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Deep brain stimulation of subthalamic neurons increases striatal dopamine metabolism and induces contralateral circling in freely moving 6-hydroxydopamine-lesioned rats

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Abstract

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) alleviates Parkinson's disease (PD) symptoms. Although widely used, the mechanisms of action are still unknown. In an attempt to elucidate those mechanisms, we have previously demonstrated that STN-DBS increases striatal extracellular dopamine (DA) metabolites in anaesthetized rats. PD being a movement disorder, it remains to be determined whether these findings are related to any relevant motor or behavioural changes. Thus, this study investigates concomitant behavioural changes during STN-DBS and extracellular striatal DA metabolites measured using microdialysis in freely moving 6-hydroxydopamine-lesioned rats. STN-DBS induced an increase of striatal DA metabolites in awake, freely moving animals. Furthermore, we observed concomitant contralateral circling behaviour. Taken together, these results suggest that STN-DBS could disinhibit (consequently activate) substantia nigra compacta neurons via inhibition of gamma-aminobutyric acid-ergic substantia nigra reticulata neurons.

Keywords: Parkinson's disease; Deep brain stimulation; Subthalamic nucleus; Striatum; Dopamine; Microdialysis; Freely moving rats

Parkinson's disease (PD) is characterized by a progressive degeneration of nigral dopamine (DA) neurons [8]. During the last ten years, deep brain stimulation (DBS) of the subthalamic nucleus (STN) has evolved as a new efficient therapeutic approach for the treatment of PD improving rigidity, bradykinesia, tremor and levodopa induced dyskinesia [1]. However, the exact mechanisms of action are still unknown. Notably, electrophysiological recordings would best reflect changes in the different structures of the basal ganglia during DBS. However, due to the stimulation artefact, recordings are limited to the time after DBS [2,3]. Therefore, other techniques such as microdialysis, which allow sampling during stimulation, are more suitable to detect changes in neuronal activity related to DBS.

Recently, it has been shown that STN-DBS increases striatal DAergic activity in naive and 6-hydroxydopamine (6-OHDA)-lesioned rats [4,11]. However, since these studies were performed in anaesthetized rats, it remains to be determined whether these findings are related to any motor or behavioural changes relevant to PD. Therefore, the present study was designed to investigate if the increase in striatal DA metabolism in the 6-OHDA-model of PD is accompanied by behavioural changes during STN-DBS.

After permission was obtained from local authorities, the study was carried out in accordance with European Communities Council Directive of 24 November 1986 (86/609/EEC) for care of laboratory animals. Male Wistar rats (Harlan-Winkelmann, Germany; 240–275 g during 6-OHDA-lesion; 300–430 g during DBS) were maintained under standard housing conditions with a 12 h dark/light cycle (lights on: 06:00–18:00 h). All experiments were done during the day period of the animals. Food and water were available ad libitum.

All procedures have been previously described in detail [11]. Twelve weeks post-striatal four-point 6-OHDA injection, a guide cannula (CMA 12 Guide Cannula, Carnegie, Sweden) and a stimulating electrode (NE-100 with connec-

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tor, RMI, USA) were stereotactically implanted into the left dorsal striatum (A: 1.0; L: 3.0; V: −6.0 [15]) and the left STN (A: −3.8; L: 2.5; V: −7.6 [15]), respectively. Animals remained in a Plexiglas bowl during microdialysis and DBS. The microdialysis probe (CMA 12, Carnegie, Sweden) was implanted into the left striatum through the fixed guide cannula 1 h prior to the beginning of sampling and perfused with artificial cerebrospinal fluid. Samples were collected every 20 min for immediate analysis of extracellular concentrations of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by high-performance liquid chromatography with electrochemical detection. For STN-DBS, the following stimulation parameters were applied: frequency, 130 Hz; pulse width, 60 μs; current intensity, 300 μA, for 20 min, in a constant current mode using an isolated stimulator (Coulbourn Instruments, USA). Control animals received no stimulation while the stimulating electrode was placed in the STN. The number of rotations (full turns/min ± SEM) were assessed in automated rotometer bowls (TSE GmbH, Kronberg, Germany) on two occasions: (i), after acute amphetamine challenge (2 mg/kg, i.p., 3 weeks after 6-OHDA-lesion); and (ii), during STN-DBS. Cresyl violet staining was performed for histological verification of STN stimulation electrode and striatal microdialysis probe placement before validation of results. In addition, tyrosine hydroxylase immunohistochemistry revealed that 9.2 ± 2.6% of substantia nigra compacta (SNc) neurons remained in the 6-OHDA-lesioned SNc in comparison with the intact side (assessed as previously described [11]). Statistical analysis of extracellular striatal concentrations of DA and its metabolites was performed using a one-way analysis of variance for repeated measures followed by post-hoc *t*-tests corrected for multiple comparisons by the method of Student–Newman–Keuls. Rotations were compared using the Student's *t*-test. A probability level of 5% ($P < 0.05$) was considered significant. Data are shown as means ± SEM.

During STN-DBS, freely moving 6-OHDA-lesioned rats expressed contralateral circling (6.7 ± 2.4 turns/min, $P < 0.05$), immediately following the onset of DBS. Control rats did not display any circling behaviour during sham stimulation. In agreement with successful retrograde lesioning of the left SNc, acute amphetamine challenge induced ipsilateral circling in all investigated animals (DBS group: 7.0 ± 1.4 turns/min, $n = 14$; controls: 7.6 ± 1.4 turns/min, $n = 6$, $P > 0.5$).

Twenty to forty minutes after DBS, striatal extracellular DOPAC and HVA significantly increased up to 121.8 ± 4.1 ($F_{(13,181)} = 10.5$, $P < 0.0001$, $n = 14$, Fig. 1A) and 119.5 ± 3.9% ($F_{(13,181)} = 6.5$, $P < 0.0001$, $n = 14$, Fig. 1B) in comparison with baseline while they remained stable in control animals (DOPAC: $F_{(5,77)} = 0.4$, $P > 0.05$, $n = 6$, Fig. 1A; HVA: $F_{(5,77)} = 0.5$, $P > 0.05$, $n = 6$, Fig. 1B). During the observation period, extracellular DA decreased in both groups (DBS: $F_{(13,181)} = 4.9$, $P < 0.0001$, $n = 14$; controls: $F_{(5,77)} = 5.3$, $P < 0.0001$, $n = 6$, Fig. 1C) as classically reported when using such an experimental paradigm [17,20]. Nevertheless, there was no significant difference of striatal extracellular DA concentrations between the DBS group and controls ($P > 0.05$). STN-DBS is therefore not responsible for this decrease which is rather a consequence of microdialysis probe insertion.

The present study shows that STN-DBS increases extracellular striatal DA metabolites in awake, freely moving rats and concomitantly induces contralateral circling in the unilaterally 6-OHDA-lesioned rat model of PD. It is well known that DAergic agents such as amphetamine and apomorphine induce circling in 6-OHDA-lesioned rats by acting on the striatal DA system [19]. Since DAergic therapy improves major motor symptoms in PD and even

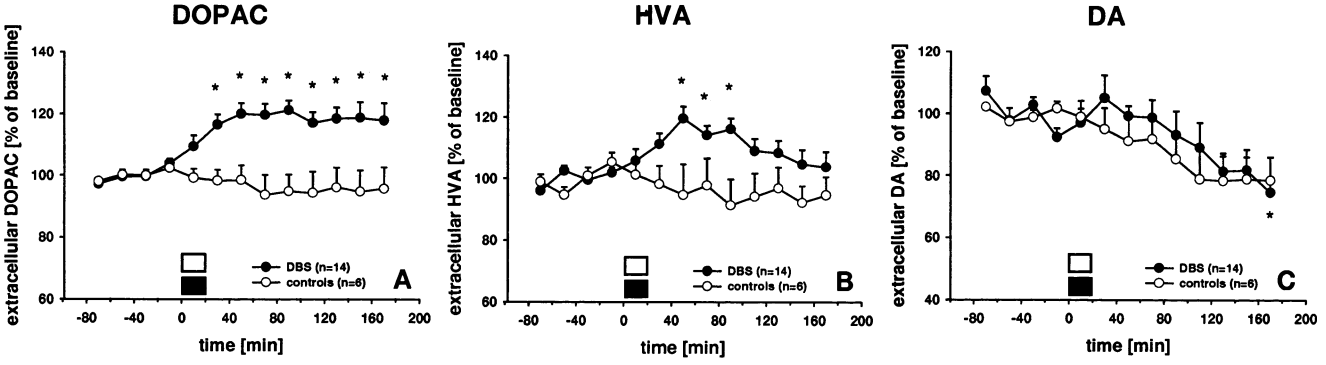

Fig. 1. DBS of the STN and striatal extracellular DA (A), DOPAC (B) and HVA (C). Closed bar represents duration of stimulation, open bars represent period of rotational behaviour assessment. Note that the dead volume in the microdialysis tubing is responsible for a lag time of 10 min. Thus, STN-DBS has been performed for either 10 min during the first and second sampling period after baseline, i.e. there is a correlation between the onset of increased DOPAC concentrations and rotational behaviour during DBS. Data are presented as means + SEM. The asterisk denotes significant differences in respect to baseline (*$P < 0.05$, post-hoc Student–Newman–Keuls test). (A) DOPAC, filled circles (-●-) represent DBS ($n = 14$); empty circles (-○-) represent controls ($n = 6$). Baseline values were 2.66 ± 0.97 pmol DOPAC/20 μl dialysate. (B) HVA, filled circles (-●-) represent DBS ($n = 14$); empty circles (-○-) represent controls ($n = 6$). Baseline values were 1.98 ± 0.67 pmol HVA/20 μl dialysate. (C) DA, filled circles (-●-) represent DBS ($n = 14$); empty circles (-○-) represent controls ($n = 6$). Baseline values were 70.04 ± 5.30 fmol DA/20 μl dialysate.

though the main effect of STN-DBS is most likely due to changes in the basal ganglia output structures, the present results suggest more distinctly that the effectiveness of STN-DBS in PD patients could, at least partially, be also mediated via the striatal DA system. However, considering that STN-DBS is applied in patients with late-stage PD [1], where almost all SNc neurons have disappeared, the observed effect would mainly be relevant in early-stage patients, i.e. in those where some nigral DA neurons remain.

An increase of DA metabolites is thought to reflect increased intraneuronal DA turnover [21]. Therefore, STN-DBS may have induced: (i), an increase of DA synthesis via activation of tyrosine hydroxylase or aromatic amino acid decarboxylase [10]; (ii), an inhibition of DA release, followed by activation of DA metabolism with increased diffusion of DOPAC to the extracellular space [18]; or (iii), an increase of DA release followed by efficient reuptake and/or metabolism. In accordance with the latter assumption and the present results, changes in movement and behaviour have been correlated with increased striatal DA metabolism, but unchanged DA release [5]. Moreover, Sabol et al. [16] reported an isolated increase of extracellular striatal DOPAC in rats turning on a treadmill while cell activity showed increased cell firing of DAergic neurons [7]. These data suggest that changes in DA metabolite concentrations may reflect underlying, but undetectable changes in DA release, which may be sufficient to induce alterations in movement and behaviour. This is further corroborated by recent findings that STN-DBS is followed by an increase in firing activity of nigral DA neurons [2]. In addition to circling behaviour induced by DAergic agents in unilaterally 6-OHDA-lesioned rats, contralateral circling has also been described in cats after local injection of the gamma-aminobutyric acid (GABA) receptor agonist muscimol in the STN [13]. Intriguingly, microinjection of muscimol into both the STN and STN-DBS decreases cell firing in the STN and the substantia nigra reticulata (SNr), whereas firing activity in the SNc is increased [2,3].

Thus, our results may be explained by a direct activation of SNc neurons via excitatory glutamatergic STN afferents. However, considering that: (i), STN neurons are already hyperactive in the parkinsonian state [6,12]; (ii), the relevance of the direct STN–SNc pathway is neglectable [14]; and (iii), STN-DBS is thought to result in an inhibition of subthalamic neurons [3], this explanation seems to be unlikely. On the other hand, it has been shown that SNc neurons are largely under inhibitory GABAergic control of the SNr [9]. Since STN-DBS is believed to inhibit these GABAergic neurons [3], an increase of striatal DA metabolism could be due to inhibition of SNr neurons.

In conclusion, this study indicates that STN-DBS is accompanied by changes in striatal DA metabolism in awake, freely moving 6-OHDA-lesioned rats with concomitant contralateral circling behaviour. Thus, STN-DBS could disinhibit (consequently activate) SNc neurons via inhibition of GABAergic SNr neurons. Finally, this would raise further evidence that STN-DBS may inhibit excitatory glutamatergic neurons.

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