Early gene mapping after deep brain stimulation in a rat model of tardive dyskinesia: Comparison with transient local inactivation

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Abstract

Deep brain stimulation (DBS) has been extensively used in Parkinson's disease and is also currently being investigated in tardive dyskinesia (TD), a movement disorder induced by chronic treatment with antipsychotic drugs such as haloperidol (HAL). In rodents, vacuous chewing movements (VCMs) following chronic HAL administration are suggested to model orofacial dyskinesias in TD. We show that 60 min of DBS (100 μA, 90 μs, 130 Hz) applied to the entopeduncular (EPN) or subthalamic (STN) nuclei significantly decreases HAL-induced VCMs. Using zif268 as a neural activity marker, we found that in HAL-treated animals EPN stimulation increased zif268 mRNA levels in the globus pallidus (+65%) and substantia nigra compacta (+62%) and reticulata (+76%), while decreasing levels in the motor cortex and throughout the thalamus. In contrast, after STN DBS zif268 levels in HAL-treated animals decreased in all basal ganglia structures, thalamus and motor cortex (range: 29% in the ventrolateral caudate-putamen to 100% in the EPN). Local tissue inactivation by muscimol injections into the STN or EPN also reduced VCMs, but to a lesser degree than DBS. When applied to the EPN muscimol decreased zif268 levels in substantia nigra (−29%), whereas STN infusions did not result in significant zif268 changes in any brain area. These results confirm the effectiveness of DBS in reducing VCMs and suggest that tissue inactivation does not fully account for DBS effects in this preparation. The divergent effects of STN vs. EPN manipulations on HAL-induced
1. Introduction

Deep brain stimulation (DBS) is a clinical procedure whereby electrical current is passed through surgically-implanted electrodes in the brain. Over the last decade, DBS applied to the subthalamic nucleus (STN) or internal globus pallidus (GPI) has been successfully used to treat several movement disorders, including Parkinson’s disease and dystonia (Blomstedt et al., 2009; Cacciola et al., 2010; Eble, 2009; Lubarr and Bressman, 2011; Moro et al., 2010; Ostrem et al., 2011; Volkmann et al., 2009). Recently, DBS of the has also been used to treat patients with tardive dyskinesia (TD) a disorder induced by chronic therapy with classical antipsychotic drugs (Damier et al., 2007; Eltahawy et al., 2004; Schrader et al., 2004; Sun et al., 2007b). TD is characterized by abnormal, involuntary movements of the head, neck, face and jaw, which can persist for a prolonged time after medication withdrawal (Casey, 1985, 2004). Patients often respond to pharmacotherapy, but a number of patients remain resistant to drug interventions and have recently been considered candidates for DBS surgery. While the GPI is the most common DBS target used in cases of TD, the STN has also been shown to alleviate motor symptoms associated with chronic classical antipsychotic use (Sun et al., 2007a; Zhang et al., 2006).

Despite its therapeutic success, mechanisms underlying the efficacy of DBS remain unclear. A leading hypothesis is that DBS is functionally equivalent to local inactivation of target nuclei (Grill et al., 2004; Obeso and Group, 2001). In line with this notion, microelectrode recordings have demonstrated that DBS can suppress output of the stimulated structure (Benazzouz and Hallett, 2000; Dostrovsky et al., 2000). Furthermore, physical or chemical lesions, as well as local tissue inactivation via infusion of the GABAAergic agonist muscimol into the STN, alleviate motor symptoms in patients with PD (Levy et al., 2001; Okun and Vitek, 2004; Patel et al., 2003; Su et al., 2001; Vitek et al., 2003) as well as in pre-clinical models of PD (Aziz et al., 1991; Mehta and Chesselet, 2005; Mehta and Chesselet, 2005; Wallace et al., 2007). However, it has also been reported that DBS influences brain regions at sites distal from the site of stimulation (Schulte et al., 2006; Su et al., 2001). One initial approach to address the role of local vs. distal processes in DBS effects would be to map neuronal activity in nodal points through the basal ganglia following DBS.

While preclinical work on DBS and dyskinesias has primarily focused on parkinsonian models, we have recently shown that DBS of the STN or entopeduncular nucleus (EPN), the dentate homologue of the GPI, effectively suppresses antipsychotic drug-induced orofacial dyskinesias (Creed et al., 2011). The target behavior, often referred to as vacuous chewing movements (VCMS), develops in the course of chronic exposure to classical antipsychotic drugs such as haloperidol (HAL)(see Turrone et al., 2002 for review) and seems to be equally susceptible to DBS applied to the EPN or STN (Creed et al., 2011). While our previous study addressed the behavioral efficacy of DBS, the mechanisms underlying this efficacy were not addressed. Therefore, the first objective of the present study was to test the hypothesis that DBS effects in the HAL VCM model are equivalent to chemical inactivation of the same areas. To this end we compared the behavioral effects of local muscimol infusions into the STN or EPN with those of DBS in these targets. A second objective was to compare regional brain changes induced by STN DBS to those induced by EPN DBS, using the immediate early gene zif268 as a marker of neuronal activation (Bozon et al., 2002; Cole et al., 1989; Creed et al., 2011) and finally to compare zif268 activity maps after DBS to those obtained after muscimol for each of the two anatonical targets.

2. Experimental procedures

2.1. Subjects

Male Sprague–Dawley rats initially weighing 200–250 g (Charles River, Quebec) were used for all experiments. All procedures were approved by the Animal Care Committee at the Centre for Addiction and Mental Health and complied with Canadian Council on Animal Care (CCAC) and NIH standards and guidelines.

2.2. Experimental design

2.2.1. DBS experiments

Fifty-four rats were used in these experiments. Twenty-seven rats underwent chronic HAL treatment prior to surgery, while the remaining twenty-seven received vehicle treatment. Within each of these treatment groups, 10 animals underwent electrode implantation into the STN, 10 had electrodes implanted in the EPN, and 7 underwent sham surgery. All animals were used for behavioral testing and zif268 analysis.

2.2.2. Muscimol infusion experiments

Eighty-eight rats were used in these experiments. Of the 48 HAL-treated rats, half had cannulae implanted above the STN, and half above the vehicle. Of the 24 rats in each condition, half had muscimol infusions, while the remaining half received saline infusion only and served as control groups for zif268 and behavioral comparisons. Likewise, of the 40 VEH-treated rats, 20 were assigned to STN and 20 to the EPN-cannulae condition. Within each condition, 12 received muscimol infusion, while 8 served as saline controls. Final group sizes after correcting for misplaced cannulae and attrition are given in Figs. 3 and 4.

2.3. Drug treatment

After one week acclimation to the housing facility, rats were randomly assigned to receive either haloperidol decanoate (HAL; 21 mg/kg i.m. N=68) or sesame oil vehicle (N=22) once every 3 weeks for 12 weeks (a total of four injections). The decanoate formulation is known to result in constant plasma levels comparable to those achieved with 1.1–1.5 mg/kg daily injections and to reliably induce progressively increasing levels of VCMs (Turrone et al., 2002).
2.4. VCM assessments

VCM assessments were conducted once a week in a quiet room, beginning 1 week before the first HAL injection. For each VCM assessment the rat was placed on a flat round surface (26 cm in diameter, 50 cm high) and allowed to acclimate for 2 min. Over the following 2 min, a trained observer recorded the number of VCMs, which were defined as jaw movements in the vertical plane not directed at specific objects accompanied or not by tongue protrusions (Tamminga et al., 1990). Discrete bursts of jaw tremors were counted as one VCM (Creed et al., 2011).

2.5. Surgical procedures

After 12 weeks of HAL treatment, rats were anesthetized with ketamine/xylazine (100/7.5 mg/kg i.p.). For DBS experiments, polymide-insulated stainless steel monopolar electrodes (250 μm diameter with 0.6 mm of surface exposed) were bilaterally implanted into the STN (AP −3.8 mm; ML +2.5 mm; DL −8.0 mm) or EPN (AP −2.3 mm; ML+2.6 mm; DL −7.8 mm) (Paxinos and Watson, 1986). Anodes were connected to a bone screw over the somatosensory cortex. We have previously demonstrated that inert electrodes implanted in either the STN or EPN had no effect on VCMs or other motor behavior. Therefore in the present study, sham DBS controls were anesthetized and had holes drilled into the skull but were not implanted with electrodes. For infusion experiments, bilateral stainless steel guide cannulae (C313G Guide 22 GA, Plastics One, Roanoke, VA) were implanted bilaterally, immediately dorsal to the STN or EPN and secured to the skull with dental cement.

2.6. DBS protocol

Starting 1 week after surgery DBS was applied using a handheld stimulator (Model 6510, St. Jude Medical, Plano, Texas) set to deliver a 100 μA current (130 Hz, 90 μs pulse width). Stimulation parameters were chosen based on achieving maximal intensity without inducing forelimb dyskinesias (in the case of the STN) or muscle contractions (in the case of the EPN), as described previously (Creed et al., 2011). During the DBS sessions, rats were initially given a 2-min acclimation period on the observation pedestal followed by a 2-min baseline VCM assessment. Thereafter, DBS was applied and VCMs were assessed immediately after DBS onset, and again after 30 and 60 min of continuous DBS. After 60 min of continuous stimulation, DBS was turned off and animals were returned to their home cages.

2.7. Muscimol infusions

Starting 1 week after surgery, muscimol (1.0 μg dissolved in 0.2 μL physiological saline; Sigma Aldrich, Oakville, CA) was infused at the rate of 0.1 μL/min using a Hamilton syringe infusion pump. The cannula was left in situ for a further 2 min. The dose of muscimol was chosen based on previous studies that have established behaviorally effective muscimol-induced inactivation of the STN (Mehta et al., 2005b) and EPN (Adachi et al., 2003; Hamann et al., 2010). Prior to the infusion, rats were given a 2-minute adaptation period followed by a 2-minute baseline assessment. VCMs were assessed immediately following muscimol infusion, and at 30 and 60 min after infusion. Controls underwent saline infusion. No gross motor abnormalities were observed after either infusion.
2.8. In situ hybridization and densitometric analyses

Hybridization was performed using \(^{35}\)S-UTP labeled riboprobes complementary to zif-268 according to Genbank # NM_012551, (bases 660–679), 5′-tcacctatactgcgcttc-3′ and (bases 1062–1043) 5′-aggtctccctgttgttgtg-3′ and consensus promoter sequences for either SP6 RNA polymerase. Using the NCBI BLAST Tool, the sequences were checked for homology with the rat genome and found to be specific for their respective transcripts. Probes were diluted to a concentration of 200,000 cpm/μl in hybridization solution containing: 50% formamide, 35% Denhardt’s solution, 10% dextran sulfate, 0.1X SSC, salmon sperm DNA (300 μg/ml), yeast tRNA (100 μg/ml), and DTT (40 μM). Slides were incubated in plastic mailers overnight at 60 °C. After hybridization, sections were rinsed in 4X SSC at 60 °C, treated in RNase A (20 μg/ml) solution at 45 °C for 40 min, washed with agitation in decreasing concentrations of SSC containing 5 g/L sodium thiosulfate, dipped in water, dehydrated in 70% ethanol, and air-dried. The slides were exposed to Kodak BioMax film for 6 days at 4 °C along with calibrated radioactivity standards. Probe specificity was confirmed by testing labeled sense and scrambled probes, both of which produced no measurable signal on film.

Densitometric analyses were performed with MCID 7.0 software (InterFocus, Lepton, UK). Prior to sampling, illumination level was adjusted for every film to ensure consistent background readings across all films. Standard curves obtained from calibrated radioactivity standards were used to convert raw optical density values to radioactivity levels in microcuries per gram of tissue (μCi/g). Basal ganglia regions were analyzed in 8 sections per animal, with brain regions defined according to the atlas of Paxinos and Watson (1986). Readings were first averaged across all sampling windows in a section and then across all sections to produce a final density value for each region for each animal.

2.9. Verification of electrode and cannula placement

Following behavioral tests, rats were deeply anesthetized with sodium pentobarbital and brains were removed, sectioned, post-fixed in 10% formalin vapor and stained with cresyl violet. Sacrifice occurred 70 min after onset of DBS or infusion of muscimol. Only animals with bilateral electrode or cannula placements within the target nuclei (Fig. 2) were included in the analyses.

2.10. Statistical analyses

Behavioral data were analyzed by repeated measures ANOVAs followed, where appropriate, by paired or unpaired t tests with false discovery rate (FDR) adjustments (Benjamini et al., 2001; Benjamini and Hochberg, 1995). Regional brain zif268 optical density measurements were first analyzed by multivariate followed by univariate ANOVAs for each brain region separately. FDR-adjusted post-hoc t tests were used for group comparisons, where appropriate.
Regression analysis between VCM behavior and \textit{zif268} expression in discrete brain areas was also performed.

3. Results

3.1. Effects on HAL on VCMs and \textit{zif268} expression

HAL induced VCMs over the course of 12 weeks, the levels of which stabilized by approximately 8 weeks of treatment (Fig. 1A). Chronic HAL treatment by itself did not induce significant changes in \textit{zif268} levels in basal ganglia, except for modest increases in the dorsolateral caudate-putamen and subthalamic nucleus ($p<0.05$) (Fig. 1B). In contrast, HAL treatment consistently increased \textit{zif268} expression in thalamic and cortical nuclei (Fig. 1B), with most pronounced effects occurring in the dorsomedial nucleus (+164%, $p<0.01$). Regression analyses revealed no significant relationship between \textit{zif268} expression in any specific brain area and VCM behavior.

Figure 3  Effects of EPN manipulations on VCMs. (A) Effects of EPN DBS. DBS suppressed HAL-induced VCMs at all time points relative to baseline and transiently induced VCMs in VEH-treated rats after 30 min of stimulation. (B) Effects of EPN Muscimol. Muscimol infusion also suppressed VCMs at all time points in HAL-treated rats and increased VCMs at 30 min in VEH-treated rats. ***$p>0.01$ in comparison with each animal’s own baseline in HAL-treated rats (paired t tests). #p<0.05; ###$p<0.01$ in comparison with HAL-SHAM or HAL-SAL groups (independent t tests). $p<0.05$, VEH-SHAM compared to VEH-EPN DBS at 30 min (independent t tests). Group Ns are indicated in parenthesis.

Figure 4  Effects of STN manipulations on VCMs. (A) Effects of STN DBS. DBS suppressed HAL-induced VCMs at all time points. (B) Effects of STN Muscimol. Muscimol infusion also suppressed at all time points but to a lesser extent than that seen with DBS. *$p<0.05$; ***$p>0.01$ in comparison with baseline in HAL-treated rats; ###$p<0.01$ in comparison with HAL-SHAM or HAL-SAL groups. Group Ns are indicated in parenthesis.
3.2. Effects of EPN manipulations on VCMs

As shown in Fig. 3, EPN DBS significantly reduced VCMs at all time points compared to baseline (2 min: −49%, p = 0.004; 30 min: −42%, p = 0.003; 60 min: −76%, p = 0.001) (Fig. 3A). Likewise, muscimol infusion into the EPN significantly reduced VCMs at all time points (2 min: −33%, p = 0.012, 30 min: −42%, p = 0.016, 60 min: −37%, p = 0.010 Fig. 3B). Across the entire 1 h trial, the overall reduction observed with EPN DBS was 56.8 ± 6.20 whereas the overall reduction after muscimol was 29.1 ± 7.17 (p < 0.007). Suppression of VCMs by DBS was significantly more pronounced 60 min after intervention compared to muscimol infusion (p = 0.007). Interestingly, in rats that had not been treated with chronic HAL, both DBS and muscimol infusions tended to increase VCMs, an effect which reached its highest level at the 30 min time point in both cases.

3.3. Effects of STN manipulations on VCMs

HAL-treated rats that received STN-DBS showed significant reductions in VCM counts (Fig. 4A). Stimulation at each time point resulted in significant decreases in VCMs compared to baseline (2 min: −72%, p = 0.005, 30 min: −80%, p = 0.003, 60 min: −72%, p = 0.009). Muscimol infusion into the STN also reduced VCM counts at all time points (2 min: −39%, p = 0.016, 30 min: −44%, p = 0.006, 60 min: −467%, p = 0.009, Fig. 4B). Across the entire 1 h trial the overall VCM reduction observed with STN DBS was 69.8 ± 11.05%, whereas the reduction seen after muscimol was 39.4 ± 5.15% (p < 0.007). Relative to muscimol infusion, the suppression of VCMs by DBS was more pronounced at 2 and 30 min after intervention (p = 0.008, p = 0.004, respectively, Fig. 4B). In VEH-treated rats, both DBS and muscimol tended to increase VCMs 60 min after each manipulation, but this effect did not reach statistical significance. Over the entire 1 h observation period, STN DBS reduced VCMs to a greater extent than did EPN DBS (p < 0.05), with peak differences occurring at the 30 min time point (p = 0.001). There were no differences between STN and EPN muscimol-treated groups.

3.4. Effect of EPN manipulations on zif268 expression

In VEH treated animals, EPN DBS increased zif268 levels in most areas examined (Fig. 5A). This increase was significant in the dorsolateral Cpu (35%, p = 0.018) and STN (30%, p = 0.015). In HAL-treated rats (Fig. 5B), EPN DBS increased zif268 expression in the GP (65%, p = 0.003), SNr (76%, p = 0.014) and SNc (62%, p = 0.009), while decreasing zif268 expression in the VP (−34%, p = 0.004).

Figure 5  Effects of EPN manipulations on zif268 levels in the basal ganglia. (A) Effects of EPN DBS in VEH rats. In VEH-treated animals, EPN DBS increased zif268 expression in most areas examined. (B) Effects of EPN DBS in HAL-treated rats. In HAL-treated rats, EPN DBS increased zif268 expression in the GPe, SNr and SNc, and decreased zif268 expression in the VP. (C) Effects of EPN muscimol in VEH rats. Muscimol infusion into the EPN increased zif268 in most areas in VEH-treated rats. (D) Effects of EPN muscimol in HAL-treated rats. In HAL-treated animals muscimol did not significantly change zif268 levels in caudate putamen areas and significantly reduced these levels in other areas. Statistical comparisons refer to adjusted t tests comparing % change from the appropriate non-stimulated or saline-infused control, following ANOVAs for each brain region. *p < 0.5; **p < 0.02; ***p < 0.01.
In VEH-treated animals, muscimol infusion into the EPN significantly increased \textit{zif268} expression in most basal ganglia areas, similarly to, but to a higher extent than the changes observed with DBS (Fig. 5C). In HAL-treated rats the effects of muscimol (Fig. 5D) were different from the effects seen in VEH rats. While in VEH-treated animals muscimol infusion elevated \textit{zif268} expression in most subdivisions of the CPu, there were no changes in CPU in HAL-treated rats. In VEH rats muscimol induced significant increases in the SNr and SNc, whereas in HAL-treated rats it decreased \textit{zif268} expression in these areas (Fig. 5D).

In the motor cortex and thalamus, both muscimol infusion and DBS increased \textit{zif268} expression in VEH-treated rats (Fig. 6A and C), whereas in HAL-treated rats a general trend towards decreasing \textit{zif268} levels was seen after both DBS and muscimol (Fig. 6B and D). Although thalamic effects were generally of the same magnitude in DBS and muscimol treated rats, statistical significance was only seen with DBS, apparently owing to smaller variability in DBS vs. muscimol treated groups.

### 3.5. Effect of STN manipulations on \textit{zif268} expression

In VEH-treated animals, DBS reduced \textit{zif268} expression in the posterior CPU (−27%, \(p=0.005\)), EPN (−92%, \(p<0.001\)), GPe (−99%, \(p<0.001\)), STN (−25%, \(p=0.033\)), SNr (−99%, \(p<0.001\)) and SNc (−89%, \(p<0.001\)) (Fig. 7A). A similar general trend toward decreased \textit{zif268} was seen after muscimol infusions in VEH rats (Fig. 7C), although these were of lesser magnitude than the DBS effects and did not reach statistical significance. In HAL-treated rats, STN DBS resulted in strong and consistent decreases in \textit{zif268} levels in all basal ganglia regions (Fig. 7B). In VEH rats, DBS tended to increase \textit{zif268} expression throughout the motor cortex and thalamus, although some thalamic nuclei showed modest decreases (Fig. 8A). Muscimol data followed a similar but less consistent pattern (Fig. 8B). In HAL-treated rats DBS had no clear effects on motor cortical regions, but consistently reduced \textit{zif268} levels in thalamic nuclei (Fig. 8B). The pattern observed after muscimol infusions was in the opposite direction, with small to moderate increases in \textit{zif268} levels seen in most thalamic nuclei (Fig. 8D).

### 4. Discussion

Recently DBS has been demonstrated to reduce antipsychotic induced dyskinesias both in patients (Damier et al., 2007; Eltahawy et al., 2004; Schrader et al., 2004; Sun et al., 2007b) and in animal models (Creed et al., 2011; Degos et al., 2005). Despite its demonstrated clinical effectiveness
in movement disorders, the mechanisms by which DBS exerts its effects remain largely unknown. In this study, we tested the hypothesis that DBS is functionally equivalent to chemical inactivation in a rodent model of TD, by comparing the effects of DBS to those of chemical inactivation on two basal ganglia targets, the EPN and the STN. Our results indicate that the suppression of VCMs by DBS applied to either the EPN or the STN was significantly more pronounced than the suppression achieved by chemical inactivation of the same nuclei (Figs. 3 and 4).

As expected, chronic haloperidol led to high and stable VCM levels in the course of 12 weeks (Fig. 1A). VCMs were countered by DBS and by muscimol infusion, an observation that is in line with reported effects of both DBS and radiofrequency lesions of the STN and GPi in TD (Damier et al., 2007; Lenders et al., 2005; Sun et al., 2007b; Wang et al., 1997). However, some important differences were found between DBS and muscimol manipulations and between the two anatomical targets.

DBS applied to the EPN suppressed HAL-induced VCMs as soon as stimulation was switched on, and VCMs were further suppressed after 60 min of continuous DBS. Likewise, muscimol infusion into the EPN caused an immediate and sustained reduction in VCMs, whereas saline infusion had no effect. In VEH-treated rats, EPN-DBS caused a transient increase in VCMs, which was not observed with muscimol infusion. Our DBS results in the EPN are in agreement with previous reports that DBS of the EPN suppresses motor symptoms in animal models of TD and dystonia (Creed et al., 2011; Haag, 2003; Harnack et al., 2004). Similarly, DBS or muscimol applied to the STN also decreased HAL-induced VCMs. However, in the STN the effects of DBS were more pronounced than those of muscimol infusion, suggesting that while tissue inactivation may have beneficial effects, it does not fully account for the effects of STN-DBS. Interestingly, both DBS and muscimol applied to the STN in VEH-treated rats caused a slight but significant induction of VCMs 60 min later. This is consistent with previously reported effects of muscimol and DBS applied to this region in normal control rats (Creed et al., 2011; Mehta and Chesselet, 2005).

Having determined that chemical inactivation and DBS have qualitatively similar effects on VCM behavior, we examined patterns of neuronal activity induced by each experimental manipulation, as measured by expression of the immediate early gene, *zif268*. HAL-treated rats exhibited higher levels of *zif268* in the DL-Cpu, STN and thalamic nuclei, relative to VEH-treated rats. The elevated expression

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**Figure 7** Effects of STN manipulations on *zif268* levels in the basal ganglia. (A) Effects of STB DBS in VEH rats. In VEH-treated animals, STN DBS increased *zif268* expression in most areas examined. (B) Effects of STN DBS in HAL-treated rats. In HAL-treated rats, STN DBS increased *zif268* expression consistently decreased *zif268* expression in all areas. (C) Effects of muscimol in VEH rats. Muscimol infusion into the STN also tended to decrease *zif268* in most areas in VEH-treated rats. (D) Effects of muscimol in HAL-treated rats. In HAL-treated animals muscimol did not significantly change *zif268* levels, except for a 70% increase in the PPTg. Statistical comparisons refer to adjusted t tests comparing % change from the appropriate non-stimulated or saline-infused control mean following ANOVAs for each brain region. *p<0.5; **p<0.02; ***p<0.01.
in the DL-CPu is consistent with the known effects of acute HAL treatment on immediate early gene expression (MacGibbon et al., 1994; Robertson et al., 1994). On the other hand, up-regulation of \textit{zif268} expression in the STN and thalamic nuclei has not been previously reported. Current models of basal ganglia functioning suggest that chronic antagonism of dopamine D2 receptors by HAL could be expected to result in increased activity of the indirect striatal output pathway, including activation of the STN.

The STN is one of the pace-making centers of the basal ganglia, and sends excitatory projections to basal ganglia output nuclei and the GP (Hamani et al., 2004; Nambu et al., 2002; Plenz and Kital, 1999). We therefore expected that STN inactivation would decrease activity throughout the basal ganglia. Consistent with this hypothesis, both muscimol and DBS applied to the STN decreased \textit{zif268} levels in thalamic nuclei. This finding may be related to the proximity and anatomical connectivity between the EPN and SNr, the two primary basal ganglia output nuclei. Muscimol-induced decreases in \textit{zif268} levels in EPN and SNr could be due to the proximity of the two structures, which are anatomically distinct but are commonly considered to be a functional unit due to similar cell composition and inputs (Lafreniere-Roula et al., 2010). By contrast, EPN DBS induced increases in \textit{zif268} expression in the SNr may suggest a decrease in inhibitory input from the EPN, increased interactions between HAL treatment and the two types of brain manipulations were reflected on \textit{zif268} expression patterns, and were more pronounced in the EPN than in the STN. In VEH-treated animals, both muscimol injections and DBS applied to the EPN tended to elevate \textit{zif268} expression in the basal ganglia (Fig. 5). However, EPN DBS increased \textit{zif268} expression in the GP and SNr, whereas EPN muscimol infusion had the opposite effect, decreasing \textit{zif268} expression in SNc and SNr (Fig. 5). This finding may be related to the proximity and anatomical connectivity between the EPN and SNr, the two primary basal ganglia output nuclei.
synaptic excitation at terminal fields of the EPN in the SNr, or compensatory up-regulation since both structures are basal ganglia output stations.

To our knowledge, this is the first study to compare muscimol infusion and DBS in the VCM model of TD. Generally, the effects of muscimol infusion on VCM behavior and zif268 expression are consistent with the known effects of ablation of these structures. We have chosen to use muscimol infusion rather than physical ablation to achieve functional inactivation without the associated tissue damage, inflammation and possible compensatory plasticity that may be associated with lesioning (Padberg et al., 2010). We also took into account the fact that changes in zif268 levels are time dependent, with peak expression occurring 60 min after the stimulus (Bozon et al., 2002; Chaudhuri et al., 2000; Farivar et al., 2004). Acute infusions of muscimol in awake, freely moving rats allowed us to accurately quantify gene expression within a time window that was relevant for assessing the motor effects of inactivation.

Our findings are consistent with previous studies in drug-naive rats, in which STN- and GP-DBS have been shown to suppress activity of local neurons (Benazzouz and Hallett, 2000; Chin and Hutchison, 2008). Our results are also in line with clinical studies that have used functional neuroimaging to explore DBS-induced changes in brain metabolism. Clinically, STN DBS and ablation have been associated with decreased activity of basal ganglia nuclei (Su et al., 2001) and increased activation of premotor cortex and SMA (Ceballos-Baumann et al., 1999; Cilia et al., 2009; Le Jeune et al., 2010). Results from our HAL-treated rats suggest a functional interaction between EPN DBS-induced changes and HAL-treatment. Altered metabolism of the basal ganglia has not been reported in patients with TD (Khiat et al., 2008; Szymanski et al., 1996; Waddington et al., 1995), while our results suggest increased basal activity of the STN and DL Cpu. One possible explanation for the discrepancy between our zif268 results and clinical imaging studies is the relatively low volume of tissue activated, which may not be easily detectable in humans using conventional neuroimaging methods (Fukuda et al., 2001). Moreover, in clinical studies, HAL-induced motor symptoms are necessarily examined in patients with underlying schizophrenia-associated brain abnormalities (Vita and De Peri, 2007), and it is not always possible to isolate the effects of chronic antipsychotic treatment from brain effects associated with schizophrenia. In the preclinical VCM model, HAL is administered to otherwise healthy rats, which eliminates confounding schizophrenia-associated pathology.

This study demonstrates the usefulness of early gene mapping in investigating the effects of focal DBS or muscimol in several brain regions simultaneously. zif268 is transcribed in response to calcium influx and, as such, is a strong indicator of neuronal activity (Chaudhuri, 1997; Chaudhuri et al., 2000; Farivar et al., 2004). However, it remains an indirect marker of neuronal activation. It has been suggested that altered firing pattern of neurons within the basal ganglia may be a signature of dyskinesias (Burkhardt et al., 2007; Lee et al., 2007) and in this regard it is important to note that examination of zif268 expression does not provide information about temporal firing rates. Further, several of brain regions examined in this study are highly heterogeneous, composed of multiple cell types that are functionally distinct. It will be of interest for future studies to identify neurochemical identities of cells in brain areas affected by STN and EPN interventions. Finally, the observation of very different effects on zif268 expression despite similar behavioral effects of our experimental manipulations highlight the fact that changes in zif268 expression alone are not predictive/correlated with functional improvement.

In summary, we have found that the behavioral effects of DBS in the HAL-induced VCM model are not fully reproduced by tissue inactivation with muscimol. Our results further indicate that chronic HAL alters regional zif268 levels, which are in turn modified by DBS or muscimol applied to either the STN or EPN. These effects however differed in the two target regions. While the overall effect of STN DBS was to decrease activity in the basal ganglia circuitry, the effects of EPN DBS were generally more restricted to afferent and efferent projection areas from the target nucleus. The divergent effects of STN vs. EPN manipulations on HAL-induced zif268 changes suggest that similar behavioral outcomes of DBS in these two areas may involve different neuroanatomical mechanisms.

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Contributors

MCC and JNN designed the experiments and wrote the manuscript. MCC conducted all of the experimental work and analyses. CH gave expert opinion, contributed to the writing and reviewed the manuscript.

The work has been entirely designed and carried out by ourselves and has not been submitted for publication elsewhere. We take full responsibility for its contents.

Conflict of interest

Dr. C. Hamani is a consultant for St. Jude Medical (Texas), the manufacturer of the stimulators used in the present study. The other authors have no conflict of interest to report.

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References


