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Brain Research

Research Report

In vivo measures of nigrostriatal neuronal response to unilateral MPTP treatment

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ARTICLE INFO

Article history:
Accepted 9 May 2014
Available online 15 May 2014

Keywords:
Nigrostriatal
MPTP
Striatal terminal field
PET

ABSTRACT

A single unilateral intracarotid infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into non-human primates causes injury to the nigrostriatal pathway including nigral cell bodies, axons and striatal terminal fields. In this model, motor parkinsonism correlates well with the loss of nigral dopaminergic cell bodies but only correlates with *in vitro* measures of nigrostriatal terminal fields when nigral cell loss does not exceed 50%. The goals of this study are to determine the relationship of motor parkinsonism with the degree of injury to nigrostriatal axons, as reflected by *in vitro* fiber length density measures, and compare *in vivo* with *in vitro* measures of striatal terminal fields. We determined axon integrity by measuring fiber length density with tyrosine hydroxylase (TH) immunohistology and dopamine transporter (DAT) density with DAT immunohistology. We then calculated the terminal arbor size and compared these measures with previously published data of quantified *in vivo* positron emission tomography (PET) measures of presynaptic dopaminergic neurons, autoradiographic measures of DAT and vesicular monoamine transporter type 2 (VMAT2), striatal dopamine, nigral cell counts, and parkinsonian motor ratings in the same animals. Our data demonstrate that *in vivo* and *in vitro* measures of striatal terminal fields correlate with each other regardless of the method of measurement. PET-based *in vivo* striatal measures accurately reflect *in vitro* measures of DAT and VMAT2. Terminal arbor size and other terminal field measures correlate with nigral TH immunoreactive (TH-ir) cell counts only when nigral TH-ir cell loss does not exceed 50%. Fiber length density was the only striatal measure that linearly correlated with motor ratings (Spearman: $r=-0.81$, $p<0.001$, $n=16$).

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http://dx.doi.org/10.1016/j.brainres.2014.05.009
0006-8993/

1. Introduction

A single unilateral intracarotid infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into non-human primates causes injury to the nigrostriatal pathway including nigral cell bodies, axons and striatal terminal fields that can cause stable hemiparkinsonism (Bankiewicz et al., 1986; Joyce et al., 1986; Palombo et al., 1990; Tabbal et al., 2006; Perlmutter et al., 1997). This animal model permits development and validation of neuroimaging biomarkers that may be useful for investigating humans with disorders such as Parkinson disease (PD). In particular this model permits comparisons of motor parkinsonism with the degree of injury to specific components of the nigrostriatal pathway. This may be important as the degree of nigral cell body injury may not correspond with the degree of striatal terminal field injury. For example, various parts of nigrostriatal pathway in PD patients are differentially involved (Burke and O'Malley, 2013; Kordower et al., 2013). In addition, in nonhuman primates given varying doses of intracarotid MPTP, motor parkinsonism correlates well with the loss of nigral dopaminergic cell bodies (Tabbal et al., 2012) but only correlates with measures of nigrostriatal terminal fields when nigral cell loss does not exceed 50%. Striatal measures in those studies included dopamine concentration (Tabbal et al., 2012) and striatal uptake of three classes of radiotracers targeting presynaptic dopaminergic neurons commonly used in positron emission tomography (PET) (Karimi et al., 2013). The goals of this study are to determine the relationship of motor parkinsonism with the degree of injury to nigrostriatal axons, as reflected by *in vitro* fiber length density and dopamine transporter (DAT) varicosity density in striatum, and compare *in vivo* with *in vitro* measures of striatal terminal fields.

We used this MPTP animal model to quantify the striatal response with *in vitro* tyrosine hydroxylase (TH) immunoreactive (ir) fiber length density, DAT varicosity density and terminal arbor size. These newly measured variables were compared with previously published data from the same animals of *in vivo* PET, quantified autoradiography, striatal dopamine, nigral cell counts and motor ratings of parkinsonism (Brown et al., 2012; Karimi et al., 2013; Tabbal et al., 2012; Tian et al., 2012). This allows us to investigate whether the measures of axonal fibers fall in between the striatal terminal field and nigral cell body measures in relative to motor parkinsonism.

2. Results

All 16 monkeys completed the study successfully with a range of motor impairment. One monkey was excluded from cell count analyses due to extensive damage to the midbrain tissue during processing procedures. Coefficients of error and coefficients of variance, calculated as estimates of precision, for TH measures in striatum and nigra as well as DAT measures in striatum were all <0.1.

In vivo PET data had a tight correlation with corresponding *in vitro* measures. The nondisplaceable binding potential (BP_{ND}) of 2-beta-[^{11}C]carbomethoxy-3-beta-4-fluorophenyltropane (CFT; reflects DAT) tightly correlated with the maximum number of binding sites (B_{max}) of DAT as both drop markedly and remain low once nigral cell loss exceeds 50% (Spearman: $r=0.95$, $p<0.001$, $n=16$) (Fig. 1A). Similarly, striatal BP_{ND} of [^{11}C]dihydrotetrabenazine (DTBZ; reflects vesicular monoamine transporter type 2, VMAT2) correlated strongly with VMAT2 B_{max} (Spearman: $r=0.90$, $p<0.001$, $n=16$) (Fig. 1B). We repeated the above correlation analyses selecting only a single point from the clustered data to ensure that the correlations were not driven by these clustered points (Spearman: $r=0.88$, $p<0.001$; $r=0.86$, $p<0.001$; $n=10$).

DAT immunhistochemistry was performed to label dopaminergic terminals; the data presented in Fig. 2A and B show brain sections from post-commissural striatum that were immunostained with DAT from both unlesioned and lesioned sides. DAT density correlated with other striatal terminal field measures including CFT BP_{ND}, DTBZ BP_{ND} and influx constant (K_{occ}) for 6-[^{18}F]fluorodopa (FD; primarily reflects decarboxylase activity), striatal dopamine, quantitative autoradiographic measures of DAT B_{max} and VMAT2 B_{max} (Spearman: $r=0.90$, $r=0.92$, $r=0.92$, $r=0.88$, $r=0.9$, and $r=0.82$, correspondingly, $p<0.001$, $n=16$) (Fig. 3A–F). Note, that all correlations remained significant when including only a

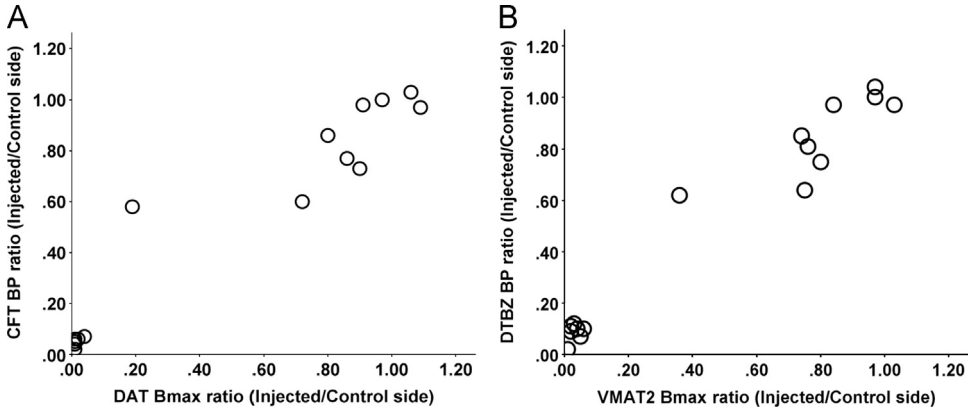

Fig. 1 – *In vivo* PET data had a tight correlation with corresponding *in vitro* measures. CFT BP_{ND} tightly correlated with DAT B_{max} (A) and striatal DTBZ BP_{ND} correlated strongly with VMAT2 B_{max} (B). The value for each monkey was expressed as the ratio of the injected side to the control side.