typical burst activity is responsible for some of the principal motor symptoms [7,8,20,31]. Both, clinical and experimental evidence support this hypothesis. For instance, intra-operatively collected electrophysiological recordings from the STN of PD patients show a typical burst pattern [6] and modulation of this pattern by deep brain stimulation (DBS) alleviates motor disability [5,22,39]. Also animal models of PD are characterized by a pathological firing pattern of STN neurons [8,19,27].

Although altered STN activity is observed in PD patients in the advanced stages of the disease as well as in animal models with severe DA lesions, it is still elusive at which stage of the disease changes in STN neuronal activity occur. In the current study, we addressed this by evaluating electrophysiological characteristics of STN neurons and the motor behaviour in an animal model mimicking early stages of PD by applying different levels of dopamine (DA) depletion by bilateral striatal 6-OHDA injections. Electrophysiological changes were assessed using *in vivo*, single-cell extracellular recordings in the STN. Motor behaviour was evaluated by reaction time task [37] and locomotor activity in by the open field task [17], and the CatWalk automated gait analysis task [40].

2. Materials and methods

2.1. Subjects

Male Lewis rats ($N = 30$, 10 weeks old) bred and housed at the Central Animal Facility of Maastricht University (Maastricht, The

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Netherlands), were 15 weeks old and weighed ~315 g at the time of surgery. All experimental procedures were approved by the Animal Experiments and Ethics Committee of Maastricht University.

2.2. Surgical procedure

Two rats died peri-operatively, and the others were divided in the following four experimental groups: (A) sham ($n = 7$); (B) 2.5 μg/μl 6-OHDA ($n = 7$); (C) 5 μg/μl 6-OHDA ($n = 7$); (D) 7.5 μg μl 6-OHDA ($n = 7$). These three different concentrations were chosen to assess changes in motor behaviour and electrophysiological activity in mild to moderate DA depleted animals mimicking different stages of PD. Details of the surgical procedure have been described earlier [35]. In brief, 1 h before surgery rats were injected with Temgesic (0.1 mg/kg, s.c.) and with desimipramine (20 mg/kg, i.p.). Throughout surgery, rats were anesthetized by 2% isoflurane inhalation. Rats were bilaterally injected in the striatum with 2 μl vehicle (0.2% ascorbic acid dissolved in 0.9% saline; *i.e.* sham), or 6-OHDA (2.5, 5.0 or 7.5 μg/μl; Sigma, Zwijndrecht, The Netherlands) at the following coordinates relative to Bregma (in mm): AP 0.7, ML ±2.8, DV −5.0 and AP −0.4, ML ±3.4, DV −5.0 [29], at 0.5 μl/min. After surgery, the rats were given a 2-week recovery period before behavioural training was started. Data from all behavioural tests were acquired 3–4 weeks after surgery.

2.3. Reaction time task

Rats were trained to perform a reaction time (RT) task preoperatively. The motor performances of the rats were tested in operant chambers as described earlier [10,37]. After training, the rats were tested for motor time performance. Post surgery the rats were trained again and were tested. The motor time was acquired before and after the injection of 6-OHDA or saline into the striatum. The average motor time (MT) was calculated over five trials. For each animal the increase in motor time and reaction time (RT) was calculated and expressed in percentages, as for the change in premature responses (PR).

2.4. Open field test

The open field was performed as described earlier [36]. Locomotor activity (distance moved and speed) was recorded using a camera and Ethovision tracking software (Ethovision®, Noldus Information Technology, Wageningen, The Netherlands).

2.5. CatWalk gait analysis

Pre- and post-surgically the rats were trained to cross the walkway (CatWalk XT8.1, Noldus, The Netherlands), *i.e.* they were motivated by food deprivation 12 h before training and rewarded with a food pellet (Noyes Precision pellets PJPPP-0045; Sandown Chemical Ltd., Hampton, UK) after a complete and continuous crossing. Training continued until crossing the walkway met the criteria for acquisition [18]. The following gait parameters were evaluated: speed, walking pattern and standing time, for more details we refer to Vlamings et al. [40]. For statistical analysis the data of 5 trials was averaged.

2.6. Electrophysiology

At the end of the behavioural experiments, rats were anesthetized with urethane (1.6 g/kg, i.p.) and fixed in a stereotactic frame (Stoelting model 51950, Stoelting Co., Wood Dale, USA). Body temperature was maintained at 37 °C (DC Temperature controller 41-90-8D, FHC Inc., Bowdoin, USA). A glass microelectrode (borosilicate glass capillaries [GC200F-10], Harvard apparatus Ltd., MA, USA), with an impedance of ±10–15 MΩ, containing 4% Pontamine sky blue dye dissolved in 0.9% NaCl was then lowered into the STN using a microdrive (Hydraulic probe drive chronic adaptor, 50-12-9 FHC Inc., Bowdoin, USA). Stereotactic coordinates in mm relative to Bregma were: AP −3.8, ML ±2.5, DV −8.0 [29]. The electrode was connected to an AlphaMap data acquisition system (AlphaOmega, Nazareth, Israel), allowing amplification, filtering for live view and recording the extracellular neuronal activity. At the end of each session, the recording site was marked by an electrophoretic injection of Pontamine sky blue using a 15 μA negative current for 20 min (Accupulser signal generator and a high current isolator [A385] with a charger [A382], WPI Inc., Sarasota, USA). Neurons were only analysed if the recording site was histochemically confirmed to be in the STN (Fig. 3a).

Raw data were analysed using Matlab (The MathWorks, Natick, MA). Spikes were detected based on a threshold that was defined as the standard deviation of the signal-median multiplied by 3.5 [16]. Classification of the spikes was based on the waveforms of the detected spikes. First, a principal component analysis was performed. Subsequently, the principal components were clustered using either *K*-means or the Gaussian mixture model and the expectation maximization algorithm [23]. Only time intervals with a stable firing rate and neurons with a signal to noise ratio greater than three were considered.

A discharge density histogram [21] was developed to assess the firing pattern as previously described [11,14]. The mean firing frequency was calculated per group.

2.7. Histology

At the end of the electrophysiological recordings, rats were transcardially perfused and prepared for immunohistochemistry. Tyrosine hydroxylase (TH) immunohistochemistry was carried out using a monoclonal mouse anti-TH (diluted 1:100, kindly supplied by Dr. C. Cuello, Canada) as primary antibody. Details of this staining have been described previously [38].

2.8. Stereological analysis

Stereological analysis was performed using a stereology workstation (CAST-GRID-Computer Assisted Stereological Toolbox, Olympus, Denmark). After exactly tracing the boundaries of the left and right substantia nigra pars compacta (SNc) 10× magnification, number of TH immunoreactive (THir) neurons was counted at 40× magnification using the Optical Fractionator [32]. All neurons that were in focus in the unbiased virtual counting spaces throughout the delineated regions were counted. The number of neurons was estimated based on the number of counted neurons and the corresponding sampling probability. The total number of THir neurons in the left and right SNc was averaged per animal.

2.9. Statistical analyses

The data from the RT task, the open field, the electrophysiology and the data on the total number of THir cells in the SNc were analysed using a one-way ANOVA followed by a least significant difference (LSD) post hoc test. Electrophysiological differences in firing pattern were evaluated by a Chi-square test. The parameters of the CatWalk were analysed using a repeated measures one-way ANOVA followed by an LSD post hoc test. All data were analysed using SPSS statistical software (SPSS version 15.0, IBM, Chicago, USA). $p < 0.05$ was considered to be statistically significant. All data are presented as means and standard errors of means (SEM).

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