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EFFECT OF INTERMITTENT WASHOUT PERIODS ON PROGRESSIVE LESIONING OF THE NIGROSTRIATAL PATHWAY WITH 1-METHYL-2-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)

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Abstract—We have previously reported that a progressively increased dose of MPTP over the course of 4 weeks induces the gradual impairment of the nigrostriatal dopamine (DA) pathway and several behaviors [Goldberg et al. (in press) Neuroscience]. To our knowledge, this is the first report of specific behavioral deficits correlated with discrete thresholds of DA loss in this pathway. In that study, MPTP was administered 5 d/wk, with behavioral and tissue analysis being carried out 3 days following the final injection at each dose. However, in order to better represent long-term progressive neurodegeneration the present study introduced a washout period of 10 days between each increased dose of MPTP. This implementation also controlled for any transient de-activation of tyrosine hydroxylase (TH), the enzyme that catalyzes synthesis of DA, caused by MPTP-induced oxidative stress which has been suggested following acute administration of the toxin [Smeyne and Jackson-Lewis (2005) Brian Res Mol Brain Res 134:57–66]. Additionally, by the end of the previous study, there was an ultimate decrease of 62% in the mean number of TH-labeled neurons/section in the substantia nigra pars compacta (SNpc) and a 74% decrease in caudate putamen (CPu) TH optical density with continuous MPTP. In the present study, we find that the washout periods lead to a final 79% decrease in the mean number of TH-labeled SNpc neurons/section, and a similar 74% decrease in CPu TH following the 32 mg/kg MPTP dose. Additionally, a dose-dependent decrease was observed in the mean number of SNpc TH-ir neurons/section in the current study which was not seen in the continuous MPTP protocol. These results suggest that a washout period following each increased MPTP dose allows for observation of continued cell death that might occur during the week following MPTP administration, and for therapeutic interventions to be applied at any of several stages during progressive neurodegeneration. Published by Elsevier Ltd on behalf of IBRO.

Key words: dopamine, progressive, MPTP, tyrosine hydroxylase, motor control, Parkinson's disease.

Several toxin- and gene-based murine models of Parkinson's disease are commonly used to explore therapeutic interventions (Potashkin et al., 2010; Meredith and Kang, 2006). Sub-acute and chronic models using MPTP currently achieve the most accurate representations of Parkinson's disease (PD) in terms of pathology and long-term decline (Blume et al., 2009; Prediger et al., 2010; Meredith et al., 2002; Bezard et al., 2001). However, none of these models have reported progressive behavioral decline correlated with distinct thresholds of dopamine (DA) loss in the nigrostriatal pathway. In fact, it has been suggested that mice become tolerant to a continuous low dose of MPTP over time (Bezard et al., 1997). Therefore, we reported in a previous study using progressively increased MPTP dosing over 4 weeks (4 mg/kg, 8 mg/kg, 16 mg/kg, and 32 mg/kg MPTP), the gradual and correlated loss of nigrostriatal DA and several behavioral measures (Goldberg et al., in press). This evidence suggests that mice do not become tolerant to MPTP over time if the dose is incrementally increased.

However, several issues arise with the weekly increase of daily-administered MPTP. It has been suggested that tyrosine hydroxylase (TH), the enzyme that converts 3,4-dihydroxyphenylalanine (DOPA) to DA, can be transiently de-activated in the substantia nigra pars compacta (SNpc) by mechanisms of oxidative stress which are stimulated by MPTP (Smeyne and Jackson-Lewis, 2005). Since continuously increased MPTP (Goldberg et al., in press) did not result in decreased DA content in the caudate putamen (CPu) until a dose of 16 mg/kg was administered, whereas TH-immunoreactivity (TH-ir) was decreased after 4 mg/kg MPTP, de-activated TH may have interfered with interpretation of nigrostriatal pathology. Therefore, in the current study, a washout period of 10 days was introduced following each week of MPTP.

Additionally, the results of this study address the effect of time on the degeneration of neurons following MPTP (Przedborski et al., 2001; Jackson-Lewis et al., 1995), and introduce a long-term progressive nigrostriatal lesioning paradigm. The mean number of TH-ir neurons/section of the SNpc and TH-ir optical density in the CPu were assessed following the 10-day washout period following each week of increased MPTP. At the same intervals, rearing behavior was assessed. These measurements were correlated and compared to the results of the continuous progressive MPTP study.

EXPERIMENTAL PROCEDURES

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. In this study, 10 week old C57Bl/6J male mice were administered pro-

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Abbreviations: CPu, caudate putamen; DA, dopamine; FSR, free-standing rears; PD, Parkinson's disease; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-ir, TH-immunoreactivity.

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gressively with increased doses of MPTP (4 mg/kg, 8 mg/kg, 16 mg/kg, and 32 mg/kg base; Sigma Aldrich, St. Louis, MO, USA) or vehicle (Veh) over the course of 8 weeks. Following 5 days of each daily dose, there was a 10 day washout period. Rearing behavior was tested at the end of each washout period in order to assess behavioral impairment due to lesioning and not resulting from any transient effects of the toxin. A sub-group of mice (n=5 ea.) were euthanized by transcardial perfusion with fixative following each dose+washout period.

Immunohistochemistry

Animals were anesthetized with 1% ketamine/0.1% mg xylazine (2.0 ml/0.1 kg, i.p.), and perfused with 1000 U/ml of heparin 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]. Consecutive 70 μm thick sections were cut using a vibratome (Ted Pella Inc., Redding, CA, USA) through the SNpc (beginning at Bregma −2.9) and CPu (beginning at +1.2), according to Paxinos and Franklin (2004). SNpc sections were counterstained with Cresyl Violet following TH-immunolabeling. Antigen retrieval, TH antibody staining (1:20,000; Immunostar, Hudson, WI, USA cat. #22941, monoclonal), and analysis were carried out as previously reported (Goldberg et al., 2011). TH-ir neurons only at the in-focus surface plane of immunolabeled SNpc tissue were counted using light microscopy (40× magnification, images analyzed using ImagePro 6.3, Media Cybernetics, Bethesda, MD, USA) by an individual blinded to the treatment group (Fig. 1A). The number of TH-ir neurons from the left and right SNpc sections were averaged for each animal for all six sections analyzed since both sides of the SNpc are affected following systemic administration of this neurotoxin. Mean numbers of TH-ir neurons/section were then determined for all animals in a given treatment group. TH-ir neurons within the SNpc were easily delineated from the few, if any, TH-ir neurons in the underlying substantia nigra pars

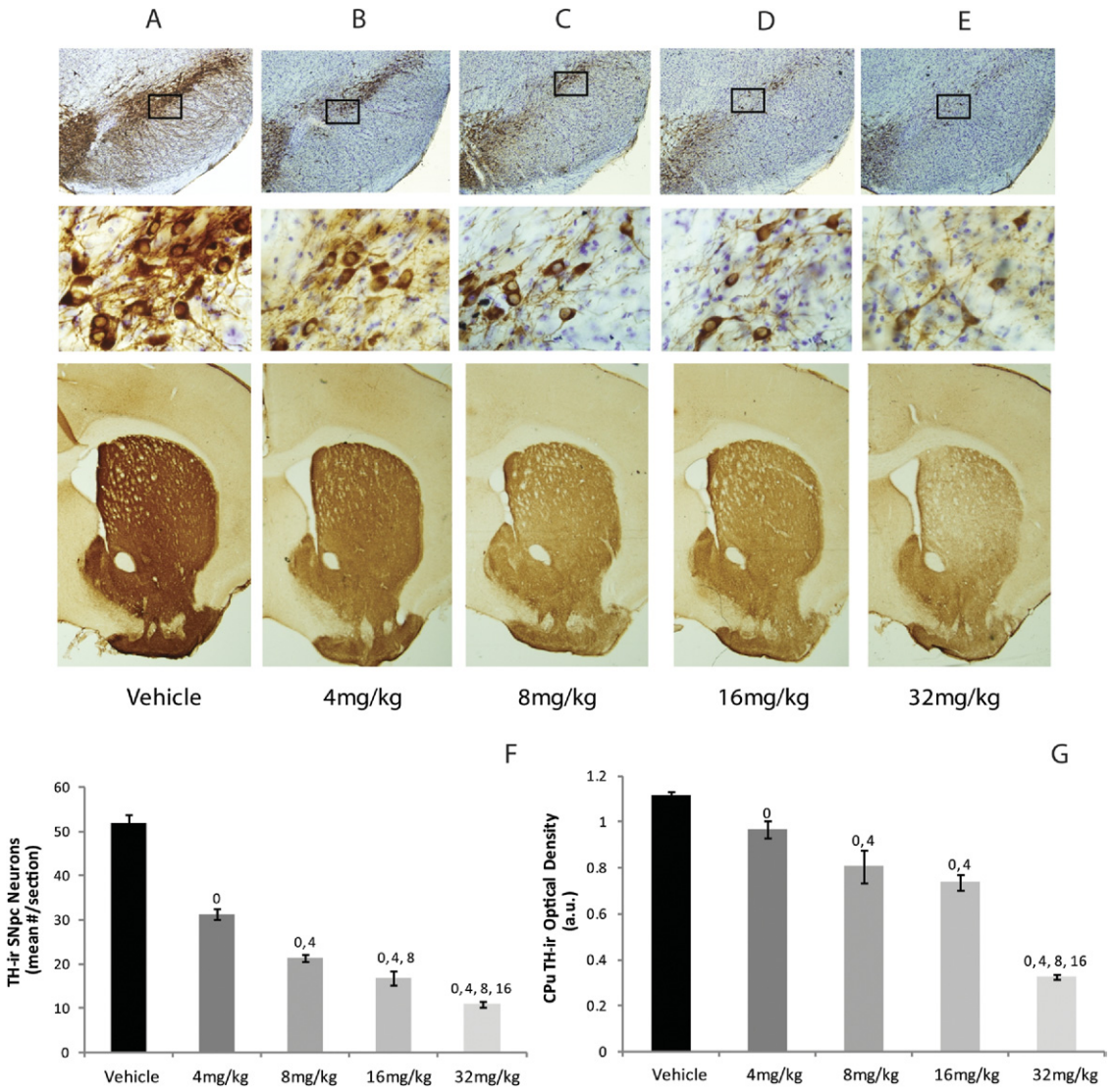

Fig. 1. Effects of progressively increased MPTP with intermittent washout periods on SNpc TH-ir neurons and CPu TH-ir terminals. Progressive loss of TH-ir is shown in the SNpc at 10× (A–E, first row) and 40× magnification (A–E, second row), and in the CPu at 5× magnification (A–E, third row). SNpc TH-ir decreased in a dose dependent manner (F), while CPu TH-ir decreased following 4 mg/kg, 8 mg/kg, and 32 mg/kg MPTP (G). Optical density is in arbitrary units (a.u.). Values are means±SEM. ^{0} P<0.02 compared to vehicle, ^{4} P<0.01 compared to 4 mg/kg, ^{8} P<0.01 compared to 8 mg/kg, ^{16} P<0.0001 compared to 16 mg/kg MPTP.