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Subthalamic deep brain stimulation alters neuronal firing in canonical pain nuclei in a 6-hydroxydopamine lesioned rat model of Parkinson's disease

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ABSTRACT

Introduction: Chronic pain is one of the most common non-motor symptoms of Parkinson's disease (PD) affecting up to 85% of patients. Previous studies have established that reduced mechanical and thermal thresholds occur in both idiopathic PD patients and animal models of PD, suggesting that changes may occur in sensory processing circuits. Improvements in sensory thresholds are achieved using subthalamic nucleus (STN) deep brain stimulation (DBS), however the mechanism by which this occurs remains unresolved.
Materials and methods: We examined unilateral medial forebrain bundle 6-hydroxydopamine (6OHDA) rat model of PD to determine whether STN DBS alters neuronal firing rates in brain areas involved in ascending and descending pain processing. Specifically, single unit in vivo recordings were conducted in the anterior cingulate cortex (ACC), the periaqueductal grey (PAG), and the ventral posteriolateral nucleus of the thalamus (VPL), before, during and after stimulation was applied to the STN at 50 or 150 Hz.
Results: Sham and 6OHDA lesioned animals have similar neuronal firing activity in the VPL, ACC and PAG before stimulation was applied ($p > 0.05$). In 6OHDA lesioned rats, both low frequency stimulation (LFS) ($p < 0.01$) and high frequency stimulation (HFS) ($p < 0.05$) attenuated firing frequency in the ACC. In shams, only LFS decreased firing frequency. A subset of neurons in the PAG was significantly attenuated in both sham and 6OHDA lesioned animals during HFS and LFS ($p < 0.05$), while another subset of PAG neuronal activity significantly increased in 6OHDA lesioned rats during HFS ($p < 0.05$). Finally, low or high frequency STN DBS did not alter neuronal firing frequencies in the VPL.
Conclusions: Our results suggest that STN DBS alters neuronal firing in descending pain circuits. We hypothesize that STN DBS attenuates excitatory projections from the ACC to the PAG in 6OHDA lesioned rats. Following this, neurons in the PAG respond by either increasing (during HFS only) or decreasing (during both LFS and HFS), which may modulate descending facilitation or inhibition at the level of the spinal cord. Future work should address specific neuronal changes in the ACC and PAG that occur in a freely moving parkinsonian animal during a pain stimulus treated with STN DBS.

1. Introduction

Chronic pain in Parkinson's disease (PD) can present from several origins simultaneously, and may involve musculoskeletal, dystonic, neuropathic and central pain symptoms (Fil et al., 2013). While the pathophysiology of pain in PD remains largely understudied (Rana et al., 2013), recent reports of improved pain symptoms and altered sensory pain thresholds following subthalamic deep brain stimulation (STN DBS) (Ciampi de Andrade et al., 2012; Smith et al., 2015; Drake et al., 2005; Kim et al., 2012; Pellaprat et al., 2014) are of particular relevance to the field. Currently, there is a lack of accurate methodology to predict the magnitude of improvement individual patients will experience, and its unclear on how to increase the efficacy of STN DBS for those who only experience minimal relief in pain. The pathophysiology of chronic pain

Abbreviations: 6OHDA, 6-hydroxydopamine; ACC, anterior cingulate cortex; BG, basal ganglia; CV, cresyl violet; DBS, deep brain stimulation; GABA, gamma-aminobutyric acid; HFS, high frequency stimulation; LAT, limb-use asymmetry test; LFS, low frequency stimulation; LPAG, lateral periaqueductal grey; MFB, medial forebrain bundle; PAG, periaqueductal grey; PD, Parkinson's disease; PFA, paraformaldehyde; RVM, rostral ventromedial medulla; SEM, standard error of the mean; SNc, substantia nigra pars compacta; SNK, student Newman–Keuls; STN, subthalamic nucleus; vlPAG, ventrolateral periaqueductal grey; VPL, ventral posteriolateral thalamus.

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in PD is complex and different studies examining PD-related pain have shown heterogeneous responses to STN DBS in PD patients (Ciampi de Andrade et al., 2012), with the majority showing improvements with high frequency stimulation. In animal models of PD, significant improvements of sensory thresholds have been reported (Gee et al., 2015). As the mechanism behind sensory threshold improvement and reduction in PD-related pain following STN DBS remains unclear, we suggest that a better understanding of changes in neural activity in sensory brain regions during STN DBS, may be a critical first step in improving potential analgesic efficacy in PD.

Information detailing specific changes in brain circuitry or firing patterns in pain processing circuits are sparse in PD. However, evidence suggests that central sensitization occurs, and thus subcortical pain nuclei are altered as a consequence of the disease (Borsook, 2012). In chronic pain rodent models, imaging studies show increased activity in the ventral posteriolateral (VPL) thalamus, and electrophysiological studies have found abnormal hyperexcitability (Syre et al., 2014; Iwata et al., 2011; Gwak et al., 2010), increased after discharge, evoked responses (Gerke et al., 2003), and bursting activity (Iwata et al., 2011; Hains et al., 2005) in the same region. Moreover, some hypothesize that desynchronized thalamocortical oscillations are a biomarker for pain in PD (Walton et al., 2010), suggesting that changes in VPL neuronal activity may occur (Walton et al., 2010; Leblanc et al., 2014). Furthermore, imaging studies suggest that heightened activation of the anterior cingulate cortex (ACC) in PD patients during the cold pressor test (Brefel-Courbon et al., 2005; Brefel-Courbon et al., 2013), and changes in cfos expression are found in the rat PAG following peripheral mechanical stimulation (Reyes & Mitrofanis, 2008).

As a first step to understanding the mechanism by which STN DBS modulates supraspinal pain circuits, we recorded in vivo single-unit activity from the VPL, ACC and PAG in 6-hydroxydopamine (6OHDA) lesioned parkinsonian and sham rats. Following this, we determined if these firing rates were altered during high or low frequency (150 or 50 Hz respectively) STN DBS. Based on the findings of other animal models with chronic pain (Iwata et al., 2011), we hypothesized that 6OHDA lesioned animals exhibit increased neuronal activity in pain nuclei such as the ACC, PAG and VPL, and that this neuronal activity is attenuated by STN DBS.

2. Materials and methods

2.1. Animals

All animal use was conducted after approval from the Institutional Animal Care and Use Committee at Albany Medical College. 44 adult male Sprague-Dawley rats (TACONIC, Germantown NY) weighing 220–250 g were used. Twenty-three rats were made parkinsonian by injecting 6-hydroxydopamine (6OHDA) in the right medial forebrain bundle (MFB) (Ungerstedt, 1968), and 21 were sham animals receiving an injection of sterile saline.

2.2. 6-Hydroxydopamine medial forebrain bundle injection surgery

Surgical procedures were conducted as previously described (Gee et al., 2015). In brief, rats were injected intraperitoneally with desipramine HCl (25 mg/kg) and pargyline (50 mg/kg) 20 min prior to craniotomy and anesthetized with 2% isoflurane (Tank Inhalant system, Harvard Apparatus, Holliston, MA, USA). Bregma was visualized, and burr holes were positioned over the right MFB at the following coordinates (with respect to bregma): −4.4 mm posterior, 1.5 mm lateral. To create the 6OHDA lesion, a 10 μL Hamilton syringe (Hamilton Company, Reno, NV, USA) was lowered 7.6 mm ventral from dura to inject 4.5 μL 6OHDA (3 μg/μL, made up with 0.1% ascorbic acid, Sigma-Aldrich, MO) at a rate of 0.5 μL/min. Sham animals were injected with 4.5 μL saline (0.9% NaCl). Post-operative procedures were similar to those used previously in our lab (Gee et al., 2015).

2.3. Behavioral assessment of parkinsonian phenotype

Two weeks following 6OHDA or saline injection, animals were placed in a clear Plexiglas cylinder and allowed to explore for 5 min to complete a limb-use asymmetry test (LAT). The number of exploratory forepaw touches was quantified for each forepaw ((number of right touches/total number of touches) ∗ 100). Marked degeneration of dopaminergic neurons in the right substantia nigra pars compacta (SNc) and terminals in the striatum coincides with a touch bias of 80% from the unimpaired right paw (Schallert et al., 2000).

2.4. In vivo single unit electrophysiological recordings in anesthetized animals

One week following LAT testing, extracellular single-unit recordings were obtained from the right VPL, PAG and ACC in 23 6OHDA lesioned and 21 sham animals, in the presence and absence of HFS and LFS. To do this, rats were anesthetized with 1.2 g/kg urethane (dissolved in saline and injected intraperitoneally) and placed in a stereotactic frame (David Kopf Instruments, Tajunga, CA, USA). Bregma was visualized and burr holes were drilled over the subthalamic nucleus and either the VPL hind paw region, or the ACC and PAG (Table 1). The STN was stimulated using a Grass S88X dual output square pulse stimulator (Grass Products, Natus neurology, Warwick, RI, USA) coupled to a PSIU6X current isolation unit (Grass Products, Natus neurology). Monophasic, cathodic stimulation was delivered through a platinum–iridium bi-concentric stimulating electrode (FHC, Bowdoin, ME) at 300 μA, 90 μs pulse width and 150 or 50 Hz, 7.6 mm ventral from the dura. Neurons were recorded with up to 5 epoxy-coated tungsten microelectrodes (500 kΩ resistance, Harvard Apparatus, Holliston, MA).

Acquisition and analysis of neuronal recordings were conducted as done previously in our laboratory (Sutton et al., 2013). In short, single-unit recordings were acquired using a multichannel acquisition system (Plexon, Dallas, TX) with a sampling rate of 40 kHz. The signal was high- and low-passed at 300 Hz and 6 kHz, respectively. Units were selected using a 2:1 signal-to-noise voltage threshold and analyzed using Offline sorter (Plexon) and NeuroExplorer (Nex Technologies, Westford MA). Finally, removal of stimulation artifacts during high and low frequency STN stimulation recordings was done using SARGE (Stimulation Artifact Removal Graphical Environment) (Erez et al., 2010).

Units of the VPL that coincided with the receptive field of the left hind paw region were functionally identified using a cotton applicator to gently brush the left dorsal hind paw(Fig. 1). Recordings were done using 4 mechanical stimuli: pressure pinch (1 per second) was applied manually using wire tweezers, and 3 different VF monofilaments (4.0 g, 15.0 g, 26.0 g) were applied with sufficient force to buckle once per second. For each recording, a 60 s baseline was obtained, followed by one type of stimuli (i.e. VF or pinch) applied three times for 10 s, with 20 s rest in between. After sensory stimuli were applied, a 60 s recovery period was recorded. These recordings were then performed during high and low frequency stimulation of the STN in a randomized order. Twenty minutes was used as a wash out period between recordings to ensure that neurons returned to their original baseline before the next recording occurred. In a separate group of animals, neuronal recordings were obtained in the ACC and PAG, units were identified by electrophysiological characteristics and STN DBS was applied at 150 Hz or 50 Hz for 30 s. These recordings were not done simultaneously due to restrictions of the stereotactic frame. Recordings consisted of a 30 s baseline, 30 s of stimulation and 2 min of recovery.

2.5. Immunohistochemistry and electrode placement

After single unit recordings, rats underwent transcardiac perfusion using heparin and 4% paraformaldehyde (PFA), followed by a 24 h post fix in 4% PFA. Brains were cryoprotected in 30% sucrose, and 60 μm free-floating sections were obtained from the SNc and striatum