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Loss of the Cortical Monoamine Projection Systems in the MPTP-treated Nonhuman Primate Model of Parkinson's Disease

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Abstract

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The condition produced by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the nonhuman primate clearly models the striatal and nigral dopamine deficiency found in Parkinson's disease (PD). However, evidence supporting its ability to mimic changes outside the nigrostriatal dopaminergic system known to occur in the idiopathic disease is controversial. In order to further assess the validity of the MPTP-treated monkey model of PD, this study investigated the changes in the serotonin and catecholamine cortical projections in nonhuman primates rendered parkinsonian following chronic exposure to MPTP. Quantitative analysis of immunohistochemical staining with antibodies raised against tyrosine hydroxylase (TH) and serotonin (5HT) in various cortical areas involved in cognitive, limbic and motor functions was compared between normal and parkinsonian animals. In normal monkeys, TH and serotonin-positive axons and terminals densely innervated all cortical areas. However, in the MPTP-treated monkeys a reduction of innervation for both transmitter systems that ranged from 17.77 to 76.08 % of control values was found in all cortical regions, but the most striking denervation was found in limbic and motor areas for TH and in the motor areas for serotonin. These findings demonstrate that the pathology of monoamine networks in the MPTP-treated nonhuman primate as a model for PD extends beyond the nigrostriatal dopaminergic system.

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Introduction

Parkinson's Disease

The chronic, progressive, neurodegenerative disorder known as Parkinson's disease ranks second only to Alzheimer's disease with a prevalence of approximately 1-3% among persons over the age of sixty (Dézsi and Vécsei, 2011). Extensive investigations support the biological mechanisms behind its distinctive motor symptoms – bradykinesia, akinesia, rigidity and tremor (Rodriguez-Oroz et al., 2009). The pathophysiological model for these motor symptoms highlights increased inhibition of motor-related thalamocortical activity by basal ganglia outflow from the globus pallidus pars interna and the substantia nigra pars reticulata caused by loss of dopaminergic neurons in the substantia nigra pars compacta (Figures 1 and 2; DeLong, 1990). Although these features are likely an accurate representation of the subcortical pathology directly responsible for some of the motor disabilities, it is becoming apparent that Parkinson's disease symptomatology goes far beyond motor dysfunctions and includes a variety of cognitive and psychiatric manifestations, for example executive dysfunction, working memory impairments, depression and anxiety (Pfeiffer, 2005).

Neuropathological changes go beyond nigrostriatal dopaminergic degeneration in PD. The existence of nonmotor symptoms of PD was outlined as early as 1817 in James Parkinson's classic article, *An Essay on the Shaking Palsy*. Although lesion of the nigrostriatal dopaminergic system has long been known as the key pathological feature of PD, pathological changes of other systems was revisited in the 1980's when it was determined that, in addition to dopamine, neurotransmitter systems including norepinephrine and serotonin are not resistant to the disease's toxic effects

(Scatton et al., 1983). A number of studies demonstrated a reduction in cortical serotonin, norepinephrine and dopamine as well as significant degeneration of the brainstem nuclei (raphe nucleus, locus coeruleus and ventral tegmental area) that give rise to these cortical projections in patients with PD (Francis, 2009; Halliday et al., 1989, 1990; Hornykiewicz, 1998; Scatton et al., 1983).

Following this discovery, numerous investigations searched to connect anatomical abnormalities in the brains of PD patients and nonmotor symptoms of the disease. The main nonmotor symptoms of PD include cognitive impairment (e.g., executive, working memory and visuospatial complications), neuropsychiatric symptoms (e.g., depression, anxiety and dementia), autonomic disturbance (e.g., gastrointestinal, urogenital, and thermoregulatory dysfunction), sleep disturbances (e.g., REM sleep behavior disorder, restless leg syndrome, and sleep apnea) and olfactory dysfunctions (Ziemssen and Reichmann, 2007). Monoaminergic projections to the dorsolateral prefrontal, orbitofrontal, anterior cingulate and sensory motor cortices have been highlighted as potential sites of neurodegeneration related to cognitive impairments and psychiatric disturbances of PD (Figure 3; Brooks and Piccini, 2006). Levodopa, a precursor to dopamine that crosses the blood brain barrier, remains the gold standard motor symptomatic therapy for PD. However, its ineffectiveness in the treatment of non-dopaminergic and nonmotor symptoms suggests the existence of different pathological substrates (Dézsi and Vécsei, 2011).

Because the common nonmotor symptoms, such as executive dysfunction, working memory impairments, depression and anxiety (Pfeiffer, 2005), are thought to involve cortical projection systems, these symptoms may be mediated by supplementary

neurochemical changes to the classic dopaminergic nigrostriatal denervation described in the current pathophysiological model. Cortical neuropathology seen in idiopathic PD may potentially be involved in disease symptomotology. Therefore, data supporting parallel cortical dysfunction in animal models of the disease is required to further investigate these changes, perhaps ultimately benefiting disease diagnosis and the search for more comprehensive treatment for afflicted patients.

The neurotoxin MPTP induces parkinsonism in humans. Produced as a byproduct in the synthesis of a meperidine analogue, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was unintentionally self administered by approximately 300 drug addicts in 1982 who subsequently developed severe motor signs of parkinsonism (Langston et al., 1983). MPTP was then shown to selectively destroy dopaminergic neurons in the substantia nigra pars compacta, resulting in a pathological condition that mimicked the physiological and clinical aspects of idiopathic PD and was highly levodopa responsive (Burns et al., 1983; Langston et al., 1984, 1999). It is now clear that MPTP itself is not toxic. However, once converted to 1-methyl-4-phenylpyridinium ion (MPP^+) by monoamine oxidase B, it is primarily taken up into dopaminergic neurons by the dopamine transporter where it accumulates in mitochondria and inhibits complex I of the electron transport chain (Figure 4; reviewed by Dauer and Przedborski, 2003).

MPTP's assault to neurons in the substantia nigra pars compacta results in striatal dopamine depletion and distinct motor symptoms including bradykinesia, akinesia, and rigidity, all cardinal features of PD (Burns et al., 1983; Langston et al., 1984). Despite these similarities, many of the early case studies of MPTP-affected patients reported none

of the cognitive, behavioral and extrastriatal changes now known to occur in idiopathic PD (Burns et al., 1983). In a review, Stern stated that MPTP-induced parkinsonism better represents a pure hypodopaminergic condition rather than the more widespread neuropathologic and neurochemical changes seen in idiopathic PD (Stern, 1990). Stern and colleagues then conducted an investigation comparing cognitive changes following different levels of MPTP exposure and found that MPTP-induced parkinkinsonian and MPTP-exposed asymptomatic individuals experienced a pattern of cognitive impairment similar to that of PD patients, including deficits in intellectual function, construction, category naming and frontal lobe function. Notably, none of the patients affected with MPTP presented with any nonmotor neurological signs other than those expected for idiopathic PD (Stern and Langston, 1985). These findings suggested that MPTP-exposure resulted in nonmotor symptoms similar to those described in idiopathic PD; however, the validity of data collected from a population with variable drug history should be considered cautiously. Due to the lack of data demonstrating extrastriatal denervation following MPTP-exposure, Stern argued that these cognitive impairments were mediated primarily by the loss of dopaminergic function in the basal ganglia (Stern et al., 1990). Although controversy remained over the comparison of idiopathic PD and MPTP-induced parkinsonism, the neurotoxin's ability to selectively destroy dopaminergic projections from the substantia nigra pars compacta into the striatum of humans led to its use in producing the present gold standard model of PD, the MPTP-treated nonhuman primate.

MPTP-treated Nonhuman Primate Model of PD

It is now generally accepted that chronic administration of MPTP in nonhuman primates results in nigrostriatal dopamine deficiency that mimics the pathology found in PD (Burns et al., 1983). The loss of striatal dopaminergic function in PD characteristically follows a gradient, with greater loss in the putamen compared to the caudate (Kish et al., 1988). Although some findings have shown caudate dopaminergic loss to be equal or greater than loss in the putamen (Alexander et al., 1992; Pifl et al., 1988), others demonstrated a striatal gradient similar to that of PD utilizing chronic administration of MPTP to nonhuman primates (Hantraye et al., 1993; Perez-Otano et al., 1994; Pifl et al., 1991). Evidence supporting this model's ability to mimic changes outside the nigrostriatal dopaminergic system is controversial. Although such networks are significantly affected in human PD (see above), it is unclear if these changes occur in the MPTP-treated nonhuman primate model.

Controversy remains over the extent of MPTP-induced neuropathology. Although nigrostriatal dopaminergic degeneration is the main anatomical feature of PD and consistently reproduced in MPTP-induced parkinsonian primates as well as cats and several rodents, conflicting evidence exists concerning the degree of additional neurochemical alterations. When considering investigations of animal models involving cortical innervation, it is crucial as always to validate similarities between animal models and humans. Patterns of cortical monoaminergic innervation in primates appear to accurately predict those in humans (Brown et al., 1979; Goldma-Rakic et al., 1992; Lewis, 1992). Therefore, MPTP-treated nonhuman primates serve as the best model to search for similar cortical dysfunction.

MPTP-induced reduction in cortical monaminergic projections has been noticed in few studies examining a limited number of brain regions. Extrastriatal neuronal degeneration induced by MPTP-treatment has been most consistently shown in the substantia nigra, but, in some studies, has also been seen in other brainstem nuclei, including the ventral tegmental area, locus coeruleus (Mitchell et al., 1985) and their catecholaminergic cortical projections, terminating in the frontal cortex (Alexander et al., 1992; Di Paolo et al., 1985), supplementary motor and cingulate cortices (Elsworth et al., 1990), frontal and cingulate cortices (Pérez-Otaño et al., 1991) and motor and cingulate cortices (Pifl et al., 1990). In contrast, others have shown these same nuclei to remain intact follow MPTP-exposure in monkeys. Reports have been made of unaffected neurons in the locus coeruleus (Kerkenham et al., 1991) and in the dopaminergic (Chiueh et al., 1985; Jacobowitz et al., 1984) and norepinephrinergic (Elsworth et al., 1987) mesolimbic pathway. In a recent review, Jenner reported that "MPTP produces an almost selective loss of nigral dopaminergic cells and does not, overall, result in the multiple pathological changes that occur in PD, for example, in the locus coeruleus, raphe nuclei or ventral forebrain with corresponding decreases in noradrenaline, serotonin and acetylcholine content of brain" (Forno et al., 1986; Jenner, 2009).

In an analysis of the neurochemical and pathological consequences of motor asymptomatic monkeys treated with a chronic low dose of MPTP (14.94-75.42 mg of MPTP over periods ranging form 5 to 13 months), findings of dopaminergic innervation of the frontal cortex were inconsistent, decreases in norepinephrine innervation were variable, and significant *increases* were found in the level of serotonin in the striatum (Schneider, 1990; Schneider and Kovelowski, 1990). In a number of other studies, only

brain DA losses were consistently observed (Brooks et al., 1987; Jacobowitz et al., 1984; Jenner and Marsden, 1986). Jacobowitz and colleagues reported a preservation of the mesolimbic dopamine system in the MPTP-treated nonhuman primate. Jenner and Marsden found that MPTP-induced pathology in nonhuman primates was limited to the nigrostriatal system and that other neurotransmitter systems appeared unaffected. These findings demonstrate the conflicting reports in the analysis of the monoaminergic projection systems of primates exposed to MPTP.

The most thorough analysis was conducted by Pifl and colleagues and examined neurochemical changes in nonhuman primates following a treatment of 2.1-7.5 mg/kg of MPTP over one to five weeks by means of high performance liquid chromatography with electrochemical detection. They reported widespread and significant loss of the three major monoamines, with the neurotoxic sensitivity ranking in the cortex as follows: norepinephrine > serotonin > dopamine. Pifl and colleagues observed loss of dopaminergic innervation in all cortical areas analyzed that reached significance in the supplementary motor (– 51%), premotor (– 49%), and motor (– 35%) cortices and loss of cortical serotonin levels that reached significance in premotor (– 41%), motor (– 39%), and prefrontal (– 61%) cortices. Interestingly, a slight increase was seen in serotonin levels in the anterior cingulate cortex (+ 5%), ventral tegmental area (+13%) and locus coeruleus (+40%); however, none of these increases reached significance (Pifl et al., 1991).

Despite the comprehensive and quantitative nature of this analysis, the use of high performance liquid chromatography limited the qualitative analytical power of the study conducted by Pifl and colleagues. The monoaminergic effects of cortical circuitry are

complex and depend highly on the laminar distribution of axon terminals (Luft and Schwarz, 2009; Seamans and Yang, 2004; Vijayraghavan et al., 2007). Consequently, a method of analysis that yields both quantitative data and qualitative description of axon terminal degeneration is crucial to the further analysis of the cortical dysfunction experienced after MPTP-treatment in the nonhuman primate. Since cortical monoaminergic dysfunction is evident in idiopathic PD and may be an important substrate in the investigation of the disease's nonmotor symptoms, the application of the MPTP-treated nonhuman primate model in the analysis of these symptoms requires further analysis to clarify the model's cortical neuropathology.

Study Objectives

In order to further assess the validity of the MPTP-treated monkey model of PD, and support its use as a tool to study nonmotor symptoms of the disease that could potentially be mediated by the loss of cortical monoamine innervation, this study investigated the changes in the serotonin and catecholamine cortical projections in nonhuman primates rendered parkinsonian following chronic exposure to MPTP. Quantitative analysis of immunohistochemical staining with antibodies raised TH and serotonin in various cortical areas involved in cognitive, limbic and motor functions was compared between normal and parkinsonian animals.

Materials and Methods

Animals

A total of three control and two MPTP-treated adult rhesus monkeys (*Macaca mulatta*; Yerkes National Primate Research Center colony) were used in this study. All animals were housed with *ad libitum* access to food and water. All experiments were

performed in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committee of Emory University.

MPTP Administration

Animals received weekly injections of the neurotoxin MPTP (0.2–0.8mg/kg, i.m., Sigma-Aldrich, St. Louis, MO) until stable parkinsonism developed (see Table 1 for individual MPTP-treatment). The severity of parkinsonian motor symptoms was quantified weekly using rating scales for parkinsonism and computer-assisted behavioral scoring methods routinely used in our laboratory (for detailed methodology, see Villalba and Smith, 2011). All MPTP-treated monkeys were stably parkinsonian for at least 6 weeks prior to tissue collection for anatomical analysis (see Figure 5 for movement analysis).

Animal Perfusion

Two MPTP-treated and three age-matched control animals were deeply anesthetized with an overdose of pentobarbital (100 mg/kg, i.v.), and then perfused transcardially with cold oxygenated Ringer's solution, followed by 2 liters of fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffered saline (PBS, 0.01 M, pH 7.4). The brains were removed from the skull and cut into 10 mm-thick blocks in the frontal plane. Frontal tissue sections (60 μm thick) were obtained with a Vibratome and collected in cold PBS (0.01 M, pH 7.4). To assess potential changes in the expression level of cortical TH and 5HT immunoreactivity between the control and parkinsonian states, sections from approximately the same antero-posterior levels of the right hemisphere of the brain (37.83 to 9.30 mm from the interaural line, according to

Paxinos et al., 2000) were selected in the three normal and two MPTP-treated parkinsonian monkeys. The sections were then treated with sodium borohydride (1% in PBS, 20 minutes) and subjected to the immunohistochemical procedures mentioned subsequently.

Immunohistochemistry

After pretreatment with 10% normal goat serum (for tyrosine hydroxylase, TH) or normal horse serum (for serotonin, 5HT) and 1% bovine serum albumin in PBS, the sections were incubated for one day at 4 °C in the primary antibody solution mouse anti-TH antibodies (1:1000, Millipore) or rat anti- 5HT antibodies (1:500, Sigma). To reveal the primary antibodies, the sections were incubated in biotinylated secondary antibodies (horse anti-mouse IgG for anti-TH (1:200; Vector, Burlingame, CA) or goat anti-rat IgG for anti-5HT (1:200, Vector) for 2 hours, and in avidin-biotin complex (ABC) solution (1:100; Vector stain Standard kit, Vector labs) for 90 minutes. The tissue was then washed in PBS and Tris buffer (0.05 M pH 7.6) before being transferred into a solution containing 0.01 M-imidazole (Fisher Scientific), 0.005% hydrogen peroxide, and 0.025% 3,3'-diami- nobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) in Tris for 10 minutes. The reaction was terminated with several rinses in PBS. After revealing the TH or 5HT antibodies binding sites, the sections were mounted on gelatin-coated slides, dehydrated in alcohol, immersed in toluene and a coverslip was applied with Cytoseal XYL (Richard-Allan Scientific, Kalamazoo, MI). The slides were digitized with an Aperio ScanScope CS system (Aperio Technologies, Vista, CA).

Digital Image Analysis

Using ImageScope viewer software (Aperio), the digital images of the stained

tissue slides were examined, and a 20×-magnification images covering the dorsolateral prefrontal cortex (DLP; Brodmann's Areas 9M, 9L, 46V, 46D), limbic cortices (Li; Brodmann's Areas 24 and 25), orbitofrontal cortex (OF; Brodmann's Area 11) and sensory motor cortices (SM; Brodmann's Areas 4 and 6) areas was obtained. Four to eight images were captured from adjacent antero-posterior tissue sections per area analyzed in each animal, depending on the size of the region of interest (Paxinos et al., 2000). The images were then imported into ImageJ (National Institutes of Health, Rasband, 1997–2009) for additional processing. For optical density measurements, the images were inverted to a dark field such that dark immunoreactive elements on a light shaded background were converted to bright immunoreactive elements on a dark background. Consistent areas of analysis were highlighted within the cortical regions of interest, and the integrated optical density within the selected area was determined. To control for differences in background staining, three optical density measurements within the highlighted area without immunoreactive elements were determined and averaged. The normalized background value was then subtracted from the initial value within each cortical region of interest.

Statistical Analysis

For statistical analysis, calculated density measurements were averaged within each area of analysis per animal. The resulting mean values for the areas of analysis from the three normal and two MPTP-treated animals were used for the statistical comparisons between normal and MPTP-treated monkeys. A two-way ANOVA was used to determine the main effect of MPTP-treatment within each antibody. When a significant main effect was found, individual t-tests were performed comparing the effect

of MPTP-treatment within the four functional areas analyzed for each antibody. To compare the cortical denervation visualized by TH and 5HT antibodies, the percent loss was determined for each animal within the areas of analysis. A two-way ANOVA was used to determine the main effect of the antibodies and individual t-tests were performed comparing the effect of the antibody within each cortical functional area.

Results

Changes in TH- and 5HT-positive Fibers in the Cortex of Normal and MPTP-treated Parkinsonian Monkeys

Dramatic loss of dopaminergic innervation revealed by TH immunostaining was visible in the striatum of the MPTP-treated animals (Figure 6). In normal monkeys, TH and 5HT-labeled fibers were distributed throughout the entire cerebral cortex, but variable intensities and laminar distribution did exist. For example, the primary motor(area 4) and limbic (areas 24 and 25) cortices contained the greatest density of TH-labeled fibers, whereas the limbic cortices had the greatest density of 5HT-labeled fibers. The orbitofrontal cortex was sparsely innervated with either TH- or 5HT-labeled fibers. The laminar pattern of innervation was more specialized for TH-labeling, with layer I receiving the densest innervation. This laminar specialization was most prominent in the primary motor cortex (see Figure 7 for examples).

MPTP-treated monkeys were characterized by a dramatic loss of TH immunostaining in the dorsal striatum and a significant loss of TH-positive cells in the substantia nigra pars compacta (Figure 6). At the cortical level, the density of both TH- and 5HT –immunoreactive fibers was reduced considerably in all areas, but to a variable extent. A quantitative assessment of the degree of cortical denervation was performed.

To do so, we quantified changes in the abundance of TH- and 5HT-immunostained fibers in different cortical areas between three normal and two MPTP-treated monkeys using the digital image analysis approach described in the Methods section. This analysis revealed a loss of TH immunostaining in all cortical areas between normal and parkinsonian animals that reached significance in the limbic and sensory motor cortices (see Table 2 for mean values and Figure 8, $t(3)=-2.366$, $p<.05$ and $t(3)=-3.511$, $p<.05$, respectively). There were decreases in 5HT immunostaining in all cortical areas analyzed that reached significance in the sensory motor cortices (see Table 2 for mean values and Figure 9, $t(3)=-2.744$, $p<.05$). The percent decrease in 5HT staining was more than TH staining in the dorsolateral prefrontal, orbitofrontal and sensory motor cortices. The percent loss in 5HT labeling was 53.6% ± 14.1% and the percent loss in TH labeling was 28.9% ± 11.2% across all areas (see Table 3 for individual mean values and Figure 10). This loss reached significance in the dorsolateral prefrontal and sensory motor cortices ($t(2)=6.170$, $p<.05$ and $t(2)=9.615$, $p<.05$, respectively).

Discussion

The results of this study demonstrate a significant loss of cortical serotonergic and catecholaminergic innervation in parkinsonian monkeys chronically treated with low doses of MPTP. Although variable across functional cortical areas, the extent of denervation was widespread affecting associative, limbic and motor cortices. These findings extend some previous studies suggesting decreases in cortical monoaminergic innervation following MPTP treatment (Jacobowitz et al., 1984; Shultz, 1988; Pifl et al., 1990). However, other studies reported no significant change in cortical monoaminergic innervation in MPTP-treated monkeys (Jenner et al., 1986; Pifl et al., 1991). Although the

exact explanation for these discrepancies remains unknown, various possibilities can be suggested including the species and age of monkeys being used, the acute versus chronic regimen of MPTP, and the depth of the quantitative analysis of immunostained fibers across studies. We suggest that the chronic MPTP-treatment used in our study spread out over multiple months models PD cortical pathology in the rhesus macaque more accurately than acute MPTP administration. Behavioral symptoms and cortical pathology of chronic MPTP-treatment in motor asymptomatic animals have been shown to mirror nonmotor symptoms and cortical degeneration of PD and may potentially model early stages of the disease (Schneider and Kovelowski, 1989; Scneider, 1990). Although some variability has been seen following chronic low dose MPTP-treatment in the rhesus macaque, the data presented in our study is comparable with that described in the cortex of PD patients (Gaspar et al., 1991; Nayyar et al., 2009) and therefore, we encourage further analysis of the extrastriatal pathology seen in the rhesus macaque PD model following chronic low dose MPTP-treatment.

The anatomical data generated through this research lay the foundation for the potential use of this MPTP-treated monkey model as a tool to understand the pathophysiology and develop better therapeutics for nonmotor symptoms of PD that could be generated through cortical monoaminergic dysfunctions. This line of research will hopefully lead to the refined understanding of the MPTP-treated nonhuman primate model of PD, and suggests that it mimics more closely the neurochemical and pathological changes observed in patients affected with the disease.

Technical Limitations

Before discussing the functional significance of the findings presented in this

thesis, I would like to highlight some of the advantages and limitations of the immunohistochemical and quantitative methods used in this study to assess the extent of monoaminergic cortical loss in parkinsonian monkeys. Due the differences in laminar distribution within monoaminergic cortical innervation (Lewis et al., 1988), the methodology used in this investigation cannot represent laminar specificity of projections lost following MPTP-treatment. Nonetheless, the global pixel density analysis employed represents decreases in every cytoarchitectonic region analyzed in the parkinsonian animals and therefore significant alteration of cortical innervation. Because TH was used as the antibody used to label catecholaminergic axon terminals, it is debatable if immunoreactive elements represent dopaminergic and or norepinephrinergic terminals, however evidence suggests that cortical TH labeling is primarily associated with dopaminergic axons and terminals (Lewis et al., 1987, 1988). Future studies using norepinephrine transporter antibodies are warranted to further address this issue. Together, our data encourage further investigation of MPTP-induced cortical dysfunction in the nonhuman primate model of PD.

Functional Implications

The cortical denervation reported in this study suggests the presence of a similar cortical monoaminergic pathology seen in idiopathic PD. The precise neuropathological dysfunction behind nonmotor symptoms of PD remains unclear. To better understand the connection between extrastriatal neuropathology, cognitive and behavioral investigations have recently employed the nonhuman primate chronically treated with low doses of MPTP. Cognitive impairment, executive dysfunction and behavioral abnormalities reminiscent of those seen in idiopathic PD have been documented.

Nonmotor symptoms of PD in nonhuman primates. Increasing awareness of the cognitive and behavioral changes associated with PD require the extended investigation of animal models of the disease beyond classic nigrostriatal pathology. Recent investigations employing a chronic low dose of MPTP-treatment to induce a nonhuman primate model of early parkinsonism have discovered parallel cognitive impairments. Decamp and Schneider report chronic low dose MPTP-treated nonhuman primates that exhibit attention and executive function deficits mirroring those seen in patients. Specifically, an inability to sustain attention, impairments in motor planning and time estimation were all described in these animals (Decamp and Schneider, 2004). Roeltgen and Schneider highlight the presence of frontal lobe-mediated task impairments and lack of non-frontal task impairments in this model. Cognitive and behavioral similarities between early parkinsonism and chronic low dose MPTP-treated nonhuman primates suggest the existence of related pathophysiological mechanisms (Roeltgen and Schneider, 1994). The neuropathological substrates for these behavioral abnormalities are unknown; however, a number of correlation studies in humans suggest a connection between areas with anatomical alterations and specific behavioral and cognitive deficits.

Neuropathological changes have been related to cognitive impairment in PD. *Abnormalities in the dorsolateral prefrontal, anterior cingulate and pre-motor cortices have been related to executive function and working memory deficits in PD patients.* A variety of experimental techniques have been utilized and report extrastriatal abnormalities involved in cognitive impairments of idiopathic PD. Cerebral glucose metabolism is reduced in the cerebral cortex demonstrating impaired metabolism in PD patients suffering from cognitive impairment (Yong et al., 2007). Cognitive and

executive deficits have been repeatedly shown to relate to attenuated innervation of the prefrontal cortex, via the mesocortical dopaminergic system (Cools et a., 2002; Dirnberger et al., 2005; Marklund P et al., 2009; Monchi O et al., 2007; Owen et al., 1998). An ^{18}F-dopa PET investigation showed significant decreases in voxel levels between early PD cases as well as advanced PD cases and age matched controls, highlighting abnormalities in the anterior cingulate, motor, and prefrontal cortices (Figure 3; Brooks and Piccini, 2006). However, some findings have been unable to detect a relationship between impaired cognitive performance and altered dopaminergic innervations of the cerebral cortex (Cropley et a., 2008; Sawamoto et al., 2008). Monchi et al. investigated this discrepancy by differentiating between cognitive tasks that do and do not involve the caudate nucleus. In an fMRI study, inter-group comparisons demonstrated a significant reduction in activation of the dorsolateral prefrontal, anterior cingulate and premotor cortices in PD patients compared to controls during a card-matching task that required significant involvement of the caudate (i.e. retrieval with a set shift) compared to a cognitive task that did not (i.e. retrieval without a set shift) (Figure 11; Monchi et al., 2007). This result follows the classic proposition that nigrostriatal dopamine depletion leads to decreased cortical activity via increased basal ganglia inhibition of thalamocortical loops (Albin et al., 1989).

Neuropathological changes have been related to neuropsychiatric symptoms. *Anxiety and depression in PD patients have been related to loss of monoaminergic innervation of the limbic, prefrontal, and orbitofrontal cortices.* Depression affects at least at least 40-50% of PD patients and precedes motor symptoms in 30% of those affected (Burn, 2002; Lemke et al., 2004). Neuropathological findings suggest that

depression in PD is predominantly caused by frontocortical dysfunction related to degeneration of monoaminergic neurotransmitter systems. Post-mortem studies have documented degeneration in the locus coeruleus and raphe, the norepinephrinergic and serotonergic cortical projection nuclei, respectively (Chan-Palay and Asan, 1989; Ziemsenn and Reichmann, 2007). Decreased levels of 5-hydroxyindolacetic acid (5-HIAA), the primary metabolite of serotonin, were found in the cerebrospinal fluid of depressed patients with PD compared with non-depressed patients with PD and healthy controls (Mann et al., 2000). PET imaging utilizing an in vivo marker for dopamine and norepinephrine transporter binding (^{11}C-RTI-32) has revealed decreased binding in depressed compared with non-depressed PD patients in limbic areas including the ventral striatum, the amygdala and the anterior cingulate cortex. In addition, the severity of anxiety in PD patients inversely correlated with ^{11}C-RTI-32 binding in these areas (Remy et al., 2005). Clinical investigations of numerous antidepressants in the therapy of depression in PD highlight the unique effectiveness of agonists targeting the dopaminergic D3- receptor, known to be involved in the loops connecting the basal ganglia to frontal cortical areas (Ziemssen and Reichmann, 2007). A recent voxel-based morphometric MRI study reports gray matter decreases in the bilateral orbitofrontal cortex and the limbic system of depressed PD patients (Feldmann et al., 2008). These neurobiological investigations suggest that depression and anxiety in PD may be mediated by changes in extrastriatal monoaminergic systems, specifically serotonergic, norepinephrinergic and dopaminergic cortical projections.

These studies all suggest the importance of cortical changes in symptoms known to afflict patients suffering from PD and therefore an animal model with parallel

dysfunction will help support the further investigation of the disease's pathology. Changes in the monoaminergic innervation of basal ganglia outflow, particularly in the limbic and association loops, are most likely involved in the nonmotor symptoms described and the use of the MPTP-treated animal model may potentially help discriminate between cortical and striatal pathologies and their resulting PD symptoms.

Future Directions

As stated, a number of investigations have suggested that the specificity of TH antibody staining in the cortex results in immunoreactive elements that are primarily dopaminergic (Lewis et al., 1987, 1988). Therefore, cortical staining with dopamine β-hydroxylase, the enzyme that converts dopamine to norepinephrine may also be included to enable the analysis of dopaminergic and norepinephrinergic axon terminals individually. Laminar specific changes could be analyzed in all cortical analyses by quantifying pixel density separately within each cortical layer. These additions may prove to be important distinctions that could reveal specific cortical dysfunction.

To complete the analysis of monoaminergic projection systems, unbiased stereological cell counts will be included for norepinephrine-containing neurons in the locus coeruleus, dopamine-containing neurons in the ventral tegmental area and serotonin-containing neurons in the raphe nuclei to determine the changes in the total number of neurons in parkinsonian monkeys. Quantification of cells in MPTP-treated and normal animals will reveal any neuronal degeneration in the different nuclei. These additions will enable comprehensive analysis of the three major monoaminergic projection systems in the parkinsonian nonhuman primate.

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Table 1

Animal, Age	Dosage	Duration	Total Dosage
MR139 8Y 9M	One 0.2mg/kg injection per week	17 weeks	5.4 mg/kg
	One 0.5mg/kg injection per week	4 weeks	
MR140 8Y 9M	One 0.2mg/kg injection per week	17 weeks	5.4 mg/kg
	One 0.5mg/kg injection per week	4 weeks	

Table 2

Cortical Area	TH Pixel Density		5HT Pixel Density	
	Control n=3 1.076E7 ± 3.967E6	MPTP n=2 5.721E6 ± 2.146E6	Control n=3 8.968E6 ± 4.289E6	MPTP n=2 4.032E6 ± 1.695E6
Dorsolateral Prefrontal	1.162E7 ± 5.150E6	5.365E6 ± 3.582E5	6.184E6 ± 1.932E6	3.385E6 ± 9.202E4
Limbic	1.306E7 ± 3.432E6	6.623E6* ± 1.770E6	1.434E7 ± 4.653E6	6.544E6 ± 2.177E5
Orbitofrontal	6.301E6 ± 2.100E6	2.885E6 ± 6.981E5	6.464E6 ± 1.431E6	3.659E6 ± 1.240E6
Sensory Motor	1.207E7 ± 1.545E6	8.010E6* ± 1.691E5	8.888E6 ± 3.075E6	2.541E6* ± 5.877E5
Main Effect (ANOVA)	$F(1, 20) = 15.580$**		$F(1, 20) = 18.531$***	

Statistical analysis: ANOVA and t-test. Mean ± Standard Deviations. * p <.05, ** p<.005, *** p<.001 versus control by independent t-test.

Table 3

Cortical Area	TH Percent Loss	5HT Percent Loss
	Control n=3; MPTP n=2 28.9% ± 11.2%	Control n=3; MPTP n=2 53.6% ± 14.1%
Dorsolateral Prefrontal	21.5% ± 5.24%	45.3%* ± 1.49 %
Limbic	37.6% ± 16.7%	54.35 ± 1.52%
Orbitofrontal	31.5% ± 16.6%	43.4% ± 19.2%
Sensory Motor	25.2% ± 1.58%	71.4%* ± 6.61%
Main Effect (ANOVA)	$F(1, 16)=19.480$. $p<.005$	

Statistical analysis: ANOVA and t-test. Mean ± Standard Deviations. * $p<.05$ larger decreases in 5HT versus TH by independent t-test.

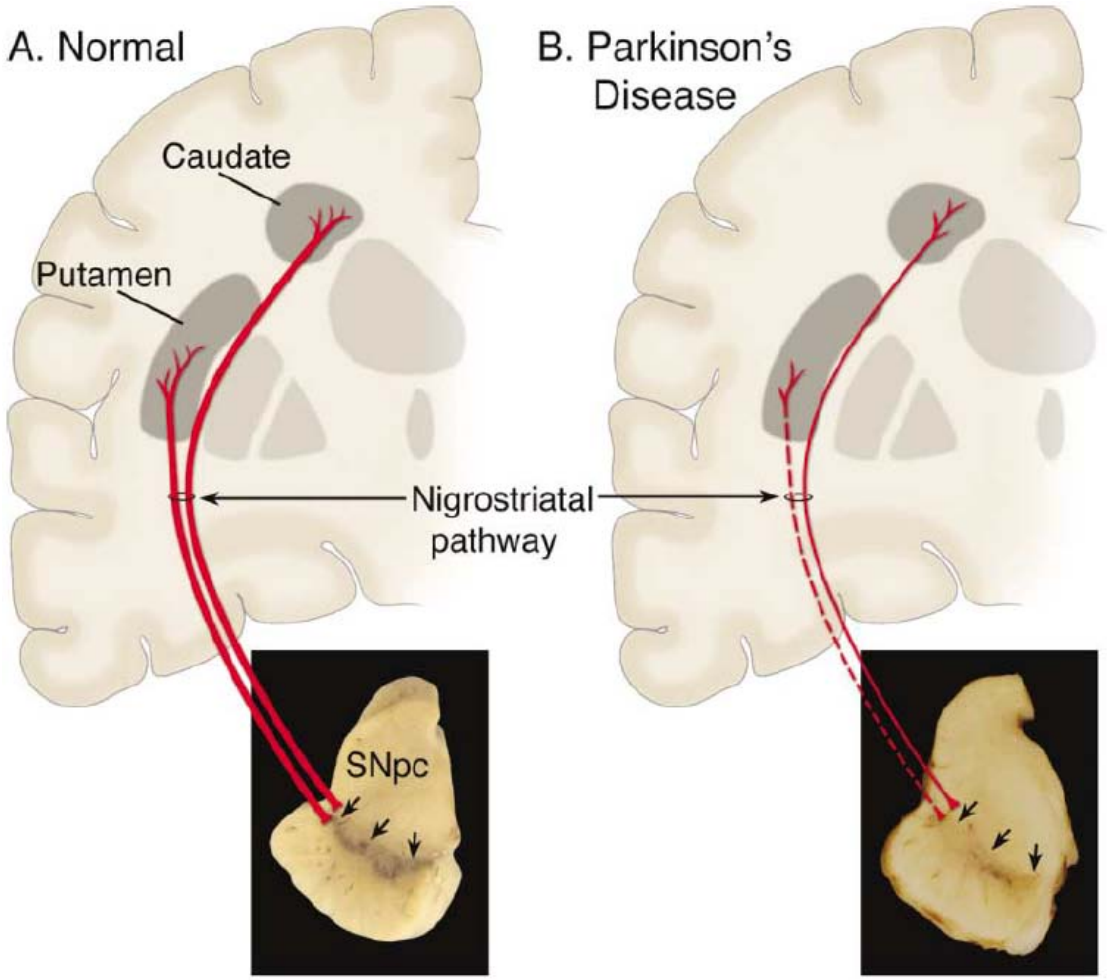

Figure 1. Dopaminergic nigrostriatal neuropathology of Parkinson's disease. (From Dauer and Przedborski, 2003). Loss of dopaminergic cells in the substantia nigra pars compacta leads to increased activity in the indirect circuit (dotted line) resulting in increased inhibition of the thalamus and reduced activation of cortical regions.

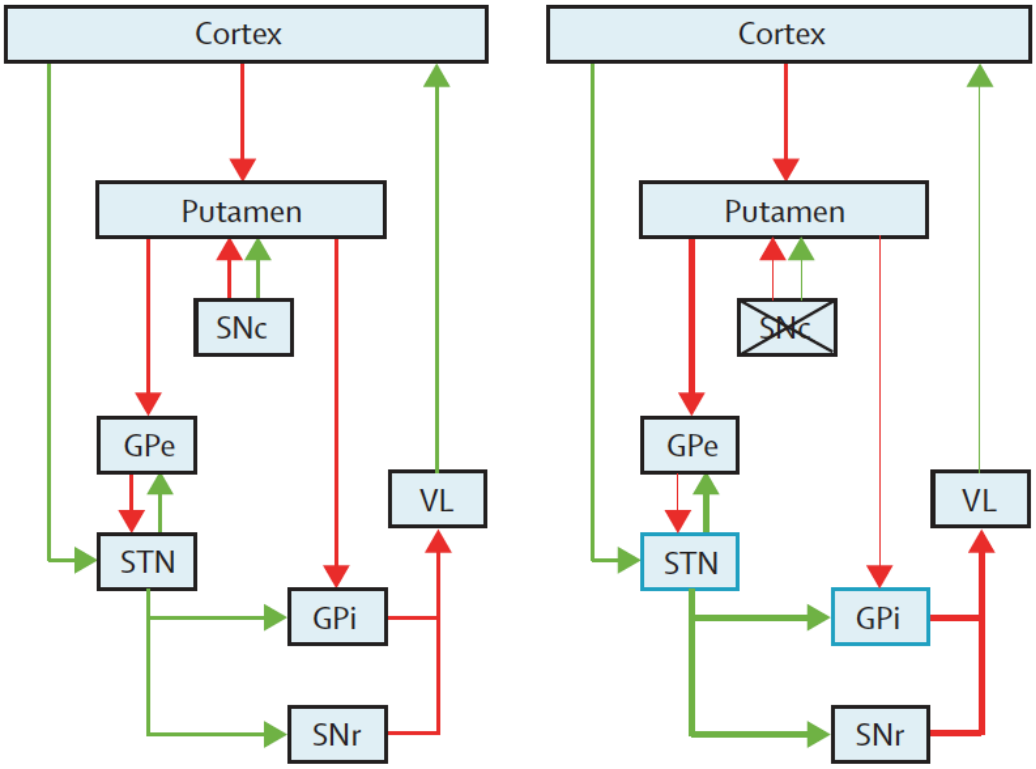

Figure 2. The classic pathophysiological model of the basal ganglia in healthy (left) and parkinsonian (right) states (From Rodriguez-Oroz et al., 2009) Direct and indirect pathway models of the basal ganglia circuitry that illustrates relative changes in firing rate activity of basal ganglia nuclei following lesion of SNc dopaminergic neurons in Parkinson's disease. Loss of SNc dopaminergic cells leads to increased activity of the indirect pathway (i.e. putamen-GPe-STN-GPi/SNr-Thalamocortical projections) and decreased activity of the direct pathway (i.e. putamen-GPi/SNr-thalamocortical projections), which result in increased inhibition of the thalamus and reduced activation of cortical regions. Green arrows indicate excitatory projections, red arrows label inhibitory projections and the thicknesses of the arrows illustrate their relative activity.

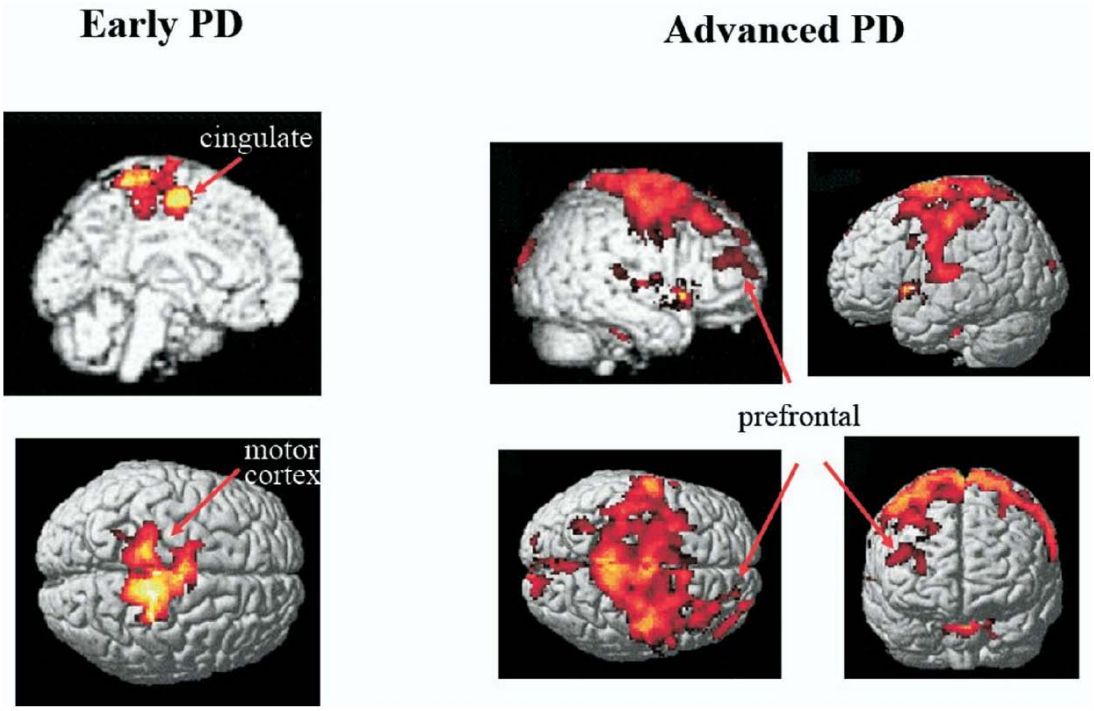

Figure 3. Statistical parametric map comparing the mean DA storage capacity at a voxel level. Areas shown in red represent significant reductions of cortical and cingulate ^{18}F-dopa uptake in early and established PD compared to age-matched controls (From Brooks and Piccini, 2006).

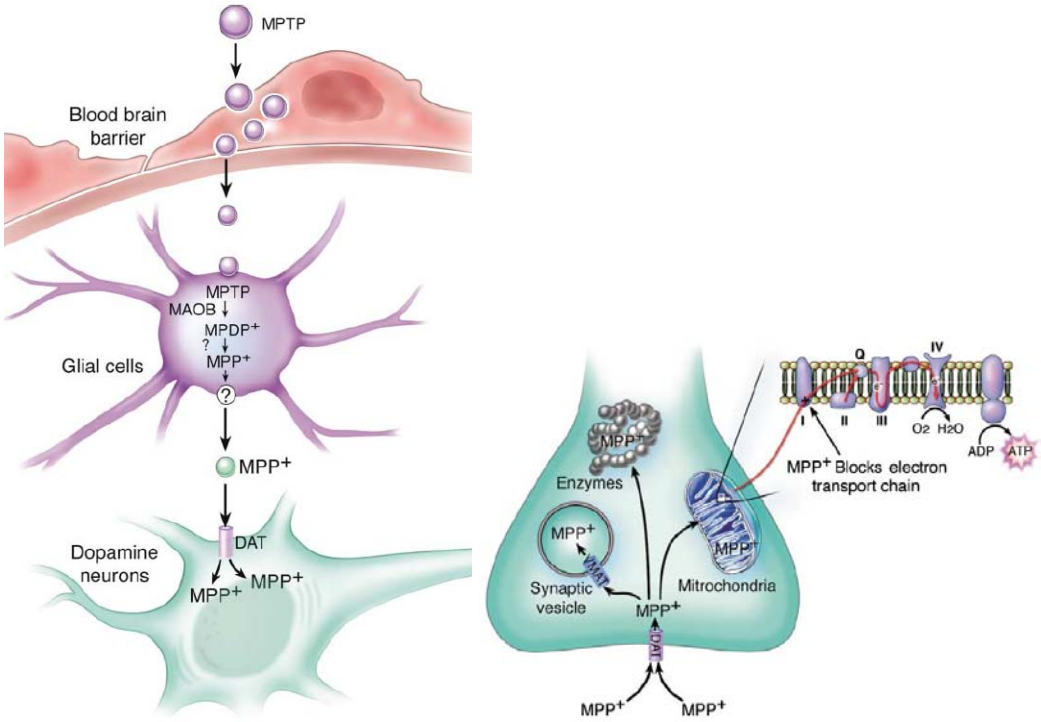

Figure 4. Schematic representation of MPTP metabolism and MPP+ intracellular pathways ultimately resulting in neuronal death primarily in the substantia nigra pars compacta (see text for details) (From Dauer and Przedborski, 2003).

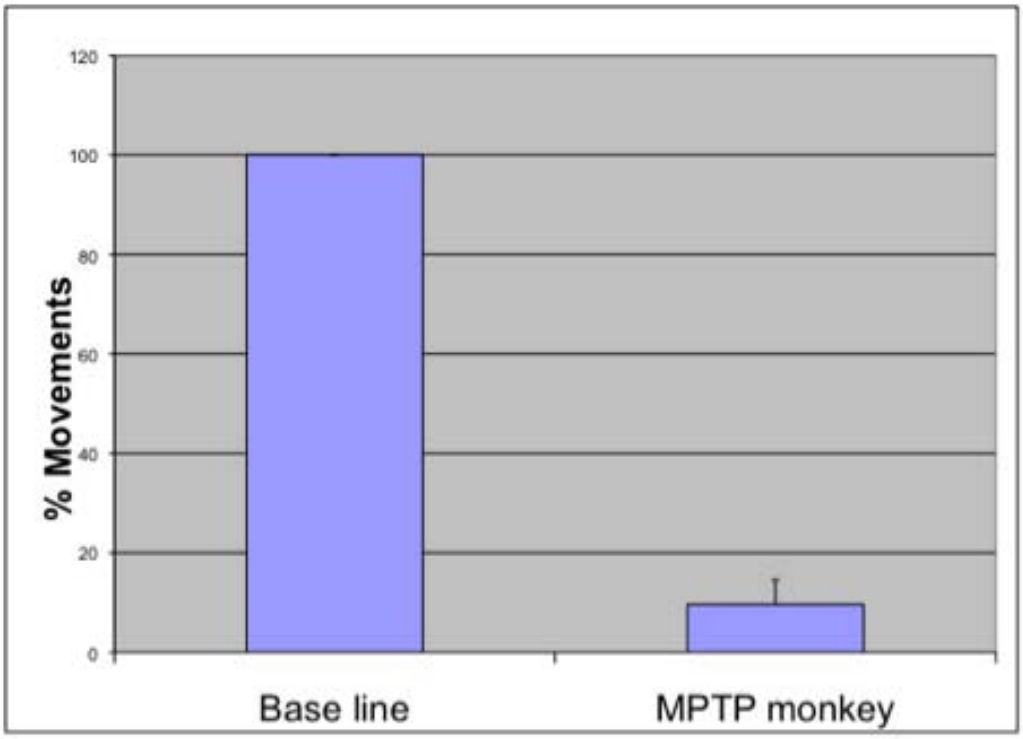

Figure 5. Mean percentage of movements in the MPTP-treated animals normalized to their pretreatment values. Changes in the animal's behavioral state were documented using observation in a behavioral observation cage equipped with infrared beams for automated activity monitoring by counting beam breaks with an attached computer. The animal's spontaneous behavior was assessed with this device for fifteen-minute periods. In addition, ongoing monkey movements were videotaped and monitored by an observer, who pressed buttons on a keypad each time the monkey moved its extremities. Counts of the number of button presses quantify the amount of movement (detailed description in Masilamoni et al., 2010).

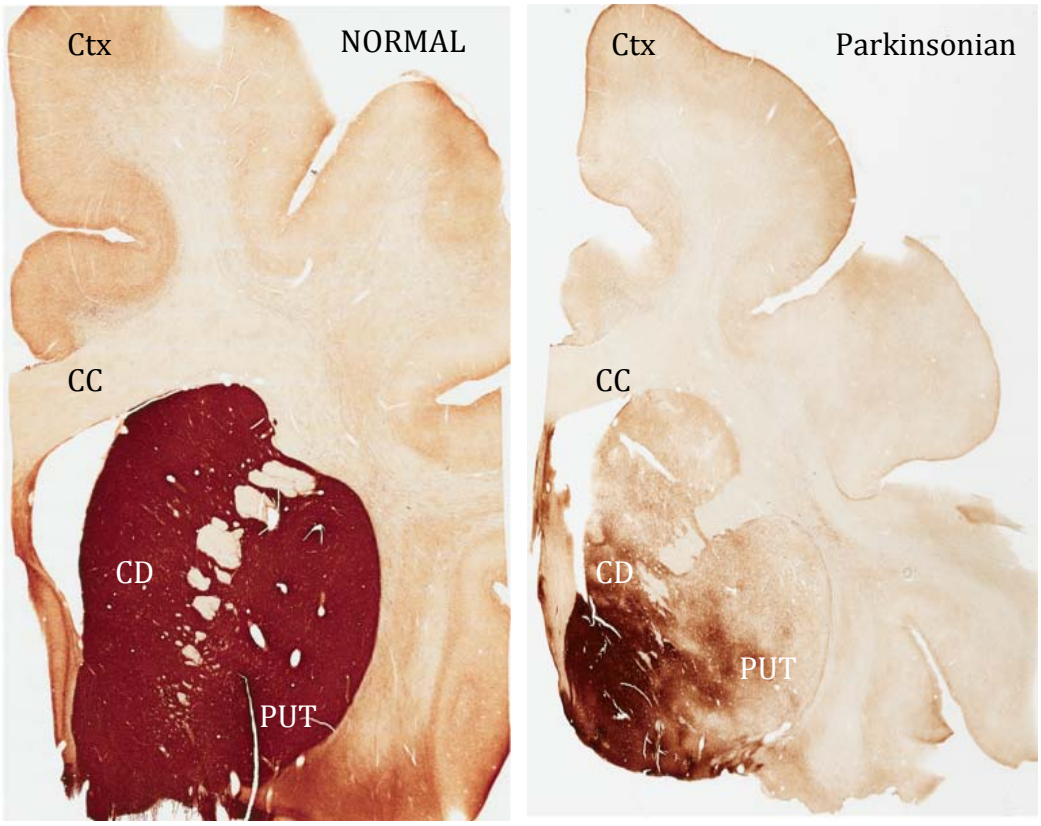

Figure 6. MPTP-induced striatal dopaminergic depletion in the dorsal caudate and putamen visualized by tyrosine hydroxylast staining. (Ctx, cortex; CC, corpus collosum; CD, caudate; PUT, putamen).

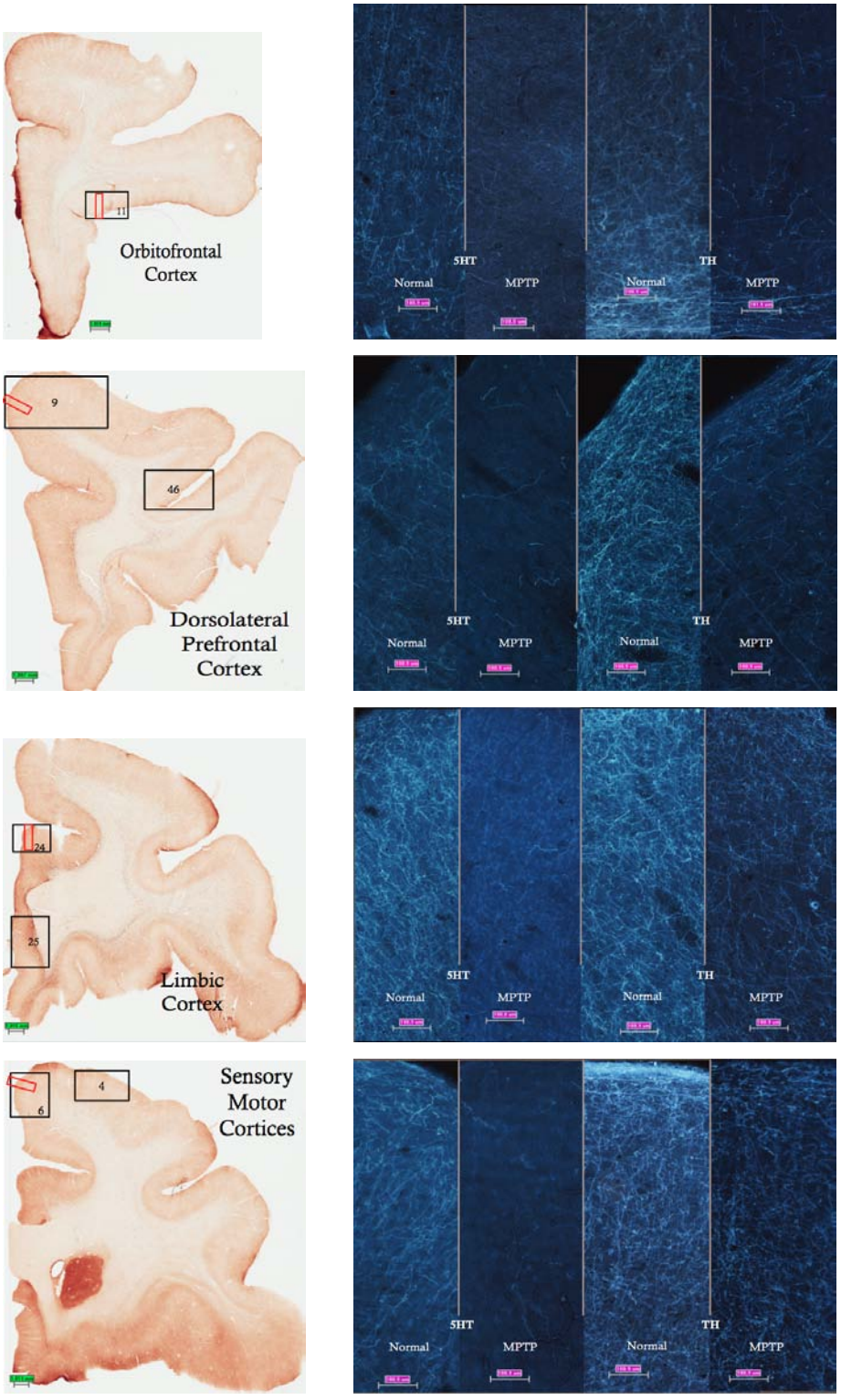

Figure 7. Example decreases in TH and 5HT antibody labeling within functional cortical areas.

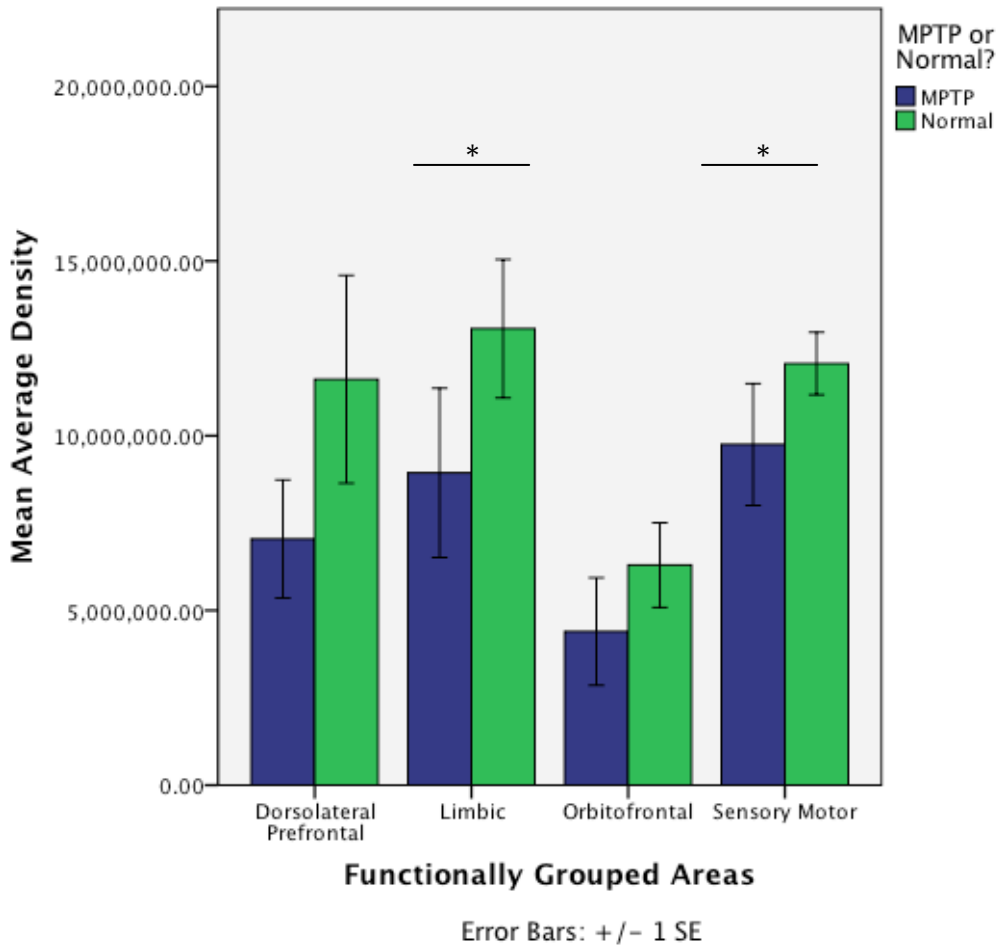

Figure 8. Pixel density analysis of TH antibody staining in MPTP-treated compared to control animals in functional areas of the cortex. There was loss in all cortical areas in TH staining between normal and parkinsonian animals (main effect $F(1, 20)=15.580$, $p<.005$) that reached significance in the limbic and sensory motor cortices ($t(3)=-2.366$, $p<.05$ and $t(3)=-3.511$, $p<.05$, respectively).

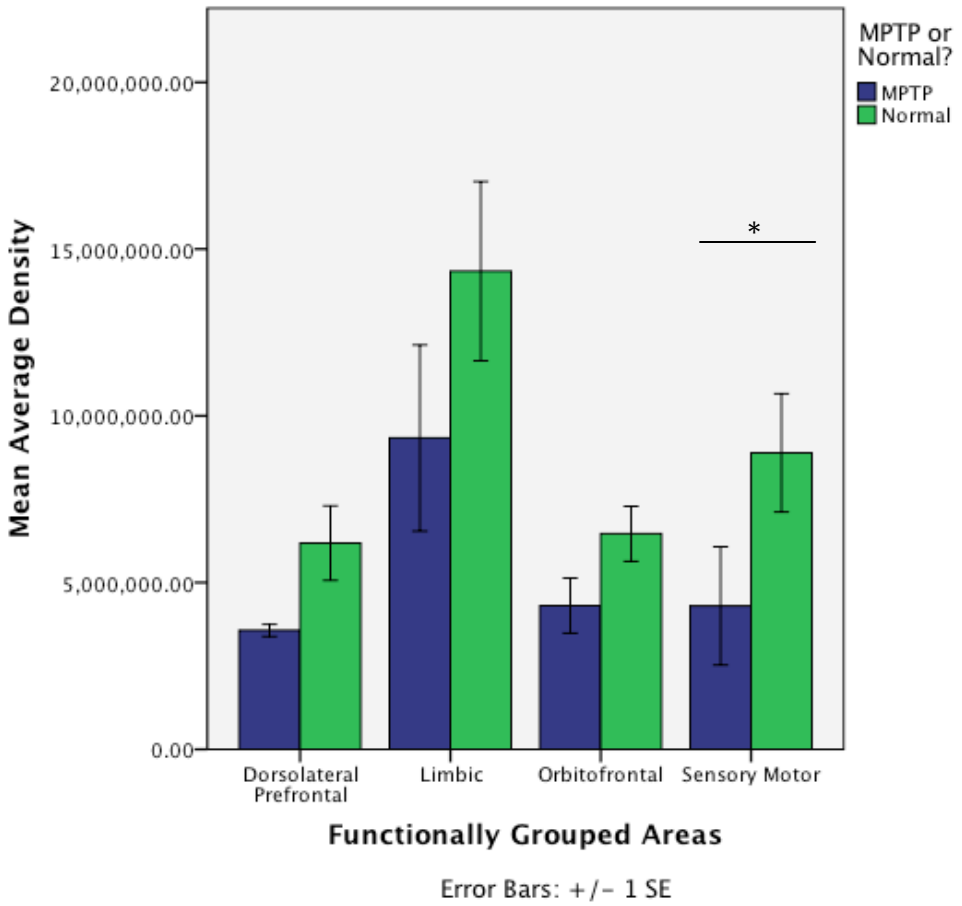

Figure 9. Pixel density analysis of 5HT antibody staining in MPTP-treated compared to control animals in functional areas of the cortex. There were decreases in 5HT staining in all cortical areas (main effect $F(1, 20)=18.531$, $p<.001$) that reached significance in the sensory motor cortices ($t(3)=-2.744$, $p<.05$).

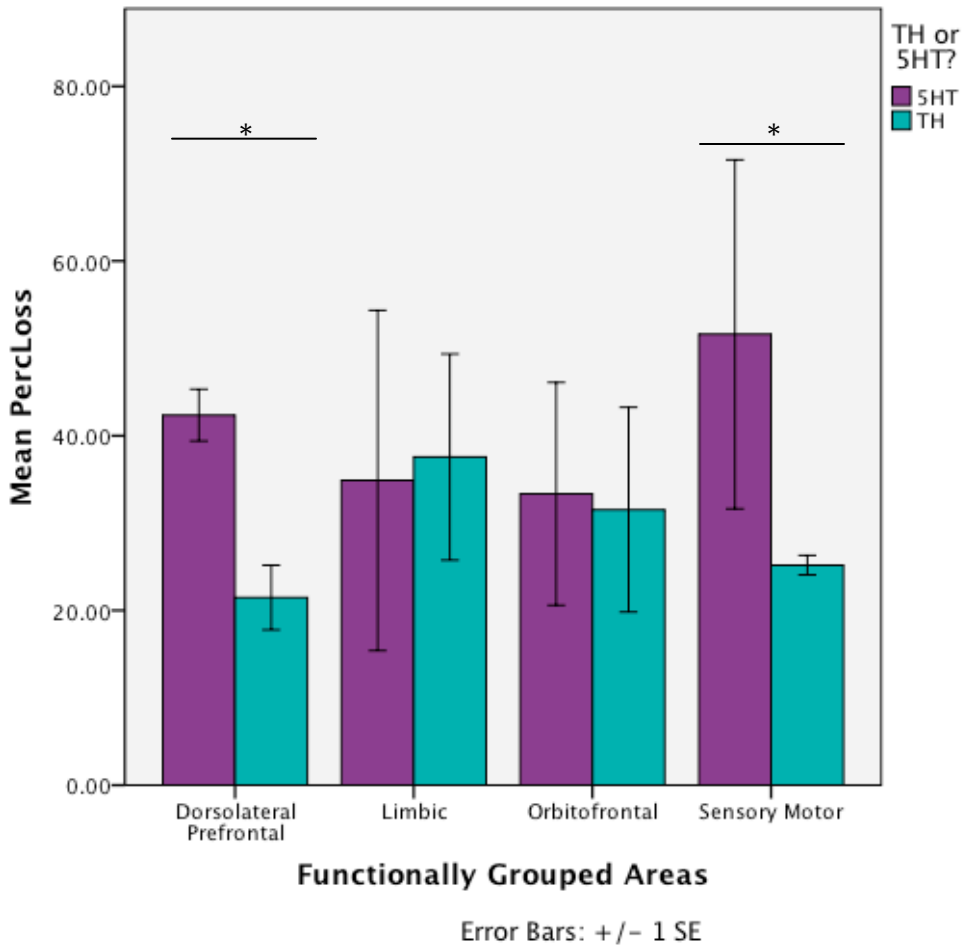

Figure 10. Percent loss of TH and 5HT antibody staining of MPTP-treated animals compared to control animals. The percent decrease in 5HT staining was more that TH staining in the dorsolateral prefrontal, orbitofrontal and sensory motor cortices with a significant main effect ($F(1, 16)=19.480$. $p<.005$). This loss reached significance in the dorsolateral prefrontal and sensory motor cortices ($t(2)=6.170$, $p<.05$ and $t(2)=9.615$, $p<.05$, respectively).

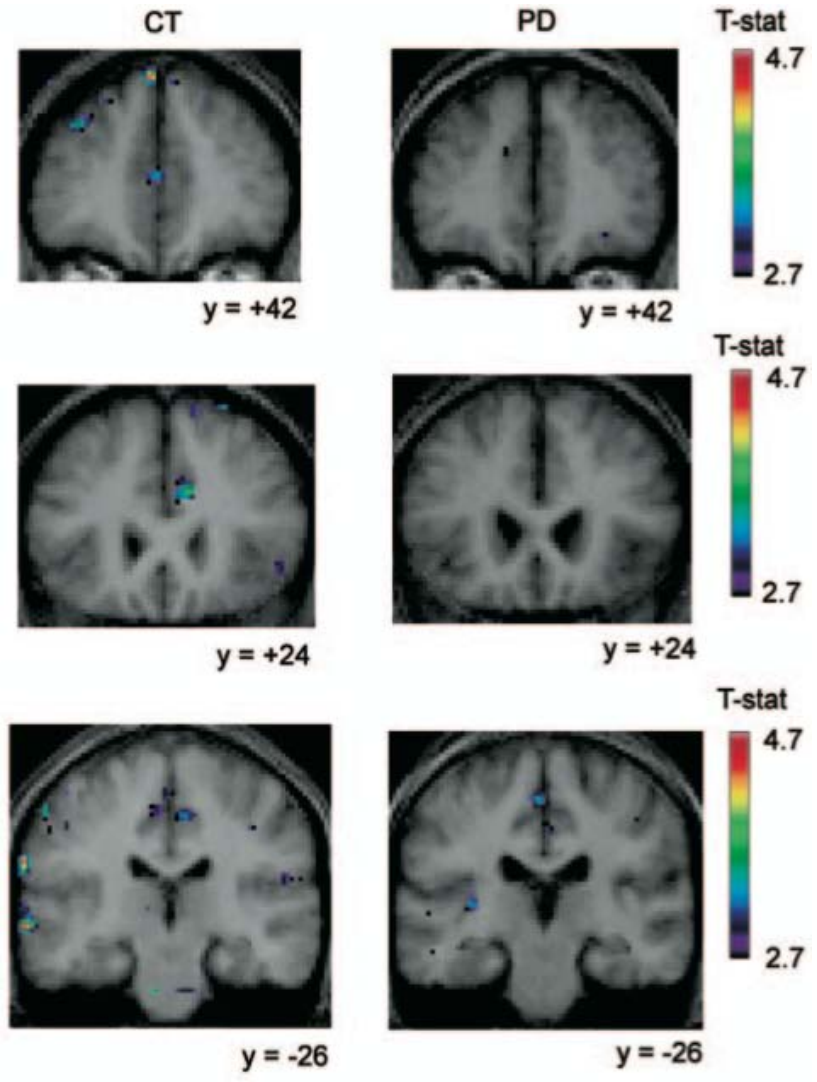

Figure 11. Functional MRI images comparing the patterns of activation between early PD patients and age-matched controls. Areas illuminated represent peaks of activation during a cognitive task involving the caudate with activation during a control task subtracted. The controls (left) display significant activation in the dorsolateral prefrontal, anterior cingulate and temporal cortices, while none is observed in the PD group (From Monchi et al., 2007).