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Research Paper

Altered somatosensory cortex neuronal activity in a rat model of Parkinson's disease and levodopa-induced dyskinesias

Mesbah Alam [a,*,1], Regina Rumpel [b,1], Xingxing Jin [a], Christof von Wrangel [d], Sarah K. Tschirner [c], Joachim K. Krauss [a,e], Claudia Grothe [b,e], Andreas Ratzka [b,2], Kerstin Schwabe [a,e,2]

[a] Department of Neurosurgery, Hannover Medical School, Hannover, Germany
[b] Institute of Neuroanatomy, Hannover Medical School, Hannover, Germany
[c] Research Core Unit Metabolomics, Institute of Pharmacology, Hannover Medical School, Hannover, Germany
[d] Department of Neurosurgery, Charité Universitätsmedizin Berlin, Berlin, Germany
[e] Centre for Systems Neuroscience (ZSN), Hannover Medical School, Hannover, Germany

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ABSTRACT

Several findings support the concept that sensorimotor integration is disturbed in Parkinson's disease (PD) and in levodopa-induced dyskinesias. In this study, we explored the neuronal firing activity of excitatory pyramidal cells and inhibitory interneurons in the forelimb region of the primary somatosensory cortex (S1FL-Ctx), along with its interaction with oscillatory activity of the primary motor cortex (MCtx) in 6-hydroxydopamine lesioned hemiparkinsonian (HP) and levodopa-primed dyskinetic (HP-LID) rats as compared to controls under urethane (1.4 g/kg, i.p.) anesthesia. Further, gene expression patterns of distinct markers for inhibitory GABAergic neurons were analyzed in both cortical regions.

While firing frequency and burst activity of S1FL-Ctx inhibitory interneurons were reduced in HP and HP-LID rats, measures of irregularity were enhanced in pyramidal cells. Further, enhanced coherence of distinct frequency bands of the theta/alpha, high-beta, and gamma frequency, together with enhanced synchronization of putative pyramidal cells and interneurons with MCtx oscillatory activity were observed. While GABA level was similar, gene expression levels of interneuron and GABAergic markers in S1FL-Ctx and MCtx of HP-LID rats differed to some extent.

Our study shows that in a rat model of PD with dyskinesias, neuronal activity in putative interneurons was reduced, which was accompanied by high beta and gamma coherence between S1FL-Ctx and MCtx, together with changes in gene expression, indicating maladaptive neuroplasticity after long term levodopa treatment.

1. Introduction

Current concepts attribute the motor symptoms of Parkinson's disease (PD) mainly to a dysfunction of the basal ganglia (BG) motor circuitry, including an increased oscillatory beta band synchronization along with altered firing rates and burst activity at the neuronal level in the globus pallidus internus (GPi) and subthalamic nucleus (STN) secondary to nigral dopamine depletion (Brown, 2003; Obeso et al., 2006; Weinberger et al., 2012; Wichmann and Dostrovsky, 2011). The motor symptoms and the abnormal neuronal activity are alleviated by levodopa treatment, which, however, is complicated by the gradual development of levodopa-induced dyskinesias (LID) after long-term treatment. During LID, enhanced oscillatory theta band activity has been observed, together with an extensive decrease in firing rate and abnormal firing patterns in both clinical (Alonso-Frech et al., 2006; Lozano et al., 2000; Obeso et al., 2000) and experimental studies (Alam et al., 2014; Jin et al., 2016).

The finding that patients rely strongly on external sensory information for movement initiation and execution supports the concept that abnormalities of sensorimotor integration may also contribute to the symptoms of PD (Abbruzzese and Berardelli, 2003; Conte et al., 2013; Quartarone et al., 2008; Sailer et al., 2007; Sailer et al., 2003). Changes in somatosensory processing in PD may be located within the dopamine-depleted cortico-striatal circuit, since altered connectivity between the putamen and somatosensory cortex (SCtx) and motor cortex (MCtx) has been described (Helmich et al., 2010). Furthermore, direct reciprocal connections between MCtx and SCtx may also play a role in the ability to update motor plans in response to changes in the sensory periphery (Rocco-Donovan et al., 2011). It has been shown that inactivation of this input causes disruption of motor function,

* Corresponding author at: Department of Neurosurgery, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany.
E-mail address: Alam.Mesbah@mh-hannover.de (M. Alam).
[1] Those authors contributed equally.
[2] Senior authors contributed equally.

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such as fine motor coordination, sustained muscle contractions and appropriate grip force, which impairs the learning of new motor skills in primates (Brochier et al., 1999; Hikosaka et al., 1985; Johansson and Westling, 1984).

Although inhibitory interneurons represent a minority of all cortical neurons (<20% in rodents), their diversity and dense axonal arborization allow them to control numerous cortical functions and behaviors (Fanselow et al., 2008; Gentet et al., 2012; Huang, 2014; Isaacson and Scanziani, 2011; Markram et al., 2004; Sohal et al., 2009). Furthermore, distinct subtypes of cortical interneurons can be subdivided based on cell morphology and the expression of calcium-binding proteins and neuropeptides, such as parvalbumin, somatostatin, and calretinin (Griffen and Maffei, 2014; Taniguchi, 2014; Zeisel et al., 2015). It has been hypothesized that disturbed plasticity of MCtx and SCtx, as well as dysfunction of the GABA and glutamate system in cortico-striatal projections and STN efferents to BG output nuclei contribute to the appearance of LID (Calabresi et al., 2015; Carta and Tronci, 2014; Cenci, 2014; Lefaucheur, 2005; Lindenbach et al., 2015; Lindenbach et al., 2016; Sgambato-Faure and Cenci, 2012; Tessitore et al., 2014). However, the interaction of SCtx and MCtx signal processing in PD and LID remains unclear.

The aim of the present study was to investigate the interaction of the firing activity in the front limb sensory region (S1FL-Ctx) with oscillatory activity of the MCtx in unilateral 6-hydroxydopamine (6-OHDA) lesioned hemi-parkinsonian rats (HP) and HP rats with levodopa-induced dyskinesias (HP-LID) after chronic treatment with levodopa. Furthermore, changes in gene expression levels of interneuron markers and GABAergic markers within these cortical regions were investigated.

2. Materials and methods

2.1. Animals and experimental design

Fifty-two adult female Sprague Dawley rats from Charles River (Germany) weighing 200–250 g at the beginning of the experiments were used in this study. Animals were housed in cages of three to four and kept in temperature- and humidity-controlled rooms on a 14 h light/10 h dark schedule with food and water available ad libitum. All experimental protocols followed the German Animal Protection Act and were approved by the local authorities (Bezirksregierung LAVES Hannover, Germany). Thirty-nine animals received a unilateral 6-OHDA lesion of the right medial forebrain bundle (MFB). Three weeks after lesion surgery, apomorphine-induced rotation was performed with all lesioned animals to assess lesion efficacy. Successfully lesioned animals were assigned to the experimental groups HP and HP-LID according to their rotation scores or were otherwise excluded from further experiments. Animals from the HP-LID group began levodopa/benserazide treatment the following day with daily injections and were scored with the abnormal involuntary movement (AIM) scale (see below) for three weeks. One week later, electrophysiological recordings were carried out or animals were sacrificed and brains were dissected for high performance liquid chromatography mass spectrometry/mass spectrometry (HPLC-MS/MS) or quantitative reverse-transcribed polymerase chain reaction (qRT-PCR) analyses (Table 1). Thirteen non-lesioned rats served as a control group.

2.2. 6-OHDA lesion surgery and apomorphine-induced rotational behavior

To induce a complete lesion of the nigrostriatal pathway, stereotaxic surgery was performed under general anesthesia using chloral hydrate (370 mg/kg; i.p., Sigma-Aldrich, Steinheim, Germany) as described previously (Rumpel et al., 2013). Briefly, animals received two injections of 6-OHDA hydrobromide (free base 3.6 μg/μl in 0.02% L-ascorbate-saline, Tocris Bioscience, Bristol, UK) into the right MFB at the following coordinates: first tract anterior-posterior (AP) −4.4, lateral (LAT) −1.2, dorso-ventral (DV) −7.8, tooth bar (TB) −2.4, injection volume 2.5 μl; second tract AP −4.0, LAT −0.8, DV −8.0, TB +3.4, injection volume 3 μl (in mm according to bregma and dura (Paxinos and Watson, 2007)). Injections were delivered using a 10-μl Hamilton syringe with an injection rate of 1 μl/min. The cannula was left in place for additional 3 min to allow diffusion before being slowly retracted. All operated rats were tested for their rotational bias in automated rotameter bowls according to Ungerstedt and Arbuthnott (1970) three weeks after lesion surgery. Animals were subcutaneously injected with apomorphine hydrochloride (0.05 mg/kg in 0.02% L-ascorbate-saline, Sigma-Aldrich), and right and left full body turns were monitored over a period of 40 min. Animals displaying a mean score of >4.0 full contralateral body turns per minute were included in the study.

Table 1
Experimental groups.

ID	Comments	Electro-physiology (*n*)	HPLC-MS/MS (*n*)	qRT-PCR (*n*)
Control	No surgery	5	3	5
HP	6-OHDA injection	5	3	5
HP-LID	6-OHDA injection, levodopa/benserazide treatment	7	3	5

n = number of analyzed animals; 6-OHDA = 6-hydroxydopamine; LID = levodopa-induced dyskinesia; HP = hemiparkinsonian; HPLC-MS/MS = high performance liquid chromatography tandem mass spectrometry; qRT-PCR = quantitative reverse-transcribed polymerase chain reaction.

2.3. Levodopa injection and AIM scoring

The day following drug-induced rotation, treatment of twelve animals in the HP-LID group was started with subcutaneous injections of 6 mg/kg levodopa methyl ester hydrochloride (Sigma-Aldrich) mixed with 12 mg/kg of benserazide hydrochloride (Sigma-Aldrich), which was dissolved in saline right before use. Daily injections continued for three weeks. Behavioral testing for AIMs was performed at the end of the induction period using the rat AIM rating scale as described previously (Lee et al., 2000). AIM scoring was taken from direct observation of animals during the testing period by an experimenter blinded to the state of the animal. Directly after injection, animals were placed into a glass cylinder and stereotypic movements induced by levodopa individually observed for 140 min. Scoring was carried out every 20 min and AIMs were classified according to their topographic distribution into locomotive, axial, orolingual, and forelimb dyskinesia subtypes. A score from zero to four was given for each of the four subtypes, respectively.

2.4. Electrophysiological recordings and data analysis

In vivo neuronal activity measurements were carried out seven weeks after 6-OHDA lesion (control $n = 5$, HP $n = 5$, HP-LID $n = 7$). Seven days after the last dose of levodopa, all HP-LID rats were recorded within a week under urethane anesthesia (1.4 g/kg i.p. with additional 25% doses as needed) as described previously (Alam et al., 2012). The temperature of the anesthetized animal was controlled with a rectal probe and maintained at 37 to 37.5 °C with a heating pad (Harvard Apparatus). S1FL-Ctx (primary somatosensory cortex, forelimb region) recordings were made at the following coordinates in mm relative to bregma: AP −0.3 to −0.4, LAT −3.5 and −4.0, and DV −1.8 to −2.0 ventral to the cortical surface. Electrocardiographic activity was monitored constantly to ensure the animals' well-being. A drop of silicon oil was applied to all areas of exposed cortex to prevent dehydration. Depth of anesthesia was monitored by examination of the reflex answer to a toe pinch.

Single unit (SU) activity and local field potentials (LFPs) were recorded in the S1FL-Ctx area by using quartz coated micro-electrodes. Additionally, an electrocorticogram (ECoG) was acquired from the