Experimental Neurology 228 (2011) 53–58

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

The effects of chronic levodopa treatments on the neuronal firing properties of the subthalamic nucleus and substantia nigra reticulata in hemiparkinsonian rhesus monkeys

Timothy P. Gilmour [a,b,c,*,1], Christopher A. Lieu [a,b,1], Mark J. Nolt [a,d], Brigitte Piallat [e], Milind Deogaonkar [f], Thyagarajan Subramanian [a,b]

[a] Department of Neurology, The Pennsylvania State University College of Medicine, Hershey, PA, USA
[b] Department of Neural and Behavioral Sciences, The Pennsylvania State University College of Medicine, Hershey, PA, USA
[c] Department of Electrical Engineering, The Pennsylvania State University, University Park, PA, USA
[d] Functional Neurosurgical Services, Sentient Medical Systems, Inc., Hunt Valley, MD, USA
[e] INSERM, Grenoble Institute of Neurosciences, Grenoble, France
[f] Center for Neurological Restoration, The Cleveland Clinic Foundation, Cleveland, OH, USA

ARTICLE INFO

Article history:
Received 2 August 2010
Revised 12 November 2010
Accepted 1 December 2010
Available online 10 December 2010

Keywords:
Parkinson's disease
Electrophysiology
MPTP
Basal ganglia
Levodopa
Macaca mulatta
Nigrostriatal degeneration
Nonmotor symptoms
Neuronal bursting
LFP

ABSTRACT

Dopamine replacement therapy with levodopa (LD) is currently the most effective pharmacological treatment for Parkinson's disease (PD), a neurodegenerative disorder characterized by dysfunction of basal ganglia electrophysiology. The effects of chronic LD treatments on the electrophysiological activity of the subthalamic nucleus (STN) and the substantia nigra reticulata (SNR) in parkinsonism are not clear. In the present study we examined the effects of chronic LD treatments on the firing rate and firing pattern of STN and SNR neurons in the stable hemiparkinsonian monkey model of PD. We also evaluated local field potentials of both nuclei before and after LD treatments. In a stable hemiparkinsonian state, STN and SNR had a mean firing rate of 42.6 ± 3.5 Hz (mean $\pm$ SEM) and 52.1 ± 5.7 Hz, respectively. Chronic intermittent LD exposure induced marked amelioration of parkinsonism with no apparent drug-induced motor complications. LD treatments did not significantly change the mean firing rate of STN neurons (41.3 ± 3.3 Hz) or bursting neuronal firing patterns. However, LD treatments induced a significant reduction of the mean firing rates of SNR neurons to 36.2 ± 3.3 Hz ($p<0.05$) and a trend toward increased burstiness. The entropy of the spike sequences from STN and SNR was unchanged by LD treatment, while there was a shift of spectral power into higher frequency bands in the LFPs. The inability of chronic LD treatments to reduce the bursty firing patterns in the STN and SNR should be further examined as a potential pathophysiological mechanism for PD symptoms that are refractory to LD treatments.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic nigrostriatal neurons leading to bradykinesia, tremor and muscle rigidity. These symptoms have been attributed to the loss of nigrostriatal dopamine, which subsequently leads to pathophysiological alterations in the basal ganglia and its intricate connections to the rest of the brain.

The classic rate model of PD basal ganglia dysfunction proposed that the foundational pathophysiological symptom was the asymmetry between the direct and indirect striatal output pathways caused by dopamine depletion (Albin et al., 1989; DeLong, 1990). The original rate model was subsequently modified to include additional details of neuronal firing pattern changes and oscillatory activity in the basal ganglia (Hammond et al., 2007; Arbuthnott and Garcia-Munoz, 2009). Additional refinements including the notion of a hyperdirect pathway and reciprocal connectivity between various basal ganglia structures have been recently added. For current reviews, see (Hammond et al., 2007; Galvan and Wichmann, 2008; Weinberger et al., 2009). Even after these refinements, the original model of the direct and indirect basal ganglia pathways and their alterations in PD remain a major working model for PD pathophysiology.

The models of normal and parkinsonian basal ganglia function have been supported by many electrophysiological studies. In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioned primate model, Bergman and colleagues demonstrated an increase in STN firing rate from normal to parkinsonian state (Bergman et al., 1994), and a similar increase in SNR neuronal firing rate (Wichmann et al., 1999). Bergman and Wichmann and colleagues also showed that the primate STN and SNR became more bursty after MPTP treatment (Bergman et al., 1994; Wichmann et al., 1999). The same increases of rate and burstiness in STN

* Corresponding author. Milton S. Hershey Medical Center, 500 University Drive, H109 Neurology, Hershey, PA 17033, USA. Fax: +1 717 531 0996.
E-mail address: timgilmour@psu.edu (T.P. Gilmour).
[1] These authors contributed equally to this work.

0014-4886/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2010.12.001

and SNR have been confirmed in a rat model of PD (Breit et al., 2001; Breit et al., 2006; Breit et al., 2007). The synchronized oscillations represented by the local field potentials (LFPs) have also been shown to have characteristic patterns in the normal and parkinsonian states. Brown and colleagues showed that normal rats have a LFP peak in the 70 Hz region, similar to human PD patients medicated with levodopa (LD) and undergoing intraoperative recordings (Brown et al., 2001, 2002). Mallet and colleagues showed that anesthetized hemiparkinsonian rats showed a prominent beta LFP peak in the activated state which normal rats did not have (Mallet et al., 2008). Together, these and other animal studies demonstrate that the neurophysiological properties of STN and SNR are altered in response to nigrostriatal denervation and dopamine depletion.

While no studies to date have examined the effects of chronic LD therapy on STN or SNR neuronal firing rates or patterns, the effects of acute dopamine agonist therapy have been examined in the human STN. Recordings in PD patients undergoing functional neurosurgery for deep brain stimulation show that apomorphine (a sopamine agonist) treatment increases burst discharges in the STN but does not alter firing rate at optimal doses. However, apomorphine treatments decreases STN firing rates in the dyskinetic state (Lozano et al., 2000). Also, the prominent beta peak in STN LFPs has been shown to be greatly reduced when the patient is acutely given apomorphine or LD treatments (Brown et al., 2001; Giannicola et al., 2010).

Although these studies in PD patients have contributed to our understanding of the neurophysiological properties of these structures, in clinical studies it is not practical for patients to be off anti-PD medications for an extended period. Clinical studies have shown that greater than 2 weeks of washout from LD is necessary to remove beneficial effects of LD therapy in PD patients (Fahn, 2005). Such long washouts are not well tolerated by patients and have potential risk of significant morbidity and mortality (Newman et al., 2009). Therefore, conducting electrophysiological studies in PD patients in the complete "off" state devoid of all influences of LD or other dopaminergic medications is virtually impossible.

To overcome these difficulties, we designed a study to evaluate the effects of chronic intermittent LD treatments at optimal doses in the MPTP-treated stable hemiparkinsonian monkey model of PD. We focused on the STN because of its important role in the indirect pathway of the corticostriatal–thalamocortical loop, and on the SNR because of its importance in mediating both motor and nonmotor symptoms of PD.

Materials and methods

Two adult female *Macaca mulatta* were housed according to standards set forth in the NIH 'Guide for the Care and Use of Laboratory Animals.' All procedures were carried out in strict compliance with the "Principles of Laboratory Animal Care" (NIH Publication No. 86-23, revised 1985) and were approved by the local institutional animal care and use committee.

Assessing parkinsonism and chronic LD therapy

Detailed information on our model and behavioral paradigm can be found elsewhere (Lieu et al., 2011). Briefly, monkeys were assessed behaviorally using a primate parkinsonism rating scale (from 0, normal, to 100, severely parkinsonian) which was modeled after Part III of the Unified Parkinson's Disease Rating Scale (mUPDRS) (Subramanian et al., 2010). Ratings were performed by a blinded investigator and were spaced at least 3 days apart.

Intra-carotid injections of MPTP were administered to render the monkeys hemiparkinsonian (HP) on the right side of their body. Both monkeys received an initial dose of 0.5 mg/kg MPTP, followed by 2 weeks of observation. If the original dose did not result in a stable unilateral HP state, repeated doses were given. The final result was a HP state which was stable for more than 6 months.

Once behavioral stability was documented, recording chambers were surgically implanted to permit chronic single cell extracellular neuronal recording from the left STN and SN in the stable HP state and on chronic LD treatment. LD/carbidopa (CD) therapy was initiated at an oral dose of 100 mg/25 mg twice a day and gradually escalated by 100 mg/25 mg every 72 h until no further improvement in mUPDRS scores was observed. This was defined to be the optimal dose and was subsequently held constant throughout the dosing period. Serum dopamine levels were measured in a single monkey to check that dopamine was being successfully delivered, showing dopamine concentrations of 1.022 ng/mL before starting LD dosing and 17.04 ng/mL while on optimal LD dosing. Occasionally, the monkeys would refuse oral intake (defined by missing three successive doses). When this occurred, injectable benserazide and methyl ester of LD (25/100) were administered SQ/IM. At the point of stable mUPDRS score improvement and steady twice daily LD/CD dosing for at least 1 week, extracellular neuronal recordings were resumed. Dyskinetic activity was not seen on this stable dosing regimen as we have previously reported (Lieu et al., 2011).

Electrophysiology

All recordings were done in awake, behaving animals. Wakefulness was monitored by eye blink reflex and responsiveness of animal to investigators while in the restraint chair. Animals were trained to allow passive limb movements by experimenters in order to examine somatotopic responses during recordings (Starr et al., 2000). Extracellular single cell recordings were carried out using glass coated platinum–iridium microelectrodes (impedance 0.5–1.0 Mega-ohms, FHC) or tungsten microelectrodes (0.5–2.0 Mega-ohms, FHC). The STN and SNR nuclei were systematically sampled, recording each neuron encountered at the target depth range, with tracts typically separated laterally from each other by 1 mm. The electrical signal was amplified (MDA-4I BAK or ISO-80 WPI), filtered (200–10,000 Hz, Krohn-Hite), monitored on an audio loudspeaker, and displayed on a digital oscilloscope to ensure good signal isolation. The signals were digitally sampled at 25000 samples per second (Spike2, CED). Simultaneously, LFPs were filtered (3–500 Hz) and digitized at 1000 samples per second.

Localization within the nuclei was confirmed in five ways. First, the depth of the electrode tip was correlated to a rhesus brain atlas. Second, firing characteristics of landmarks in the brain were monitored as reported previously (Starr et al., 2000). Third, on some tracts, after a neuron had been recorded for at least 60 s for later analysis, somatotopic responses were examined by flexing and extending the monkey's arm or leg during the recording (for example, see supplement Fig. S2). Fourth, recording tracts were histologically confirmed in the STN and SNR. Fifth, the root mean square (RMS) was calculated on the activity recorded along each track. RMS has been used clinically to determine the borders of STN, as the overall activity in STN is higher than that superior and inferior to STN (Moran et al., 2006; Snellings et al., 2009).

Data analysis

During offline analysis, interspike intervals (ISIs) were generated using Spike2's template matching spike sorting algorithm. Neuron sorting and isolation was further refined using principal component analysis on the spike waveforms. In each case, records were comprised of at least 400 spikes and had duration between 60 and 120 s.

In addition to firing rates, seven measures of the firing patterns were employed. First, the coefficient of variation (CV) of the ISIs was computed for each recording. A low CV indicates a regularly firing cell. Second, the burst index was computed as the mean of the ISI distribution