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Research Report

Multiscale imaging characterization of dopamine transporter knockout mice reveals regional alterations in spine density of medium spiny neurons

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ABSTRACT

The dopamine transporter knockout (DAT KO) mouse is a model of chronic hyperdopaminergia used to study a wide range of neuropsychiatric disorders such as schizophrenia, attention deficit hyperactivity disorder (ADHD), drug abuse, depression, and Parkinson's disease (PD). Early studies characterizing this mouse model revealed a subtle, but significant, decrease in the anterior striatal volume of DAT KO mice accompanied by a decrease in neuronal cell body numbers (Cyr et al., 2005). The present studies were conducted to examine medium spiny neuron (MSN) morphology by extending these earlier reports to include multiscale imaging studies using correlated light microscopy (LM) and electron microscopy (EM) techniques. Specifically, we set out to determine if chronic hyperdopaminergia results in quantifiable or qualitative changes in DAT KO mouse MSNs relative to wild-type (WT) littermates. Using Neurolucida Explorer's morphometric analysis, we measured spine density, dendritic length and synapse number at ages that correspond with the previously reported changes in striatal volume and progressive cell loss. Light microscopic analysis using Neurolucida tracings of photoconverted striatal MSNs revealed a highly localized loss of dendritic spines on the proximal portion of the dendrite (30 μm from the soma) in the DAT KO group. Next, thick sections containing MSN dendritic segments located at a distance of 20–60 μm from the cell soma, a region of the dendrite where spine density is reported to be the highest, were analyzed using electron microscope tomography (EMT). Because of the resolution limits of LM, the EM analysis was an extra measure taken to assure that our analysis included nearly all spines. Spine density measurements collected from the EMT data revealed only a modest decrease in the DAT KO group (n=3 mice) compared to age-matched WT controls (n=3 mice), a trend that supports the LM findings. Finally, a synaptic quantification using unbiased stereology did not detect a difference between DAT KO mice (n=6 mice) and WT controls (n=7 mice) at the EM level, supporting

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the focal nature of the early synaptic loss. These findings suggest that DAT KO mice have MSNs with highly localized spine loss and not an overall morphologically distinct cell shape. The characterization of morphological changes in DAT KO mice may provide information about the neural substrates underlying altered behaviors in these mice, with relevance for human neurological disorders thought to involve altered dopaminergic homeostasis. Results from this study also indicate the difficulty in correlating structural changes across scales, as the results on fine structure revealed thus far are subtle and non-uniform across striatal MSNs. The complexities associated with multiscale studies are driving the development of shared online informatics resources by gaining access to data where it is being analyzed.

1. Introduction

The dopamine transporter knockout (DAT KO) mouse lacks the plasma membrane dopamine transporter (DAT) protein that allows cells to recycle dopamine (DA) from extracellular space (Giros et al., 1996). As a result, DA clearance from the synaptic cleft is greatly reduced, resulting in a 5-fold increase in extracellular levels of DA (Gainetdinov et al., 1998) but a marked reduction of DA in vesicles of DA neurons (Jones et al., 1998). In this mouse model, extracellular DA availability is high in the striatum and can lead to prolonged activation of DA receptors or altered pre- and post-synaptic DA receptor expression and function (Dumartin et al., 2000; Fauchey et al., 2000; Giros et al., 1996; Jones et al., 1998). Dopamine receptor-mediated behavioral abnormalities exhibited by the DAT KO mouse include elevated baseline locomotor activity (Giros et al., 1996), prepulse inhibition deficits of the startle response (Ralph et al., 2001), and deficits in executive function (Morice et al., 2007). The behavioral disturbances exhibited by these mice may reflect underlying differences in their neurocircuitry, as a result of their chronically elevated extracellular DA levels. Accordingly, striatal cell loss (Cyr et al., 2005) as well as changes in projections from the prefrontal cortex to the mesocortical limbic system have been observed (Zhang et al., 2010) suggesting a functional reorganization of striatal cell circuits.

DAT KO mice have a 5-fold increase in basal extracellular DA concentrations (Gainetdinov et al., 1998; Jones et al., 1998) and exhibit 5–6 times more locomotor activity than wild-type (WT) animals (Giros et al., 1996). Repeated administration of cocaine or amphetamine also produces a hyperdopaminergic state in the striatum causing increases in locomotor activity, similar to the DAT KO mouse, and, thus, the DAT KO mouse is a putative animal model for long-term drug use (Jones et al., 1998). The hyperdopaminergic state induced by long-term drug use has been shown to cause robust morphological changes in the cellular networks of striatal medium spiny neurons (MSNs) (Lee et al., 2006; Robinson and Kolb, 1999). A detailed examination of striatal cell morphology in the DAT KO mouse has not previously been reported. Therefore, the present study analyzed the morphology of MSNs from the DAT KO mouse and WT littermate controls using correlated light microscopy (LM) and electron microscopy (EM) techniques to determine whether quantifiable or qualitative neuroadaptive changes in MSNs were taking place as a result of the chronic hyperdopaminergic environment that is known to exist in the striatum of this mouse model.

Although spine density measurements are typically quantified at the LM level using classical Golgi staining, photoconversion of Lucifer yellow was used in the present study to isolate a single cell and its entire dendritic field so that dendritic segments (analyzed in thick sections for EM) could be successfully mapped back to the original neuron for correlated analysis at the EM level. Furthermore, a comparison of previous LM and EM studies (Gioia et al., 1998; Harris and Stevens, 1988) on spine density demonstrates that spine density quantifications at the LM level typically underestimate spine measurements at the EM level. Because the photoconverted MSNs traced and analyzed in this study are susceptible to the same resolution limits at the LM level as classical Golgi staining, these neurons were further processed for analysis at the EM level, to ensure that many spines were not going undetected.

This project is an extension of earlier studies examining magnetic resonance imaging (MRI) volumes of DAT KO animals. These studies reported that a decrease in the volume of the anterior striatum in DAT KO mice correlated to a loss in neuron number as compared to age-matched controls (Cyr et al., 2005). Such changes at the cellular level suggest that alterations in the striatal microcircuitry may also be occurring. This reduction in neuron numbers could translate into changes in dendritic sprouting, synapse number, and overall synaptic connectivity known to accompany alterations in cell morphology (Holtmaat et al., 2008; Knott et al., 2006).

Changes in cell morphology can also come about as a result of increases in DA neurotransmission (Lee et al., 2006). Giros et al. previously reported that extracellular DA persists 100 times longer in DAT KO mice (1996). Therefore, prolonged alterations in DA receptor expression and/or function likely persist as well. In fact, some DA receptors target synaptic scaffolding proteins and other structurally-related cytoskeletal proteins (Allen et al., 2006). Thus, prolonged DA availability in DAT KO mice could potentially induce changes in cell morphology in DA-rich brain areas, such as the striatum.

Based on the previous reports of cellular changes in the DAT KO mouse, and the effects of DA on dendritic structures mediating synaptic transmission in striatal MSNs, we conducted a detailed structural investigation of MSNs using correlated LM and EM to investigate whether quantitative or qualitative alterations in MSN spine density, dendritic length