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DIFFERENTIAL DEGRADATION OF MOTOR DEFICITS DURING GRADUAL DOPAMINE DEPLETION WITH 6-HYDROXYDOPAMINE IN MICE

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Abstract—Parkinson's disease (PD) is a movement disorder whose cardinal motor symptoms arise due to the progressive loss of dopamine. Although this dopamine loss typically progresses slowly over time, currently there are very few animal models that enable incremental dopamine depletion over time within the same animal. This type of gradual dopamine depletion model would be useful in studies aimed at the prodromal phase of PD, when dopamine levels are pathologically low but motor symptoms have not yet presented. Utilizing the highly characterized neurotoxin 6-hydroxydopamine (6-OHDA), we have developed a paradigm to gradually deplete dopamine levels in the striatum over a user-defined time course – spanning weeks to months – in C57BL/6 mice. Dopamine depletions were achieved by administration of five low-dose injections (0.75 μg) of 6-OHDA through an implanted intracranial bilateral cannula targeting the medial forebrain bundle. Levels of dopamine within the striatum declined linearly with successive injections, quantified using tyrosine hydroxylase immunostaining and high-performance liquid chromatography. Behavioral testing was carried out at each time point to study the onset and progression of motor impairments as a function of dopamine loss over time. We found that spontaneous locomotion, measured in an open field, was robust until ~70% of striatal dopamine was lost. Beyond this point, additional dopamine loss caused a sharp decline in motor performance, reaching a final level comparable to that of acutely depleted mice. Similarly, although rearing behavior was more sensitive to dopamine loss and declined linearly as a function of dopamine levels, it eventually declined to levels similar to those seen in acutely depleted mice. In contrast, motor coordination, measured on a vertical pole task, was only moderately impaired in gradually depleted mice, despite severe impairments observed in acutely depleted mice. These results demonstrate the importance of the temporal profile of dopamine loss on the magnitude and progression of behavioral impairments. Our gradual depletion model thus establishes a new paradigm with which to study how circuits respond and adapt to dopamine loss over time, information which could uncover important cellular events during the prodromal phase of PD that ultimately impact the presentation or treatability of behavioral symptoms.

Key words: basal ganglia, Parkinson's disease, gradual depletion, compensation.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease characterized by a progressive loss of dopamine neurons in the substantia nigra pars compacta (SNc) (Fearnley and Lees, 1991; Morrish et al., 1996; Damier et al., 1999). This results in decreased dopamine signaling in the striatum, a major input nucleus of the basal ganglia involved in the control of voluntary movement (Marsden and Obeso, 1994; Mink, 1996; Olanow and Tatton, 1999; Dauer and Przedborski, 2003). Motor symptoms such as tremors, rigidity, bradykinesia, freezing, and balance instability typically do not become overt enough to diagnose PD until dopaminergic loss exceeds 70% in the striatum (Bernheimer et al., 1973; Riederer and Wuketich, 1976; Betarbet et al., 2002; Deumens et al., 2002; Fahn, 2003). Unfortunately by this time, patients have likely been living with chronically low levels of dopamine for years and dysfunction in neural circuits may have already passed a point of no return. It has been proposed that the pre-symptomatic phase of PD, called the prodromal phase, is an ideal time to begin therapies (Schapira and Tolosa, 2010; Olanow and Obeso, 2012). Treatments administered prior to complete dopamine loss may prevent further neurodegeneration or delay the onset of motor deficits. In addition, further understanding of how and when motor systems begin to break down during this phase may lead to techniques for early detection and more effective therapies aimed at restoring circuit function (Little and Brown, 2014).

Current standard animal models of PD typically involve acute, rapid degeneration of dopamine neurons which does not recapitulate PD disease progression (for reviews see Betarbet et al., 2002; Schober, 2004; Bove et al., 2005; Terzioglu and Galter, 2008; Hisahara and Shimohama, 2010). These models preclude the

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Abbreviations: 3-MT, 3-methoxytyramine; 6-OHDA, 6-hydroxydopamine; ANOVA, analysis of variance; DOPAC, 3,4-dihydroxyphenylacetic acid; HPLC, high-performance liquid chromatography; HVA, homovanillic acid; MFB, medial forebrain bundle; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NE, norepinephrine; PD, Parkinson's disease; SNc, substantia nigra pars compacta; TDL, turn down latency; TH, tyrosine hydroxylase.

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development of pathogenic mechanisms and prevent studies of various stages of PD. This need for chronic models of dopamine degeneration has led to an increase in the number and availability of genetic models of PD, but only 5% of human PD cases are inherited and these models come with their own set of limitations, discussed at length in a number of reviews (Betarbet et al., 2002; Dauer and Przedborski, 2003; Chesselet et al., 2008; Meredith et al., 2008; Terzioglu and Galter, 2008; Dawson et al., 2010; Potashkin et al., 2010). In contrast to genetic strategies, well-characterized neurotoxin models, and more recently, AAV-induced overexpression of α-synuclein (Decressac et al., 2012a,b, Lundblad et al., 2012), have been adapted to create alternative chronic models of dopamine depletion (Greenamyre et al., 2003; Fleming et al., 2005; Meredith et al., 2008; Goldberg et al., 2011; Goldberg et al., 2012). However, models using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) require increased safety precautions when handling mice which has proven to be a deterrent for widespread application of these models. Furthermore MPTP has had questionable toxicity in various mouse strains and does not always result in persistent and progressive motor symptoms (Bezard et al., 1997a,b; Schwarting et al., 1999; Przedborski et al., 2001; Betarbet et al., 2002; Meredith et al., 2002; McNaught et al., 2004; Schober, 2004; Blume et al., 2009; Blesa et al., 2010). Other adapted models utilize 6-hydroxydopamine (6-OHDA), the neurotoxin used in the first animal model of PD associated with SNc dopaminergic neurodegeneration (Ungerstedt, 1968). In most models utilizing 6-OHDA, animals are dopamine depleted unilaterally or given various acute doses to model different stages (Przedborski et al., 1995, Schwarting and Huston, 1996a,b, Kirik et al., 1998; Ferro et al., 2005; Fleming et al., 2005; Truong et al., 2006). While dopamine loss in human PD can be asymmetrical, it ultimately results in dopamine loss in both hemispheres (Deumens et al., 2002; Simola et al., 2007). There are also concerns regarding contralateral compensation in unilateral depletions and lack of compensatory mechanisms in using acute doses to model different stages of the disease (Schwarting and Huston, 1996a, Meredith and Kang, 2006; Potashkin et al., 2010). Thus, there is lack of behavioral data available for the prodromal phase, where dopamine is being depleted bilaterally and gradually within the same animal.

In this study, we adapted the traditional 6-OHDA model to produce a gradual, bilateral dopamine loss over a user-defined time course. By administering low doses of 6-OHDA bilaterally through an intracranial cannula targeting the medial forebrain bundle (MFB), we gradually depleted dopamine levels within the same animal over 2–7 weeks rather than 2–3 days. Using this technique, we were able to slowly deplete dopamine over a prolonged time course to study the effects of gradual vs. acute depletion on the onset and progression of motor impairments. We found that certain aspects of motor behavior are altered differentially as dopamine is depleted. Furthermore, some behaviors were differentially affected in gradually vs. acutely depleted mice, suggesting the engagement of compensatory plasticity during gradual depletion that is not engaged with the more traditional, acute paradigm. This study demonstrates that the time course of dopamine loss can influence the final behavioral state of the animal and provides a paradigm with which to study how motor systems adapt to chronically low levels of dopamine over time.

EXPERIMENTAL PROCEDURES

Animals

Experiments were conducted in accordance with the guidelines from the National Institutes of Health and with approval from Carnegie Mellon University Institutional Animal Care and Use Committee. Male and female P30–P80-day-old mice on a C57BL/6J background were used for experiments. Acutely depleted animals were P42–P59 at the time of injection and were P47–P81 when they completed their last behavioral testing. Gradual (3 day) depleted animals and littermate saline controls were P34–P61 at the time of their first injection and were P44–P80 when they completed their last behavioral testing. Gradual (7 day) depleted animals and littermate controls were P41 at the time of their first injection and were P90 when they completed their last behavioral testing. After surgical implantation of the cannula, animals were housed separately to prevent damage to the cannula. Animals were provided with dishes of crushed high-fat food pellets moistened with water, additional hard food pellets on the floor of the cage, as well as access to a water bottle. All cages were placed on half-on/half-off heating pads following surgery and each subsequent infusion of 6-OHDA. Cages remained on heating pads unless animals were observed resting mainly in the unheated portion of the cage. Each infusion of saline or 6-OHDA was performed while animals were lightly anesthetized on a heating pad, and all animals were injected with 0.1 cc of saline i.p. before being returned to their home cage. Animals' weights were tracked regularly and extra i.p. saline and softened food or trail mix were provided to encourage weight gain and proper hydration when appropriate.

Implantation of bilateral cannulas and 6-OHDA injection in the MFB

Under ketamine/xylazine (100 mg/kg: 30 mg/kg, i.p.) anesthesia, the animals were placed on a stereotaxic frame (David Kopf Instruments, Tujunga, California, USA) and maintained throughout surgery using 1–2% isoflurane. Bilateral internal cannulas (Plastics One, Roanoke, Virginia, USA) cut to target ±1.1 mm lateral and −5.0 mm ventral were implanted 0.45 mm posterior to bregma and secured using superglue. 6-OHDA was prepared at a concentration of 5 μg/μL in 0.9% NaCl for acute depletions and diluted further with 0.9% NaCl to 0.75 μg/μL for gradual depletions (Sigma–Aldrich H116 6-OHDA hydrobromide). Injections were performed using a 33-gauge cannula (Plastics One, Roanoke, Virginia, USA) attached to a 10 μL Hamilton syringe within a