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RESPIRATORY DEFICITS IN A RAT MODEL OF PARKINSON'S DISEASE

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Abstract—Parkinson's disease (PD) is a neurodegenerative disease characterized by loss of the dopaminergic nigrostriatal pathway. In addition to deficits in voluntary movement, PD involves a disturbance of breathing regulation. However, the cause and nature of this disturbance are not well understood. Here, we investigated breathing at rest and in response to hypercapnia (7% CO_2) or hypoxia (8% O_2), as well as neuroanatomical changes in brainstem regions essential for breathing, in a 6-hydroxydopamine (6-OHDA) rat model of PD. Bilateral injections of 6-OHDA (24 μg/μl) into the striatum decreased tyrosine hydroxylase (TH^+)-neurons in the substantia nigra pars compacta (SNpc), transcription factor phox2b-expressing neurons in the retrotrapezoid nucleus and neurokinin-1 receptors in the ventral respiratory column. In 6-OHDA-lesioned rats, respiratory rate was reduced at rest, leading to a reduction in minute ventilation. These animals also showed a reduction in the tachypneic response to hypercapnia, but not to hypoxia challenge. These results suggest that the degeneration of TH^+ neurons in the SNpc leads to impairment of breathing at rest and in hypercapnic conditions. Our data indicate that respiratory deficits in a 6-OHDA rat model of PD are related to downregulation of neural systems involved in respiratory rhythm generation. The present study suggests a new avenue to better understand the respiratory deficits observed in chronic stages of PD.

Key words: Parkinson's disease, breathing, hypercapnia, hypoxia, brainstem.

INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, characterized by resting tremor, rigidity, bradykinesia and postural instability, as well as non-motor features, including abnormalities of the autonomic nervous system. Although the motor symptoms of PD are considered a pathological hallmark of the disease (Fearnley and Lees, 1991), multiple debilitating symptoms are related to the non-motor aspects of PD (Wolters, 2009). These include sleep disturbances, neuropsychiatric and cognitive deficits, autonomic and sensory dysfunction and breathing instability (Bassetti, 2011; Chaudhuri et al., 2011).

Breathing depends on a sophisticated neural network in the lower brainstem that controls respiratory rhythm and pattern generation (Feldman and Ellenberger, 1988; Nogués et al., 2002; Feldman and Del Negro, 2006; Feldman et al., 2013). Research efforts are escalating to understand the underlying causes of neuronal respiratory dysfunction, which is symptomatic of many diseases that can occur at almost any time during life (Axelrod et al., 2006; Weese-Mayer et al., 2006, 2008). Breathing deficits, including prolonged and frequent apneas during sleep, are present in a number of neurodegenerative diseases such as PD, multiple system atrophy and amyotrophic lateral sclerosis (Benarroch, 2003, 2007; Benarroch et al., 2003; Schwarzacher et al., 2011). Therefore, it becomes important to evaluate how these conditions can modify breathing activity. The aim of this manuscript was to investigate breathing at rest and in response to hypercapnia (7% CO_2) or hypoxia (8% O_2), as well as neuroanatomical changes in brainstem regions responsible for the neural control of breathing. We selected a widely used rat model of PD created by bilateral injections of 6-hydroxydopamine (6-OHDA) into the striatum.

RESULTS

Animal model of PD: bilateral intra-striatal 6-OHDA selectively destroyed tyrosine hydroxylase (TH)-expressing-neurons of the substantia nigra (SN)

The 6-OHDA neurotoxic lesion within the nigrostriatal dopaminergic system is one of the most widely used for modeling PD in rodents (McDowell and Chesselet, 2012). In this study, 6-OHDA (6, 12 or 24 μg/μl) was administered into the dorsal striatum of three groups of rats. The size and specificity of the lesion were determined by TH-immunoreactivity (TH-ir) in the SN. Compared to vehicle-infused control rats (906 ± 17

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Abbreviations: 6-OHDA, 6-hydroxydopamine; cVRG, caudal ventral respiratory group; fR, respiratory frequency; LC, locus coeruleus; MV, minute ventilation; PD, Parkinson's disease; phox2b-ir, phox2b immunoreactivity; preBötC, pre-Bötzinger complex; RTN, retrotrapezoid nucleus; rVRG, rostral ventral respiratory group; SN, substantia nigra; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-ir, TH-immunoreactivity; VRC, ventral respiratory column; VT, tidal volume.

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neurons), 6-OHDA (6, 12 and 24 μg/μl) dramatically and dose-dependently reduced the number of TH-ir neurons in the SN (722 ± 15, 204 ± 18 and 135 ± 23, respectively, $p < 0.001$; AP −5.32 to −6.04 mm relative to Bregma, counted in one-in-six series of 40-μm brain sections per rat) (Fig. 1a, b, e). We also analyzed TH-ir in A6 (locus coeruleus: LC) and intra-striatal 6-OHDA did not change the number of TH neurons in A6 compared to control rats (Fig. 1c, d, e). Previous studies have shown that intrastriatal injection of 6-OHDA did not cause degeneration of catecholaminergic neurons in the LC (Miguelez et al., 2011).

6-OHDA selectively destroyed neurons involved in respiratory control

To further assess the effect of bilateral intrastriatal 6-OHDA on neurons involved in respiratory control, phox2b immunoreactivity (phox2b-ir) was examined within the retrotrapezoid nucleus (RTN) at 60 days after injection. The number of phox2b-ir nuclei counted in the RTN was unchanged after vehicle or 6 μg of 6-OHDA (one-in-six series of 40-μm brain sections per rat) (Fig. 2e). A massive reduction of phox2b-ir nuclei was found after 12 and 24 μg 6-OHDA (51 ± 3 and 30 ± 4,

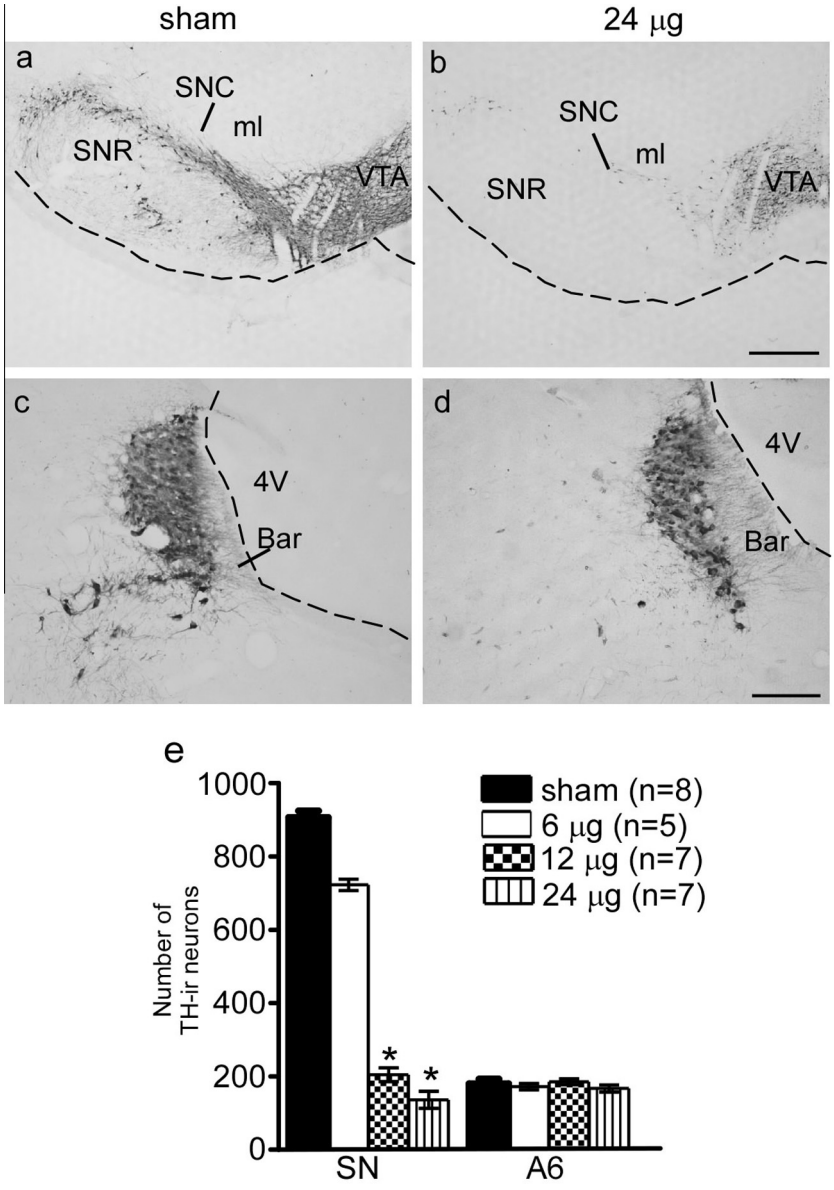

Fig. 1. Bilateral intrastriatal injections of 6-OHDA destroy TH^+ neurons within the substantia nigra (SN). Photomicrographs from a control animal that received bilateral intrastriatal injections of vehicle (left column, panels a, c) or 24 μg of 6-OHDA (right column, panels b, d). Compared to vehicle, 6-OHDA caused an almost complete loss of TH-immunoreactivity at the level of the SN (a, b), but A6 neurons (locus coeruleus) were intact (c, d). (e) Group data. Each column represents the total number of neurons of a given type in five consecutive 40-μm-thick coronal sections, separated by 240 μm. Scale bar in b = 500 μm for panels a, b. Scale bar in *d* = 200 μm for panels c, d. *Abbreviations:* SNR, substantia nigra reticulata; SNC, substantia nigra pars compacta; ml, medial lemniscus; VTA, ventral tegmental area; 4V, fourth ventricle. Bar: Barrington's nucleus. $^*p < 0.05$ relative to vehicle (sham).