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**High frequency stimulation of the subthalamic nucleus modulates neuronal activity in  
the lateral habenula nucleus**

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## **Abstract**

High frequency stimulation (HFS) of the subthalamic nucleus (STN) is often used to treat movement disability in advanced Parkinson's disease, but some patients experience debilitating psychiatric effects including depression. Interestingly, HFS of the STN modulates 5-HT neurons in the dorsal raphe nucleus (DRN) which are linked to depression, but the neural substrate of this effect is unknown. Here we tested the effect of STN stimulation on neuronal activity in the lateral habenula nucleus (LHb), an important source of input to DRN 5-HT neurons and also a key controller of emotive behaviours. LHb neurons were monitored in anaesthetised rats using single unit extracellular recording, and localisation within the LHb was confirmed by juxtacellular labelling. HFS of the STN (130 Hz) evoked rapid changes in the firing rate of the majority of LHb neurons tested (38 of 68). Some LHb neurons (19/68) were activated by HFS while others (19/68), distinguished by a higher basal firing rate, were inhibited. LHb neurons that project to the DRN were identified using antidromic activation and collision testing (n=17 neurons). Some of these neurons (5/17) were also excited by HFS of the STN, and others (7/17) were inhibited although this was only a statistical trend. In summary, HFS of the STN modulated the firing of LHb neurons, including those projecting to the DRN. The data identify that the STN impacts on the LHb-DRN pathway. Moreover, this pathway may be part of the circuitry mediating the psychiatric effects of STN stimulation experienced by patients with Parkinson's disease.

## Introduction

High frequency stimulation (HFS), often referred to as deep brain stimulation (DBS), of the subthalamic nucleus (STN) is currently the neurosurgical treatment of choice to manage the disabling motor symptoms of advanced Parkinson's disease (Krack *et al.*, 2003; Deuschl *et al.*, 2006; Weaver *et al.*, 2009). However, some patients experience unpleasant and debilitating psychiatric side-effects including depression, suicidal thoughts and actions, and cognitive deficits (Temel *et al.*, 2006; Appleby *et al.*, 2007). These adverse effects are apparent in large scale, multi-centre studies of suicide rates, and the depressive effects can be acute and dramatic in individual patients (Soulas *et al.*, 2008; Voon *et al.*, 2008). Consequently, a history of major depression is now an exclusion criterion for DBS, which deprives many patients of this treatment option as Parkinson's disease and mood disorder are highly co-morbid. There are analogous motor and non-motor effects associated with STN stimulation at the high frequencies used clinically (>100 Hz) in animals (Temel *et al.*, 2005; Temel *et al.*, 2007; Gubellini *et al.*, 2009).

The neural circuits underpinning the non-motor behavioural effects of HFS of the STN might include re-entrant cortico-basal ganglia-thalamic loops connecting the STN with forebrain limbic circuits (Turner *et al.*, 2001; Temel *et al.*, 2005). However, the diversity of behavioural effects of STN HFS might be better explained by alterations in a global neuromodulatory system. Recent work has demonstrated unexpected actions of STN HFS on the midbrain 5-hydroxytryptamine (5-HT) system, which is a well-known mediator of depression and other psychiatric symptoms. In particular, in rodents HFS of the STN (>100 Hz) modulated 5-HT cell firing in the dorsal raphe nucleus (DRN) (Temel *et al.*, 2007; Hartung *et al.*, 2011), decreased 5-HT release in the forebrain (Navailles *et al.*, 2010; Tan *et al.*, 2012), and evoked 5-HT-dependent depressive-like behavioural effects (Temel *et al.*, 2007). The neural circuitry

mediating these effects is unknown since there is little if any direct projection from the STN to the DRN (Ogawa *et al.*, 2014; Pollak Dorocic *et al.*, 2014).

Recently, we found that HFS of the STN increased c-Fos expression in the lateral habenula nucleus (LHb) of the epithalamus (Tan *et al.*, 2011). This putative interaction between the STN and LHb is interesting because the LHb is an important source of input to 5-HT neurons in the DRN (Wang & Aghajanian, 1977; Varga *et al.*, 2003). Moreover, the LHb and its connectivity with the DRN are fast emerging as a key node in neural circuitry that regulates motivational and emotive behaviours (Hikosaka *et al.*, 2008; Li *et al.*, 2011; Li *et al.*, 2013; Zhao *et al.*, 2015). Here we investigated the effect of STN HFS on the firing of neurons in the LHb, including LHb neurons identified as projecting to the DRN.

## Methods and Materials

### *Animals*

Recordings were obtained from a total of 25 male Sprague Dawley rats (265-310 g; Harlan, Bicester, UK). Experiments were used in accordance with the Animals (Scientific Procedures) Act of 1986 (United Kingdom), and approved by local ethical review at the University of Oxford. Rats were housed with water and food *ad libitum* on a 12 h light/dark cycle (lights on 07:00 am).

### *Electrophysiological recordings*

Extracellular recordings were carried out as described previously (Hartung *et al.*, 2011; Tan *et al.*, 2012). Rats were anaesthetised using chloral hydrate (460 mg/kg i.p. with supplementary doses as necessary) and placed in a stereotaxic frame (David Kopf). General anaesthesia was monitored by recording of electrocorticograms (high frequency-pass filtered at 0.1 Hz with x2000 amplification) via steel skull screws located above the left prefrontal cortex, with the contralateral cerebellum serving as reference. Body temperature was maintained at 37° C with a thermoregulated heating blanket (Harvard Apparatus Ltd., Edenbridge, UK).

A burr-hole was drilled in the skull and a stimulating electrode (gold-coated, concentric bipolar coaxial design with an platinum-iridium inner wire, 50 µm tip diameter, 250 µm shaft diameter; Technomed, The Netherlands) was implanted unilaterally into the STN (coordinates (mm) from bregma; anteroposterior -3.8, mediolateral  $\pm 2.5$ , dorsoventral -8.0 (Paxinos & Watson, 1998). STN stimulations were unilateral because the close proximity of the LHb and STN made stereotaxic bilateral electrode placement into the STN technically challenging, even more so for experiments also involving stereotaxically implanted DRN electrodes (see below).

The STN electrode was connected via a constant-current isolator (A360, World Precision Instruments, UK) to a stimulus generator (Master 8, AMPI, Israel) for high frequency stimulation. A second burr-hole was drilled for implantation of a glass recording microelectrode (12-25 M $\Omega$  impedance *in vivo*) filled with 0.5 M NaCl and neurobiotin (1.5 % w/v; Vector Laboratories, UK) that was lowered into the ipsilateral LHb (coordinates from bregma: anteroposterior -3.6, mediolateral  $\pm$ 0.5 and dorsoventral -3.5 to -4.0 (Paxinos & Watson, 1998) using a piezoelectric microdrive (Inchworm, EXFO). Mineral oil and saline solution (0.9 % sodium chloride) were applied to all areas of exposed cortex to prevent tissue dehydration.

Biopotentials were amplified (x10) through the active bridge circuitry of a Neurodata amplifier (Cygnus Technologies), AC-coupled, and further amplified (x100; NL106 AC-DC Amp; Digitimer), before being filtered between 0.3 and 5 kHz (NL125; Digitimer). Signals were digitized online using a Micro1401 Analog-Digital converter (Cambridge Electronic Design, UK) and a computer running Spike2v6 acquisition and analysis software (Cambridge Electronic Design, UK). Single unit activity and electrocorticograms were sampled at 20 kHz.

#### *Identification of LHb-DRN neurons*

LHb neurons projecting to the DRN were identified by antidromic activation and collision testing. For this procedure, a stimulating electrode (stainless steel bipolar, 200  $\mu$ m tip diameter) was lowered into the DRN (AP -7.8 mm, ML 0 mm, DV -5-6 mm (Paxinos & Watson, 1998) and antidromic spikes were evoked by low frequency stimulation (0.67 Hz, 50-700  $\mu$ A, 300  $\mu$ s). For collision testing, delayed stimulus pulses were triggered by spontaneous spikes using a custom written script (Spike2). Collisions occurred when the timing of the spontaneous spike was shorter than the antidromic spike latency, and was consistent over consecutive trials using supra-threshold stimulus intensities. Antidromically activated neurons were classified according to the following criteria: i) evoked spikes of constant latency, ii) all-or-none property

of antidromic spikes as determined by sub- and suprathreshold stimulus intensities, iii) collision of antidromic and spontaneous spikes.

### *Experimental protocol*

Following single unit detection and 5 min of stable baseline recording, the STN was stimulated at high frequency (130 Hz, 100  $\mu$ A, 60  $\mu$ s) for 5 min, and recordings continued for 5 min. In some experiments, after recording the effect of HFS, the STN was stimulated again at low frequency (10 Hz, 100  $\mu$ A, 60  $\mu$ s) for 5 min. Typically, the effect of STN HFS was tested on 3-7 neurons per rat, and attempts were made to juxtacellular label each recorded neuron (see below). In the final analysis, the order in which a cell was recorded had no effect on the response to STN HFS observed.

### *Processing of spike trains*

Spike trains were analysed as described previously (Hartung *et al.*, 2011; Tan *et al.*, 2012). To isolate single unit activity in the spike trains, stimulation artefacts were removed using standard spike-sorting techniques, including template-matching, principal component analysis and supervised clustering (Spike2, Cambridge Electronic Design, UK). Spike trains were also visually inspected to ensure that no spikes were missed during spike sorting. Baseline firing rate was calculated for the last 60 s prior to STN stimulation. Interspike interval (ISI) histograms were plotted for the baseline period and regularity of firing was assessed by coefficient of variation (COV; standard deviation of ISI/mean ISI). Spike waveforms were averaged over the baseline period, and spike width was defined as the time between a 5 % deviation from baseline to return of baseline after the negative phase (Allers & Sharp, 2003).

Firing rate was calculated at baseline, during the 2<sup>nd</sup> and 5<sup>th</sup> min of stimulation, and during the 5<sup>th</sup> min after cessation of stimulation. The response of individual neurons was categorized by plotting firing rate histograms (10 s bin size). Neurons were counted as responding to stimulation, if 3 consecutive bins during the 2<sup>nd</sup> or 5<sup>th</sup> min of stimulation, were below the mean baseline firing rate minus one standard deviation of the mean (inhibition), or above the mean baseline firing rate plus one standard deviation of the mean (excitation). Neurons with low (<0.2 Hz) or highly variable baseline firing rates typically resulted in an uninterpretable response to STN stimulation, and were excluded from the data set.

### *Juxtacellular labelling*

Juxtacellular labelling was carried out as described previously (Pinault, 1996; Allers & Sharp, 2003; Hartung *et al.*, 2011). To eject neurobiotin from the recording electrode and facilitate uptake, neurons were entrained for 30 s to 5 min using 1-10 nA, 200 ms on/off current pulses passed through the electrode. Typically, 1-2 neurons were labelled per animal, and could be unambiguously identified post-mortem on the basis of the automatically monitored stereotaxic coordinates of the recording electrode. After juxtacellular labelling, animals were maintained under anaesthesia for 1-3 h, and then perfused via the left ventricle with 0.9 % saline solution, followed by 300 ml of 4 % paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were removed and cryo-protected in 30 % sucrose in 0.1 M phosphate buffer for at least 48 h prior to sectioning.

### *Immunocytochemistry*

Sections (30-40  $\mu$ m) were cut on a sliding, freezing microtome, and then rinsed in phosphate buffered saline (PBS) 5 x 7 min, then incubated in 1% sodium borohydride in PBS for 10 min. After 5 alternating washes with PBS or PBS containing 0.25 % Triton-X100 (PBS-T) each for



7 min, sections were blocked for 1 h in PBS-T containing 5 % normal donkey serum and 5 % human serum, before being incubated for 24-72 h (room temperature) in goat anti-EAAC1 antibody (1:500, Millipore, U.K.) in PBS-T containing 1% normal donkey serum and 1% human serum. Next, sections were rinsed alternately in PBS-T and PBS five times (each for 7 min) and then incubated overnight (4 °C) in Alexa Fluor 488 donkey anti-goat (1:1000, Invitrogen, U.K.) and Alexa Fluor 594 conjugated streptavidin (1:1000, Invitrogen, U.K.) in PBS-T with 1 % normal donkey serum and 1 % human serum. Finally, sections were rinsed 5 x 7 min in PBS and mounted on gelatine-coated slides and cover-slipped.

Sections were analysed using an epifluorescence microscope (Zeiss Imager M2, Carl Zeiss AG, Germany) with a fluorescent light source. Photomicrographs were taken with a monochrome digital camera (ORCA-ER, Hamamatsu Photonics K.K., Japan) and collected using Axiovision software (Carl Zeiss AG). Photomicrographs were processed and false colour applied using Adobe Photoshop software.

### *Statistical analysis*

To determine the effect of **STN HFS** on the firing of LHb neurons, grouped data were analysed statistically (SPSS or GraphPad Prism). Within group analyses were also carried out using repeated measures one-way ANOVA followed by post-hoc Dunnett's t-tests, particularly to determine the timing of the effect of **STN HFS**. Differences between the groups were also assessed using repeated measures two-way ANOVA, followed by post-hoc Dunnett's t-tests. Selected between group differences in baseline firing were tested using Student's unpaired t test. Probability levels of  $\leq 0.05$  were considered statistically significant. Data are presented as mean  $\pm$  s.e.m. values.

## Results

### *In vivo electrophysiological and chemical properties of LHb neurons*

Stable recordings were obtained from a total of 82 LHb neurons (25 rats in total). LHb neurons were spontaneously active with firing rates in the range 0.2 - 23 Hz and showed a variety of firing properties (Table 1). The majority of LHb neurons (91 %) fired broad spikes ( $2.01 \pm 0.06$  ms) tonically in an irregular firing pattern (COV  $1.14 \pm 0.06$ , Fig.1a). A minority of LHb neurons were regular firing (Fig. 1b) or fired short, adapting bursts intermittent with periods of low activity (Fig. 1c).

Of the 82 LHb neurons recorded, 19 were successfully juxtacellular-labelled (collected from 16 of 25 rats) as evident by the somatic localisation of neurobiotin (Fig. 1). All juxtacellular-labelled cells included in the analysis were located in the medial subdivision of the LHb or at the border between the medial and lateral subdivisions (Fig. 2e). Dense immuno-labelling for EAAC1 which marks glutamatergic neurons (Li *et al.*, 2011) was localised in soma, dendrites and axons throughout the habenula region (Fig. 1). Seventeen of the 19 juxtacellular labelled neurons were judged EAAC1 immunopositive. The electrophysiological properties of EAAC1 immunopositive neurons were heterogeneous (Table 1) and included cells with tonic-irregular, regular and burst-firing patterns, as well as neurons with low (< 1Hz) and high firing rates (< 20 Hz) (Fig.1). We had too few examples of juxtacellular-labelled EAAC1 immunonegative neurons to allow firm conclusions regarding their electrophysiological properties.

### *Modulation of LHb neurons by HFS of the STN*

We tested the response of 65 stable LHb neurons to HFS (130 Hz, 5 min) of the STN. Of these neurons, 19/65 (29 % of total tested) met the criterion of increased firing during the stimulation

period ( $+73.7 \pm 17.3$  % increase in firing rate above baseline). In many cases (70 %) this effect did not reverse on cessation of the stimulation. Recordings from examples of individual LHb neurons demonstrating increased firing are shown in Fig. 2 and grouped data are shown in Fig. 3. This increase in firing was statistically significant when compared to both pre-stimulus firing rate (repeated measures one-way ANOVA  $F_{2,36}=10.81$   $P=0.0002$ , followed by Dunnett's post-hoc test 2<sup>nd</sup> min  $P=0.03$ , 5<sup>th</sup> min  $P=0.0001$ ) and when compared against another group of neurons ( $n=27$ ) identified as non-responding to STN HFS (repeated measures two-way ANOVA interaction group x time  $F_{4,120}=14.297$   $P=1.42 \times 10^{-9}$  followed by Dunnett's post-hoc test activated vs non-responders  $P=1 \times 10^{-6}$ : one-way ANOVA for individual time points 2nd min  $F_{2,62}=13.47$   $P=1.4 \times 10^{-5}$  followed by Dunnett's post-hoc test activated vs non-responders  $P=0.002$ , 5th min  $F_{2,62}=27.81$   $P=2.39 \times 10^{-9}$  followed by Dunnett's post-hoc test activated vs non-responders  $P=3.99 \times 10^{-7}$ ).

This excitatory effect was specific to a subpopulation of LHb neurons in that a further group of neurons (19/65; 29 % of total neurons) met the criterion of being inhibited by HFS of the STN ( $-28.4 \pm 4.9$  % decrease in firing rate from baseline). Recordings from an individual LHb neuron demonstrating decreased firing is shown in Fig. 2, and grouped data are shown in Fig. 3. This decrease in firing was statistically significant when compared to pre-stimulus values (repeated measures one-way ANOVA  $F_{2,36}=10.66$   $P=0.0002$ ; followed by Dunnett's post-hoc test 2<sup>nd</sup> min  $P=0.0033$ , 5<sup>th</sup> min  $P=0.0002$ ) and the effect returned to baseline values on cessation of the stimulus (Fig. 3a). It should be noted that this inhibitory effect was of borderline statistical significance when compared to non-responding neurons (repeated measures two-way ANOVA followed by Dunnett's post-hoc test,  $P=0.063$ ). Nevertheless, overall, HFS of the STN modulated more than half (38/65) of the LHb neurons tested, with the remainder (27/65) being non-responsive (Fig. 3a).

A selection of neurons that were either activated or inhibited by high frequency (130 Hz) stimulation did not respond to low frequency (10 Hz) stimulation (Fig. 3b). Moreover, there was no relationship between the response of the LHb neurons and the localisation of the stimulating electrode within sub-regions of the STN (Fig. 3c). Of the LHb neurons responding to HFS of the STN, 7 neurons were successfully labelled by juxtacellular application of neurobiotin, of which 6 neurons were immunopositive for EAAC1. Three non-responsive LHb neurons were also juxtacellular labelled and all were EAAC1-immunopositive.

The baseline firing rate of LHb neurons that were activated by HFS of the STN was significantly lower than LHb neurons that were inhibited ( $T_{36}=2.83$ ,  $P=0.0075$   $n=19$ , Student's unpaired t-test; Table 1), and thus might be a specific subpopulation of LHb neurons. LHb neurons responding to HFS of the STN were otherwise heterogeneous in terms of their firing properties, and indistinguishable from non-responsive neurons (Table 1).

#### *Modulation of LHb-DRN neurons by HFS of the STN*

Further experiments were carried out on LHb-DRN projecting neurons identified by antidromic activation and collision testing. These experiments were technically challenging since they involving the accurate *in vivo* stereotaxic placement of a recording electrode (LHb) and two stimulating electrodes (STN and DRN) in confined space. A total of 17 LHb neurons (collected from 15 of 25 rats) were identified that demonstrated antidromic spikes to electrical stimulation (0.67 Hz) of the DRN; antidromic spikes had a low threshold (80-450  $\mu$ A), short latency (3-15 ms) with little jitter (Fig. 4a), and they demonstrated collision with spontaneous spikes (Fig. 4b). LHb neurons demonstrating antidromic spikes were most frequently detected when the tip of stimulating electrode was located in the ventral DRN (Fig. 4c).

LHb neurons antidromically activated by the DRN were tested for their response to HFS (130 Hz) of the STN. Of 17 LHb-DRN neurons tested, 5/17 (29.4 % of total) met the criterion for activation ( $+31.6 \pm 10.5$  % increase in firing rate from baseline). An individual example of an activated LHb-DRN neuron as well as the grouped data are shown in Fig. 5. This increase in firing was statistically significant when compared to a group of LHb-DRN neurons ( $n=5$ ) identified as non-responding (repeated measures two-way ANOVA, interaction group x time;  $F_{4,24}=6.98$ ,  $P=0.001$  followed by Dunnett's post-hoc test activated vs non-responders  $P=0.051$ : one-way ANOVA for individual time points; 2nd min  $F_{2,14}=1.14$ ,  $P=0.27$ , 5th min  $F_{2,14}=14.74$ ,  $P=0.0003$  followed by Dunnett's post-hoc test activated vs non-responders  $P=0.007$ ).

In addition, 7/17 (41.2 % of total) of LHb-DRN neurons met the criterion of being inhibited by STN HFS ( $-21.8 \pm 6.2$  % decrease in firing rate from baseline) but this effect did not reach statistical significance when compared to pre-stimulus values (repeated measures one-way ANOVA  $F_{2,12}=2.58$   $P=0.12$ ) nor when compared to LHb-DRN neurons classified as non-responding (repeated measures two-way ANOVA followed by Dunnett's post-hoc test inhibited vs non-responders,  $P=0.136$ ). Eleven LHb-DRN neurons were juxtacellular-labelled and confirmed to be located in the LHb, and 10 were judged EAAC1-immunopositive.

The baseline firing properties of LHb-DRN neurons were heterogeneous and not distinguishable from other LHb neurons recorded (Table 1). LHb-DRN neurons that were activated by HFS of the STN tended to be slower firing than those that were inhibited (Table 1), but this difference was not statistically significant in the small sample tested.

## Discussion

Patients with advanced Parkinson's disease treated with so called DBS of the STN often experience disabling psychiatric symptoms, which currently are unexplained. The current study observed in a rodent model (anaesthetised rat) that stimulation of the STN stimulation at a high frequency used clinically (130 Hz) modulated the firing of neurons in the LHb, a key node in the circuitry regulating motivational and emotive behaviours (Hikosaka *et al.*, 2008; Proulx *et al.*, 2014). Thus, HFS of the STN modulated the firing of about 60% of the total LHb neurons tested (50/82, including LHb-DRN neurons); some LHb neurons responded to HFS of the STN with activation, consistent with evidence that the same stimulation increased Fos expression in the LHb (Tan *et al.*, 2011), while other LHb neurons were inhibited. Moreover, it was observed that LHb neurons projecting to the DRN were also modulated by HFS of the STN. Taken together, the current findings identify an interaction between the STN and the LHb-DRN pathway, which may be relevant to psychiatric effects of STN stimulation.

### *Electrophysiological and chemical properties of LHb neurons*

Neurons recorded in the LHb were spontaneously active and demonstrated heterogeneous firing properties that could be accommodated into three main categories as previously described (Kowski *et al.*, 2009) in one of few *in vivo* electrophysiological studies of rat LHb neurons to date (Zhao & Rusak, 2005; Sharp *et al.*, 2006; Kowski *et al.*, 2009); the majority of LHb neurons (91 %) fired broad spikes in an irregular firing pattern whereas a minority were either regular firing or fired bursts intermittent with periods of low activity. Here, the localisation of neurons within the borders of the LHb was confirmed with juxtacellular labelling, and post-mortem immunocytochemistry. Furthermore, double labelling experiments demonstrated the presence of immunolabelling for the glutamate transporter EAAC1 in the majority of juxtacellular-labelled neurons examined, which is consistent with the largely glutamatergic

nature of LHb neurons (Li *et al.*, 2011). LHb neurons identified as projecting to the DRN (see below) were indistinguishable from other LHb neurons in terms of their electrophysiological properties, and all but one such neurons were judged EAAC1 immunopositive after juxtacellular-labelling. The latter finding agrees with earlier immunohistochemical, pathway tracing and electrophysiological evidence that the major pathway from the LHb to the DRN is glutamatergic (Wang & Aghajanian, 1977; Ferraro *et al.*, 1996; Varga *et al.*, 2003; Pollak Dorocic *et al.*, 2014; Weissbourd *et al.*, 2014).

#### *Specificity of effects of HFS of the STN*

Although the reaction of LHb neurons to HFS of the STN comprised of a combination of excitatory and inhibitory responses, this effect is likely to be specific given that i) LHb neuron firing rate was stable prior to commencing stimulation, ii) not all LHb neurons were modulated, and iii) low frequency (10 Hz) stimulation did not alter the firing of LHb neurons that responded to high frequency (130 Hz) stimulation. Furthermore, this finding accords with reports that basal ganglia neurons also respond to HFS of the STN with both excitatory and inhibitory responses (Gubellini *et al.*, 2009).

The mixed effects of HFS of the STN on LHb neurons might reflect diversity in the circuitry connecting the STN and LHb (see below). This would fit with the current evidence that HFS of the STN influenced two distinct populations of LHb neurons; LHb neurons activated by HFS of the STN had a slower firing rate than LHb neurons that were inhibited. In addition to circuit diversity, the STN itself may also be a source of heterogeneous effects in the LHb; thus, STN neurons show both excitatory and inhibitory responses when HFS is applied directly to this nucleus (Deniau *et al.*, 2010).

From the grouped data, on cessation of STN HFS the firing rate of LHb neurons was not statistically different from pre-stimulus levels, although there is a clear upwards trend and for individual neurons firing did remain increased. We have previously observed that some DRN 5-HT neurons were inhibited beyond cessation of STN HFS (Hartung et al. 2011). Furthermore, changes in firing rates of neurons in other brain regions including the STN itself have been reported to continue after HFS of the STN is terminated (Li et al. 2010). This suggests that STN HFS may evoke plastic changes that endure after cessation of the stimulus.

#### *Pathways mediating effect of HFS of the STN on LHb neurons*

Various neural circuits could mediate the effects of HFS of the STN on the firing of LHb neurons. A direct projection from the STN to the LHb has not been reported, but the STN sends axon collaterals to various regions which have direct and/or indirect connectivity with the LHb, including the globus pallidus (GP), ventral pallidum and cerebral cortex (Herkenham & Nauta, 1977; Hammond *et al.*, 1983; Alexander & Crutcher, 1990; Parent & Hazrati, 1995; Shabel *et al.*, 2012). Thus, there is the potential for the involvement of multiple polysynaptic circuits in the modulation of LHb neurons by the STN. However, involvement of the GP internal segment (GPi; entopeduncular nucleus in rodents) is likely since there is a substantial glutamatergic projection from the STN to GPi. Moreover, HFS of the STN modulates neuron firing (Hashimoto *et al.*, 2003; Shi *et al.*, 2006; Hahn *et al.*, 2008), glutamate release and fos expression in the GPi (Windels *et al.*, 2000). It is estimated that ~10% of GPi neurons project to the LHb (Parent *et al.*, 2001). These GPi neurons have been found to utilise GABA, acetylcholine or glutamate (Oertel *et al.*, 1984; Moriizumi & Hattori, 1992; Hong & Hikosaka, 2008; Shabel *et al.*, 2012), and some of these neurons may control the activity of LHb neurons through co-release GABA and glutamate (Shabel *et al.*, 2014). Thus, HFS of the STN may target the GPi to influence a mixture of inhibitory/excitatory pathways that project from the GPi to LHb, and thereby generate the combination of inhibitory and excitatory effects on LHb neurons observed herein.



### *HFS of the STN modulates LHb neurons projecting to the DRN*

Previously we demonstrated that HFS of the STN (>100 Hz) inhibited the firing of many 5-HT neurons in the DRN, with a small number being excited (Temel *et al.*, 2007; Hartung *et al.*, 2011; Tan *et al.*, 2012). Moreover, HFS of the STN decreased extracellular 5-HT in forebrain regions (Navailles *et al.*, 2010; Creed *et al.*, 2012; Tan *et al.*, 2012). To investigate whether HFS of the STN modulated LHb neurons projecting to the DRN, these neurons were identified by antidromic activation. Interestingly, HFS of the STN also modulated LHb-DRN neurons, with some neurons being activated and others inhibited (although the latter effect was only a trend on subsequent statistical analysis). Thus, the effect of HFS of the STN on DRN 5-HT neurons is likely mediated by the glutamatergic pathway from the LHb to the DRN that targets 5-HT directly and also indirectly via local GABAergic neurons (Wang & Aghajanian, 1977; Ferraro *et al.*, 1996; Varga *et al.*, 2003; Pollak Dorocic *et al.*, 2014; Weissbourd *et al.*, 2014). Overall these findings are consistent with the idea that the effect of HFS of the STN on the midbrain 5-HT system is mediated (at least in part) via the LHb.

All LHb-DRN neurons were antidromically identified using stimulating electrodes located in the DRN, with many of the successful experiments having electrode tips placed in the ventral DRN which is rich in 5-HT neurons. Low stimulation currents and concentric bipolar electrodes were used to restrict current spread to the DRN. Despite this, the LHb also innervates the nearby median raphe nucleus (MRN) (Bernard & Veh, 2012) and the possibility that a small number of the antidromically activated LHb neurons projected to the MRN cannot be excluded. Nevertheless, even if LHb-MRN neurons contribute to our recordings, the MRN is also a source of 5-HT neurons to the forebrain. Although the present study emphasises the interaction between the LHb and 5-HT system, the LHb also targets dopamine neurons in the ventral tegmental area (Bernard & Veh, 2012). Thus, it is plausible that some LHb neurons influenced by HFS of the STN projected to the ventral tegmental area, amongst other LHb targets.

Given the important role of the LHb and the LHb-DRN pathway in numerous behavioural and physiological processes including reward and motivation, impulse control and sleep regulation (reviewed by Hong & Hikosaka, 2008; Zhao et al, 2015), the above data suggest that HFS of the STN would evoke such behavioural effects. Indeed, in animals HFS of the STN evokes a range of effects on emotional responses, motivation and cognition (Temel *et al.*, 2005; Temel *et al.*, 2007; Gubellini *et al.*, 2009). Moreover, there is evidence that depressive-like behavioural effects of HFS of the STN are dependent on 5-HT (Temel *et al.*, 2007), and the current data predict that such effects may also be dependent on the LHb although this needs to be tested experimentally.

#### *Clinical relevance of effects of HFS of the STN on LHb function*

The current data are clinically relevant in that high frequency (130 Hz) STN stimulation is used in the therapeutic management of patients with Parkinson's disease. However, in the present experiments the period of stimulation was brief (minutes) whereas clinically, HFS of the STN is applied over longer periods (hours, days, weeks and even longer). A further limitation of this study is that the effects of HFS of the STN were not tested in a Parkinson's disease model. However, previous work demonstrated that effects of HFS of the STN in naive animals translate to such models (Temel et al, 2007; Tan et al, 2011); for example, HFS of the STN inhibits 5-HT neurons in both treatment naïve and dopamine-depleted rats (Temel et al, 2007). Despite these drawbacks, it is tempting to speculate that the interaction between the STN and LHb-DRN connectivity is associated with the psychiatric effects of STN stimulation in Parkinson's disease patients. This point is supported by the depressive-like behavioural effects of HFS of the STN noted above, and the fact that LHb-DRN connectivity is highly conserved across species (Aizawa *et al.*, 2011; Stephenson-Jones *et al.*, 2012).

A final general point, altered STN-LHb-DRN connectivity might also be associated with the psychiatric symptoms such as depression and apathy that are often comorbid with Parkinson's disease, because abnormal neural activity in the STN is a hallmark of Parkinson's disease. Thus, STN neurons display hyperactivity and burst activity as well as increased oscillatory activity in both Parkinson's disease models (Bergman *et al.*, 1994; Tai *et al.*, 2003) and patients (Lopez-Azcarate *et al.*, 2010; Hirschmann *et al.*, 2013). LHb function in depressed Parkinson's disease patients is, to our knowledge, not yet reported but in animal models of Parkinson's disease the LHb demonstrates metabolic hyperactivity (Wooten & Collins, 1981; Trugman & Wooten, 1987) that has also been detected in depression models and depressed patients (Caldecott-Hazard *et al.*, 1988; Morris *et al.*, 1999). Furthermore, it is reported that LHb lesions were able to attenuate depressive-like behaviours in an animal model of Parkinson's disease, thereby emphasising the link between the LHb, basal ganglia and depressive-like behaviours (Sourani *et al.*, 2012). Finally, electrical or pharmacological manipulation of the LHb has antidepressant effects in animals and clinical cases (Hikosaka *et al.*, 2008; Li *et al.*, 2011; Li *et al.*, 2013). Interestingly, other basal ganglia disorders including dystonia, Huntington's disease and Tourette's syndrome are characterized by co-morbid motor and psychiatric disorders (Robertson, 2006; Miller *et al.*, 2007; van Duijn *et al.*, 2007; Zurowski *et al.*, 2013), and there is evidence for disruption of the STN in these patients (Baym *et al.*, 2008; Schrock *et al.*, 2009; Vlamings *et al.*, 2012).

### *Summary*

In conclusion, the current study reveals evidence that HFS of the STN modulates the firing of LHb neurons including those that connect to the midbrain raphe 5-HT system. These data thereby provide functional evidence for a neural circuit by which STN is able to influence the midbrain 5-HT system. These findings also suggest a mechanism by which DBS might evoke psychiatric effects in Parkinson's disease patients, and also a mechanism by which

dysfunctional basal ganglia circuitry might disturb mood more generally. Tractable drug targets in the LHb including receptors for monoamine, neuropeptides and amino acid transmitters are now emerging (Aizawa *et al.*, 2012) which offer the future prospect of LHb-targeted pharmacological interventions.

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## References

- Aizawa, H., Amo, R. & Okamoto, H. (2011) Phylogeny and ontogeny of the habenular structure. *Front Neurosci*, **5**, 138.
- Aizawa, H., Kobayashi, M., Tanaka, S., Fukai, T. & Okamoto, H. (2012) Molecular characterization of the subnuclei in rat habenula. *J Comp Neurol*, **520**, 4051-4066.
- Alexander, G.E. & Crutcher, M.D. (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci*, **13**, 266-271.
- Allers, K.A. & Sharp, T. (2003) Neurochemical and anatomical identification of fast- and slow-firing neurones in the rat dorsal raphe nucleus using juxtacellular labelling methods in vivo. *Neuroscience*, **122**, 193-204.
- Appleby, B.S., Duggan, P.S., Regenberg, A. & Rabins, P.V. (2007) Psychiatric and neuropsychiatric adverse events associated with deep brain stimulation: A meta-analysis of ten years' experience. *Mov Disord*, **22**, 1722-1728.
- Baym, C.L., Corbett, B.A., Wright, S.B. & Bunge, S.A. (2008) Neural correlates of tic severity and cognitive control in children with Tourette syndrome. *Brain*, **131**, 165-179.
- Bergman, H., Wichmann, T., Karmon, B. & DeLong, M.R. (1994) The primate subthalamic nucleus. II. Neuronal activity in the MPTP model of parkinsonism. *J Neurophysiol*, **72**, 507-520.

- Bernard, R. & Veh, R.W. (2012) Individual neurons in the rat lateral habenular complex project mostly to the dopaminergic ventral tegmental area or to the serotonergic raphe nuclei. *J Comp Neurol*, **520**, 2545-2558.
- Caldecott-Hazard, S., Mazziotta, J. & Phelps, M. (1988) Cerebral correlates of depressed behavior in rats, visualized using 14C-2-deoxyglucose autoradiography. *J Neurosci*, **8**, 1951-1961.
- Creed, M.C., Hamani, C., Bridgman, A., Fletcher, P.J. & Nobrega, J.N. (2012) Contribution of decreased serotonin release to the antidyskinetic effects of deep brain stimulation in a rodent model of tardive dyskinesia: comparison of the subthalamic and entopeduncular nuclei. *J Neurosci*, **32**, 9574-9581.
- Deniau, J.M., Degos, B., Bosch, C. & Maurice, N. (2010) Deep brain stimulation mechanisms: beyond the concept of local functional inhibition. *Eur J Neurosci*, **32**, 1080-1091.
- Deuschl, G., Schade-Brittinger, C., Krack, P., Volkmann, J., Schafer, H., Botzel, K., Daniels, C., Deutschlander, A., Dillmann, U., Eisner, W., Gruber, D., Hamel, W., Herzog, J., Hilker, R., Klebe, S., Kloss, M., Koy, J., Krause, M., Kupsch, A., Lorenz, D., Lorenzl, S., Mehdorn, H.M., Moringlane, J.R., Oertel, W., Pinsker, M.O., Reichmann, H., Reuss, A., Schneider, G.H., Schnitzler, A., Steude, U., Sturm, V., Timmermann, L., Tronnier, V., Trottenberg, T., Wojtecki, L., Wolf, E., Poewe, W., Voges, J. & German Parkinson Study Group, N.S. (2006) A randomized trial of deep-brain stimulation for Parkinson's disease. *N Engl J Med*, **355**, 896-908.

Ferraro, G., Montalbano, M.E., Sardo, P. & La Grutta, V. (1996) Lateral habenular influence on dorsal raphe neurons. *Brain Res Bull*, **41**, 47-52.

Gubellini, P., Salin, P., Kerkerian-Le Goff, L. & Baunez, C. (2009) Deep brain stimulation in neurological diseases and experimental models: from molecule to complex behavior. *Prog Neurobiol*, **89**, 79-123.

Hahn, P.J., Russo, G.S., Hashimoto, T., Miocinovic, S., Xu, W., McIntyre, C.C. & Vitek, J.L. (2008) Pallidal burst activity during therapeutic deep brain stimulation. *Exp Neurol*, **211**, 243-251.

Hammond, C., Shibasaki, T. & Rouzaire-Dubois, B. (1983) Branched output neurons of the rat subthalamic nucleus: electrophysiological study of the synaptic effects on identified cells in the two main target nuclei, the entopeduncular nucleus and the substantia nigra. *Neuroscience*, **9**, 511-520.

Hartung, H., Tan, S.K., Steinbusch, H.M., Temel, Y. & Sharp, T. (2011) High-frequency stimulation of the subthalamic nucleus inhibits the firing of juxtacellular labelled 5-HT-containing neurones. *Neuroscience*, **186**, 135-145.

Hashimoto, T., Elder, C.M., Okun, M.S., Patrick, S.K. & Vitek, J.L. (2003) Stimulation of the subthalamic nucleus changes the firing pattern of pallidal neurons. *J Neurosci*, **23**, 1916-1923.

- Herkenham, M. & Nauta, W.J. (1977) Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study, with a note on the fiber-of-passage problem. *J Comp Neurol*, **173**, 123-146.
- Hikosaka, O., Sesack, S.R., Lecourtier, L. & Shepard, P.D. (2008) Habenula: crossroad between the basal ganglia and the limbic system. *J Neurosci*, **28**, 11825-11829.
- Hirschmann, J., Hartmann, C.J., Butz, M., Hoogenboom, N., Ozkurt, T.E., Elben, S., Vesper, J., Wojtecki, L. & Schnitzler, A. (2013) A direct relationship between oscillatory subthalamic nucleus-cortex coupling and rest tremor in Parkinson's disease. *Brain*, **136**, 3659-3670.
- Hong, S. & Hikosaka, O. (2008) The globus pallidus sends reward-related signals to the lateral habenula. *Neuron*, **60**, 720-729.
- Kowski, A.B., Veh, R.W. & Weiss, T. (2009) Dopaminergic activation excites rat lateral habenular neurons in vivo. *Neuroscience*, **161**, 1154-1165.
- Krack, P., Batir, A., Van Blercom, N., Chabardes, S., Fraix, V., Ardouin, C., Koudsie, A., Limousin, P.D., Benazzouz, A., LeBas, J.F., Benabid, A.L. & Pollak, P. (2003) Five-year follow-up of bilateral stimulation of the subthalamic nucleus in advanced Parkinson's disease. *N Engl J Med*, **349**, 1925-1934.



Li, B., Piriz, J., Mirrione, M., Chung, C., Proulx, C.D., Schulz, D., Henn, F. & Malinow, R.

(2011) Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature*, **470**, 535-539.

Li, K., Zhou, T., Liao, L., Yang, Z., Wong, C., Henn, F., Malinow, R., Yates, J.R., 3rd & Hu, H.

(2013) betaCaMKII in lateral habenula mediates core symptoms of depression. *Science*, **341**, 1016-1020.

Lopez-Azcarate, J., Tainta, M., Rodriguez-Oroz, M.C., Valencia, M., Gonzalez, R., Guridi, J.,

Iriarte, J., Obeso, J.A., Artieda, J. & Alegre, M. (2010) Coupling between beta and high-frequency activity in the human subthalamic nucleus may be a pathophysiological mechanism in Parkinson's disease. *J Neurosci*, **30**, 6667-6677.

Miller, K.M., Okun, M.S., Fernandez, H.F., Jacobson, C.E.t., Rodriguez, R.L. & Bowers, D.

(2007) Depression symptoms in movement disorders: comparing Parkinson's disease, dystonia, and essential tremor. *Mov Disord*, **22**, 666-672.

Moriizumi, T. & Hattori, T. (1992) Choline acetyltransferase-immunoreactive neurons in the

rat entopeduncular nucleus. *Neuroscience*, **46**, 721-728.

Morris, J.S., Smith, K.A., Cowen, P.J., Friston, K.J. & Dolan, R.J. (1999) Covariation of

activity in habenula and dorsal raphe nuclei following tryptophan depletion. *Neuroimage*, **10**, 163-172.

- Navailles, S., Benazzouz, A., Bioulac, B., Gross, C. & De Deurwaerdere, P. (2010) High-frequency stimulation of the subthalamic nucleus and L-3,4-dihydroxyphenylalanine inhibit in vivo serotonin release in the prefrontal cortex and hippocampus in a rat model of Parkinson's disease. *J Neurosci*, **30**, 2356-2364.
- Oertel, W.H., Nitsch, C. & Mugnaini, E. (1984) Immunocytochemical demonstration of the GABA-ergic neurons in rat globus pallidus and nucleus entopeduncularis and their GABA-ergic innervation. *Adv Neurol*, **40**, 91-98.
- Ogawa, S.K., Cohen, J.Y., Hwang, D., Uchida, N. & Watabe-Uchida, M. (2014) Organization of monosynaptic inputs to the serotonin and dopamine neuromodulatory systems. *Cell Rep*, **8**, 1105-1118.
- Parent, A. & Hazrati, L.N. (1995) Functional anatomy of the basal ganglia. II. The place of subthalamic nucleus and external pallidum in basal ganglia circuitry. *Brain Res Brain Res Rev*, **20**, 128-154.
- Parent, M., Levesque, M. & Parent, A. (2001) Two types of projection neurons in the internal pallidum of primates: single-axon tracing and three-dimensional reconstruction. *J Comp Neurol*, **439**, 162-175.
- Paxinos, G. & Watson, C. (1998) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.

- Pinault, D. (1996) A novel single-cell staining procedure performed in vivo under electrophysiological control: morpho-functional features of juxtacellularly labeled thalamic cells and other central neurons with biocytin or Neurobiotin. *J Neurosci Methods*, **65**, 113-136.
- Pollak Dorocic, I., Furth, D., Xuan, Y., Johansson, Y., Pozzi, L., Silberberg, G., Carlen, M. & Meletis, K. (2014) A whole-brain atlas of inputs to serotonergic neurons of the dorsal and median raphe nuclei. *Neuron*, **83**, 663-678.
- Proulx, C.D., Hikosaka, O. & Malinow, R. (2014) Reward processing by the lateral habenula in normal and depressive behaviors. *Nat Neurosci*, **17**, 1146-1152.
- Robertson, M.M. (2006) Mood disorders and Gilles de la Tourette's syndrome: An update on prevalence, etiology, comorbidity, clinical associations, and implications. *J Psychosom Res*, **61**, 349-358.
- Schrock, L.E., Ostrem, J.L., Turner, R.S., Shimamoto, S.A. & Starr, P.A. (2009) The subthalamic nucleus in primary dystonia: single-unit discharge characteristics. *J Neurophysiol*, **102**, 3740-3752.
- Shabel, S.J., Proulx, C.D., Piriz, J. & Malinow, R. (2014) Mood regulation. GABA/glutamate co-release controls habenula output and is modified by antidepressant treatment. *Science*, **345**, 1494-1498.

- Shabel, S.J., Proulx, C.D., Trias, A., Murphy, R.T. & Malinow, R. (2012) Input to the lateral habenula from the basal ganglia is excitatory, aversive, and suppressed by serotonin. *Neuron*, **74**, 475-481.
- Sharp, P.E., Turner-Williams, S. & Tuttle, S. (2006) Movement-related correlates of single cell activity in the interpeduncular nucleus and habenula of the rat during a pellet-chasing task. *Behav Brain Res*, **166**, 55-70.
- Shi, L.H., Luo, F., Woodward, D.J. & Chang, J.Y. (2006) Basal ganglia neural responses during behaviorally effective deep brain stimulation of the subthalamic nucleus in rats performing a treadmill locomotion test. *Synapse*, **59**, 445-457.
- Soulas, T., Gurruchaga, J.M., Palfi, S., Cesaro, P., Nguyen, J.P. & Fenelon, G. (2008) Attempted and completed suicides after subthalamic nucleus stimulation for Parkinson's disease. *J Neurol Neurosurg Psychiatry*, **79**, 952-954.
- Sourani, D., Eitan, R., Gordon, N. & Goelman, G. (2012) The habenula couples the dopaminergic and the serotonergic systems: application to depression in Parkinson's disease. *Eur J Neurosci*, **36**, 2822-2829.
- Stephenson-Jones, M., Floros, O., Robertson, B. & Grillner, S. (2012) Evolutionary conservation of the habenular nuclei and their circuitry controlling the dopamine and 5-hydroxytryptophan (5-HT) systems. *Proc Natl Acad Sci U S A*, **109**, E164-173.

- Tai, C.H., Boraud, T., Bezard, E., Bioulac, B., Gross, C. & Benazzouz, A. (2003)  
Electrophysiological and metabolic evidence that high-frequency stimulation of the subthalamic nucleus bridges neuronal activity in the subthalamic nucleus and the substantia nigra reticulata. *FASEB J*, **17**, 1820-1830.
- Tan, S.K., Hartung, H., Visser-Vandewalle, V., Steinbusch, H.W., Temel, Y. & Sharp, T. (2012) A combined in vivo neurochemical and electrophysiological analysis of the effect of high-frequency stimulation of the subthalamic nucleus on 5-HT transmission. *Exp Neurol*, **233**, 145-153.
- Tan, S.K., Janssen, M.L., Jahanshahi, A., Chouliaras, L., Visser-Vandewalle, V., Lim, L.W., Steinbusch, H.W., Sharp, T. & Temel, Y. (2011) High frequency stimulation of the subthalamic nucleus increases c-fos immunoreactivity in the dorsal raphe nucleus and afferent brain regions. *J Psychiatr Res*, **45**, 1307-1315.
- Temel, Y., Boothman, L.J., Blokland, A., Magill, P.J., Steinbusch, H.W., Visser-Vandewalle, V. & Sharp, T. (2007) Inhibition of 5-HT neuron activity and induction of depressive-like behavior by high-frequency stimulation of the subthalamic nucleus. *Proc Natl Acad Sci U S A*, **104**, 17087-17092.
- Temel, Y., Kessels, A., Tan, S., Topdag, A., Boon, P. & Visser-Vandewalle, V. (2006) Behavioural changes after bilateral subthalamic stimulation in advanced Parkinson disease: a systematic review. *Parkinsonism Relat Disord*, **12**, 265-272.
- Temel, Y., Visser-Vandewalle, V., Aendekerk, B., Rutten, B., Tan, S., Scholtissen, B., Schmitz, C., Blokland, A. & Steinbusch, H.W. (2005) Acute and separate modulation

- of motor and cognitive performance in parkinsonian rats by bilateral stimulation of the subthalamic nucleus. *Exp Neurol*, **193**, 43-52.
- Trugman, J.M. & Wooten, G.F. (1987) Selective D1 and D2 dopamine agonists differentially alter basal ganglia glucose utilization in rats with unilateral 6-hydroxydopamine substantia nigra lesions. *J Neurosci*, **7**, 2927-2935.
- Turner, M.S., Lavin, A., Grace, A.A. & Napier, T.C. (2001) Regulation of limbic information outflow by the subthalamic nucleus: excitatory amino acid projections to the ventral pallidum. *J Neurosci*, **21**, 2820-2832.
- van Duijn, E., Kingma, E.M. & van der Mast, R.C. (2007) Psychopathology in verified Huntington's disease gene carriers. *J Neuropsychiatry Clin Neurosci*, **19**, 441-448.
- Wang, R.Y. & Aghajanian, G.K. (1977) Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science*, **197**, 89-91.
- Varga, V., Kocsis, B. & Sharp, T. (2003) Electrophysiological evidence for convergence of inputs from the medial prefrontal cortex and lateral habenula on single neurons in the dorsal raphe nucleus. *Eur J Neurosci*, **17**, 280-286.
- Weaver, F.M., Follett, K., Stern, M., Hur, K., Harris, C., Marks, W.J., Jr., Rothlind, J., Sagher, O., Reda, D., Moy, C.S., Pahwa, R., Burchiel, K., Hogarth, P., Lai, E.C., Duda, J.E., Holloway, K., Samii, A., Horn, S., Bronstein, J., Stoner, G., Heemskerk, J., Huang, G.D. & Group, C.S.P.S. (2009) Bilateral deep brain stimulation vs best medical

therapy for patients with advanced Parkinson disease: a randomized controlled trial. *JAMA*, **301**, 63-73.

Weissbourd, B., Ren, J., DeLoach, K.E., Guenther, C.J., Miyamichi, K. & Luo, L. (2014) Presynaptic partners of dorsal raphe serotonergic and GABAergic neurons. *Neuron*, **83**, 645-662.

Windels, F., Bruet, N., Poupard, A., Urbain, N., Chouvet, G., Feuerstein, C. & Savasta, M. (2000) Effects of high frequency stimulation of subthalamic nucleus on extracellular glutamate and GABA in substantia nigra and globus pallidus in the normal rat. *Eur J Neurosci*, **12**, 4141-4146.

Vlamings, R., Benazzouz, A., Chetrit, J., Janssen, M.L., Kozan, R., Visser-Vandewalle, V., Steinbusch, H.W., von Horsten, S. & Temel, Y. (2012) Metabolic and electrophysiological changes in the basal ganglia of transgenic Huntington's disease rats. *Neurobiol Dis*, **48**, 488-494.

Voon, V., Krack, P., Lang, A.E., Lozano, A.M., Dujardin, K., Schupbach, M., D'Ambrosia, J., Thobois, S., Tamma, F., Herzog, J., Speelman, J.D., Samanta, J., Kubu, C., Rossignol, H., Poon, Y.Y., Saint-Cyr, J.A., Ardouin, C. & Moro, E. (2008) A multicentre study on suicide outcomes following subthalamic stimulation for Parkinson's disease. *Brain*, **131**, 2720-2728.

Wooten, G.F. & Collins, R.C. (1981) Metabolic effects of unilateral lesion of the substantia nigra. *J Neurosci*, **1**, 285-291.

Zhao, H. & Rusak, B. (2005) Circadian firing-rate rhythms and light responses of rat habenular nucleus neurons in vivo and in vitro. *Neuroscience*, **132**, 519-528.

Zhao, H., Zhang, B.L., Yang, S.J. & Rusak, B. (2015) The role of lateral habenula-dorsal raphe nucleus circuits in higher brain functions and psychiatric illness. *Behav Brain Res*, **277**, 89-98.

Zurowski, M., McDonald, W.M., Fox, S. & Marsh, L. (2013) Psychiatric comorbidities in dystonia: emerging concepts. *Mov Disord*, **28**, 914-920.



**Table 1:** Baseline electrophysiological properties of LHb neurons in relation to their response to high frequency stimulation of the STN. The electrophysiological properties of neurons identified as EAAC1 immunopositive neurons are included. \*P<0.05 versus LHb inhibited neurons.

	Mean firing rate ± SEM (Hz)	Mean COV ± SEM	Mean action potential duration ± SEM (ms)
LHb activated (n=19)	3.79 ± 0.64*	1.16 ± 0.12	2.29 ± 0.18
LHb inhibited (n=19)	10.56 ± 2.30	1.18 ± 0.15	1.92 ± 0.09
LHb no response (n=27)	8.57 ± 2.38	1.25 ± 0.13	1.92 ± 0.06
DRN-LHb excited (n=5)	3.89 ± 1.8	1.14 ± 0.22	2.51 ± 0.38
DRN-LHb inhibited (n=7)	12.7 ± 6.7	0.92 ± 0.12	1.61 ± 0.09
DRN-LHb no response (n=5)	18.25 ± 5.47	0.71 ± 0.08	1.93 ± 0.16
EAAC1- immunopositive neurons (n=17)	12.25 ± 3.04	1.03 ± 0.14	2.10 ± 0.16

## Figure legends

### Figure 1

Examples of in vivo extracellular recording and juxtacellular labelling of neurons in the rat LHb. (a) neurobiotin (NB) labelled neuron (arrow), immunopositive for EAAC1, located in the medial LHb (red circle) together with the spike train from the same neuron and corresponding ECoG trace. The spike train is typical of a tonically firing irregular neuron, which was the most common firing pattern of LHb neurons recorded. (b) Images and recordings for a regular firing LHb neuron. (c) Images and recordings for a burst-firing LHb neuron. Scale bars in a-c = 10  $\mu$ m.

### Figure 2

Effect of high frequency stimulation of the STN on firing rate of single neurons recorded in the LHb of the anaesthetised rat. Examples of firing rate traces (a,b,d: 15 s bins, c: 5s bins) of LHb neurons that responded to STN stimulation (130 Hz, 100  $\mu$ A, 5 min) with excitation (a and b), inhibition (c) and no response (d). The images (e) illustrate the location of juxtacellular-labelled neurons within the habenula complex at different anteroposterior levels (Paxinos and Watson, 1998). Open symbols, not-antidromically tested; closed symbols, antidromically activated from the DRN. Response to STN stimulation; circles - activated, triangles - inhibited, squares - non-responding.

### Figure 3

Summary of effect of STN stimulation on LHb neurons. a) Time course changes in firing rate of LHb neurons in response to high frequency stimulation (130 Hz, 100  $\mu$ A, 5 min). b) Changes

in firing rate of LHb neurons in response to low frequency stimulation (10 Hz, 100  $\mu$ A, 5 min). Neurons shown in b) responded with either activation or inhibition to high frequency stimulation (data not shown). Data are percentage of baseline firing rate 60 s prior to stimulation. \* $p < 0.01$ , \*\* $p < 0.001$  versus non-responders at individual time points, one-way ANOVA followed by post-hoc Dunnett's t-test. Mean  $\pm$  SEM values are shown (number of neurons in brackets). c) Localisation of stimulation sites within the STN resulting in either excitation or inhibition. Abbreviations; ZI - Zona incerta, CP - cerebral peduncle.

#### Figure 4

Electrophysiological identification of LHb neuron projecting to the DRN. a) Raster plot and peri-stimulus time histogram demonstrating antidromic response to DRN stimulation (0.67 Hz, 500-700  $\mu$ A, 2 min). b) Illustration of collision test. When the latency between spontaneous spike (triangle) and stimulus pulse (downward arrow) is long ( $>20$ ms) an antidromically activated spike is triggered (star), hence the spontaneous and antidromic spikes do not collide (left). However, if the latency between spontaneous spike and stimulus pulse is shorter ( $<5$ ms), the antidromic and spontaneous spikes collide and no spike is triggered by the stimulus (right). c) Localisation of tips of stimulation electrodes at different anteroposterior levels of the DRN (Paxinos and Watson, 1998) that evoked antidromic spikes on LHb neurons.

#### Figure 5

Effect of high frequency stimulation of the STN on the firing rate of LHb neurons antidromically activated from the DRN. a) Juxtacellular labelling with neurobiotin (NB) of LHb neuron antidromically activated by DRN stimulation (scale bar = 10  $\mu$ m), and its localisation in the LHb (red spot; MHb =medial habenula nucleus). b) On the left, example of corresponding antidromic spike (starred) of the same neuron illustrated in a) elicited by DRN activation

(stimulus artefact, downward arrow) 20 ms following a spontaneous spike (upward arrow). On the right, collision of the antidromic spike elicited by DRN activation (stimulus artefact, downward arrow) 4 ms following a spontaneous spike (upward arrow). c) Firing rate response of the same neuron illustrated in a) and b) to high frequency stimulation (130 Hz, 100  $\mu$ A, 5 min) of the STN. d) Time course changes in firing rate of groups of DRN-projecting LHb neurons in response to high frequency stimulation (130 Hz, 100  $\mu$ A, 5 min) of the STN. Neurons were classified as excited, inhibited or non-responding on the basis of electrophysiological criteria (see Methods). Data are percentage of baseline firing rate 60 s prior to stimulation. \*\* $p < 0.01$  versus non-responders at individual time points, one-way ANOVA followed by post-hoc Dunnett's t-test. Mean  $\pm$  SEM values are shown (number of neurons in brackets).