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Research Paper

Short- and long-term dopamine depletion causes enhanced beta oscillations in the cortico-basal ganglia loop of parkinsonian rats

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ABSTRACT

Abnormally enhanced beta oscillations have been found in deep brain recordings from human Parkinson's disease (PD) patients and in animal models of PD. Recent correlative evidence suggests that beta oscillations are related to disease-specific symptoms such as akinesia and rigidity. However, this hypothesis has also been repeatedly questioned by studies showing no changes in beta power in animal models using an acute pharmacologic dopamine blockade. To further investigate the temporal dynamics of exaggerated beta synchrony in PD, we investigated the reserpine model, which is characterized by an acute and stable disruption of dopamine transmission, and compared it to the chronic progressive 6-hydroxydopamine (6-OHDA) model. Using simultaneous electrophysiological recordings in urethane anesthetized rats from the primary motor cortex, the subthalamic nucleus and the reticulate part of the substantia, we found evidence for enhanced beta oscillations in the basal ganglia of both animal models during the activated network state. In contrast to 6-OHDA, reserpine treated animals showed no involvement of primary motor cortex. Notably, beta coherence levels between primary motor cortex and basal ganglia nuclei were elevated in both models. Although both models exhibited elevated beta power and coherence levels, they differed substantially in respect to their mean peak frequency: while the 6-OHDA peak was located in the low beta range (17 Hz), the reserpine peak was centered at higher beta frequencies (27 Hz). Our results further support the hypothesis of an important pathophysiological relation between enhanced beta activity and akinesia in parkinsonism.

1. Introduction

There is a growing amount of evidence indicating that pathologically enhanced beta oscillations (13–35 Hz) in the cortico-basal ganglia loop play a paramount role in the system level pathophysiology of Parkinson's disease (PD) and other parkinsonian disorders (Brown, 2007; Oswal et al., 2013; Stein and Bar-Gad, 2013). For this reason, beta oscillations are currently investigated as a potential biomarker for closed loop deep brain stimulation treatment of PD patients (Little et al., 2013a; Priori et al., 2013; Little et al., 2015).

Enhanced oscillatory synchrony in the beta frequency band has been found in recordings of local field potentials and spiking neuron activity of the motor cortex and basal ganglia of humans and in different animal models of Parkinson's disease (Brown et al., 2001; Levy et al., 2002; Kuhn et al., 2004; Kuhn et al., 2005; Sharott et al., 2005; Leblois et al., 2007; Shimamoto et al., 2013). It has been hypothesized that beta oscillations are directly linked to disease symptoms (Eusebio and Brown, 2009; Timmermann and Fink, 2011). A key element underlying this theory is based on the fact that effective symptomatic treatments with levodopa and deep brain stimulation both reduce abnormal beta power (Doyle et al., 2005a; Kuhn et al., 2006a; Kuhn et al., 2009; Eusebio et al., 2011). In addition, this reduction of beta correlates with the alleviation of the parkinsonian motor symptoms akinesia and rigidity (Kuhn et al., 2006a; Weinberger et al., 2006; Ray et al., 2008; Kuhn et al., 2009). Furthermore, deep brain stimulation of the subthalamic nucleus (STN) at beta frequencies worsens motor symptoms in PD patients (Eusebio et al., 2008; Chen et al., 2011). Despite these and many other important findings advocating a central importance of abnormal beta oscillations, their role in the pathophysiology of PD has been put into question by several studies finding no direct correlation to disease severity in human patients and in animal models of PD (Weinberger et al., 2006; Leblois et al., 2007; Kuhn et al., 2009). Since beta oscillations in the basal ganglia of humans can only be studied in patients already in advanced disease states treated with deep brain stimulation, little is known so far about signaling in the cortico-basal ganglia loop early in the disease and in healthy human subjects. Furthermore, the direct relationship between reduced basal ganglia dopamine levels and the emergence of enhanced beta oscillations remains unclear. Experimental

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animal studies investigating this question by using an acute pharmacologic blockade of the dopamine receptors D_1 and D_2 showed seemingly ambiguous results: while some studies found evidence for increased beta band oscillations under D_1-D_2-receptor antagonists (Costa et al., 2006; Dejean et al., 2011), others showed no changes in this regard (Degos et al., 2005; Mallet et al., 2008b). Thus, it still remains unclear if beta oscillations are either directly linked to a lack of dopamine, or if they might be simply an epiphenomenon developing late in a state of chronic dopamine depletion.

The aim of our study was to further clarify this question by investigating cortico-basal ganglia circuit abnormalities in two animal models of PD which differ substantially in the time course until their maximum phenotype is reached: We opted to investigate the reserpine model, which results in an acute, long-lasting dopamine depletion due to an irreversible blockade of the vesicular monoamine transporter (VMAT2) of monaminergic neurons (Leao et al., 2015), and compared it to the chronic progressive 6-hydroxydopamine MFB-model (medial forebrain bundle) where the dopaminergic deficits develop a few days after the lesion progressively over several weeks (Blandini and Armentero, 2012). Thus, we wanted to clarify the differential effects of short- and long-term dopamine depletion on signaling characteristics in the cortico-basal ganglia loop. The 6-OHDA model is well known to exhibit enhanced beta oscillations (Sharott et al., 2005; Mallet et al., 2008b; Delaville et al., 2015), while this has not been studied so far in the reserpine model. We performed simultaneous extracellular electrophysiological recordings from the primary motor cortex (ECoG, LFP), the subthalamic nucleus and the reticulate part of substantia nigra (both LFP and multi-unit activity) under urethane anesthesia and assessed the animals motor behavior and histology.

2. Methods

2.1. Animals and materials

Experimental procedures were carried out on 27 male Wistar-rats (Harlan Winkelmann, Germany), and were conducted in accordance with the German Animal Welfare Act (last revised in 2014) and European regulations (2010/63/EU). Experiments were approved by the local animal welfare authority (LaGeSo, Berlin), and conformed to local department and international guidelines. Every effort was made to minimize the number of animals and to reduce the animal's harm caused by the experimental setting. Animals were kept on a 12 h light cycle in standard housing conditions and received food and water ad libitum. Unless indicated otherwise, materials were obtained from Sigma-Aldrich, Germany. All stereotactic coordinates were measured in relation to the bregma (Paxinos and Watson, 2006).

2.2. Experimental groups

Three groups of animals (n = 9 each) were formed in this study: healthy control (285–363 g), reserpine-treated (305–377 g) and 6-OHDA lesioned (304–355 g) animals.

2.3. Unilateral 6-hydroxydopamine lesions of dopaminergic neurons

The neurotoxin 6-OHDA-hydrochloride was dissolved in NaCl 0.9% containing 0.02% ascorbic acid at a final concentration of 8 μg/μl of the free base. The solution was stored at −80 °C and defrosted directly before injection. Anesthesia was induced and maintained with a combination of fentanyl (5 μg/kg, s.c., Rotexmedica, Germany), medetomidine (150 μg/kg, s.c., Domitor ®, Provet AG, Germany) and midazolam (2 mg/kg, s.c., Hameln Pharma, Germany). Rats were placed in a stereotactic frame (David Kopf Instruments, CA, USA) with heads fixed with atraumatic ear bars. Ophthalmic ointment (Bepanthen™, Bayer, Germany) was applied to prevent corneal dehydration. Body temperature of 37 ± 0.5 °C was maintained throughout surgery using a self-adjusting heating pad (CMA, Sweden). After incision of the skin, the head was aligned to the flat skull position using the rat alignment tool (David Kopf Instruments, CA, USA). Following a small craniotomy, a 33-gauge blunt-tip cannula fixed on a 10 μl Hamilton syringe (World Precision Instruments, FL, USA) was inserted into the left medial forebrain bundle (MFB, AP: −2.6, ML: +1.6, DV: −8.4 mm) and 1 μl of 6-OHDA was injected at a pace of 0.125 μl/min via a precision syringe pump (Micro 4™, World Precision Instruments, FL, USA). After completion of the injection the cannula remained in place for 5 more minutes to avoid a reflux of the liquid. After wound closure, anesthesia was reversed using a combination of naloxone (120 μg/kg, s.c., B. Braun Melsungen AG, Germany), flumazenil (200 μg/kg, s.c., Inresa, Germany) and atipamezole (750 μg/kg, s.c., cp-pharma, Germany). On the first three days after surgery the animals received the analgesic carprofen (5 mg/kg, s.c., Pfizer, Germany) to minimize their distress. The efficacy of the 6-OHDA lesion was assessed behaviorally and post-mortem using immunochemistry for TH (both see below). The electrophysiological experiments were conducted 20–30 days following the injection of the neurotoxin, at a time when a maximal lesion of the dopaminergic system can be expected (Sharott et al., 2005; Blandini and Armentero, 2012).

2.4. Pharmacologic dopamine depletion with reserpine

To achieve an acute and widespread dopamine depletion, known to last at least 48 h, (Leao et al., 2015), rats received systemic injections of reserpine (3 mg/kg, i.p., Sigma-Aldrich). Reserpine was dissolved in 0.9% NaCl and dimethyl-sulfoxide (4 mg/l). Efficiency of the pharmacologic dopamine depletion was assessed behaviorally (see below). Furthermore, body weight was monitored carefully, since it has been demonstrated before that a weight reduction exceeding 5% predicts widespread reserpine-induced amine depletion (Halaris and Freedman, 1975). All animals reached this criterion. Eighteen hours after the injection reserpine-treated rats underwent electrophysiological recordings. At this point in time, an almost total dopamine depletion (>95%) can be expected in the basal ganglia (Leao et al., 2015).

2.5. Behavioral testing

Motor testing was performed in all animals prior to the electrophysiological recordings. Additionally, reserpine-treated and 6-OHDA-lesioned animals were evaluated in a baseline testing before reserpine application or 6-OHDA lesion. All behavioral examinations were videotaped (Canon Legria HF R506, Canon, Japan) for offline data analysis. Since the two examined animal models are known to show different motor phenotypes, hemiakinesia in case of 6-OHDA and bilateral akinesia in case of reserpine (Bezard and Przedborski, 2011; Leao et al., 2015), we conducted different tests to quantify the animal's motor performance. To assess the unilateral motor deficit in the 6-OHDA-lesioned animals we performed the limb use asymmetry test and the drag test (Meredith and Kang, 2006). For the limb asymmetry use test (cylinder test) rats were placed in a transparent acrylic glass cylinder (height: 45 cm, diameter: 20 cm) without prior habituation (Schallert et al., 2000). During the rat's vertical exploration of the walls, full contacts of the right and the left forepaw were counted. The rats were left in the cylinder until at least one paw had touched the walls a minimum of 15 times. For the drag test the rat's hind limbs were raised off the ground leaving the forelimbs in touch with the ground (Olsson et al., 1995). Then the animal was dragged backwards for the distance of one meter and the adjusting steps of each forepaw were counted separately. Results for both tests are presented as a ratio of right to left forelimb uses. The test was repeated until a minimum of 15 steps was made by at least one of the paws. Reserpine-treated animals were examined with the bar test (Sanberg et al., 1988). For this test the rat's forelimbs were placed on a small pedestal (height: 8 cm, width: 10 cm) and the time needed for removal of both forelimbs was measured. The test was also completed when a time limit of 300 s was exceeded. Control