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Neuronal Pathways Involved in Deep Brain Stimulation of the Subthalamic Nucleus for Treatment of Parkinson's Disease

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Abstract— **In this study, fixed potential amperometry was used to examine several pathways by which Deep Brain Stimulation (DBS) of the subthalamic nucleus (STN) or dopamine axons within the dorsal forebrain bundle (DFB) release striatal dopamine, thus potentially providing therapeutic benefits for Parkinson's Disease patients. In urethane anesthetized mice, electrical stimulations (20 monophasic pulses at 50 Hz every 30 sec) were applied to the STN or DFB while infusing the local anesthetic lidocaine (4%) into the substantia nigra compacta (SNc) or pedunculopontine tegmental nucleus (PPT). Findings suggest that DFB stimulation activates ascending SNc dopamine axons, while STN stimulation evokes striatal dopamine release directly via excitatory glutamatergic inputs to SNc dopamine cells and indirectly via excitatory cholinergic/glutamatergic STN-PPT-SNc pathways.**

I. Introduction

Despite unclear mechanisms, deep brain stimulation (DBS) targeting the subthalamic nucleus (STN) is an established neurosurgical approach for effectively treating the motor symptoms of Parkinson's disease. Therapeutic effects of DBS are similar to those of a lesion, thus DBS has been thought to act by silencing neuronal activity at the site of stimulation [1]. However, DBS has been shown to excite neurons within the subthalamic nucleus (STN) [2,3], increase activity in dopaminergic neurons of the substantia nigra pars compacta (SNc) [4,5], and enhance dopamine release in the striatum [6]. These findings suggest that STN DBS improves motor symptoms related to Parkinson's disease by activating surviving nigrostriatal dopamine neurons and subsequently increasing striatal dopamine release.

There are several neuronal pathways by which STN DBS could elicit dopamine release in the striatum. First, stimulation of the glutamatergic input that projects from the STN to the SNc has been shown to activate nigrostriatal dopaminergic pathways directly [7]. Second, stimulation of the glutamatergic neurons of the STN projecting to the pedunculopontine tegmental nucleus (PPT) indirectly activates nigrostriatal dopamine neurons via both reciprocal excitatory innervation back to the STN which leads to subsequent SNc activation by the aforementioned glutamatergic inputs [8] and by activating excitatory cholinergic and glutamatergic inputs from the PPT to the SNc [9,10]. Lastly, STN DBS may be activating nigrostriatal dopamine neurons via direct stimulation of the dorsal forebrain bundle (DFB) within the region of the zona incerta. Therapeutic outcomes of DBS have suggested that optimal improvements in symptoms are obtained when stimulating the white matter dorsal to the STN [11], which is comprised of dopaminergic axons within the DFB that project directly from the SNc to the striatum. Thus, DBS of the DFB may be superior to STN DBS in enhancing dopamine release in the striatum [6]. The present study investigated these pathways using *in vivo* fixed potential amperometry with carbon fiber microelectrodes in the striatum to record striatal dopamine release evoked by electrical stimulation of the DFB or STN, while infusing the local anesthetic lidocaine into the SNc or PPT.

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II. Methods

Thirty-two adult male C57BL/6J mice were anesthetized with urethane (1.5 g/kg, i.p.) and mounted in a stereotaxic frame. Stereotaxic coordinates (AP from bregma, ML from midline, and DV from dura, all in mm) were determined from the mouse atlas of Paxinos and Franklin (2001) [12]. As illustrated in Fig. 1, a concentric bipolar stimulating electrode was implanted into the left DFB (coord.: anterior-posterior (AP) -2.0, medial-lateral (ML) +1.1, and dorsal-ventral (DV) -4.0) or STN of each mouse (coord.: AP -2.0, ML +1.5, and DV -4.25). A 31 g stainless-steel guide infusion cannula was implanted into the left SNc (coord.: AP -3.1, ML +1.5, and DV -3.6) or PPT (coord.: AP -4.7, ML +1.25, and DV -2.25). An Ag/AgCl reference and stainless-steel auxiliary electrode combination was placed in surface contact with contralateral cortical tissue, and a carbon fiber recording microelectrode (250 µm in length by 10 µm o.d.) was then implanted into the left striatum (coordinates: AP +1.4, ML +1.4, and DV -2.5). All AP and

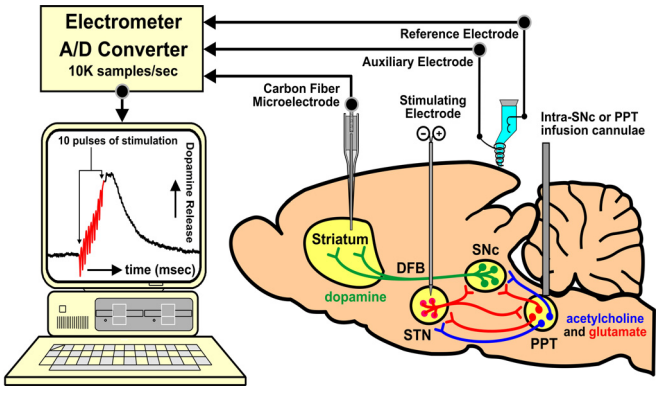

Fig. 1. Fixed potential amperometry set-up for evaluation of the effects of intra-SNc or PPT lidocaine infusions on STN or DFB electrical stimulation-evoked dopamine release in the striatum.

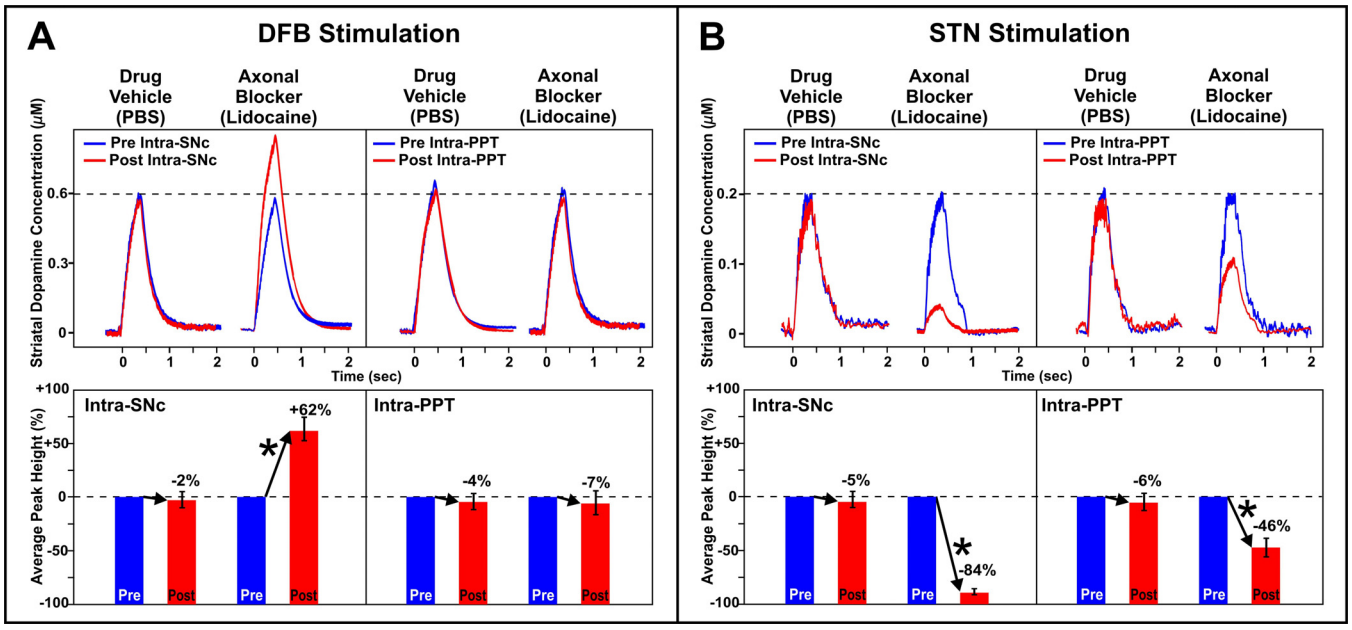

Fig. 2. Mean amperometric recordings of DFB (A) or STN (B) electrical stimulation-evoked dopamine release in the striatum following intra-SNc (left panel of A and B) or PPT (right panel of A and B) infusions of the axonal blocker lidocaine or drug vehicle (PBS) and corresponding mean peak percentages (bottom of A and B). * Significant change in dopamine concentrations post-infusion.

DV coordinates were with respect to skull landmark bregma and dura brain surface. A fixed potential (+0.8 V) was applied to the recording electrode and oxidation current was monitored continuously (10K samples/sec) with an electrometer filtered at 50 Hz. A series of 0.5 ms duration cathodal monophasic current pulses (20 pulses at 50 Hz applied every 30 sec) was delivered to the stimulating electrode. Intensity levels were set at 800 μAmps in the DFB and 400 μAmps in the STN as determined by preliminary studies to be optimal for each target site. Fixed potential amperometry coupled with carbon fiber microelectrodes has been confirmed as a valid technique for real-time monitoring of striatal dopamine oxidation current evoked by relatively brief (e.g., 20 pulses) electrical stimulation of ascending nigrostriatal dopaminergic projections to the striatum and afferent inputs to the SNc, such as those arising from the STN or PPT [6,10,13].

After a 10 min baseline recording period, intra-SNc or PPT 1.0 μl lidocaine (4%) microinfusions were made over a 1.5 min period. Infusion of lidocaine into these sites effectively blocks all neuronal transmission through these nuclei, thus acting as a reversible lesion (~15 min in duration). Intra-SNc or PPT infusions of 1.0 μl of sterile phosphate-buffered saline (PBS, pH~7.4) were also performed as control for the axonal blocking effects of lidocaine. The mean change in dopamine oxidation current, corresponding to stimulation-evoked dopamine release, was converted to a mean dopamine concentration (μM) by post-experiment *in vitro* calibration of the carbon fiber microelectrode in solutions of dopamine (0.2-10 μM) using a flow injection system [14].

Upon completion of data collection, an iron deposit was made in the STN or DFB stimulation sites by passing direct anodic current (100 μA for 10 sec) through the stimulating electrodes. Micro-lesions of the striatal recording sites were performed by passing direct anodic current (1 mA for 1 sec) through the recording microelectrodes. SNc and PPT infusion sites were marked by infusions of 1.0 μl cresyl violet stain into the cannula sites. Mice were then euthanized with a 0.25 ml intracardial injection of urethane (0.345 g/ml). Brains were removed immersed overnight in 10% buffered formalin containing 0.1% potassium ferricyanide, and then stored in 30% sucrose/ 10% formalin solution until sectioning. After fixation, 30 μm coronal sections were cut in a cryostat at -30 °C, with a Prussian blue spot resulting from a redox reaction of ferricyanide marking the stimulation sites. Placements of stimulating electrodes, recording microelectrodes and drug infusion cannulae were determined under a light microscope and recorded on representative coronal diagrams [12].

Urethane and lidocaine hydrochloride were purchased from Sigma-Aldrich (St Louis, MO, USA). With the exception of urethane (distilled water), chemicals were dissolved in sterile PBS (pH~7.4).

III. Results

A. Effects of intra-SNc or PPT lidocaine on DFB-evoked striatal dopamine release

As illustrated in Fig. 2A, with respect to pre-infusion baseline levels, DFB-evoked striatal dopamine release was not significantly altered 5 min following PBS infusion into the SNc (-2%) or PPT (-4%). In contrast, intra-SNc infusion of lidocaine significantly increased DFB-evoked striatal dopamine levels from pre-infusion baseline levels (+62%) with the peak increase occurring 5 min post-infusion. However, lidocaine infused into the PPT had no significant

effect on DFB-evoked dopamine release in the striatum assessed 5 min following infusion (-7%).

B. Effects of intra-SNc or PPT lidocaine on STN-evoked striatal dopamine release

As illustrated in Fig. 2B, with respect to pre-infusion baseline levels, STN-evoked striatal dopamine release was not significantly altered at 5 min following PBS infusion into the SNc (-5%) or PPT (-6%). In contrast, STN-evoked striatal dopamine release was significantly attenuated by lidocaine infused into either the SNc (-84%) or the PPT (-46%) compared to pre-infusion baseline levels with the peak decrease occurring 5 min post-infusion.

C. Stereotaxic placements of infusion cannulae, recording, and stimulating electrodes

Placements of the recording microelectrode surfaces were confined to the striatum (ranging from 1.34 to 1.54 mm anterior to bregma, 1.30 to 1.60 mm lateral to midline, and 2.40 to 2.70 mm ventral to dura; Fig. 3A). The tips of the stimulating electrodes were positioned within the anatomical boundaries of the DFB in the region of the zona incerta (ranging from 1.94 to 2.18 mm posterior to bregma, 1.00 to 1.20 mm lateral to midline, and 3.80 to 4.10 mm ventral to dura; Fig. 3B) or the STN (ranging from 1.94 to 2.18 mm posterior to bregma, 1.50 to 1.70 mm lateral to midline, and 3.90 to 4.20 mm ventral to dura; Fig. 3B). Infusion cannula tip placements were localized within the SNc (ranging from 2.92 to 3.16 mm posterior to bregma, 1.40 to 1.60 mm lateral to midline, and 3.60 to 3.90 mm ventral to dura; Fig. 3C) or PPT (ranging from 0.39 to 0.63 mm posterior to lambda, 1.15 to 1.40 mm lateral to midline, and 2.60 to 2.90 mm ventral to dura; Fig. 3D).

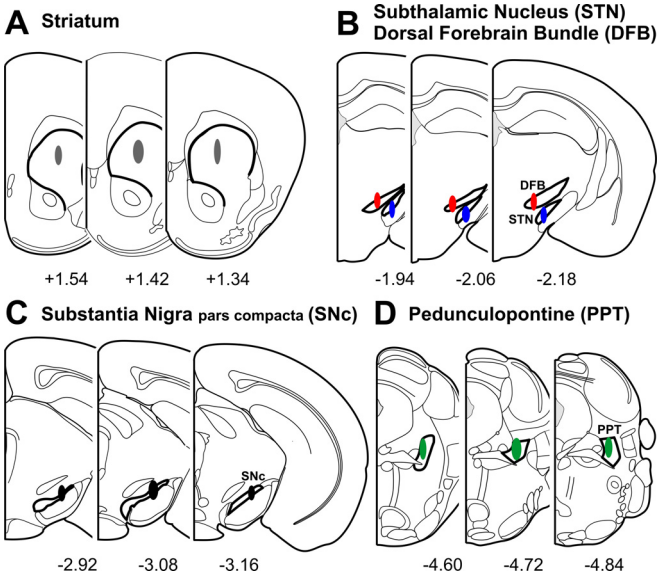

Fig. 3. Representative coronal sections of the mouse brain [12], with colored areas indicating the placements of (A) carbon fiber recording microelectrodes in the striatum (gray), (B) stimulating electrodes in the dorsal forebrain bundle and subthalamic nucleus (red and blue, respectively), and drug infusion cannulae in the (C) substantia nigra pars compacta (black) and (D) pedunculopontine tegmental nucleus (green). Numbers correspond to mm from skull landmark bregma.

IV. Conclusions

As measured by *in vivo* fixed potential amperometry, brief electrical stimulation of the DFB or STN elicits dopamine release in the striatum. The present findings suggest that DFB stimulation is mediated predominately by activation of ascending SNc dopamine neuronal axons, as lidocaine infusions into the SNc or PPT did not reduce DFB-evoked striatal dopamine release. Previous studies have shown that pharmacological denervation of dopamine axonal transmission promotes a rapid compensatory mechanism that dramatically enhances synthesis and storage of dopamine in terminal vesicles [15]. These findings are consistent with our observation of an enhancement (+62%) in DFB stimulation-evoked striatal dopamine release following lidocaine inactivation of the SNc. Altogether, these data may help to explain the clinical improvements in motor symptoms of Parkinson's patients following stimulation of the white matter dorsal to the STN [11].

The present results show that STN stimulation is dependent upon activities within the SNc and PPT, as inactivation of neuronal transmission in the SNc or PPT with the axonal blocker lidocaine lead to significant decreases in STN-evoked striatal dopamine release compared to pre-infusion baseline responses. As summarized in Fig. 4, these findings suggest that direct excitatory STN projections to dopamine cells in the SNc contribute at least 38% to striatal dopamine release evoked by DBS of the STN, while at least 46% of this response is mediated indirectly via excitatory STN-PPT projections that, in turn, provide excitatory PPT inputs to SNc dopamine cells. However, the present findings do not provide information as to the nature of these excitatory pathways (i.e., glutamatergic and cholinergic). As well, they cannot distinguish the extent to which STN stimulation is activating dopamine cells in the SNc via reciprocal excitatory innervation between the STN to the PPT. Future experiments incorporating intra-SNc. PPT, and STN infusions of selective glutamate and acetylcholine receptor antagonists will address these issues.

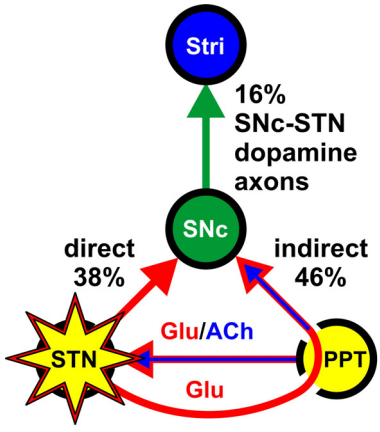

Fig. 4. Representations of the direct and indirect contributions of neuronal pathways involved in mediating striatal dopamine release following subthalamic nucleus (STN) electrical stimulation. Substantia nigra compacta (SNc), pedunculopontine tegmental nucleus (PPT), striatum (Stri), glutamate (Glu) and cholinergic (ACh) neuronal projections.

STN-evoked striatal dopamine release was significantly reduced but not completely diminished by lidocaine inactivation of the SNc. The relatively small remaining response (16%) can either be attributed to incomplete inactivation of the SNc or slight stimulation of adjacent dopamine axons within the DFB that project dorsal to the striatum; the latter of which supports the notion that STN DBS may act by directly stimulating axons passing near or through the STN [16].

The present data also confirms the significance of the PPT in basal ganglia functioning as the neuronal pathways running through the PPT provided nearly half of the observed STN-evoked striatal dopamine release. These findings are of particular importance as the PPT is an emerging target for DBS [17]. Although these studies were conducted in intact animals, future experiments using the present neurochemical recording procedures in SNc dopamine-lesioned mice will provide further knowledge of the involvement of these pathways in an animal model of Parkinson's disease. Understanding the underlying mechanisms of DBS of the STN, adjacent DFB, and interconnected PPT could lead to improvements in stimulation locations and parameters, which may prove invaluable in improving DBS procedures and enhancing the clinical efficacy of DBS for the treatment of Parkinson's disease.

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