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Acute nigro-striatal blockade alters cortico-striatal encoding: An *in vivo* electrophysiological study

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ABSTRACT

Spreading of slow cortical rhythms into the basal ganglia (BG) is a relatively well-demonstrated phenomenon in the Parkinsonian state, both in humans and animals. Accordingly, striatal dopamine (DA) depletion, either acute or chronic, drives cortical-globus pallidus (GP) and cortical-substantia nigra pars reticulata (SNr) slow wave coherences in urethane-anesthetized rats. This paper investigates the striatal dynamics following acute DA depletion by tetrodotoxin (TTX) injection in the medial forebrain bundle (MFB) with respect to the transmission of slow cortical rhythms throughout the BG in more detail. The acute DA depletion offers the advantage of detecting electrophysiological changes irrespectively of chronically developing compensatory mechanisms. We observed that the acute blockade of the dopaminergic nigro-striatal pathway reshapes the firing rate and pattern of the different striatal neuron subtypes according to cortical activity, possibly reflecting a remodeled intrastriatal network. The observed alterations differ amongst striatal neuronal subtypes with the striatal medium spiny neurons and fast-spiking neurons being the most affected, while the tonically active neurons seem to be less affected. These acute changes might contribute to the diffusion of cortical activity to BG and the pathophysiology of Parkinson's disease (PD).

Introduction

Micro-electrode recordings during functional neurosurgery in PD have allowed the demonstration of abnormally synchronized oscillatory activity at multiple structures of the BG-cortical loop (Brown, 2003; Hammond et al., 2007). This excessive synchronization might represent an electrophysiological trait of the disease being directly correlated to the clinical state and susceptible to levodopa therapy or deep-brain stimulation (DBS; Eusebio et al., 2011; Kühn et al., 2006). Similar findings have been observed in chronically as well as in acutely dopamine-depleted animals (Fuentes et al., 2009; Galati et al., 2009, 2010; Magill et al., 2001; Sharott et al., 2005). In these animals, cortical slow wave activity (SWA) spreads into the BG network providing evidence of a perturbed mechanism of cortical input processing in the dopamine-depleted status (Fuentes et al., 2009; Galati et al., 2009, 2010; Magill et al., 2001). These features have been ascribed to changes of intrinsic voltage-gated conductance in the nucleus subthalamicus (STN) (Beurrier et al., 1999; Nambu and Llinaś, 1994) and/or to an impaired interplay between the STN and the GP, leading to oscillatory behavior (Plenz and Kitai, 1999). In agreement with these data, we recently described that intra-GP haloperidol or bicuculline injection generates cortical-GP synchronization supporting the presence of local mechanisms in pathological oscillatory neuronal behavior (Galati et al., 2009).

Besides, several lines of evidence have suggested an involvement of the striatum in the pathogenesis of excessive synchronization in PD, probably due to an enhanced corticostriatal glutamatergic excitatory drive and/or to decreased interneuronal inhibition. Three different neuronal cell types in the striatum may be implicated in this electrophysiological phenomenon. It has been quite consistently proven that the activity of the GABAergic medium spiny neurons (MSN) is increased (Blume et al., 2009; Galarraga et al., 1986; Liang et al., 2008; Tang et al., 2001; Tseng et al., 2001; Zold et al., 2012). Accordingly, abnormal MSN hyperactivity and synchronization in 6-OHDA animals is reduced by intrastriatal blockade of glutamatergic transmission. However, also the application of the $GABA_A$ receptor blocker bicuculline facilitated the electrophyiological consequences of dopamine depletion indicating decreased interneuronal inhibition and hypothetically reflecting a reduced activity of the GABAergic fast-spiking interneurons (FSI) (Carrillo-Reid et al., 2008; Costa et al., 2006; Jáidar et al., 2010; Taverna et al., 2008;

Abbreviations: PD, Parkinson's disease; BG, basal ganglia; DA, dopamine; MFB, medial forebrain bundle; TTX, tetrodotoxin; ECoG, electrocorticogram; SWA, slow wave activity; SNr, substantia nigra pars reticulate; STN, nucleus subthalamicus; SNc, substantia nigra pars compacta; ISI, inter-spike interval; CV, coefficient of variation; AutoCrl, autocorrelograms; MSN, medium spiny neuron; FSI, fast-spiking interneuron; TAN, tonically active large aspiny interneuron.

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Tecuapetla et al., 2009). These GABAergic interneurons show a coordinated high-frequency firing coherent with the cortex in normal animals (Sharott et al., 2009). However, in the chronic DA depletion state the activity of the FSI is rather spared. FSI provide a strong feed-forward inhibition upon the MSN and their stable activity in the DA depletion state worsened the striatal imbalance between MSN projecting to the GP or the substantia nigra pars reticulata (Mallet et al., 2006). The GABAergic effect may also be explained by increased interneuronal inhibition of MSN that conversely leads to an increased activity of these neurons (McCarthy et al., 2011). Besides the cholinergic tonically active large aspiny interneurons (TAN) have to be taken into consideration as proposed by the intrastriatal infusion of the cholinergic agonist carbachol (McCarthy et al., 2011).

So far, the role of the diverse systems controlling the MSN activity remains poorly defined and has been investigated in a severe chronic DA depletion state in which many histological alterations have already occurred. The real impact of the substantia nigra pars compacta (SNc) DA system upon the striatum and its consequences on the BG network is not well documented. To address this question we adopted a model based on an acute block of the medial forebrain bundle (MFB) by tetrodotoxin (TTX). In previous observations we recently showed that TTX-induced MFB impairment considerably changes the interaction between STN and GP and the BG output (Galati et al., 2009, 2010).

Methods

Animals

Experimental procedures were carried out on 49 adult male Wistar rats weighing 250–300 g in compliance with Swiss laws on animal experimentation and with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

Surgery

Rats were anesthetized with urethane (1.4 g/kg, i.p.) (Sigma Chemical Co., St. Louis, MO, USA) and mounted on a stereotaxic instrument (Stoelting Co., Wheat Lane Wood Dale, IL, USA). Body temperature was maintained at 37–38 °C with a heating pad placed beneath the animal. A midline scalp incision was made and the skull was almost completely drilled on the left side or both. The *dura* was then removed to expose the cortical surface. All wound margins were infiltrated with a local anesthetic (bupivacaine).

Electrophysiology

The electrophysiological methods are extensively described elsewhere (Galati et al., 2009). Briefly, electrocorticogram (ECoG) recordings coupled with striatal single unit extracellular recordings were performed. The ECoG was recorded via silver chloride screw electrodes placed on the cortical surface above the ipsilateral frontal cortex (3.0 mm anterior of the bregma and 2.0 lateral to the midline) and referenced against an indifferent electrode. Raw EEG was band-pass-filtered (0.1–100 Hz), amplified (2000×; model 12A5 amplifier, Grass Instrument Company, Quincy, MA), sampled (1000 Hz) on-line and stored on a computer connected to an analog/digital interface (micro1401 mk II, Cambridge Electronic Design, Cambridge, UK). During ECoG recording, extracellular action potentials of striatal neurons were acquired using ~15 MΩ glass electrodes (tip diameter ~1.5 μm) containing saline solution (2 M NaCl). Electrode signals were amplified (10.000×; ISO-DAM8; World Precision Instruments, Hertfordshire, UK), band-pass filtered (300–1000 Hz), sampled (60 kHz) on-line and stored on a computer connected to the Cambridge Electronic Design (CED) 1401 interface (see Galati et al., 2006, 2008, 2009, 2010).

Pharmacological blockade of the MFB

During ECoG and extra-cellular sampling, TTX (5 μM in NaCl) was infused in the MFB (stereotaxic coordinates: 2.56 mm posterior to the bregma, 2 mm lateral to the midline, and 8.6 mm below the cortical surface; Figs. 1A, B) by a 30 gauge stainless steel tube (external ∅ 0.2 mm) connected via a tubing to a 25 μl pump-driven syringe (CMA 400 syringe pump) at an infusion rate of infusion rate of 1 μl min^{-1} for 2–5 min (Galati et al., 2009, 2010).

Data analysis

Single-unit activity and ECoG were analyzed off-line by Spike 2 software (CED, Cambridge, UK). During urethane-induced deep anesthesia frontal ECoG was characterized by regularly occurring slow-waves of large amplitude (>500 μV) in which a smaller and faster activity (<200 μV) overlaid specific portions (Galati et al., 2009, 2010; Magill et al., 2000; Steriade et al., 1993). ECoG was assessed and epochs of robust cortical SWA were identified before and after MFB injection in conjunction with a portion of the coincident striatal spike trains of 500 events. These spikes were utilized for the subsequent characterization of cellular subtypes. Spike sorting was made by applying a principal component analysis in order to select the spikes belonging to a putative neuron subtype. We used several parameters from the average waveform of the collected spikes. Specifically, we measured the total and peak amplitude and the total and peak duration. On the basis of K-means analysis we identified three clearly separate neuronal subtypes by using the peak/total amplitude ratio and peak length (Fig. 1).

The inter-spike interval (ISI) and related parameters such as the mean ISI and its reciprocal, mean firing rate, coefficient of variation (CV), skewness and kurtosis were further analyzed. Autocorrelograms (AutoCrl) were used to detect the rhythmic neuronal activity by plotting 2000 ms intervals with 1 ms bin width. Phase histograms were constructed by using the corresponding Spike 2 (CED) script in order to determine the relationship between spike discharge and ECoG. The phase histogram was used to show how striatal spikes were distributed with respect to the cyclical process identified by the peaks of SWA (60 bins). The phase histogram indicates the firing probability of a neuron with regard to the ECoG. The correlation was recognized by putting a threshold level above noise (mean threshold SD, 3.2 ± 1.2) with a visually pre-set width ranging between 0.5 and 2 s.

Statistics

Statistical analysis was performed by using a statistical software (IBM SPSS). Statistical comparisons of firing rates and ISI parameters were conducted using the Mann–Whitney U-test. The comparison within the values of each phase (60 bins, from 0 to 360°) was performed using the non-parametric Friedman ANOVA whilst the comparison between the power of coherence of pre-TTX and post-TTX was performed by the Mann–Whitney U-test. The Bonferroni correction was applied for the multiple (60 bins) comparison leading the *P* value threshold up to 0.0008.

Results

Electrophysiological classification of striatal neuronal types

We recorded the extracellular activity of striatal neurons from forty-nine rats. The spike waveform of all cells (n = 86) recorded in the striatum showed a waveform characterized by a biphasic (−/+) action potential. As previously described, two neuronal types were clearly distinguishable on the basis of discharge frequency pattern (Galati et al., 2006; Kawaguchi, 1993; Mallet et al., 2005; Sharott et al., 2009; Wilson et al., 1990). In urethane-anesthetized rats, the striatal activity is characterized by TAN with a sustained firing rate and by neurons with sporadic activity.