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ENRICHED ENVIRONMENT PROMOTES SIMILAR NEURONAL AND BEHAVIORAL RECOVERY IN A YOUNG AND AGED MOUSE MODEL OF PARKINSON'S DISEASE

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Abstract—Environmental enrichment has been shown to be neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease (PD). Because PD patients are not typically diagnosed until later neuropathological stages, the current study investigated the capacity of an enriched environment (EE) to stimulate restoration of neurons in the substantia nigra pars compacta (SNpc) and locomotor recovery after lesioning, as opposed to before. A low-dose chronic MPTP regimen was used to achieve a partial, less severe lesion of the nigrostriatal pathway not seen in acute MPTP models. Both young adult (10 weeks) and aged (12 months) C57BL/6J male mice were used to assess the effects of aging on recovery with EE intervention. After the first week of either MPTP (7 mg/kg/d in young; 5 mg/kg/d in aged) or saline injection, animals from both groups were housed in a standard environment (SE) or an EE for 3 weeks, with continued daily administration of MPTP. We are the first to report that following 3 weeks exposure to an EE, young and aged MPTP-lesioned mice showed a significant 53% and 52% restoration of tyrosine hydroxylase (TH)-labeled neurons in the SNpc, respectively. This increase in TH-labeled cells in the MPTP+EE group was correlated with recovery of free-standing rear (FSR) behavior in both age groups; however, improved locomotor control as measured by foot faults (FF) per total activity was only seen in the aged MPTP+EE group. Our data demonstrate that an EE promotes neurorestoration in TH protein expression in SNpc neurons as well as some locomotor recovery in both young and aged animals in this mouse model of PD. Published by Elsevier Ltd on behalf of IBRO.

Key words: enriched environment, aging, Parkinson's disease, dopamine, MPTP, neurorestoration.

Parkinson's disease (PD) is a progressive, neurodegenerative condition associated with the specific loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc) resulting in the dramatic decrease in the levels of this neurotransmitter within the striatum. The consequence of this loss of activity in the nigrostriatal pathway is an impairment of locomotor control exhibited in tremor, poorly coordinated movement and bradykinesia. Previous studies have investigated the amelioration of the above symptoms through pharmacotherapy and transplantation of nigral dopaminergic neurons (Geraerts et al., 2007; Van Kampen and Eckman, 2006; Piccini et al., 1999). However, these methods of neuron replacement can be short-lasting, and might be accompanied by severe side effects such as dyskinesias (Jenner, 2008; Olanow et al., 2003; Freed et al., 2001). Their immediate effects, though, suggest the tremendous capacity for plasticity and augmented repair of the SNpc in animal models of PD. Therefore, it is important to investigate non-invasive interventions as stimulants of this plasticity.

It has been shown that previous exposure for up to several months to an enriched environment (EE) is neuroprotective, reducing the loss of DA neurons in the SNpc of several animal models of PD (Bezard et al., 2003; Faherty et al., 2005). However, no studies have been reported to date on the potential of an EE to stimulate SNpc plasticity post-lesioning. Because patients have already lost at least 60% of the DA neurons by the earliest stages of diagnosis (Mori et al., 2006; Pakkenberg et al., 1991) it is important to investigate the restorative effects of an EE following the loss of nigrostriatal DA. Therefore, in the current study, we hypothesized that subsequent exposure to an EE following administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) would result in partial restoration of DA neurons in the SNpc, as measured by the number of tyrosine hydroxylase-immunoreactive (TH-ir) neurons.

It is established that, in general, older individuals have a higher rate of developing PD than younger individuals (Quinn et al., 2004; Mayeux et al., 1988). It is also known that DA SNpc neurons in older mice are more sensitive to the toxic effects of MPTP than those of younger mice (Ricaurte et al., 1987b; Jarvis and Wagner, 1985), and do not recover from acute doses over time as do younger mice (Ricaurte et al., 1987a). This is reflective of the slower rate at which young-onset Parkinson's patients develop motor symptoms than older patients (Schrag and Schott, 2006; Alves et al., 2005), suggesting that age is an important factor in nigrostriatal vulnerability. Therefore, we speculated in this study that the MPTP-induced locomotor symptoms would be more severe in aged mice, and that

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Abbreviations: ANOVA, analysis of variance; BB, beam breaks; DA, dopamine; EE, enriched environment; FF, foot faults; FSR, free-standing rears; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PRAC, parallel rod activity chamber; SE, standard environment; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-ir, tyrosine hydroxylase-immunoreactive; Veh, vehicle animals.

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restorative effects of an EE would be less robust than in younger mice.

Many acute models present limitations such as rapid neurodegeneration, which does not allow for development of pathological mechanisms characteristic of this progressive disease, and which does allow the compensatory recovery of striatal DA over time (Chesselet et al., 2008; Schneider et al., 2008; Ricaurte et al., 1987a,b). However, variations of chronic exposure have been the only models to replicate multiple pathological and motor aspects of human Parkinsonism in a single model (Schintu et al., 2009; Fornai et al., 2005; Petroske et al., 2001; Bezard et al., 2003). The partial lesion achieved by sub-chronic MPTP allows for the study of an early rather than late stage model, as seen in the 6-hydroxydopamine and acute MPTP models of PD. We have used a similar, but higher dose sub-chronic MPTP model in which animals receive daily injections for the duration of the experiment, based on an earlier study by Bezard et al. (1997).

In the present study, we examined the effects of 3 weeks of EE on the mean number of TH-ir neurons/section in the SNpc of both young adult (10 weeks) and aged (12 months) C57BL/6J male mice, starting 1 week following daily administration of MPTP. The effects of MPTP and subsequent exposure to an EE on exploratory and motor behavior were quantified by changes in free-standing versus wall-assisted rears in a cylinder. Motor deficits and amelioration were assessed with an additional behavioral task, the Parallel Rod Activity Chamber (PRAC) test, as modified from a previously described ethanol sensitivity apparatus (Kamens and Crabbe, 2007). Our results are the first to show that both the decrease in the number of TH-ir labeled neurons/section in the SNpc and motor impairments following daily treatment with MPTP are partially restored by the third week of exposure to an EE. These observations are discussed with respect to the capacity of an EE intervention to reverse deficits in young adult as compared to aged mice.

EXPERIMENTAL PROCEDURES

Animals and environments

Young adult (10 weeks) and aged (12 months) male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA) had *ad libitum* access to standard lab chow and water, and were maintained on a 12:12 light-dark cycle. Each treatment group was comprised of $n=7–8$ (young adult), or $n=20–23$ (aged) male mice. All procedures were approved by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals, and conducted at the Portland Veterans Affairs Medical Center. The standard environment (SE) housing was comprised of a standard lab cage with four animals in each. The EE was arranged as previously reported (Cao et al., 2010; Bezard et al., 2003; Van Praag et al., 2000). In brief, the EE consisted of a large cage ($53.6\times43.9\times37.6$ cm^3) equipped with regularly exchanged toys (e.g. ladders, running wheels, balls, jungle gyms rotated between cages every 3 days) to promote gross motor and sensorimotor activities, and housed 8–10 animals each. It should be noted that the social and novel physical components of an EE have not yet been shown to have discrete or additive effects, but we are currently conducting experiments to address that question. Following 1 week of daily MPTP or vehicle injections, mice were housed in either the SE or EE for 3 weeks, 24 h/d, 7 d/week.

Parkinsonian model

In order to develop a sub-chronic rodent model displaying partial loss of TH-ir neurons in the SNpc, the DA neuron sensitive toxin, MPTP (Sigma Aldrich, St. Louis, MO, USA) was administered following the acquisition of baseline behaviors (see below). We have found, consistently with previous reports, that C57Bl/6J mice tolerate half to two-thirds the dose of MPTP when over 6 months old (Irwin et al., 1992). Therefore, the doses were 7 mg/kg in normal saline, i.p., young adult; and 5 mg/kg, i.p., aged. The drug was administered once daily, 5 days/week followed by 2 days off for a total of 4 weeks. Vehicle animals (Veh) were injected with vehicle (0.1 ml/kg in normal saline). Treatment groups were: Veh+SE, Veh+EE, MPTP+SE and MPTP+EE. The degenerative progression was allowed to proceed for 7 days prior to exposure to an EE.

Tyrosine hydroxylase immunohistochemistry

Samples were taken from 3 days after week 1 of MPTP or vehicle injection to assess initial TH-ir before treatment, and 3 days following the last day of EE exposure. All animals euthanized for immunohistochemical analysis at week 4 were also behaviorally tested; because a power showed that a larger number of animals was needed for the behavioral tests than for immunohistochemistry (IHC), half of the behaviorally tested animals were used for further analysis that was not reported in the current study. Animals ($n=6–8$ per group) were anesthetized with ketamine/xylazine (2.0 ml/0.1 kg, I.P; Sigma, St. Louis, MO, USA), and euthanized by transcardial perfusion with 1000 U/mL of heparin (APP Pharmaceuticals, LLC, Germany) in 0.1 M phosphate buffer (3 ml total) followed by fixative [2% paraformaldehyde, 1% acrolein in 0.1 M phosphate buffer; pH 7.4; ~35–50 ml]. Brains were removed and washed for 24 h in 0.1 M phosphate buffer at 4 °C, followed by cutting the SNpc in 70 μm thick sections using a vibratome (Ted Pella Inc., Redding, CA, USA). Alternate sections of the rostrocaudal extent of the SNpc were incubated in 10 mM sodium citrate (pH 6) for 5 min at 500 W for antigen retrieval, using a PELCO BioWave® Pro microwave (Ted Pella Inc.). Sections were incubated with 1% sodium borohydride in phosphate buffer for 30 min, then for 1 h in blocking solution [10% goat serum/0.5% Triton-X 100/0.1 M phosphate buffer, pH 7.4] at room temperature, followed by TH antibody (1:20,000; Immunostar, Hudson, WI, USA # 22941 monoclonal) at 4 °C overnight. The following incubations were carried out in the PELCO microwave between 150 and 200 W: sections were washed for 2 min in 0.1 M phosphate buffer, incubated with biotinylated goat anti-mouse 2 antibody (1:250; Vector, Burlingame, CA, USA) for 12 min under vacuum, and washed with avidin-biotin complex (ABC, diluted according to manufacturer instruction; Vector) solution for 12 min under vacuum. Sections were then stained with Diaminobenzidine (DAB kit, Vector), mounted on gel-coated slides, and cover-slipped using Pro-Texx® medium (Lerner, Pittsburgh, PA, USA). Tissue from all treatment groups was processed on the same day, and all reacted with DAB for the same length of time. TH-ir neurons only at the surface of immunolabeled SNpc tissue were counted using light microscopy (40× magnification, images analyzed using ImagePro 6.3, Media Cybernetics) by an individual blinded to the treatment group. TH-ir neurons from the left and right SNpc were averaged for each tissue section. The sections were matched anatomically in each of the animals, verifying that the coronal sections of the SNpc were similar in all treatment groups. The mean number of TH-ir neurons/section was determined for each of the six sections representing the rostrocaudal extent of the SNpc for each animal, and a grand mean was calculated for each treatment group. From these tissue section counts, the total number of labeled neurons was re-evaluated using the Abercrombie correction, which accounts for fragmented nuclei within each section and provides an accurate estimate when tissue thickness exceeds soma thickness

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