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Research Report

Similar L-dopa-stimulated motor activity in mice with adult-onset 6-hydroxydopamine-induced symmetric dopamine denervation and in transcription factor Pitx3 null mice with perinatal-onset symmetric dopamine denervation

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ABSTRACT

The transcription factor Pitx3 null mutant (Pitx3Null) mice have a constitutive perinatal-onset and symmetric bilateral dopamine (DA) loss in the striatum. In these mice L-3,4-dihydroxyphenylalanine (L-dopa) induces apparently normal horizontal movements (walking) but also upward movements consisting of the vertical body trunk and waving paws that are absent in normal animals and in animals with the classic unilateral 6-hydroxydopamine (6-OHDA) lesion-induced DA denervation. Thus, a concern is that the perinatal timing of the DA loss and potential developmental abnormalities in Pitx3Null mice may underlie these upward movements, thus reducing the usefulness as a DA denervation model. Here we show that in normal wild-type (Pitx3WT) mice with adult-onset symmetric, bilateral 6-OHDA-induced DA lesion in the dorsal striatum, L-dopa induces normal horizontal movements and upward movements that are qualitatively identical to those in Pitx3Null mice. Furthermore, after unilateral 6-OHDA lesion of the residual DA innervation in the striatum in Pitx3Null mice, L-dopa induces contraversive rotation that is similar to that in Pitx3WT mice with the classic unilateral 6-OHDA lesion. These results indicate that in Pitx3Null mice, the bilateral symmetric DA denervation in the dorsal striatum is sufficient for expressing the L-dopa-induced motor phenotype and the perinatal timing of their DA loss is not a determining factor, providing further evidence that Pitx3Null mice are a convenient and suitable mouse model to study the consequences of DA loss and dopaminergic replacement therapy in Parkinson's disease.

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1. Introduction

In the classic 6-OHDA unilateral nigrostriatal DA lesion model of Parkinson's disease (PD) model, L-dopa stimulates a striking, contraversive or asymmetric rotation toward the DA intact side (Schwarting and Huston, 1996; Ungerstedt, 1971), providing a motor phenotype for studying PD pathophysiology and testing new therapeutic drugs (Lane et al., 2006; Marin et al., 2006; Thiele et al., 2011). However, 6-OHDA lesion requires manual stereotaxic intracranial injection that is not only time-consuming but also has unavoidable variations in the surgery among individual animals, and postmortem neurochemical and immunohistochemical examinations are needed to verify the extent and severity of the DA lesion (Thiele et al., 2011), another time-consuming procedure. Thus a simpler animal model with a predefined and consistent DA denervation would make more effective use of research resources.

The transcription factor Pitx3 null mutant (Pitx3Null) mice are a potentially excellent genetic alternative to the unilateral 6-OHDA model. Pitx3Null mice have an autogenous, bilaterally symmetric and severe DA denervation in the dorsal striatum that is consistent among every Pitx3Null mouse (Nunes et al., 2003; Smidt et al., 2004; Smits et al., 2006; van den Munckhof et al., 2003; Ding et al. 2015). These mice are fertile and easy to maintain. Additionally, in Pitx3Null mice, L-dopa stimulates both normal horizontal movements and also upward movements not seen in Pitx3WT mice (Ding et al., 2007, 2011; Hwang et al., 2005; Li and Zhou, 2013; Solís et al., 2015; Won et al. 2014). However, since most nigral DA neurons are already lost during the perinatal period in Pitx3Null mice (van den Munckhof et al., 2003), there is a possibility that a developmental abnormality may be responsible for the L-dopa-induced upward movements, potentially confounding data interpretation and limiting the utility of this mouse model. Additionally, this possibility is difficult to prove or disprove because developmental abnormalities may be absent or hard-to-detect.

Another possibility is that the DA loss pattern and severity, not its perinatal timing and the potential developmental abnormalities, if any, determine L-dopa's motor response; hence the upward movements may be a type of symmetric movements resulting from symmetric supersensitive DA responses in the striata of the two hemispheres. This possibility can be tested by a set of straightforward experiments: To mimic the autogenous DA loss in Pitx3Null mice, we can produce bilateral symmetric DA denervation in the dorsal striatum in normal adult WT mice, and then determine if L-dopa in the adult-onset DA deficient context is sufficient to trigger upward movements similar to those in the perinatal-onset DA deficient context of Pitx3Null mice. We have performed these experiments and the results are reported below.

2. Results

2.1. *Asymmetric rotation in wild type mice with 6-OHDA-induced unilateral DA denervation*

For comparing with our new observation described below, here we reproduced the classic unilateral 6-OHDA lesion model (Schwarting and Huston, 1996; Thiele et al., 2011; Ungerstedt, 1971). The surgery and the procedure to inject 6-OHDA into the right median forebrain bundle (MFB) of the mouse are described below in Section 4.2.1. After 2 weeks of recovery from the lesion surgery, mice ($n=5$) were tested for their motor response to L-dopa [10 mg/kg+5 mg/kg benserazide, injected intraperitoneally (IP)]. Although already complex, the response to the first dose of L-dopa is more directly related to the DA loss-induced DA receptor supersensitivity (Li and Zhou, 2013); whereas, after repeated L-dopa injection, additional complex processes such as priming are also activated (Calabresi et al., 2010; Ding et al., 2011; Nadjar et al., 2009; Won et al. 2014). Thus, this study focused on the motor response to the first injection of L-dopa.

During the baseline, the 5 mice that received an injection of 6-OHDA into their right MFB displayed some ipsilateral (ipsiversive) turning toward the lesioned side, but no other overt abnormality in their natural locomotion, fully consistent with the literature (Schwarting and Huston, 1996). To test

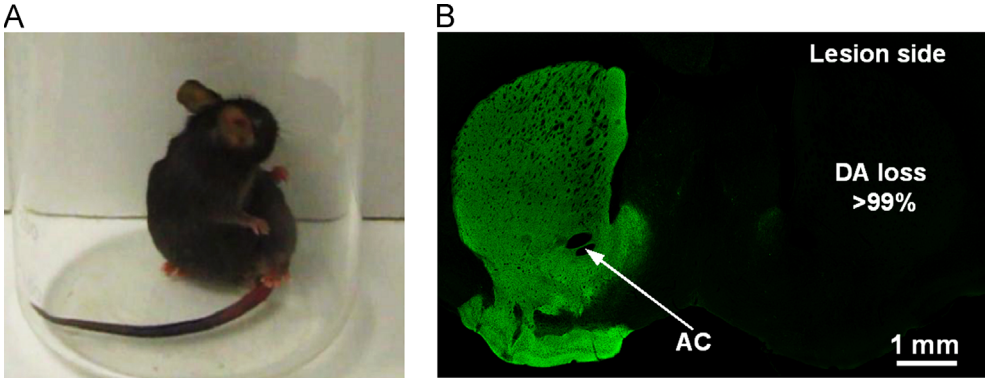

Fig. 1 – Unilateral MFB 6OHDA injection-induced DA lesion induces motor function asymmetry in Pitx3WT mice. (A) The 1st injection of 10 mg/kg L-dopa induced rotations toward the DA-intact left side in the Pitx3WT mice ($n=5$) with a right MFB 6-OHDA injection. The mouse was in a glass cylinder. (B) A confocal image of 4 μm optical section showing the dense DA axons as labeled by tyrosine hydroxylase (TH) immunostain in the left striatum in a Pitx3WT mouse, whereas almost all DA axons are lost in the right striatum due to the unilateral right MFB 6-OHDA injection. AC, anterior commissure.