Mechanisms of pallidal deep brain stimulation: Alteration of cortico-striatal synaptic communication in a dystonia animal model

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A B S T R A C T

Pallidal deep brain stimulation (DBS) is an important option for patients with severe dystonias, which are thought to arise from a disturbance in striatal control of the globus pallidus internus (GPI). The mechanisms of GPI-DBS are far from understood. Although a disturbance of striatal function is thought to play a key role in dystonia, the effects of DBS on cortico-striatal function are unknown.

We hypothesised that DBS, via axonal backfiring, or indirectly via thalamic and cortical coupling, alters striatal function. We tested this hypothesis in the \textsuperscript{d}dt\textsuperscript{ed} hamster, an animal model of inherited generalised, paroxysmal dystonia.

Hamsters (dystonic and non-dystonic controls) were bilaterally implanted with stimulation electrodes in the GPI. DBS (130 Hz), and sham DBS, were performed in unanaesthetised animals for 3 h. Synaptic cortico-striatal field potentials, as well as miniature excitatory postsynaptic currents (mEPSC) and firing properties of medium spiny striatal neurones were recorded in brain slice preparations obtained immediately after EPN-DBS.

The main findings were as follows: a. DBS increased cortico-striatal evoked responses in healthy, but not in dystonic tissue. b. Commensurate with this, DBS increased inhibitory control of these evoked responses in dystonic, and decreased inhibitory control in healthy tissue. c. Further, DBS reduced mEPSC frequency strongly in dystonic, and less prominently in healthy tissue, showing that also a modulation of presynaptic mechanisms is likely involved. d. Cellular properties of medium-spiny neurones remained unchanged.

We conclude that DBS leads to dampening of cortico-striatal communication, and restores intrastratal inhibitory tone.

1. Introduction

Primary dystonias were first recognised to be linked to brain, particularly to basal ganglia dysfunction by Charles Marsden and his group (Berardelli et al., 1998; Marsden et al., 1975) only as late as in the 1970s. By now, it is being recognised that in dystonic patients, a pathological cortico-striatal function, and subsequent disturbance of striatal control of GPI, are likely to be important factors of dystonic dysfunction (Berardelli et al., 1998). This disturbance is characterised by increased synaptic plasticity within the cortico-basal ganglia network (Quartarone and Hallett, 2013; Quartarone and Pisani, 2011; Schirinzi et al., 2018), and speculated to result in a shift of the balance toward the direct pathway (Wichmann and Dostrovsky, 2011) (Fig. 6A). Beyond the basal ganglia, such network disturbances are reflected in a reduction of cortical inhibition (Meunier et al., 2012; Beck and Hallett, 2011; Beck et al., 2009), and a relative persistence of \(\beta\)-band synchronisation during...
movement initiation (Crowell et al., 2012), as well as dominant low-frequency pallidal activity in the α-band at rest (Kuhn et al., 2008; Silverstein et al., 2003). Further, recent publications show that lower frequency oscillations (especially theta and alpha oscillations) correlate with dystonia and are indeed modulated by DBS (Neumann et al., 2017; Neumann et al., 2015; Barow et al., 2014). Beta oscillations seem be of lesser importance. This should be added here. Indeed, a reduction of inhibitory tone within the extended network is being discussed (Tisch et al., 2007a), although it is still unresolved whether the entire network or parts of it would be affected (Balint et al., 2018). Although a contribution of cerebellar dysfunction is being assumed (Schrizzi et al., 2018; Prudente et al., 2014), an altered striatal function is likely a major causal factor in primary dystonias.

Deep brain stimulation (DBS) is clearly the most important innovation for the treatment of dystonias (Bledsoe et al., 2020), and often the “only option for symptom reduction” (Krack et al., 2019). As a consequence, clinical trials implementing DBS in dystonia (Vollmann et al., 2014; Vollmann et al., 2012) show that it is largely successful in patient groups particularly with e.g. genetic isolated dystonias as well as with mobile dystonias, in younger patients and those without other comorbidities (Bledsoe et al., 2020). However, as much as the pathomechanisms of dystonia are still not fully understood, this knowledge gap extends even more so to the mechanisms underlying the effects of DBS in dystonia for several reasons: One is that DBS has most widely been investigated in patients and in animal models of Parkinson’s disease (PD) (see reviews (Udupa and Chen, 2015; Herrington et al., 2016)), which allows inference on DBS mechanisms in dystonia only in a limited way, also since the target nuclei (GPI vs. nucleus subthalamicus) are usually not the same. A second is that most of the hypotheses on this question are derived either from DBS in normal primates, or from DBS-like stimulation in vitro in normal rodent tissue, or from cortical or basal ganglia recordings of e.g. local fields in patients which obviously limit the extent to which the entire network can be assessed. Importantly, the effect of DBS in dystonia, in contrast to most motor symptoms of PD, require at least hours of stimulation, indicating that functional network changes likely occur (Herrington et al., 2016). To summarise the findings so far, the data from PD patients suggest that pathological oscillatory activity prominent in the β-band can be reduced by subthalamic DBS (Kuhn et al., 2008; Kuhn et al., 2006; Quinn et al., 2015). As for dystonia, pallidal DBS has been demonstrated to reduce low-frequency (4–12 Hz) activity (speculated to be a marker of dystonia severity (Scheller et al., 2019)), and also coherence between pallidal and cortical activity (Barow et al., 2014). What is also known is that pallidal DBS in dystonic patients does have network effects interpreted by the authors as inhibitory – increased cortical excitability and synaptic plasticity tested by e.g. paired associative stimulation or so-called cortical silent period using motor evoked potentials seem to be normalised (Tisch et al., 2007a; Ruge et al., 2011; Tisch et al., 2007b; Bocek et al., 2016) and firing of thalamic neurones is altered, albeit differentially (reduced in the majority of neurones, increased in a minority) (Montgomery Jr., 2006). It is thus safe to conclude that cortical excitability is somehow reduced by pallidal DBS, but nothing is known on alterations in the extended network, in particular regarding cortico-striatal functional connectivity. Looking at animal studies, in one investigation in normal primates, pallidal DBS completely silenced neuronal firing in this nucleus – presumably via activation of GABAergic afferents to the nucleus (Chiken and Nambu, 2013). In contrast to this, a study on DBS-like stimulation in normal rat brain in anaesthetised and artificially respirated subjects, with high-frequency stimulation leading to prolonged afterdepolasurations mediated by cholinergic inputs, and no silencing of neurones (Luo and Kiss, 2016) – thus the issue remains undecided. More importantly, animal studies so far were mainly conducted on healthy controls. In studies using animal models of dystonia, in turn, DBS-stimulation was delivered only under deep anaesthesia with urethane (Leblois et al., 2010; Reese et al., 2009), that is known to distort cortico-striatal connectivity (Paasonen et al., 2018). Data investigating the mechanisms of DBS in dystonia models in awake and behaving animals are completely lacking.

In view of the scant knowledge on the mechanisms of DBS, and in particular on excitability changes in the nuclei presumably being strongly involved in dystonic pathophysiology, i.e. the corpus striatum, we set out to test the lasting effect of prolonged (3 h) pallidal DBS, presenting the first study so far conducting DBS in freely moving dystonic animals. For this, we chose an animal model which at least in many ways resembles the human situation of generalised paroxysmal dystonia, the dt46 hamster, which we have extensively characterised in the past (Bode et al., 2017; Gernert et al., 1999; Gernert et al., 2000; Hamann et al., 2007; Richter and Löschner, 1998; Richter and Richter, 2014; Bennay et al., 2001). Although the generalisability to human primary dystonia is unclear, there are important similarities: This strain shows spontaneous paroxysmal dystonic attacks which can also be provoked by handling and stress. As speculated for at least some human dystonias (Quartarone and Hallett, 2013; Quartarone and Pisani, 2011; Schrizzini et al., 2018), this animal model is also associated with increased cortico-striatal excitability (Avchalumov et al., 2013), on the basis of reduced intra-striatal GABAergic signalling, resulting in overall increased EPN/GPi inhibition (Gernert et al., 2000; Gernert et al., 2002). Interestingly, this is in line with disturbed cortico-striatal communication (DeSimone et al., 2016) and increased pallidal inhibition also in DYT1 mouse (Chiken et al., 2008; Sciamanna et al., 2020). As we could show recently (Paap et al., 2021), the DBS protocol used in the current study effectively reduces dystonic attacks. Importantly, in the present study we used the same DBS protocol in freely moving dt46 hamsters to elucidate underlying mechanisms in electrophysiological studies on the cortico-striatal network. In this paper, we propose as a possible mechanism of DBS a dampening of cortico-striatal synaptic communication possibly due to an increase in intrastratial inhibitory tone and presynaptic changes mediated anterogradely via thalamo-striatal or thalamo-cortical projections.

2. Methods

2.1. Animals

The experiments were carried out using two groups of age-matched dystonic dt46 mutant hamsters (inbred; total n = 84), obtained by selective breeding (Institute of Pharmacology, University of Leipzig) as described previously (Richter and Löschner, 1998), and two groups of age-matched non-dystonic control hamsters (Mesocricetus auratus, outbred, total of n = 43) provided by a commercial breeder (JANVIER LABS; origin: Central Institute for Laboratory Animal Breeding, Hannover, Germany). Dystonic dt46 hamsters display spontaneous dystonic attacks particularly after stress, as described below (Richter and Löschner, 1998; Richter and Richter, 2014). The animals were kept under controlled environmental conditions with a 14 h/10 h light/dark cycle and an ambient temperature of 23 °C. Standard diet and water were supplied ad libitum.

After weaning at the age of 21 days, all groups of hamsters were screened for dystonic symptoms three times every 2–3 days by mild stress (triple stimulation technique), as described previously (Richter and Löschner, 1998). All dt46 hamsters used in this study exhibited severe dystonia with at least stage 5 at an age of 21 days. Healthy control hamsters were treated equally. Due to this screening process, experimenters could not be blinded to the status of the animals. All animal experiments were carried out in accordance with the guidelines of the EU Directive 2012/63/EU and the federal laws for the protection of animals under licence Az: 7221.3-1-053/17.

2.2. Surgical procedure for deep-brain stimulation electrode implantation and stimulation protocol

Animals (32–40 days old) were fixed in a stereotactic frame...
(Narishige, Japan) under deep anaesthesia with isoflurane (Isoflurane, Baxter, Deerfield, IL, USA; Univentor 1200 Anaesthesia Unit + Univentor 2010 Scavenger Unit, Biomedical Instruments, Zöllnitz, Germany). The periostea were additionally treated with the local anaesthetic bupivacaine (bupivacaine 0.25% JENAPHARM®). Two concentric bipolar electrodes (platinum-iridium Pt/Ir; SNEX-100, Microprobes, Gaithersburg, MD, USA) were placed bilaterally in the entopeduncular nucleus (EPN; corresponding to GPi in humans; stereotaxic coordinates AP: −0.6 mm, ML: ±2.2 mm, DV: −0.6 mm relative to Bregma from the golden hamster atlas (Morin and Wood, 2000)). To provide firm fixation of the electrodes, two screws were anchored in the skull behind the electrodes and enclosed with dental adhesive (Helio Bond + Compo glass flow, Schaan, Liechtenstein; SDR® flow+, Dentsply DeTrey GmbH, Konstanz, Germany). After 3–5 days of recovery, the electrode wires were connected to an external programmable stimulator (Institute of Applied Microelectronics, Faculty of Computer Science and Electrical Engineering, University of Rostock) generating charge-balanced rectangular current pulses. DBS (130 Hz, 50 μA, 60 μs pulse duration) was performed for 3 h on awake and freely moving animals. These parameters were chosen with regard to the proven antidystonic efficacy as shown in a parallel study (Paap et al., 2021). Every second dt³ or control hamster was used for sham stimulation (i.e. electrode implantation but inactive stimulation) to be able to compare effects with and without stimulation. These sham-stimulated groups received the same treatment as the stimulated groups, but with the stimulator turned off, which indeed does not exert any effect on the severity of dystonia (Paap et al., 2021). In a parallel set of experiments, DBS resulted in reduction of dystonia severity by 1.6 stages on average (Paap et al., 2021). Since the handling procedure necessary for staging can itself change cortico-striatal network properties (Avchalumov et al., 2013), we chose to sacrifice the animals immediately after DBS.

To gauge the impedance of the electrode in vivo, we measured the voltage drop over a 400 Ω shunt, checking that stimulus amplitude remained stable at 50 μA. Calculating the ratio of control voltage and current amplitude showed a mean impedance of 31.4 ± 2.3 kΩ (covering both, electrode and tissue components).

### 2.3. Brain slice preparation for analysis of striatal network excitability and inhibitory tone

Immediately after bilateral DBS or sham stimulation, the animals were decapitated under deep anaesthesia. The electrodes were carefully removed from the skull, without causing shearing movements, and the brain was quickly removed and chilled in ice-cold sucrose solution, containing (in mM): NaCl 87, NaHCO₃ 25, KCl 2.5, NaH₂PO₄ 1.25, CaCl₂ 0.5, MgCl₂ 7, glucose 10 and sucrose 75. The brain was then cut dorsally at an angle of 40–45° to the horizontal axis to maintain cortico-striatal connections (Kawaguchi et al., 1988; Schlösser et al., 1999), and glued with the cut off surface to the microtome table (VT1200S, Leica Biosystems Nussloch, Germany) (Fig. 1A). The angled brain was cut horizontally into para-horizontal slices of 400 μm or 300 μm (field or patch clamp recordings, respectively), maintaining synaptic connections between motor cortex and striatum. After cutting, the slices (total of n = 120 from control and of n = 174 from dt³ hamsters) were incubated for 60 min in sucrose solution at room temperature, before transferral to an interface-type recording chamber (BSC-BU, Harvard Apparatus Inc., March-Hugstetten, USA) perfused with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, NaHCO₃ 26, KCl 3, NaH₂PO₄ 1.25, CaCl₂ 2.5 and glucose 10, kept constant at 32 °C (TC-10, npi electronic GmbH, Tamm, Germany).

### 2.4. Field potential recordings

Field excitatory postsynaptic potentials (fEPSP) were recorded from the dorso-medial striatum (where dt³ hamsters show most prominent alterations (Avchalumov et al., 2013)) using an ACSF-filled glass pipette with a silver/silver chloride wire as described before (Avchalumov et al., 2013; Avchalumov et al., 2014). To activate cortical projections to the striatal network, a bipolar stimulation electrode (PT-T 2 T, Science Products GmbH, Hofheim, Germany) was placed above the underlying white matter of the adjacent cortex (Fig. 1B). Current-controlled stimulation was delivered by a Master-8 pulse stimulator (AMPI, Jerusalem, Israel) connected to a stimulus isolator (A365, WPI Inc., Sarasota, USA). To gauge cortico-striatal excitability, evoked input-output responses were characterised by increasing stimulus intensity stepwise until reaching saturating responses (remaining always below maximal intensity of 400 μA). For further investigations, stimulus strength was set to 50% of saturating response intensity to maintain dynamic range of responses. To test for synaptic facilitation and/or depression, paired-pulses were delivered at 40 ms inter-pulse-intervals (IPI) every 30 s. The paired-pulse ratio (PPR) was calculated by dividing the slope of the second response by the slope of the first response (s-fEPSP2/s-fEPSP1). In addition, we calculated the coefficient of variation of synaptic evoked responses from the first 40 responses under baseline conditions in each group to be able to compare response variability. To determine the degree of inhibitory tone controlling PPR, the GABAA receptor antagonist gabazine (SR 95531 hydrobromide, Tocris, Wiesbaden-Nordenstadt, Germany, 5 μM) was bath-applied for 50 min after having recorded a minimum of 20 min baseline responses. Signals were recorded using EXT-10-2F field-potential amplifier in AC mode (low pass filter at 1 kHz, npi, Tamm, Germany). Signals were processed and digitized at 10 kHz with Power1401 A/D converter (Cambridge Neurobiol.
Electronic Design, Cambridge, UK).

2.5. Patch-clamp recordings

Patch-clamp recordings from medium spiny striatal neurons (MSN) were obtained to assess frequency and kinetic properties of spontaneous miniature excitatory postsynaptic currents (mEPSCs) as a measure of presynaptic cortico-striatal functional modulations, and to gauge neuronal properties of MSN, identified by their characteristic firing temperature in cortico-striatal slices submerged in recording ACSF with borosilicate pipettes (3.1–8.5 MΩ, mean 5.6 ± 0.1 MΩ, n = 65, pulled with DMZ Zeitz puller, Zeitz-Instrumente Vertriebs GmbH, Martinsried, Germany) filled with a solution containing (in mM): K-gluconate 115, MgCl2 2, HEPES 10, Na2-ATP 2, Mg-ATP 2, GTP 0.3; pH set to 7.3 and osmolarity to 280 ± 5 mosmol/L. MSN were visualized via differential interference contrast microscopy and a CCD camera (Till Photonics, Gräfelfing, Germany) enabling visual differentiation between MSN and other striatal neurones by cell shape and size (Step et al., 2002). Visual classification was further re-checked by electrophysiological characterization of MSN showing specific passive and active membrane properties. The MSN recording seals were > 1 GΩ (6.0 ± 1.5 MΩ, n = 65); junction potentials (12.97 mV) as well as series resistance (18.0 ± 0.7 MΩ, n = 65) were not compensated. Voltage- and current-clamp data were recorded with an EPC-10 amplifier (HEKA, Lambrecht, Germany), filtered at 1 kHz, digitized at 20 kHz and stored via Patchmaster v2.20 software (HEKA, Lambrecht, Germany). The resting membrane potential was measured initially after establishing whole cells configuration. The number of action potentials, the threshold current (rheobase) and the latency of the first action potential at rheobase were achieved at 0 pA holding current by depolarizing current injections of 500 ms duration from 0 to at least 300 pA (50 pA increments). Cellular input resistance was calculated from the slope of the steady state current–voltage relation resulting from voltage steps (2 mV increments, 1 s duration) of −60 mV to −80 mV from a holding potential of −70 mV. For measurements of miniature excitatory post-synaptic currents (mEPSC) the membrane potential was clamped at −70 mV, and TTX (1 μM) as well as gabazine (5 μM) were added to the ACSF. mEPSC events were low-pass filtered at 1 kHz and detected within 5 min with a signal to noise ratio of 5:1 using the software MiniAnalysis v.6.0.7 (Synaptosoft, Decatur, USA). Mean mEPSC frequency of each MSN was calculated by dividing the detected events by observation time (300 s). Cumulative probability plots of mEPSC-intervals were generated for each MSN using 50 ms bins. The cumulative plots (Fig. 4) therefore represent the mean probability of all MSN within the experimental group (in favor of clarity without indicating SEM). Off-line analysis of patch-clamp data was performed using FitMaster v2.11 software (HEKA), Office Excel 2003 (Microsoft, Redmond, USA) und SigmaPlot 10.0 (Systat Software GmbH, Erkrath, Germany).

2.6. Data analysis

Before initiating the study, sample sizes were projected by assuming 20% differences in means, and an alpha-error of <5%, and a beta error of <20%, and a data variability of 12% using the online calculation program Sample Size Calculator (https://www.clinicalcalc.com/stats/samplesize.aspx). The resulting ideal n was >6. In all experiments involving dystonic animals, constituting the main emphasis of this study, n was hence chosen ≥11, and ≥ 6 in experiments on control animals. Data of extra-cellular recordings were analysed using Signal 2.16 software (Cambridge Electronic Design, Cambridge, UK). All values are given as means ± SEM; n refers to numbers of slices unless otherwise stated. Statistical analysis was performed with SigmaStat and SigmaPlot software (Systat Software Inc., San Jose, CA, USA). The significance of difference between the median values of the input-output activity of stimulated and sham-stimulated dtz mutant and control groups were evaluated using a two-way repeated measures analysis of variance (ANOVA, two factor repetition) and a post-hoc multiple comparison procedure (Holm-Sidak method). Kolmogorov–Smirnov test (K–S) was used for statistical analysis of cumulative probability plots. For all other analyses, statistical significance was tested using the Wilcoxon Rank Sum Test for paired data and the Wilcoxon-Mann-Whitney Rank Sum Test for unpaired data. A probability value of p < 0.05 was considered significant, indicated by asterisks (*) unpaired test and by hash (#) paired test) respectively. Whiskers in all figures denote limits of 5th and 95th percentiles.

2.7. Data sharing

All data are available as Excel files at doi:10.17632/tmmcbbzg6p6.1 in Mendeley Data.

3. Results

The aim of this study was to explore the possible mechanisms underlying the antidystonic effect of 3 h-DBS delivered to freely moving animals reported recently by our group (Paap et al., 2021). Our focus was on exploring changes in cortico-striatal communication, since dystonias are thought to involve a disturbance in the balance of striatal control of the GPi (Berardelli et al., 1998; Wichmann and Dostrovsky, 2011), the equivalent of the entopeduncular nucleus (EPN) in rodents.

3.1. Input-output relationship of cortico-striatal synaptic connections

We were first interested whether EPN-DBS changed the overall efficacy of synaptic connectivity between motor cortex and striatum. To gauge this, we explored the so-called input-output relationship of evoked field potential responses in the dorso-medial striatum to cortical activation via local afferent fibre stimulation. For this, stimulation intensity was stepwise increased from threshold to saturating response. As Fig. 2A illustrates, the resulting field excitatory postsynaptic potentials (fEPSP) increased in amplitude with cumulative rising stimulus in both groups, healthy (control; triangles) and dtz (circles). Of note, without DBS (empty symbols), the magnitudes of the responses in dtz were significantly smaller than in healthy tissue, with maxima at around 0.8 V/s (control) to 0.5 V/s (dtz). As Fig. 2A shows, EPN-DBS (filled symbols) altered the responsiveness of this cortico-striatal synapse, but only in healthy tissue, where it essentially enhanced the slope of the responses to approximately 150% (p < 0.05, ANOVA). Specifically, the mean values of field potential slopes (in V/s) were −0.56 ± 0.06 (dtz, n = 65) and −0.86 ± 0.09 (control, n = 41) for sham-stimulated tissue, and −0.61 ± 0.08 (dtz, n = 42) and −1.22 ± 0.13 (control, n = 44) for EPN-DBS-stimulated groups, as a response to the highest cortico-striatal stimulus intensity of 400 μA. Thus, DBS enhances synaptic efficiency only in healthy, non-dystonic control tissue.

3.2. Variability of cortico-striatal synaptic responses

Since DBS is speculated to normalise bursting oscillatory activity (Ashkan et al., 2017) and to disrupt aberrant synaptic transmission (Chiken and Nambu, 2016), we hypothesised that cortico-striatal responses would show greater variability in dystonic tissue, which should be reduced by DBS. As shown in Fig. 2C, the coefficients of variation of the synaptic responses (all in the range of 0.13–0.16, see Table 1 for details) did not differ between control and dystonic tissue, and remained unaffected by EPN-DBS. Hence, our results provide no direct support for these hypotheses, although – these being investigations in slices and not in vivo – an effect on oscillations cannot be ruled out.

3.3. Paired-pulse ratio (PPR)

We were also interested in the effect of EPN-DBS on short-term plasticity at the cortico-striatal synapse, since this plasticity governs...
the fidelity of transmission of repetitive synaptic events. For this, we gauged paired-pulse responses at the cortico-striatal synapse using fEPSP as before, now elicited in short succession twice at 40 ms inter-stimulus intervals. Paired-pulse response changes (facilitation or depression of the second response) are generally thought to be based on presynaptic release probability alterations, with paired-pulse depression (PPD) probably reflecting a presynaptic Ca\(^{2+}\)-dependent effect on release probability which however is under modulatory tone of GABA and can thus be reduced with loss of inhibition (Kirischuk et al., 1999). Paired-pulse facilitation (PPF), in turn, is supposed to be caused by an initially low release probability, which increases with residual presynaptic Ca\(^{2+}\) (Dittman and Regehr, 1996; Dittman et al., 2000). PPF was present in all groups (Fig. 2B). Thus, the ratio 2nd/1st pulse was 1.14 ± 0.03 (control tissue, sham DBS; n = 38), 1.20 ± 0.04 (control tissue, DBS; n = 45), 1.12 ± 0.04 (dsz tissue, sham DBS; n = 46) and 1.30 ± 0.09 (dsz tissue, DBS; n = 32); these values did not differ significantly, even though the values under DBS were always higher than those without – an effect of DBS thus cannot be ruled out completely.

### 3.4. Inhibitory control of cortico-striatal synaptic communication

With regard to evidence of reduced GABAergic inhibition of striatal projection neurones, probably based on deficient striatal GABAergic interneurones in dystonic hamsters (Bode et al., 2017; Germert et al., 1999; Germert et al., 2000) and considering the postulated GABAergic disinhibition in patients with dystonia, where a shift of balance toward the indirect pathway is speculated to occur (Wichmann and Dostrovsky, 2011), we were interested whether the synaptic responses evoked in the striatum by cortical afferents would be under the control of GABAergic inhibition, and whether this GABAergic control might change after DBS. We therefore explored the reaction of evoked fEPSP under the blockade of GABA\(_A\) receptors using gabazine (5 μM) application. As illustrated in Fig. 3A/B (dot plot of fEPSP responses during continuing gabazine application) and Fig. 3C (example traces of fEPSP before and after DBS).

### Table 1

|          | Control | dsz       | Sham      | dsz       |
|----------|---------|-----------|-----------|-----------|
| Sham     | 12.7 ± 1.0% (n = 11) | 12.7 ± 1.6% (n = 12) | 15.9 ± 1.4% (n = 6) | 13.9 ± 3.0% (n = 9) |
| DBS      |         |           |           |           |

Values are means ± SEM.

![Image](image_url)

**Fig. 2.** DBS enhances synaptic efficiency in control, but not in the dystonic groups. A: The dot plot shows the effect of DBS on input-output behaviour in the cortico-striatal network. Average data of pooled fEPSP slopes in response to increasing stimulation of the four experimental groups are indicated by dots and slashed lines. Data are presented as mean ± SEM of stimulated (filled symbols) and sham-stimulated (empty symbols) control (triangles) and dsz hamsters (circles). The number of slices is given in parentheses (in each experiment, usually only one slice per animal was obtained). Asterisks indicate significant differences (p < 0.01; ANOVA). Representative traces (top) illustrate series of fEPSP in response to increasing stimulus strengths of the respective groups as indicated. B: Box and whisker plot of paired-pulse ratio (PPR) of evoked field potential slopes in dsz and control slices, without DBS (sham), and after EPN-DBS (DBS). A PPR > 1 demonstrates facilitation, and < 1 depression of the second of a pair of responses evoked at an interval of 40 ms. Medians: straight lines, means: hashed lines. Single dots represent means of one experiment (slice). C: Box and whisker plot of coefficient of variation (CV) of evoked field potential slopes of 40 consecutive responses under baseline conditions in dsz and control slices, without DBS (sham), and after EPN-DBS (DBS). Means: straight lines, medians: hashed lines. Single dots represent the CV of one experiment (slice).
GABA-block), suppressing GABA<sub>A</sub>-receptor activation indeed had the effect of increasing the striatal field responses by 2–3 fold. Notably, this was more significant in control than in dystonic, dt<sup>2z</sup> tissue (to 309.8 ± 34.3% vs. 250.4 ± 34.5% of basal values, respectively, p < 0.05, means ± SEM of values of last 5 min, controls n = 6, dt<sup>2z</sup> n = 11), suggesting inhibitory control of synaptic activity to be higher in non-dystonic controls than in dystonic animals. Importantly, this inhibitory control as evidenced by the gabazine effect was significantly modulated by DBS, and differentially so in control vs. dystonic group: While in non-dystonic controls, DBS was associated with a significantly lower increase in fEPSP slope compared to sham-DBS stimulation (p < 0.05; two-way repeated measurement ANOVA), the converse was the case in dystonic, dt<sup>2z</sup> tissue, where DBS led to a higher increase in fEPSP (p < 0.05, ANOVA). Thus, in the control group, the slope fell from 309.8 ± 34.3% to 263.6 ± 56.1%. In slices from dt<sup>2z</sup>-hamsters, by contrast, the slope rose from 250.4 ± 34.5% to 282.3 ± 44.0% (controls DBS n = 9, dt<sup>2z</sup> DBS n = 12, means ± SEM of values of last 5 min).

We were also interested in which way the PPR would be modulated by GABA<sub>A</sub>-receptor inhibition (5 μM gabazine, 20 min application). In all groups, the PPR was reduced during GABA<sub>A</sub>-receptor blockade using gabazine (5 μM) from time-point 0, as indicated, after 20 min of stable baseline conditions. The data points represent cumulative means of relative fEPSP changes as percentage of baseline values before GABA-receptor block. Relative increases of fEPSP slope under GABA-receptor block hence indicate degree of inhibitory tone controlling synaptic transmission. fEPSP slopes were measured at 50% saturating stimulus intensity. Data are presented as mean ± SEM of EPN-DBS stimulated (filled symbols) and sham-stimulated (empty symbols) in dt<sup>2z</sup> (A) and control hamsters (B). The number of slices is given in parentheses. Asterisks indicate significant differences (p < 0.05; two-way repeated measurement ANOVA) and refer to comparisons of EPN-DBS stimulated (DBS) and sham-stimulated (sham) animals. C: Representative traces illustrate fEPSP in slices of dt<sup>2z</sup> and control slices, without DBS (sham), and after EPN-DBS (DBS). In all plots, filled symbols represent data from animals having undergone EPN-DBS, open symbols those of animals with sham stimulation only. In all box plots, medians are represented by straight lines and means by dashed lines. Single dots represent means of one experiment (slice).

### 3.5. Spontaneous cortico-striatal synaptic activity

The field potential investigations so far remained on a compound network level. We therefore strove to look at glutamatergic cortico-striatal synapses in more detail, i.e. on the single-cell level, by analysing miniature excitatory postsynaptic currents (mEPSC), reflecting spontaneous release activity from cortical projections. As shown by the cumulative probability plots (Fig. 4A and C) EPN-DBS indeed affected the occurrence of mEPSC by shifting the cumulative probability to longer mEPSC intervals in both healthy and dystonic animals. Accordingly, EPN-DBS also reduced the frequency of mEPSC. Interestingly, this reduction was stronger in dystonic tissue and in fact significant only in this case. Thus, the frequency of mEPSC after EPN-DBS fell from 4.11 ± 0.39 Hz to 2.26 ± 0.51 Hz (p < 0.05, MWRS-test) in dystonic tissue, and from 3.30 ± 0.39 to 2.27 ± 0.52 in control (n.s.) (Fig. 4A and C). At the same time, neither mean amplitudes, nor rise or decay times of the mEPSC (Fig. 4B and D) differed among the groups, even though the peak incidence in dystonic tissue shifted from 6 to 8 pA after EPN-DBS.
Fig. 4. DBS decreases miniature EPSC (mEPSC) occurring spontaneously at the cortico-striatal synapse of medium spiny neurones. Original traces of mEPSCs recorded from medium spiny neurones in dt\(^{sz}\) (A) and control (C) slices from animals having undergone EPN-DBS (DBS) or sham stimulation (sham). Corresponding cumulative probability histograms as well as box and whisker plots of the same groups (sham: light curves and open box plots, DBS: dark curves and filled boxes) show that DBS significantly shifts the interval distribution to the right in dystonic and control animals and therefore reduces frequency of mEPSC, with a significant reduction in dystonic tissue (asterisk in A, \(p < 0.05\), MWRS test). By contrast, amplitudes of mEPSC are not altered by DBS with respect to sham stimulation in either dystonic (dt\(^{sz}\), B) or control slices (control, D), as illustrated by amplitude distribution histograms, as well as box and whisker plots of mean mEPSC amplitudes (B, D). Neither are there any differences among the groups regarding the kinetics of mEPSC, as illustrated in the box and whisker plots of mean mEPSC rise and decay times. In all plots, filled symbols represent data from animals having undergone EPN-DBS, open symbols those of animals with sham stimulation only. In all box plots, medians are represented by straight lines and means by dashed lines. Single dots represent means of one experiment (slice).
(Fig. 4B), while it slightly declined from 8 pA to 7 pA after DBS in healthy tissue (Fig. 4C) (for details on the values, see Table 2). EPN-DBS thus obviously dampens spontaneous presynaptic glutamate release at cortico-striatal synapses, and this again differentially stronger in dystonic than in healthy tissue.

### 3.6. Neuronal properties

Last, we were interested in the effect of EPN-DBS on the postsynaptic level, i.e. on cellular properties of medium spiny neurones receiving cortical input. We thus tested the firing properties of these neurones upon intracellular current injection at increasing strengths (Fig. 5A, C), rheobase and latency to first AP (which characteristically is >50 ms in these neurone types) (box plots in Fig. 5A and C) as well as input resistance, membrane capacitance and resting membrane potential (Fig. 5B and D). There were no differences, neither between healthy (control) and dystonic (dt\(^{sz}\)) tissue, as already reported before in this dystonia model (Köhl et al., 2004), and actually also a mouse DYT1 model (Sciamanna et al., 2009), nor between conditions without (sham) or with EPN-DBS (DBS) (for details on the values, see Table 3). Hence, we can conclude that EPN-DBS does not alter striatal medium spiny neurone properties.

### 4. Discussion

In this study we could show that DBS of the EPN for 3 h in awake animals, which leads to alleviation of dystonic symptoms in mutant hamsters as recently demonstrated by the same DBS protocol (Paap et al., 2021), is associated with functional changes in cortico-striatal synaptic communication.

#### 4.1. Properties defining the dystonic condition

Studies in patients suggest that a pathological cortico-striatal function, and subsequent disturbance of striatal control of GPe, are likely to be important factors of dystonic dysfunction (Berardelli et al., 1998), possibly resulting in a shift of the balance toward the direct pathway (Wichmann and Dostrovsky, 2011). In this sense, the animal models of dystonia, and in particular the dystonic dt\(^{sz}\) mutant hamster, mirror this condition: It is spontaneously dystonic, and, as speculated for at least some human dystonias (Quarantone and Hallett, 2013; Quarantone and Pisani, 2011) (Schirinzì et al., 2018), displays increased cortico-striatal excitability, as a result of reduced intra-striatal GABAergic signalling (Gernert et al., 2000; Avchalumov et al., 2013). This is corroborated by findings in humans, where reduced cortical and striatal inhibition were reported (Levy and Hallett, 2002). Even though there is an apparent in contrast to monogenetic dystonias such as in non-dystonic DYT1 models, where GABAergic transmission was actually found to be reduced (Sciamanna et al., 2009), the fact that instead inhibition via cholingeric interneurones was reverted to excitation in these models (Martella et al., 2014) also substantiates a deficit in inhibition, albeit via a different cell type. However, one can hypothesise that the dystonic phenotype arises from a functional shift in basal ganglia circuitry, which originates from disinhibited striatal projection neurones (schematically shown in Fig. 6A), which in turn results in a more prominent pallidal/entope-duncular inhibition, as demonstrated in dystonic hamsters (Bennay et al., 2001).

#### 4.2. DBS alters cortico-striatal communication: synaptic efficacy

Our experiments demonstrate that EPN-DBS increases cortico-striatal evoked compound synaptic potentials, but only from healthy, control animals. How does DBS then change this circuitry? From patient studies, comparatively few data exist on activity changes brought about by pallidal DBS within the basal ganglia network. Pallidal stimulation suppresses low-frequency activity in the pallidum itself, and this low-frequency activity is a persistent marker of disease severity (Scheller et al., 2019). In addition, pallidal neurones react either with a persistent increase of activity, or with a sequence of events comprising initial increase, and prolonged decrease of spiking (Luo et al., 2018). From a case study on one patient, we know that pallidal DBS in turn generates complex downstream effects on thalamic neuronal firing, with close to 48% of neurones showing a decrease in discharge frequency, and the rest an increase (8%) or no change (44%) (Montgomery Jr., 2006). Further downstream, pallidal DBS also seems to increase motor cortical inhibition (Bociek et al., 2016). Since there is both a thalamic, cortical and indeed pallidal, retrograde axonal connection to the striatum, effects on cortico-striatal communication have been speculated on (Dostrovsky et al., 2000), but have not been reported so far. Also animal studies do not directly address the question: Pallidal spiking changes have been confirmed in healthy rodent tissue in vitro, with the biphasic responses being attributed to cholineric modulation (Luo et al., 2018). Again, animal studies directly investigating cortico-striatal communication are lacking. We are hence left to speculate that the enhancing effect of EPN-DBS exclusively in healthy animals is related to the apparently differential effects on GABAergic tone (see below).

#### 4.3. DBS alters striatal inhibitory tone

In our study, we could show that EPN-DBS differentially affects inhibitory tone in healthy (relative reduction in tone) and dystonic tissue (relative increase in tone). This differential effect certainly is highly interesting, and could constitute one important factor in the mechanism of DBS. It is tempting to speculate that this inhibitory tone modulation results in a normalisation of the intrastriatal inhibition (which in dystonic tissue was shown to be abnormal) (Sciamanna et al., 2020) (Fig. 6B), although obviously we cannot rule out that also changes in feed-forward inhibition coming from the cortex contributes to this effect. Specifically, the observation that EPN-activity is reduced in dt\(^{sz}\) hamsters (Gernert et al., 2000) is very likely due to striatal overactivity in dystonic animals, and previous findings showing that intrastriatal injection of GABA blockers worsens dystonia (Hamann and Richter, 2002) also stress the pivotal role of intrastriatal GABAergic control. Interestingly, such a reduction in striatal activity control seems to play a pivotal role also in another dystonia model, the DYT mouse, where this leads to excessive inhibition of pallidal activity via the indirect pathway (and consecutive alterations of nig pallidal cells) (Sciamanna et al., 2020). From our data, we speculate that the inherent loss of parvalbumin-positive interneurones (Bode et al., 2017) is functionally alleviated by DBS, but alternatively, also a possible pathological contribution of cholingeric interneurones being overactive and hence activating medium-spiny neurones (Eskow Jaunrajas et al., 2015) might be normalised. At any rate, the paired-pulse experiments support this notion: facilitation reverted to depression in control sham, and DBS dystonic tissue, under GABA block: Again, this corroborates that GABAergic tone controls synaptic transmission, and that EPN-DBS seems to reinstate a

### Table 2

Characteristics of spontaneous miniature excitatory currents on medium-spiny neurones.

|       | mEPSC frequency (Hz) | mEPSC amplitude (pA) | mEPSC rise time (ms) | mEPSC decay time (ms) |
|-------|----------------------|----------------------|----------------------|----------------------|
| Sham  | 4.11 ± 0.39          | 9.20 ± 0.56          | 3.18 ± 0.08          | 13.33 ± 0.75         |
| DBS   | 2.26 ± 0.51          | 10.84 ± 0.67         | 3.20 ± 0.06          | 13.80 ± 0.94         |
| control | 3.30 ± 0.39          | 10.87 ± 0.40         | 3.18 ± 0.04          | 13.76 ± 0.01         |
| n = 10 | 2.27 ± 0.52          | 11.43 ± 0.94         | 3.18 ± 0.15          | 13.76 ± 0.41         |
| n = 10 | 0.75 ± 0.50          | 0.67 ± 0.40          | 0.33 ± 0.01          | 0.74 ± 0.01          |

Values are means ± SEM.
A

B

C

D

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Fig. 5. DBS does not influence intrinsic neuronal properties of medium spiny neurones. Original traces of membrane potential recordings illustrating firing properties of medium spiny neurones in dt (A) and control (C) slices from animals having undergone EPN-DBS (DBS) or sham stimulation (sham). Firing was elicited by depolarising current injection at increasing amplitudes. The corresponding input-output curve is displayed below the original traces (at 300 pA step current), as dot diagram of action potential number plotted against current injection. Box and whisker plots of rheobase and of latency to first action potential after depolarising current injection in combination with truncated sample trace displaying no effect of EPN-DBS in tissue from dystonic (A) and control (C) animals. Voltage-current relationship used to determine input resistance exhibit characteristic rectification of medium spiny neurones in dystonic (B) and control tissue (D). Box and whisker plots of input resistance, membrane capacitance and resting membrane potential provide comparison of data from animals having undergone EPN-DBS with data from animals with sham stimulation. In all plots, filled symbols represent data from animals having undergone EPN-DBS, open symbols those of animals with sham stimulation only. In all box plots, means are represented by straight lines and medians by dashed lines. Single dots represent means of one experiment (slice).

Table 3

Properties of medium spiny striatal neurones.

|                          | dt | control |
|--------------------------|----|---------|
|                          | Sham | DBS   | Sham  | DBS |
| maximum no. of AP (at 300 pA) | 4.0 ± 1.0 | 5.1 ± 1.6 | 6.9 ± 1.9 | 7.8 ± 1.4 |
|                          | n = 20 | n = 14 | n = 10 | n = 10 |
| rheobase (pA)            | 260.0 ± 35.8 | 203.1 ± 35.8 | 205.0 ± 175.0 | 25.7 |
|                          | n = 20 | n = 16 | n = 10 | n = 10 |
| Latency of 1st AP (ms)   | 212.2 ± 176.8 | 152.7 ± 39.1 | 178.7 ± 194.5 | 10 |
|                          | n = 13 | n = 14 | n = 10 | n = 10 |
| Input resistance (MO)    | 142.5 ± 85.5 | 178.7 ± 194.5 | 15.6 ± 28.9 | 23.2 |
|                          | n = 24 | n = 20 | n = 11 | n = 10 |
| membrane capacitance (pF) | 76.1 ± 5.6 | 82.9 ± 4.2 | 85.1 ± 7.8 | 87.3 ± 5.9 |
|                          | n = 23 | n = 20 | n = 11 | n = 10 |
| resting membrane potential (mV) | –72.6 ± –11.1 | –72.8 ± –72.6 | –72.6 ± –72.6 | 1.1 ± 1.7 |
|                          | n = 22 | n = 20 | n = 11 | n = 10 |

Values are means ± SEM.

Fig. 6. Hypothetical effect of DBS on basal ganglia circuitry. The graph shows a schematic and reduced representation of basal ganglia circuitry under dystonic conditions before (A) and after DBS (B). A: Previous findings in animal dystonia (Gernert et al., 2000; Chiken et al., 2008) models and data from human studies (Tisch et al., 2007) suggest a loss of inhibitory tone within the striatal network, possibly based on a reduction of interneurone function, which at least in the dt hamster has been documented by transient loss of parvalbumin-positive interneurones (Gernert et al., 2000). We hypothesise that this leads to a disinhibition within the striatum (red hatching), and hence a more prominent inhibitory projection onto the Gpi (bold lines in blue). B: We hypothesise that DBS (red lightning arrow) normalises this state, possibly via activating backfiring axons into the striate (dotted light grey arrow), or axons of the hyperdirect pathway (dotted lines; this pathway is in close proximity to the stimulation site) backfiring in to the cortex. The effect could also be mediated anterogradely, via signalling of thalamic projections onto striatum or pallido-thalamo-cortical loop (dark grey arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.4. DBS alters spontaneous release in cortico-striatal synapses on medium spiny neurones

A prominent effect of DBS in the current study is the decrease of mEPSC frequency in tissue from dystonic animals, and to a lesser degree from healthy animals having undergone EPN-DBS. From studies in dystonia, no reports are available on this phenomenon. However, the reaction bears similarities to an effect of very high frequency spinal cord stimulation, which reduces mEPSC frequency in lamina II dorsal horn neurones to normal values (Liao et al., 2020). How is this effect mediated? Regarding our data on intrinsic properties of the postsynaptic, medium spiny neurones, which remained unaltered by EPN-DBS, a retrograde effect of the axons projecting to the EPN backfiring into the striate (as indicated by the arrow in Fig. 6B) is unlikely, although it cannot be fully excluded. Another retrograde effect is possible: The stimulation electrode, actually lies close to the fibres of the hyperdirect pathway. It is conceivable that the stimulation causes the changes we observed by changing the neuronal properties in the cortex. Another plausible hypothesis would be that either direct thalamic projections to the striatum or indirect projections via the pallido-thalamo-cortical loop are responsible. Again, these issues await exploration in the future by exploring thalamic and cortical changes.

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Declarations of interest

The authors declare that there are no conflicts of interest.

Author contributions

RK and AR designed the study, FP, CN and DT designed and constructed the DBS stimulator, MP,DF,FP,CN,DT,CB and UvR proposed the layout of the stimulator features, FP,CN,DT as well as MZ, DF, VN, MP, SP, AL, AR and RK tested it in pilot conditions, MH, MZ, and DF conducted in vivo DBS for this study, and conducted the electrophysiological experiments, together with JH; finally, MZ,MH,DF and RK wrote the paper, and verified the underlying data, and all authors contributed to discussion of the manuscript.

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