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Bursting activity of substantia nigra pars reticulata neurons in mouse parkinsonism in awake and anesthetized states

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ABSTRACT

Electrophysiological changes in basal ganglia neurons are hypothesized to underlie motor dysfunction in Parkinson's disease (PD). Previous results in head-restrained MPTP-treated non-human primates have suggested that increased bursting within the basal ganglia and related thalamic and cortical areas may be a hallmark of pathophysiological activity. In this study, we investigated whether there is increased bursting in substantia nigra pars reticulata (SNpr) output neurons in anesthetized and awake, head-restrained unilaterally lesioned 6-OHDA mice when compared to control mice. Confirming previous studies, we show that there are significant changes in the firing rate and pattern in SNpr neuron activity under urethane anesthesia. The regular firing pattern of control urethane-anesthetized SNpr neurons was not present in the 6-OHDA-lesioned group, as the latter neurons instead became phase locked with cortical slow wave activity (SWA). Next, we examined whether such robust electrophysiological changes between groups carried over to the awake state. SNpr neurons from both groups fired at much higher frequencies in the awake state than in the anesthetized state and surprisingly showed only modest changes between awake control and 6-OHDA groups. While there were no differences in firing rate between groups in the awake state, an increase in the coefficient of variation (CV) was observed in the 6-OHDA group. Contrary to the bursting hypothesis, this increased CV was not due to changes in bursting but was instead due to a mild increase in pausing. Together, these results suggest that differences in SNpr activity between control and 6-OHDA lesioned mice may be strongly influenced by changes in network activity during different arousal and behavioral states.

Introduction

Parkinson's disease is the second most common neurodegenerative disease, affecting nearly 1% of the population (Huse et al., 2005; Tanner and Goldman, 1996). The disease is typically diagnosed when several dopamine-dependent motor signs are present, such as bradykinesia, muscle rigidity, and resting tremor. Current hypotheses of the neurological mechanisms underlying these motor deficits implicate changes in the electrical activity patterns of basal ganglia neurons after dopamine depletion, specifically increased oscillatory firing in the beta band, increased neural synchronization, and abnormally increased bursting (Galvan and Wichmann, 2008; Rubin et al., 2012).

Electrophysiological studies have suggested that abnormal bursting may play a role in the pathophysiology of PD (reviewed by Lobb (2014)). Several studies in MPTP-treated non-human primates have found an increase in the percentage of 'bursty' basal ganglia neurons or the proportion of spikes in bursts in the internal segment of the globus pallidus (GPi) and the subthalamic nucleus using a variety of burst detection methods. However, inconclusive results have been found with neurons in the external segment of the globus pallidus (Bergman et al., 1994; Boraud et al., 1998, 2000; Sanders et al., 2013; Soares et al., 2004; Wichmann et al., 1999). Increased bursting has also been seen in human PD patients during neurosurgical procedures targeting the STN and globus pallidus (Starr et al., 2005; Steigerwald et al., 2008; Tang et al., 2005, 2007) in comparison to recordings from other neurological diseases (e.g. dystonia, essential tremor, and Huntington's disease).

One of the most widely used animal models of parkinsonism is the unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rodent (Schwarting and Huston, 1996a,b). Basal ganglia neurons recorded in vivo under anesthesia undergo significant changes in the firing pattern after a 6-OHDA lesion, developing a strong preference to fire in-phase or anti-phase with cortical SWA (Belluscio et al., 2003; Hollerman and Grace, 1992; MacLeod et al., 1990; Magill et al., 2001; Mallet et al., 2012; Murer et al., 1997; Seeger-Armbruster and von Ameln-Mayerhofer, 2013; Tseng et al., 2001a,b, 2005; Walters et al., 2007; Zold et al., 2007). Several studies in awake rodents have demonstrated that the spike trains of SNpr neurons in 6-OHDA-lesioned rats can become entrained to movement-related episodes of high beta in the local field potential (LFP) (Avila et al., 2010; Brazhnik et al., 2012,

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2014); however, these studies have not addressed the hypothesis of whether there is an abnormally increased baseline level of bursting in rodent parkinsonism. To investigate this hypothesis, we recorded basal ganglia output neurons in the SNpr in control and unilaterally 6-OHDA-lesioned, behaviorally impaired mice. SNpr neurons were recorded in both the anesthetized and the awake state of head-restrained mice. We found that the SNpr neuron firing in 6-OHDA lesioned but not control anesthetized mice was heavily influenced by cortical SWA activity. In contrast, in awake, head-restrained mice, SNpr neurons had a single-spiking pattern in both control and 6-OHDA groups, with no change in the firing rate, and only subtle changes in the firing pattern including increased coefficient of variation and pausing metrics.

Materials and methods

All experiments were performed in accordance with protocols reviewed and approved by the Animal Care and Use Committee of Emory University. A full description of many of the methods employed here has been previously published (Lobb et al., 2013). A ?thyc=5?> total of 37 adult mice (14 non-injected controls, 7 saline-injected controls, 16 6-OHDA-injected mice; all C57BL/6J and non-infected Slc32a1$^{tm2(Cre)/Lowl}$/J on a C57BL/6J background) were used in this study. After accommodating mice to handling, behavioral testing (see below for details) was conducted and then either saline or 6-OHDA administered. The behavior of the mouse was reassessed 4 weeks later. No effect of saline injections was seen when compared to the non-injected hemisphere (0.86 ± 3.11% loss of striatal TH, n = 6). Therefore, non-injected and saline-injected mice were pooled. Of the 16 6-OHDA-injected mice, 2 died after surgery and one had an insufficient lesion (only 40% striatal TH loss). The remaining animals had successful lesions (>80%; average of 94.0 ± 1.69% loss in striatal TH staining, n = 13, see below). The animals were used either in the anesthetized or in the awake state. Recordings were made acutely from anesthetized (15; 8 controls, 7 6-OHDA) or awake (19; 13 controls, 6 6-OHDA) mice. At the end of the final recording session, mice were perfused transcardially with a 4% paraformaldehyde solution in phosphate-buffered saline, and the brains removed for histological processing.

Saline/6-OHDA administration

Lesions in the nigrostriatal dopamine system were made using standard methods (Cenci and Lundblad, 2007). Briefly, 1 μl of 6-OHDA (3.0 mg/ml free base 6-OHDA prepared in saline containing 0.02% ascorbic acid) or saline (also containing 0.02% acetic acid) was injected into the medial forebrain bundle (MFB) of the right hemisphere (bregma: −1.2 mm AP, +1.1 mm ML, 4.75 mm DV (Franklin and Paxinos, 2008)) of isoflurane-anesthetized mice. Mice were fed chocolate Pediasure daily until they recovered their pre-surgical weight.

Behavioral testing

Open-field test

Mice were placed in a 20 × 12 × 9.5 inch white plastic rectangular box for 5 min and were videotaped throughout. Automated behavioral analysis was carried out with a custom Matlab script (adapted from Gomez-Marin et al. (2012)). The mouse was detected as an elongated shape using the Matlab 'regionprops' command and tracked across successive video frames. The distance traveled and number of rotations in 5 min were measured.

Electrophysiological recordings

Recordings in the anesthetized state

Recordings were performed as previously described (Lobb et al., 2013). Mice were anesthetized with urethane (1.7 g/kg) and placed in a stereotaxic apparatus. A craniotomy was made over the SNpr recording site (bregma: −3.2 mm AP, 1.6 mm ML). Additionally, a depth LFP wire (100–200 kΩ) was implanted into the primary motor cortex (+1.1 mm AP from bregma, 1.2 mm ML, 0.8 mm DV) to record cortical LFP signals. Next, glass pipettes (tip size, approximately 3 μm) filled with 1 M NaCl and 1% pontamine sky blue were lowered into the SNpr (3.9–4.5 mm DV). Monopolar recordings were then made in the SNpr with reference to a silver chloride wire placed under the scalp near the temporal musculature. The final recording site for each mouse was marked by iontophoretic ejection of pontamine sky blue (−10 to −15 μA, 20–25 min).

Awake, head-fixed recordings

During surgical preparation, mice were anesthetized with isoflurane and placed in a stereotaxic apparatus. A craniotomy was made over the future SNpr recording site and covered with Kwik-cast (WPI Inc.). The dura was left intact. A custom headpost weighing 1–2 g was implanted on the skull with dental cement (RelyX Unicem 2, 3 M). In some experiments, skull screws were used to enhance the fixation of the implant to the skull. The mice were allowed at least 3 days to recover.

After recovery, the mice were given 3 days to acclimate to being head-fixed (one session per day, 60 min per session) to a custom-made head-fixation apparatus. The apparatus consisted of a plastic hemitube (1 5/8″ OD, 1 3/8″ ID, 3.5″ long) with head holder bars that allowed the wing-like headpost to be secured and a detachable flat top to ensure that the mouse stayed in the tube. The size of the tube was large enough that although the mouse was not immobilized, it could not turn or contort its body. Once the head was stabilized, mice showed no overt signs of stress and appeared relaxed. During recording sessions mice were maintained on a fixed interval reward paradigm (FI: 60 s, 10% sucrose via a lick spout) to encourage quiet wakefulness during most of the session. Since mice in the normal and lesioned groups generally drank almost all of liquid provided, this indicates that mice remained awake for the entire session.

Following acclimation, mice were head-fixed and the Kwik-cast cover over the craniotomy was removed. Glass pipettes (as above) were lowered into the SNpr. We found that the pipettes penetrated the dura without causing damage to the dura or the pipette. Monopolar recordings were then made in the SNpr with reference to a second glass pipette (~20 μm tip size which is too large to pick up single units) placed just underneath the dura in the craniotomy. For final recording sessions, the site of the recordings was marked by iontophoretic injection of pontamine sky blue as before. After each session, the craniotomy was covered with Kwik-cast. After completion of the recording sessions, the mouse was perfused with PBS followed by perfusion with 4% paraformaldehyde and 15% sucrose. The brain was then removed and transferred to a 4% paraformaldehyde/30% sucrose solution for later histological processing.

Histological verification, TH immunohistochemistry and densitometry

Coronal sections of fixed brains (50 μm thick) were cut on a microtome and mounted onto slides. Sections with blue recording dots were counterstained with Neutral Red (Lobb et al., 2013). Every sixth section was stained for tyrosine hydroxylase (TH), scanned with an Aperio ePathology microscope (Leica Biosystems), and analyzed for TH staining intensity with ImageJ (Lobb et al., 2013).

Data analysis

Spiking–LFP relationship

To determine if spiking activity occurred at a preferred phase of an oscillation, circular analysis was performed (Lobb et al., 2013). Briefly, the ranked distribution of the Hilbert-transformed instantaneous phase (low-pass filtered at 1.5 Hz) at each spike time was tested for

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