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Deep brain stimulation changes basal ganglia output nuclei firing pattern in the dystonic hamster

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ABSTRACT

Dystonia is a heterogeneous syndrome of movement disorders characterized by involuntary muscle contractions leading to abnormal movements and postures. While medical treatment is often ineffective, deep brain stimulation (DBS) of the internal pallidum improves dystonia. Here, we studied the impact of DBS in the entopeduncular nucleus (EP), the rodent equivalent of the human globus pallidus internus, on basal ganglia output in the dt^{sz}-hamster, a well-characterized model of dystonia by extracellular recordings. Previous work has shown that EP-DBS improves dystonic symptoms in dt^{sz}-hamsters. We report that EP-DBS changes firing pattern in the EP, most neurons switching to a less regular firing pattern during DBS. In contrast, EP-DBS did not change the average firing rate of EP neurons. EP neurons display multiphasic responses to each stimulation impulse, likely underlying the disruption of their firing rhythm. Finally, neurons in the substantia nigra pars reticulata display similar responses to EP-DBS, supporting the idea that EP-DBS affects basal ganglia output activity through the activation of common afferent fibers.

Introduction

Dystonia is a heterogeneous syndrome of movement disorders characterized by involuntary muscle contractions leading to abnormal twisting movements and postures (Fahn, 1988). A modification of neuronal activity in the basal ganglia (BG), the thalamus, the cerebellum and different cortical areas, and a loss of inhibition of brainstem and spinal reflexes have been related to dystonia (Berardelli et al., 1998; Vitek, 2002). As predicted by the Albin and Delong model of BG pathophysiology (Albin et al., 1989), pallidal neurons of dystonic patients display a reduced mean spontaneous firing rate in comparison to parkinsonian patients and healthy monkeys (Hutchison et al., 2003; Starr et al., 2005; Tang et al., 2007; Vitek et al., 1999). In addition, pallidal local field potentials are excessively synchronized (Chen et al., 2006; Sharott et al., 2008; Silberstein et al., 2003).

Medical treatment of dystonia is often ineffective (Bressman, 2000). By contrast, deep brain stimulation (DBS) of the globus pallidus internus (GPi) improves dystonia (Coubes et al., 2000; Kupsch et al., 2006; Vidailhet et al., 2005). The mechanisms underlying the therapeutic effects of DBS in other movement disorders such as Parkinson's disease are likely the result of a complex action of the electrical stimulus on neurons and fibers in the vicinity of the stimulating electrode (Gradinaru et al., 2009; Hammond et al., 2008). Following stimulation, the activation of efferent and afferent axons can give rise to sustained neurotransmitter release (Windels et al., 2000, 2005) modifying local, up- and downstream neuronal activity. Hitherto, only few studies have investigated the mechanisms of action of DBS in dystonica. While previous studies have pointed to changes in the mean activity of single GPi neurons (Pralong et al., 2007) and in downstream thalamic activity (Montgomery, 2006) during GPi-DBS in dystonic patients, more complex changes in BG output remain to be determined.

The dt^{sz}-hamster is a well-characterized model of non-kinesiogenic paroxysmal dystonia (Löscher et al., 1989; Richter and Löscher, 1998, 2002). In agreement with the Albin and Delong model (Albin et al., 1989) and similar to differences between dystonic patients and parkinsonian patients or healthy monkeys (Starr et al., 2005; Vitek et al., 1999), firing rates in the entopeduncular nucleus (EP), the rodent equivalent of the GPi, are reduced in dystonic dt^{sz}-hamsters (Bennay et al., 2001; Gernert et al., 2000, 2002). Similar to human dystonia, the severity of dystonic symptoms is decreased during EP-DBS in dt^{sz}-hamsters (Harnack et al., 2004). Moreover, EP-DBS in this model modifies transcriptional activity in striatum and thalamus (Reese et al.,

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2009), while GPi-DBS modulates cerebral blood flow in the same regions in dystonic patients (Detante et al., 2004).

The dt^{sz}-hamster seems therefore to be a suitable model to further study DBS effects and mechanisms of action in dystonia. Here, we report for the first time changes in neuronal activity induced during DBS in dystonic animals. Using extracellular single-unit recordings, we characterize the impact of EP-DBS on the activity of EP and substantia nigra pars reticulata (SNr) neurons in dt^{sz}-hamsters.

Materials and methods

Animals

All experiments were carried out in accordance with the European Community Council Directive of 24 November 1986 (86/09/EEC) for the care of laboratory animals. Sex- and age matched dystonic ($n=10$) and control hamsters ($n=12$) were obtained by breeding pairs from an inbred line by selective breeding as described before (Löscher et al., 1989). Male and female dt^{sz}-hamsters show age-dependently dystonic symptoms that can be triggered by external stressing stimuli (Löscher et al., 1989). Dystonic attacks last for several hours and can be rated on a six-point rating system (Löscher et al., 1989; Richter and Löscher, 1998). Animals were exposed to standardized external stressors at the age of day 21 and again at the age of day 30. Animals were taken from their home cage, swayed and intraperitoneally injected with sterile physiologic saline (5 ml/kg). They were then separately placed in a new plastic cage for 3 h. The severity of dystonic symptoms was rated as following: (stage 1): flat body posture, (stage 2): facial contortions, rearing with forelimbs crossing and disturbed gait with hyperextended forepaws, (stage 3): hyperextended hindlimbs so that the animals appear to walk on tiptoes, (stage 4): twisting movements and loss of balance, (stage 5): hindlimbs hyperextended caudally, (stage 6): immobilization in a twisted, hunched posture with hind- and forelimbs tonically extended forward. As previously described, only animals showing severe dystonia (at least stage 3) were subjected to further experiments (Reese et al., 2009). The age of the animals ranged between 31–38 days and their weight between 60–100 g. Animals were housed in small groups under controlled environmental conditions: 12/12 h light–dark-cycle (light on at 5:00 AM), temperature 23 °C. Food and water were permitted ad libitum. All experiments were done during daytime.

Extracellular single-unit recordings in the EP and SNr before, during and after acute EP-DBS

The impact of EP-DBS on neuronal activity in the EP and SNr of dt^{sz}-hamsters and controls was assessed by extracellular single-unit recordings before, during and after acute EP-DBS in anesthetized (urethane 20%, 0.8 ml/100 g, Sigma, Les Ulis, France) dt^{sz}-hamsters and controls. Body temperature was maintained at 37 °C using a heating pad, which was connected to a rectal probe (CMA150, Sweden). Under deep anesthesia, animals were fixed in a stereotaxic frame (Narishige, Japan). The calvarium above the left hemisphere was partially removed and two monopolar tungsten microelectrodes (8–12 MΩ, FHC, Bowdoin, ME, USA), fixed together with nail varnish, were oriented along the anterior–posterior axis and lowered to the EP. Electrode tips were positioned 400–500 µm apart under a microscope, with a slight shift (50 µm) in ventro-dorsal positioning. The posterior and slightly deeper electrode served as stimulation electrode, while EP neuronal activity was recorded with the anterior electrode. The electrodes were lowered up to a depth of 5 mm under brain surface and then more slowly under continuous recording of neuronal activity until single-neuron EP activity could be distinguished. Stereotaxic coordinates were: −0.6 to −0.9 mm anterior and 2.1 to 2.3 mm lateral (relative to Bregma) (Harnack et al., 2004). Once a stable single-unit activity was detected, the signal was recorded before, during and after EP-DBS, the latter being applied by using a stimulator (WPI, Sarasota, USA). As shown in a previous study, bilateral EP-DBS significantly reduces the severity of dystonic symptoms in dt^{sz}-hamsters (Harnack et al., 2004). To ensure that the stimulation would have similar clinical efficiency if the animals were awake, we used similar stimulation parameters (frequency 130 Hz, intensity 100 µA, impulse width 60 µs) as the parameters used in the latter study. Extracellular single-unit recordings were also performed in the SNr before, during and after acute EP-DBS after having assessed EP neuronal signals in the same animal. The electrodes were left in the EP and another one of equal configuration was placed within the SNr. The electrode was lowered up to a depth of 4 mm under brain surface and then more slowly under continuous recording of neuronal activity until single-neuron SNr activity could be distinguished. Stereotaxic coordinates were: −3.2 to −3.4 mm anterior and 2.0 to 2.2 mm lateral (relative to Bregma) (Gernert et al., 2002).

Neuronal signals were preamplified and analogically filtered (band-pass 300 Hz–3 kHz; Digitimer Ltd. NeuroLog System, Hertfordshire, UK), amplified (PowerLab, ADInstruments Ltd., Oxfordshire, UK), and visualized and stored using a personal computer equipped with Chart5 software (ADInstruments). Spike sorting was performed off-line using a custom version of the Spike-O-Matic software (Pouzat et al., 2004). Although we did not use any sophisticated feature of this spike-sorting program, it allowed us to get a satisfactory classification of extracellular spikes and electrical artifacts induced by DBS. Indeed, spikes were sometimes overriding the late component of the stimulation artifact (an overshoot lasting 2 to 3 ms), making spike discrimination inaccurate with most spike-sorting algorithms. On the contrary, our custom version of Spike-O-Matic allowed properly classifying events corresponding to the superposition of two different waveforms occurring simultaneously or with a small time delay. This feature allowed us to reduce the shadowing effect of the stimulation artifact to 1 ms.

Data analysis

Firing rate and pattern analysis

The mean firing rate was calculated for baseline (2 min), DBS (2 min), and recovery (post-DBS, 4 min). During DBS, the adjusted mean firing rate excluding the saturation time of the amplifier by the stimulation artifact was also calculated. To this end, the spike count over time windows excluding saturation time was divided by the sum of the length of these windows (number of impulse*(inter-impulse interval − saturation time)). In many cases, the stimulation artifact was shorter than the duration of the electrical pulse plus an action potential of EP neurons. Thus, it seems to be very unlikely that a spike might have been triggered by the stimulus. However, we cannot rule out that some evoked spikes might have been obscured by the artifact.

Regular, burst and random patterns of neuronal activity have been analyzed by using a further refinement (Labarre et al., 2008) of the method proposed by Kaneoke and Vitek (1996). The density histogram was estimated from each spike train and compared to three standard probability density functions (PDF). A Gaussian PDF with mean 1 was considered to represent a regular activity. A Poisson PDF with mean 0.2 was considered to reflect a bursting activity and a Poisson PDF with mean 1 was considered to comply with a random activity. The PDF with the smallest distance to the estimated density histogram of the assessed neuron determined its firing pattern.

ISI analysis

The distributions of inter-spike intervals (ISI) were calculated with a 1 ms resolution (interval max: 500 ms) during baseline, DBS and post-DBS. The coefficient of variation (CV) and the asymmetry index of the ISI distribution were calculated for each neuron. The asymmetry index is defined as the ratio of the mode (most frequent ISI) to the mean ISI. A Gaussian distribution displays an asymmetry index of 1,