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Using Fast-Scan Cyclic Voltammetry to Evaluate Striatal Dopamine Release Elicited by Subthalamic Nucleus Stimulation

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Abstract— **Deep brain stimulation (DBS), an effective neurosurgical therapy for Parkinson's disease (PD), may act via eliciting neurotransmitter release. However, the precise relationships between DBS and neurotransmitter release are not established. One issue in these studies may be analytical limitations of microdialysis and positron emission tomography, the primary measurement technologies employed. Limitations may be overcome by microsensors, which exhibit improved temporal and spatial resolution. Here we assess fast-scan cyclic voltammetry (FSCV) at a carbon-fiber microelectrode (CFM) for monitoring a putative DBS neurotransmitter, dopamine, during stimulation of the subthalamic nucleus (STN), a DBS target for PD. These results, obtained in the anesthetized rat, suggest that real-time microsensors are a suitable approach for testing the neurotransmitter release hypothesis of DBS action.**

I. Introduction

Parkinson's disease (PD) is associated with the loss of nigrostriatal dopamine originating in the substantia nigra ("nigro") and terminating in the striatum ("striatal") [1]. Drugs such as levodopa and dopamine agonists reduce cardinal symptoms by restoring dopamine control over motor circuits, but long-term use is associated with the loss of efficacy and debilitating side effects such as "on-off" phenomena and dyskinesias [2]. Deep brain stimulation (DBS), a functional neurosurgical approach based on applying high-frequency electrical pulses to an implanted electrode, has proven effective in these patients [3].

Despite well established clinical efficacy, the mechanism of DBS action is not well understood. The initial hypothesis that pathologically hyperactive neurons are quieted may be giving way to the alternative that DBS exerts complex effects, including both inhibition of cell bodies and activation of axonal projections at the target site [4]. Based on neuroanatomy, such efferent neuronal activation could lead to neurotransmitter release in multiple regions distal to the implanted stimulating electrode. Establishing the relationship(s) between DBS and neurotransmitter release is thus critical for understanding DBS mechanism of action.

The two primary chemical measurement techniques used for investigating the neurotransmitter release hypothesis of DBS are microdialysis in experimental animals and positron emission tomography (PET) in humans. Microdialysis samples neurochemistry by removing analyte from brain extracellular fluid via an implanted probe, whose open tip is covered by a semi-permeable membrane [5]. The dialysate is then collected externally and analyzed by highly sensitive and selective analytical methods. PET, a noninvasive imaging technique, monitors neurochemistry indirectly via exogenously administered radiolabeled compounds [6]. Displacement of a radiolabeled ligand at postsynaptic receptors by the release of endogenous neurotransmitter is used as an index of brain neurotransmitter levels.

Electrochemical microsensors, which directly monitor neurotransmitters *in situ*, are another class of chemical measurement techniques [7]. In voltammetry, a potential is applied to the sensing electrode in order to oxidize analyte. Non-electroactive neurotransmitters are measured by a biosensor, which utilizes a biological recognition element, such as an enzyme, to act on analyte and generate an electroactive product that is subsequently monitored electrochemically. By affording chemical measurements with micron spatial and millisecond temporal resolution, microsensors may afford analytical advantages for testing the neurotransmitter release hypothesis of DBS action.

Dopamine release, evoked by electrical stimulation of the subthalamic nucleus (STN), a DBS target for PD, provides a basis for comparing measurement modalities. Because the STN sends direct and indirect projections to dopamine cell bodies in the substantia nigra, it is conceivable that STN stimulation activates surviving nigrostriatal dopamine neurons in PD, thereby increasing striatal dopamine levels. Interestingly, most studies using microdialysis in the intact [8] or parkinsonian [9] rat do not show increased dialysate dopamine with STN stimulation. Similarly, PET has failed to demonstrate enhanced striatal dopamine levels with STN DBS in humans [e.g., 10]. On the other hand, a carbon-fiber microelectrode (CFM) combined with the electrochemical technique of amperometry readily measured robust STN-evoked dopamine release in the intact rat striatum [11].

The failure to demonstrate changes in striatal dopamine release with STN stimulation in parkinsonism could be due to insufficient dopamine neurons. Another possibility is analytical limitations of microdialysis and PET. For example, the large microdialysis probe damages adjacent brain tissue and impairs the measurement of dopamine elicited by electrical stimulation [12]. PET may also lack the requisite sensitivity to detect changes in striatal dopamine levels during STN DBS [13]. By virtue of directly sampling dopamine near its release site in real-time, chemical microsensors may be better suited for this task.

Here we extend the microsensor approach for assessing STN-evoked striatal dopamine release in the intact rat with

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fast-scan cyclic voltammetry (FSCV), an electrochemical technique with better chemical resolution than amperometry.

II. FSCV AT A CFM

As opposed to fixed in amperometry, the potential of the sensor is linearly scanned in FSCV. This novel detection scheme generates a voltammogram (i.e., a plot of measured current versus the applied potential), which serves as a chemical signature to identify the analyte detected.

Figure 1 conceptually describes FSCV at a CFM. A "cylinder CFM" is shown in the micrograph in Panel A. This microelectrode is fabricated by aspirating a single carbon fiber (r = 2.5 µm) into a glass capillary and pulling to a tip. The sensing region is the exposed carbon fiber, extending tens of microns beyond the glass insulation. During the positive sweep of the voltage scan, dopamine is oxidized to dopamine-ortho-quinone by giving up two electrons. This electroformed quinone is reduced back to dopamine during the negative sweep by the addition of two electrons.

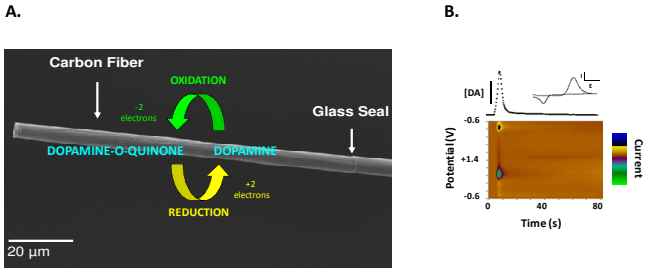

Figure 1. FSCV at a CFM for real-time dopamine monitoring in the striatum of the anesthetized rat.

Panel B shows data collected in the anesthetized rat. The CFM potential was scanned with a triangle waveform from -0.6 to +1.4 V and back again at a rate of 400 V/s. The potential rested at a bias of -0.6 V between scans, which were applied at 10 Hz. For these FSCV parameters, dopamine oxidizes around +0.6, whereas the quinone reduces at around -0.2 V. Several waveform parameters have been employed for FSCV. In general, extending the potential range of the triangle waveform increases sensitivity, but at the expense of response time [14]. Both positive and negative outcomes, respectively, are due to enhanced dopamine adsorption to the surface of the CFM.

The top trace in Panel B is current recorded in the striatum at the peak oxidation potential for dopamine and plotted for each scan. When converted to concentration, this signal represents the change in dopamine levels with time. With a scan application rate of 10 Hz, dopamine changes are recorded with 100 ms resolution. The signal increase is coincident with electrical stimulation of dopamine axons within the medial forebrain bundle (MFB). The INSET shows a voltammogram, determined at signal zenith, clearly displaying an upward peak for dopamine oxidation and a downward peak for quinone reduction. A large charging current is actually recorded at the CFM during the scan. However, this signal is stable for short times and can be removed by a background subtraction procedure to reveal the smaller faradic current arising from dopamine.

The color plot in Panel B shows every background-subtracted voltammogram collected in the trace above. The x-axis is time, the y-axis is applied potential, and the z-axis or pseudo-color scale is measured current. Zero or background-subtracted current is represented in the color plot by brown. Two prominent color features coincide with the signal increase in the top trace. These features represent dopamine oxidation (green-purple) and quinone reduction (yellow-black). While exhibiting less resolution than the individual voltammogram shown in the INSET above the trace, the color plot has the advantage of displaying all electrochemical data collected during the recording. Combined, the electrochemical data clearly demonstrate that dopamine is the major contributor to the signal measured in the striatum during electrical stimulation of the MFB.

As described next, FSCV at a CFM was employed to compare striatal dopamine release elicited, in the anesthetized intact rat, by electrical stimulation of the STN to that elicited by direct stimulation of dopamine neurons at the level of the MFB or substantia nigra. To improve the accuracy of stimulating the STN, which is quite small in the rat, electrophysiology was first use to target this region.

III. EXPERIMENTAL DESIGN AND METHODS

Adult, male Sprague-Dawley rats (~300 to 400 g, Harlan Industries, Indianapolis, IN USA) were anesthetized with urethane (1.5 g/kg) and immobilized in a stereotaxic apparatus (David Kopf Instruments, Tajunga, CA USA) [15]. Holes were drilled through the skull for placement of reference, stimulating, and recording electrodes. The Ag/AgCl reference electrode, a chloridized silver wire, was positioned in superficial cortex contralateral to recording and stimulation. FSCV consisted of a triangle waveform (-0.4 to 1.3 V) applied at 400 V/s and 10 Hz. Electrochemistry was performed by instrumentation built by the Department of Chemistry at the University of North Carolina (Chapel Hill, NC USA). LabVIEW-based software (ESA Biosciences, Chelmsford, MA USA) controlled electrochemisty and stimulation, which was optically isolated and constant current (Neurolog, Medical Systems, Great Neck, NY USA). Biphasic stimulus pulses, 300 µA and 2 ms each phase, were applied as 2 s, 60 Hz trains.

Figure 2 shows the experimental design. Stereotaxic coordinates are given in mm. Bregma was the reference point for anteroposterior (AP) and mediolateral (ML) positioning and dura for dorsoventral (DV) positioning. Panel A shows a sagittal view depicting overall electrode placements (EPHYS, electrophysiological recording; ES, electrical stimulation). Coronal views are shown in the following panels. The design was to measure FSCV at a CFM in the striatum (Panel B; var, variable) during electrical stimulation of the MFB or STN (Panel C). Prior to stimulation, electrophysiological recordings were collected to target the STN specifically. At the end of the experiment, a second stimulating electrode was lowered into the substantia nigra (SN; Panel D) to assess whether the nigrostriatal dopamine projection was intact following the

various experimental manipulations.

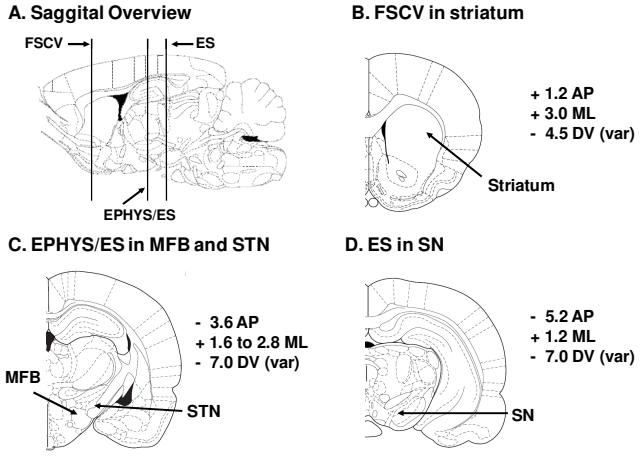

Figure 2. Experimental Design.

Two different stimulation electrodes were employed. A small, concentric bipolar electrode (Rhodes SNE 100; David Kopf Instruments, Tajunga, CA USA) was used to stimulate the MFB and STN selectively. A larger, twisted bipolar electrode (MS 303/2, Plastics One, Roanoke, VA USA) was used to stimulate the substantia nigra. Extracellular electrophysiological recordings were collected at a CFM, filtered, and analyzed with Spike 2 software (Cambridge Electronic Design Ltd., Cambridge, UK).

IV. In Vivo Results

A. *Electrophysiological Targeting*

Figure 3 shows electrophysiological targeting of the STN in the rat prior to implantation of the stimulating electrode. Two electrode tracks (B, 2.0 ML and C, 2.4 ML), separated by 0.4 mm, are shown. As can be seen, the STN exhibits a unique electrophysiological signature, ~10 Hz regular firing, that can be readily distinguished from that in surrounding areas. Because the cerebral peduncle (CP) is devoid of recordable activity and lies just below the STN, this fiber track provides a valuable landmark to identify the STN. The MFB is shown to the left of track B for reference.

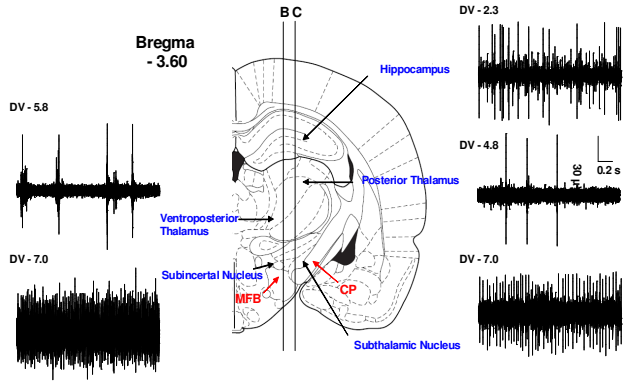

Figure. 3. Electrophysiologically targeting of the STN. Recordings were filtered with a high pass of 300 Hz and low pass of 3 KHz.

B. *Striatal Dopamine Release*

Figure 4 shows the effects of electrical stimulation on striatal dopamine release. The stimulating electrode was lowered along four tracks (A, B, C, and D; left panel), separated by 0.4 mm. Tracks A and C targeted the MFB and STN, respectively. Tracks B and D were used as negative controls, to assess the spatial resolution of the small, concentric bipolar stimulating electrode for selectively activating the MFB and STN. Tracks B and C were electrophysiologically established (see Figure 4).

Similar to our previous findings [15], directly stimulating dopamine fibers traversing the MFB (ML 1.6, Track A) elicited robust dopamine release in the striatum (Fig. 4A). For these stimulation parameters (60 Hz, 120 pulses, 2 ms and 300 µA each phase of the biphasic stimulus pulse), extracellular dopamine levels increased during the pulse train (see line underneath trace), and quickly returned to baseline after cessation of the stimulation. Action-potential evoked dopamine release increases the recorded signal, whereas re-uptake of dopamine by dopamine terminals underlies its decrease. The near immediate increase in dopamine levels time-locked to the initiation of stimulation indicates that the CFM was positioned close, within a few microns, to dopamine release sites in the striatum [16].

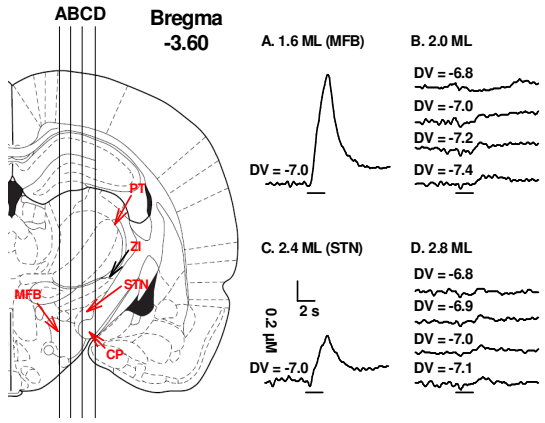

Figure 4. Striatal dopamine release elicited by electrical stimulation of the MFB and STN. ZI, zona incerta.

Striatal dopamine release was also elicited by stimulating the STN (Fig. 4C, ML 2.4, Track C). The depth of the electrode (-7.0 DV) that elicited dopamine release in this recording was consistent with the dorsoventral location where the STN electrophysiological signature was collected (Fig. 3), suggesting that the STN was indeed stimulated. Because the position of the CFM in the striatum was not changed between MFB and STN stimulations and identical stimulus parameters were used, direct comparisons can be made regarding signal amplitude. On average (n=8), the magnitude of STN-evoked dopamine release in the striatum was about 3-fold less than that evoked by MFB stimulation.

No dopamine release was observed in the striatum when Tracks B (Fig. 4B, ML 2.0) or C (Fig. 4D, ML 2.8) were stimulated, even at depths comparable to that eliciting dopamine release with MFB or STN stimulation. The lack of dopamine release evoked by stimulation of Track B indicates that the small, concentric bipolar electrode has sufficient spatial resolution to discriminate the MFB and STN. No dopamine release with stimulation of Track D, which targeted the lateral STN, suggests that the medial, but

not the lateral, STN regulates striatal dopamine levels.

C. *Stimulation of the Substantia Nigra*

Because several recording and stimulation tracks were made in the vicinity of the MFB and STN, it was important to establish that the nigrostriatal dopamine pathway was intact at the end of the experiment. To assess the integrity of this projection, a large, twisted bipolar stimulating electrode was positioned in the substantia nigra to activate dopamine cell bodies directly. The position of the CFM in the striatum was not changed from MFB and STN stimulation. As shown in Figure 5, stimulation of the MFB (Panel A) or substantia nigra (Panel C) elicited similar levels of dopamine release, whereas STN-evoked dopamine release was smaller. These results indicate that the nigrostriatal dopamine projection was intact, suggesting that the lower amplitude dopamine release elicited by STN, as compared to the MFB, is not due to tissue damage.

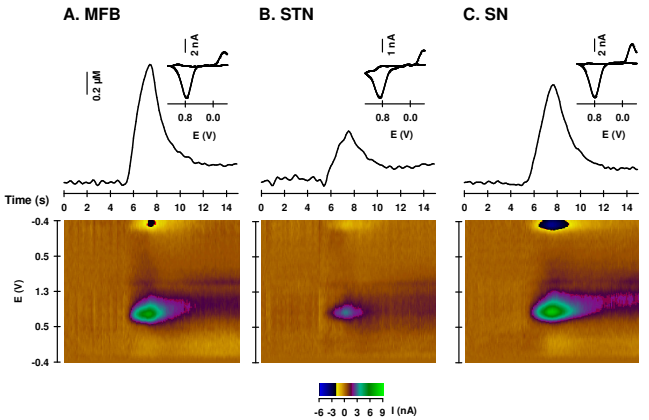

Figure 5. Comparison of striatral dopamine release elicited by electrical stimulation of the MFB, STN, and substantia nigra.

Positively identifying the evoked striatal signal as dopamine is made possible by the voltammograms collected during stimulation of each region. The INSET to each trace shown in Figure 5 displays the signature background-subtracted voltammogram for dopamine, with two prominent current peaks, one (positive) for dopamine oxidation and the other (negative) for quinone reduction. Similarly, the sequential background–subtracted voltammograms displayed in the color plot below each trace, and on the same time scale as the trace, clearly show that the signature current features for dopamine electrochemistry coincide with the increase in signal evoked by stimulation of each region.

V. Discussion and Conclusion

We reported that FSCV at a CFM was capable of monitoring striatal dopamine release elicited by electrical stimulation of the STN. The amplitude of these signals was, on average, about 3-fold less than dopamine release elicited by direct stimulation of dopamine neurons ascending through the MFB and recorded at the same striatal location by the same CFM. Despite making several electrode tracks in the vicinity of the MFB and STN for electrophysiological recording and electrical stimulation, subsequent activation of dopamine cell bodies in the substantia nigra elicited similar dopamine release as evoked by MFB stimulation, suggesting that the nigrostriatal dopamine pathway was intact during the entire experiment. Overall, these results suggest that FSCV at a CFM is well suited for testing the dopamine release hypothesis of STN DBS and underscore the general utility of the chemical microsensor approach.

Several future directions can be highlighted. The most immediate is to assess DBS-like stimulation, because parameters used herein were optimized to release dopamine. Another critical experiment is to record dopamine levels in an animal model of Parkinson's disease. While FSCV at a CFM may be a suitable technique for testing the dopamine release hypothesis of STN DBS, it could very well be that the sparse dopamine innervation in the parkinsonian striatum simply does not support the generation of physiologically relevant dopamine concentrations. The use of DBS-like electrodes and large-animal models, with more human-like neuroanatomy, would additionally be very beneficial.

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