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Pharmacological MRI (phMRI) as a tool to differentiate Parkinson's disease-related from age-related changes in basal ganglia function

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Abstract

The prevalence of both parkinsonian signs and Parkinson's disease (PD) per se increases with age. While the pathophysiology of PD has been studied extensively, less is known about the functional changes taking place in the basal ganglia circuitry with age. To specifically address this issue, three groups of rhesus macaques were studied: Normal middle-aged animals (used as controls), middle-aged animals with MPTP-induced parkinsonism, and aged animals (>20 years old) with declines in motor function. All animals underwent the same behavioral and phMRI procedures to measure changes in basal ganglia function in response to dopaminergic drug challenges consisting of apomorphine (APO) administration followed by either a D1 (SCH23390) or D2 (raclopride) receptor antagonist. Significant, functional changes were predominantly seen in the external segment of the globus pallidus (GPe) in aged animals and in the striatum (caudate nucleus and putamen) in MPTP-lesioned animals. Despite significant differences seen in the putamen and GPe between MPTP-lesioned versus aged animals, a similar response profile to dopaminergic stimulations was found between these two groups in the internal segment of the globus pallidus (GPi). In contrast, the pharmacological responses seen in the control animals were much milder compared with the other two groups in all examined areas. Our phMRI findings in MPTP-lesioned parkinsonian and aged animals suggest that changes in basal ganglia function in the elderly may differ from those seen in parkinsonian patients and that phMRI could be used to distinguish PD from other age-associated functional alterations in the brain.

Keywords

pharmacological MRI; aging; MPTP; parkinsonism; apomorphine; SCH23390; raclopride

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1. INTRODUCTION

Human aging is a universal phenomenon. Parkinson's disease (PD), an important example of an age-related movement disorder, is clinically characterized by slowness of movement, rigidity, tremor, postural and balance instability. The prevalence of PD rises with increasing age from 0.6% in 65–69 year old individuals to 3.6% in 80 years old individuals (de Rijk et al., 1997). In normal aging, nearly 15% of individuals between the ages of 65–75 year old were found to display two or more parkinsonian signs, and the incidence rose to over 50% in those more than 85 years old (Bennett et al., 1996; Buchman et al., 2012). Bennett and coauthors (1996) reported that motor symptoms with the highest prevalence included bradykinesia (37%), gait disturbance (51%) and rigidity (43%) while resting tremor, a cardinal symptom of idiopathic PD, had the lowest prevalence (5%) in the elderly. Although the expression of movement dysfunctions, often called “mild parkinsonian signs” is more prevalent in older people who otherwise have no definite neurological disease (Louis and Bennet, 2007), those so-called “mild PD signs” are in fact not benign. Rather, they are associated with a wide range of adverse health outcomes including an increased risk of death and the development of disability, mild cognitive impairment, Alzheimer's disease and cognitive decline (Buchman et al., 2012). Furthermore, dopaminergic replacement therapies, in contrast to their use in PD, are ineffective at relieving the burden associated with age-related parkinsonism (for a review, see Darbin, 2012).

For over half a century, it has been hypothesized that age-associated decline in movement functions is caused by changes in “central processes initiating, shaping and monitoring movements” (Welford, 1958). Because the primary cause of PD is the degeneration of the nigrostriatal dopaminergic system (Hornykiewicz and Kish, 1987), there has long been a suspicion that the mechanisms underlying motor decline in normal aging also involve the central dopaminergic system (for a review, see Darbin, 2012). Although the movement disorders seen with advancing age resemble those seen in PD, it is still not clear whether similar changes in the CNS circuitry underlie these similar behavioral impairments. Emerging evidence demonstrates that the basal ganglia dopaminergic system, which degenerates in PD, is also altered in normal aging processes in humans but with some distinct differences. For instance, dopamine (DA) cell loss in PD is more severe and mainly occurs in the ventral tier of the substantia nigra pars compacta (SNc), while it is milder and located in the dorsal tier of the SNc in normal aging. Also, DA levels are higher in the SNc and putamen in normal age-matched controls than in PD patients (Bokobza et al., 1984; Kish et al., 1988). Thus, dopaminergic pathways are changing in the basal ganglia in PD as well as in normal aging, but the underlying mechanisms may be different. While the effects of PD on basal ganglia functions have been extensively studied, much less is known about the functional consequences due to normal aging. As a result, the treatments for age-associated motor dysfunctions are even more limited than for PD (Buchman et al., 2012). Differentiating idiopathic PD from atypical PD syndromes, especially in the early disease stages, has proven to be difficult due to an overlap of clinical signs and symptoms. This may explain, at least in part, the high rate of misdiagnosis for PD (for a review, see Mahlknecht et al., 2010).

PET and SPECT imaging with radioactive tracers such as [^{11}C] SCH23390 and [^{11}C] raclopride have been increasingly used clinically to study PD (Sioka et al., 2010). Striatal DA receptor binding has been investigated *in vivo* with PET in patients with early PD using the D1 receptor antagonist [^{11}C] SCH23390 as well as the DA D2 receptor antagonist [^{11}C] raclopride (Rinne et al., 1990). In that study, abnormal bindings of D2 but not D1 receptors were found in early PD. In general, idiopathic PD patients usually show a normal or unregulated postsynaptic DA D2 receptor profile, whereas atypical parkinsonian syndromes like multiple system atrophy (MSA) or progressive supranuclear palsy (PSP) present with decreased postsynaptic binding (Schreckenberger et al., 2004). However, alternative, non-invasive imaging methods that avoid ionizing radiation would be preferable for screening a large number of individuals at risk for developing PD and/or to differentiate PD from other neurological disorders (Zhang et al., 2006). After carefully examining previously published studies using PET and SPECT in PD research, we hypothesize that pharmacological MRI (phMRI) could be used to investigate the underlying mechanism of age-associated parkinsonism and propose to explore the feasibility of using this imaging modality for the differential diagnosis of PD in future clinical trials.

The present study was designed to use pharmacological MRI to investigate the differences in basal ganglia function in aged and MPTP-lesioned rhesus monkeys in response to dopamine receptor agonists and antagonists. For example, compared to young rhesus monkeys, dopamine agonists including apomorphine and d-amphetamine significantly increased neuronal activity in the GPe of aged animals indicating the altered responses in the aged GPe may contribute significantly to the motor dysfunctions characterizing advanced age (Zhang et al., 2001). Based on our previously published studies (Zhang et al., 2001, 2006, Hardy et al., 2005, Cass et al., 2007), we hypothesized that age-related pathophysiological changes would be concentrated in the pallidal regions, particularly in the GPe while changes related to dopamine denervation induced by MPTP would be dominant in the nigrostriatal regions.

2. METHODS AND MATERIALS

2.1 Animals

A total of 10 female rhesus monkeys (*Macaca mulatta*) ranging in age from 15 to 22 years old and weighing between 5.5 and 7.5 kg were obtained from a commercial supplier (Covance, Alice, TX) and used for this study. All animals were housed in individual cages in a temperature-controlled room and maintained on a 12-hour light and 12-hour dark cycle. Throughout the entire study, water was available *ad libitum*. They were divided into three test groups: normal middle-aged (12–14 years old, n=3), middle-aged (15–16 years old, n=4) animals with unilateral MPTP lesions and normal aged (> 20 years old, n=3) animals. The four parkinsonian animals received unilateral administration of 0.12 mg/kg 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) via the right carotid artery 12 months prior to entering the present study using previously described surgical procedures (Ding et al., 2008). All procedures were conducted in the Laboratory Animal Facilities of the University of Kentucky, which are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. All experimental protocols were

approved by the University of Kentucky Animal Care and Use Committee and followed NIH and USDA guidelines.

2.2 Standardized rating of movement dysfunctions

As per previously described procedures (Zhang et al., 2000), all animals were videotaped to assess motor functions and the MPTP-treated animals were video-recorded pre and post MPTP administration before entering the present study. Briefly, the monkeys were transferred into a customized videotaping cage at 9:00am on the day of testing and allowed to adapt to the environment for a few hours. The videotaping segment started at 1:00pm and 2:00pm, and each video segment lasted 45 minutes. Motor dysfunctions were rated independently in quarter-point increments by two experienced observers (RCG and ZZ) using our previously published rating scale (Zhang et al., 2000). Motor dysfunctions including bradykinesia, rigidity, postural and balance instability were rated from 0 (normal) to 3 (severe disability). The rating from the middle-aged controls was considered to be the normal baseline (Rating as “0”).

2.3 Pharmacological MRI procedure

The complete methodology for conducting MRI scanning and minimize head motion in alert rhesus monkeys has been detailed elsewhere (Andersen et al., 2002). The methodology includes: 1) acclimating awake animals to the MRI environment prior to scanning, 2) using custom head-frame and head-pins to secure the head, 3) using earbars to reduce ambient scanning noise levels, and 4) using multivariate methods of image data analysis suitable for detecting and deleting outlying observations due to motion artifacts. The phMRI scans were conducted in fully conscious and alert animals on a Siemens VISION 1.5 T clinic scanner using the body coil to transmit radio frequency and an 8 cm diameter surface coil for signal reception. The anatomical structures of interest were visualized using a 3D FLASH sequence with 1 mm isotropic resolution (TR/TE=21/6 ms, flip angle = 30°, image matrix size = 128×128×90, field of view = 128 mm). The functional MR images from pharmacological challenges were acquired continuously at a temporal sampling rate of 30 sec using a FLASH 2D multiple gradient-recalled-echo (MGRE) navigator sequence (Chen et al., 1996). Slices were acquired interleaved for three noncontiguous coronal slices: the first slice covering the area of the putamen and the caudate, the second covering the globus pallidus (GP), and the third including the substantia nigra (SN). An 11-echo MGRE sequence was used to map, on a pixel-by-pixel basis, the local transverse relaxation rate R_2^* , an intrinsic MRI parameter sensitive to changes in local cerebral blood flow and oxygenation associated with changes in cerebral activity. The last TE-images were acquired without phase encoding gradients to serve as a navigator echo for detection of head motion during the acquisition of data for a single calculated R_2^* image. The FLASH 2D acquisition parameters were: TR=250 ms, TE=7–75 ms, TE=6.5 ms, image matrix size = 112×128, field of view = 128 mm, slice thickness = 3 mm, flip angle = 40°, bandwidth = 156 Hz/pixel. Brain activation in BOLD-based imaging is associated with a decrease in the transverse relaxation rate ($R_2^* < 0$), whereas deactivation or inhibition is associated with an increase ($R_2^* > 0$). By convention, pharmacological MRI (phMRI) studies have traditionally measured the response to a drug challenge in terms of changes in the transverse relaxation rate, R_2^* (Chen et al., 1996; Zhang et al., 2000, 2001; Jenkins et al., 2004; Chen et al.,

2005). In comparison, functional MRI (fMRI) studies typically report the fractional signal change, S/S_0 , associated with activation or deactivation. The two measures are related as $S/S_0 = 1 - R_2^* TE$, where TE is the echo time of the acquisition (Zhang et al., 2000).

The changes in response to dopaminergic stimulations were averaged spatially across pixels within individual regions of interest (ROI) selected in the caudate nucleus, putamen, internal and external segment of GP (GPi, GPe) and SN. The ROI dimensions were $3 \times 3 \times 3$ mm, each representing a 27-mm^3 volume and selected manually by ZZ in the left and right hemispheres as previously described by our group (Zhang et al., 2000, 2001, 2006). In normal middle-aged control and aged animals, the combined data from the two hemispheres was used for the final analysis, since an initial analysis did not reveal any effect of laterality in either the current study or in a previous pHMRI study (Zhang et al., 2001). After collection of baseline images over an initial period of 15 min preceding drug challenge, the DA D1/D2 receptor agonist Apomorphine (APO, 0.15 mg/kg i.m.) was administered followed 15 min later by either the DA D2 receptor antagonist Raclopride (RAC, 0.05 mg/kg i.m.) or in a separate scan by the DA D1 receptor antagonist SCH23390 (SCH, 0.1 mg/kg i.m.). The total scan time per session was 45 minutes. Two replicate scans for each of the drugs RAC and SCH were conducted at least one week apart. Activation/deactivation is quantified in terms of the drug-induced change in relaxation rate R_2^* relative to baseline.

2.4 Statistical analysis

The behavioral data was analyzed by using Welch's adaptation of the Student t-test for independent samples of possibly unequal variance. Differences of $P < 0.01$ for behavioral changes were considered significant. Due to the small number of animals per group, variability in the pHMRI data was estimated from the time series observations of the R_2^* response in a particular region rather than between animals. For each time point of the measured pHMRI response, R_2^* values were averaged across animals and repeated scans within a group and collapsed into a single representative observation. For the normal middle-aged and aged animals, average values of left- and right-hemisphere responses combined were used for each brain region. Mean and standard deviation were calculated from the 20 observations of the time series response collected during the 5–15 minutes time interval following drug administration. Z-score values were computed for each comparison of drugs within a group or for differences between groups for a given drug as

$z = (m_1 - m_2) / \sqrt{s_1^2 + s_2^2}$, where m_1 , m_2 , s_1 , and s_2 are the estimates of group/drug means and standard deviations. Significance levels were determined in turn based on a t-distribution with 38 degrees of freedom. This approach reflects a conservative fixed-effect analysis particular to the animals used in the study. Because of the large number of multiple comparisons, a more conservative threshold of $P < 0.01$ was used in individual comparisons for establishing statistical significance of the pHMRI data. It should be noted that pHMRI data from only 8 of the animals was used for the final analysis because of incomplete data in one of the MPTP-treated and one of the aged animals. For comparison of the temporal evolution of the pHMRI responses (Figs. 2B, 3B), the mean and standard deviation were estimated separately from each 5-minute segment. Z-score values of differences were computed as stated above and statistical significance established based on a t-distribution with 18 degrees of freedom.

3. Results

3.1 Motor deficits in MPTP-treated and aged animals

The three normal middle-aged animals (Group 1) did not show any clinically observable motor deficits, so they were considered as controls with a baseline rating of “0”. Compared with the normal middle-aged monkeys, both the MPTP-treated and aged (>20 years old) animals exhibited bradykinesia and rigidity (on both sides in aged animals and on the affected side in MPTP-treated animals), along with postural and balance instability. As shown in Figure 1, limb movements in the three aged rhesus monkeys were bradykinetic and rigid. Overall, the motor deficits appeared greater in aged animals than in those with mild MPTP lesions with the exception of postural instability (Fig. 1). Motor dysfunctions observed in the three aged animals were comparable to those seen in aged animals used in a previous study (Zhang et al., 2000). Agreement between the motor dysfunction ratings of the two observers (RCG and ZZ) was judged to be good with an intraclass correlation coefficient of 0.83. Combined scores from the two raters were used in the final analyses. As expected, counterclockwise rotations were observed in MPTP-lesioned animals administered APO. Other side effects usually associated with APO like stereotypic behavior and vomiting were not seen in animals used in this study.

3.2 phMRI responses: APO followed by either SCH or RAC

3.2.1 Similarities ($P>0.1$) between MPTP-treated and aged animals—Comparable responses ($P>0.1$) to the initial APO administration and the subsequent injection of SCH were observed in the putamen between the two groups (APO, $z=1.42$, $P=0.1641$; APO +SCH, $z=-1.41$, $P=0.1675$) (Table 1, 3–4th columns, Fig 2A). Notably, almost equal R_2^* values were found in the GPi of both MPTP-lesioned and aged animals regardless of which dopaminergic agents were used (Table 1, Fig. 3A), i.e., similar amplitudes of activations were seen after the initial administration of APO ($z=0.76$, $P=0.45$) or APO+RAC ($z=0.62$, $P=0.53$), as well as equal deactivations after the administration of APO+SCH ($z=-0.49$, $P=0.62$) (Table 1, Fig. 3A). Furthermore, the initial activations produced by APO in the GPi were equally potentiated in both groups of animals shortly following the subsequent injection of RAC as illustrated in Figure 3A. Contrasting with the responses seen in MPTP-treated and aged animals, moderate deactivations ($R_2^* >0$) and very mild activations ($R_2^* <0$) were found in the putamen after the initial APO and the subsequent injection of RAC, respectively (Fig. 2A), in middle-aged healthy controls.

3.2.2 Differences ($P<0.01$) between MPTP-treated and aged animals—After the initial administration of APO, significant differences were detected in the caudate nucleus (CD), and GPe between MPTP-treated and aged animals (Table 1, 3rd column, Fig. 4A&B). Similar differences were also observed in these two brain areas as well as in the SN following the subsequent administration of SCH23390 (Table 1, 4th column, Fig. 4A–C). The most remarkable and significant differences were found in the GPe between the two groups in their responses to dopaminergic stimulations (Fig. 4B). An almost 9-fold stronger activation profile was seen in the GPe of the aged animals versus that seen in the MPTP-lesioned animals after the initial administration of APO ($z=-3.38$, $P=0.0017$). This was followed by even statistically larger differences with the subsequent injection of SCH ($z=$

–5.46, $P < 0.0001$) or RAC ($z = -4.8$, $P < 0.0001$) (Table 1, 4–5th columns, Fig. 4B). In the CD, the initial APO injection produced significant ($z = 3.39$, $P = 0.0017$) differences between MPTP-treated and aged animals, i.e., mild deactivations in MPTP-treated animals and mild-moderate activations in the aged animal (Fig. 4A). Interestingly, the subsequent injection of the dopamine D1 receptor antagonist SCH turned R_2^* signals from negative (activation) to positive (deactivation) values only in the aged animals but not in MPTP-treated animals (Fig. 4A). Differences between the two groups were highly significant post SCH administration ($P < 0.001$) (Table 1). Similarly, significant ($z = -6.31$, $z < 0.001$) responses induced by the subsequent injection of SCH were also seen in the SN of MPTP-treated animals (Table 1, 6th column, Fig. 4C). In the GPe, the pharmacological responses to the initial APO administration subsequently followed by either injection of SCH or RAC were much smaller ($P < 0.001$) in MPTP-treated animals than for their aged counterparts (Fig. 4B). In the putamen, the only significant difference between MPTP-treated and aged animals was found after the injection of RAC. The R_2^* value in the MPTP-lesioned putamen were changed from -1.13 ± 0.1 (after APO) to -2.94 ± 0.3 (after RAC), which indicated that the APO-induced activations were significantly ($P < 0.001$) potentiated (Fig. 2A). The time course of responsiveness to the sequential dopaminergic drug administration in the putamen and GPi is presented in Figures 2B and 3B, respectively.

3.2.3 Similarities and differences between normal middle-aged controls and MPTP-treated or aged animals—As illustrated in Table 1, the overall responses to the initial APO administration followed by subsequent administration of either of the dopamine antagonists (SCH or RAC) in the normal middle-aged controls were much milder than for the other two groups, while statistically significant differences could be found in some regions between the groups. For example, after the initial APO administration, moderate responses were detected in the CD of both MPTP-treated and aged animals whereas only minor changes were found in the middle-aged controls (Table 1). The differences between middle-aged controls and aged or MPTP-treated animals were very significant regarding responses to APO administration (controls vs. aged, $P = 0.0029$; controls vs. MPTP, $P < 0.001$). Significant differences between the middle-aged controls and MPTP-treated animals could be detected in the CD, putamen, and SN in the responses to all dopaminergic stimulations and in the GPe between middle-aged controls and aged animals (Table 1).

4. DISCUSSION

The present study has used pHMRI in groups of awake, MRI-adapted monkeys to map neurophysiological responses to APO, combined with dopamine D1 and D2 receptor antagonists. The most notable differences between animals with MPTP-induced and aged-related motor dysfunctions were found in the striatum (in parkinsonian animals) and the GPe (in aged animals). Specifically, the initial APO-induced activation was strongly potentiated in the MPTP-treated putamen by the subsequent injection of the D2 receptor antagonist RAC (Fig. 2A&B). By contrast, the initial APO-induced activations in the aged GPe were maintained after the subsequent administration of either SCH or RAC (Fig. 4B). In addition to the putamen and GPe regions, significant neuromodulatory differences were also found at various sites within the basal ganglia such as in the CD (Fig. 4A) and SN (Fig. 4C) between

aged, MPTP-treated and middle-aged controls. Changes in MRI signals in MPTP-treated and aged animals following APO administration were likely due to activation of the dopamine receptors and not to its vasoactive properties as no significant MR signal changes were seen in the control animals post APO treatment. This is consistent with the observation that L-dopa at therapeutic doses increases rCBF in the basal ganglia of parkinsonian patients, but has no effect on rCBF in patients with progress supranuclear palsy (Kobari et al., 1992).

The results from the present study support the feasibility of translating phMRI protocols to clinical use in patients to differentiate PD from age-associated parkinsonism because: 1) all MRI scans were conducted on a regular clinical MRI scanner using FDA-approved drugs; 2) there were no anesthetic procedures involved in any of the phMRI scans for any of the tested animals used in the current research protocol; and 3) the results from this non-human primate study parallel previous findings from patients with early PD. For example, the main neuromodulatory effects (drug-induced activations) found in DA-depleted striatal regions and in the GP and SN in the aged animals were comparable to previous human PET data (Shinotoh and Calne, 1995; Schreckenberger et al., 2004). However, it is worth noting that, due to the small number of animals in each group, the statistical analyses of the phMRI data were carried out as fixed-effect analyses particular to animals used in the study with the limitation that our results may not generalize to a larger population.

In the GPi, an important structure in the direct pathway, similarities rather than differences in responses to all dopaminergic stimulations were observed between the MPTP-treated and aged animals. Although numerous clinical studies have shown that either pallidotomy or pallidal stimulation can improve the symptoms of PD (Hyam et al., 2011), less evidence is available demonstrating the GPi's involvement in normal aging. By contrast, significant differences ($P < 0.001$) were seen in the GPe, which has long been described as a structure with a relatively homogenous population of GABAergic neurons that forms a requisite component of the indirect pathway of the basal ganglia. Published data suggest that dysfunctions of the GPe have been implicated as one of the mechanisms underlying PD (Obeso et al., 1997; Prensa et al., 2000) and could also contribute to the motor declines of normal aging (Wisco et al., 2008). In addition, the GPe receives dopaminergic inputs as well, although the functional significance of these inputs is not fully understood (Parent et al., 2001). Data collected from both rodent and primate studies indicate that a lower average firing rate has been observed in the rodent GP compared to the primate GPe, while firing patterns were very similar (Benhamou et al., 2012). The phMRI responses observed in the GPe following dopaminergic stimulations appear to parallel previously reported neurophysiological data. In the present study, activity levels following dopaminergic stimulations were much smaller in the MPTP-treated than in the aged GPe (Fig. 4B). With the discovery of massive GPe to GPi projections (Hazrati et al., 1990; Sato et al., 2000), it has been suggested that the GPe is not just a mere relay station in the indirect pathway of the basal ganglia. Rather, the GPe is probably a central nucleus in the basal ganglia circuitry, which is reciprocally connected to the striatum and the subthalamic nucleus (STN), and is a major source of innervation to the GPi and the substantia nigra pars reticulata (SNr) (for reviews, see Goldberg and Bergman, 2011; Tachibana et al., 2008). Therefore, anatomically, there is a unidirectional flow of information in the basal ganglia system from the input

structures (striatum and STN) to the basal ganglia output structures (GPi and the SNr), through the central networks of the GPe, which could be altered by aging or other neurodegenerative conditions like PD (Figure 5). The results of the present study appear to parallel those of an earlier phMRI study (Zhang et al., 2001). In that study, BOLD activations induced by apomorphine were significantly increased in the GPe of aged versus younger animals. In addition, the variance of the responses to the dopaminergic drug was greater in the aged monkeys, suggesting a more heterogeneous response. Although the GPe has been considered a key brain area regulating the motor output of the basal ganglia system, it still remains speculative whether hypoactivity of the GPe leads to hyperactivity in the STN in parkinsonism (for reviews, see Levy et al., 1997; Parent and Hazrati, 1995; Prensa et al., 2000; Obeso et al., 2008a,b). Regarding the role of the GPe in age-associated motor impairment, available data are scarce supporting that further translational studies are warranted.

Numerous studies have shown that the GP (including both GPi and GPe) as a whole is preferentially affected by many neurotoxins in both human and nonhuman primates including manganese (Olanow et al., 1996; Cersosimo and Koller 2006); carbon monoxide (Bhatia and Marsden, 1994; Kinoshita et al., 2005), hypoxia (Laplane et al., 1989), and MPTP (Zhang et al., 1999). One possible explanation could be that the GP has the highest iron levels in aged adults (Zecca et al., 1996) and aged primates (Hardy et al., 2005). Available evidence suggests that free iron is very reactive and thus highly toxic (for a review, see Yantiri and Andersen, 1999). High concentrations of ferrous iron can exacerbate the effects of neurotoxin such as MPTP (Mochizuki et al., 1994), suggesting that there may be synergistic effects between iron and the neurotoxicity, which may attribute to the vulnerability of the GP to any neurophysiological challenges. Interesting enough, levels of iron accumulation in the striatum can also be used to predict aging-related decline in motor function in rhesus monkeys (Cass et al., 2007). That study showed a strong correlation between decreases in motor performance, decreases in striatal DA release, and increases in striatal iron levels in aged monkeys.

Similar to the “mild parkinsonian signs” previously described in humans, the aged rhesus monkeys used in this study displayed parkinsonian-like symptoms including rigidity, bradykinesia, postural and balance instability. The age-associated motor deficits seen in the current study were comparable to those previously reported in rhesus macaques (Zhang et al., 2000) suggesting that aged rhesus monkeys could be used as an appropriate model to analyze age-associated changes in motor functions and “mild parkinsonian signs” with advancing age. The progressive impairment of motor abilities seen in aging includes bradykinesia, stooped posture and shuffling gait, all of which resemble the clinical features of PD (Bennett et al., 1996; Smith et al., 1999). It has been hypothesized that the similarities in movement dysfunction in aging and PD may arise in part from alterations of the same neuronal circuitry, namely the nigrostriatal dopamine pathway (Cass et al., 2007; Hämmeler and Eppinger, 2012). Results from the present study may partially change the view in this regard. Indeed, functional changes of the nigrostriatal dopaminergic system have been demonstrated in both PD and aging. PD is characterized by an extensive, >60% loss of midbrain dopaminergic neurons in SNc and >80% loss in DA content in the striatum (Fearnley and Lees, 1991). In older adults, there is evidence for a widespread decline of

dopaminergic neuromodulation in cortical as well as subcortical regions such as the basal ganglia (for reviews, see Bäckman et al., 2010; Darbin, 2012). However, major changes in dopaminergic neurons (i.e., >50% loss) do not occur during normal aging in either human or in animal models of aging suggesting that DA cell loss alone cannot account for age-related motor deficits (Irwin et al., 1994; Emborg et al., 1998; Gerhardt et al., 2002). Emerging evidence suggests that other functional alterations occur in the nigrostriatal dopaminergic pathway during aging including decreases of DA receptors, changes in DA release and uptake and changes in DA transporter (DAT) markers (Bannon et al., 1992; Gerhardt et al., 1995, 2002). The result from this study suggests that age-associated changes, which cause motor dysfunction in the elderly, could be more in the pallidal rather than striatal regions (Fig. 5). Meanwhile, the involvement of the dopaminergic system can still not be ruled out. Neuroimaging studies have consistently found an age-related decrease of D2 receptor marker at a rate of 5–10% per decade, starting in early adulthood (Antonini et al., 1993; Rinne et al., 1993; Wang et al., 1995; Wong et al., 1997; Pohjalainen et al., 1998; Volkow, Wang et al., 1998; Bäckman et al., 2000; Ishibashi et al., 2009).

Raclopride (RAC) administration has been widely used not only in PD-related research but also in a number of aging studies that were primarily focused on age-related changes in the nigrostriatal system of humans (Antonini et al., 1993; Rinne et al., 1990, 1993; Volkow et al., 1996, 1998) as well as of non-human primates (Harada et al., 2002). However, an outstanding question from the present study is why RAC did not inhibit APO-induced activations but instead potentiated the activation responses even further in the MPTP-treated putamen (Fig. 2A). A possible explanation of these potentiated effects seen after the subsequent administration of RAC may be attributed to the pharmacological effects of RAC on the DA D2 pre-synaptic autoreceptors. Because the pre-synaptic autoreceptor sites are considered to be more sensitive than the post-synaptic DA D2 receptor sites (Carey et al., 2008), one possibility is that the dose of RAC used in the present study could preferentially antagonize the autoreceptors and increase DA availability (Cooper et al., 1996).

In summary, similar motor dysfunctions were expressed in both aged and MPTP-lesioned monkeys. However, most notable differences in response to dopaminergic stimulations were shown in different anatomical regions, i.e., in aged GPe and MPTP-treated putamen, although relatively smaller but significant differences were also seen in the CD and SN between aged and MPTP-treated monkeys. Results from the present study and others point to the GPe as an attractive target for investigations of both aging and PD, and for future therapies, potentially involving direct pharmacological targeting. In addition to these differences, similar responses were found in the GPi of aged versus MPTP-treated animals in response to the initial APO challenge and the subsequent stimulations either by SCH or RAC (Fig. 3A). Overall, these findings strongly indicate that the GPe could be the primary treatment target for age-related movement. Treatments may take aim at the accumulation of excess iron in the brain during normal aging. Furthermore, the present study demonstrates that phMRI is a non-invasive, safe, and objective tool that can be used to longitudinally investigate functional alterations in the brain.

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Highlight

- Pharmacological MRI was used to study the central dopaminergic system in awake aged and parkinsonian rhesus monkeys.
- MPTP-treated and aged animals had similar behavioral features of motor dysfunction.
- Apomorphine and D1 (SCH23390) or D2 (raclopride) receptor antagonist were used as dopaminergic stimuli.
- The key differences in responses to the dopaminergic stimuli were found in the aged GPe and MPTP-lesioned striatum.

Figure 1. Ratings of parkinsonian features of motor dysfunction in MPTP-treated and aged animals. Normal control animals have a rating of zero on all features. ***P<0.001 MPTP vs aged animals.

Figure 2.

phMR responses in the putamen. (2A) Similar pharmacological actions were found in responses to either APO or the subsequent administration of SCH between MPTP-treated and aged animals (left and middle columns); highly significant differences were detected between the two groups after the subsequent injection of RAC ($P < 0.001$). (2B) Illustration of the evolution of the temporal phMRI response to dopaminergic challenges with APO followed by RAC in the putamen. Each time point represents the average value of R_2^* over a 5-minute period relative to the overall 15-minute baseline level; a, $P < 0.01$ compared with normal healthy controls; b, $P < 0.01$ compared with MPTP; c, $P < 0.01$ MPTP vs. aged animals.

Figure 3.

phMRI responses in the GPi. (3A) Similar pharmacological responses to either APO or the subsequent administration of dopamine receptor antagonists were found between MPTP-treated and aged animals. (3B) Illustration of the evolution of the temporal phMRI response to dopaminergic challenges with APO followed by either SCH or RAC in the GPi. Each time point represents the average value of R_2^* over a 5-minute period relative to the overall 15-minute baseline level. No statistically significant differences were found between groups. a, $P < 0.01$ compared with normal healthy controls.

Figure 4.

phMRI responses in the CD, GPe and SN. (4A) Significant ($P < 0.01$) differences were found in the caudate between MPTP-treated and aged animals with APO and the subsequent administration of SCH (left and middle columns); (4B) Significant ($P < 0.01$) differences were found in the GPe, between MPTP-treated and aged animals in responses to all dopaminergic stimulations i.e. strong activations were seen in the aged animals while much milder responses were seen in the MPTP-treated animals; (4C) In the SN, the only statistically significant differences were seen after the subsequent administration of SCH. Slight differences were also observed in responses to APO and the subsequent RAC, but they failed to reach statistically significant levels (left and right columns). a, $P < 0.01$ compared with normal healthy controls; b, $P < 0.01$ compared with MPTP.

Figure 5.

Age- and parkinsonism-related changes in the basal ganglia. Compared with normal middle-aged animals, activity associated with dopaminergic stimulations were seen in the aged GPe and MPTP-treated striatum. Much smaller and/or no differences were observed between aged and MPTP-treated monkeys in the SN and GPi, respectively. The schematic diagram was modified based on Galvan and Wichmann (2008). Black arrows indicate inhibitory connections; gray arrows indicate excitatory connections. The thickness of the arrows corresponds to their presumed activity. Abbreviations: CM, centromedian nucleus of thalamus; CMA, cingulate motor area; Dir., direct pathway; D1, D2, dopamine receptor subtypes; Indir., indirect pathway; M1, primary motor cortex; Pf, parafascicular nucleus of the thalamus; PMC, premotor cortex; PPN, pedunculopontine nucleus; SMA, supplementary motor area. See text for other abbreviations.

Table 1

ROI Responses to Dopaminergic Stimulations

ROI Target	Group	R_2^* APO	R_2^* APO+SCH	R_2^* APO+RAC	Comparisons		
					APO vs APO+SCH	APO vs APO+RAC	APO+SCH vs APO+RAC
					<i>z-score</i>	<i>z-score</i>	<i>z-score</i>
Caudate Nucleus (CD)	Middle-Aged	0.1116±0.059	0.291 ±0.059	-0.255±0.072	-2.10 ^{ns}	3.99 ^{***}	5.87 ^{***}
	MPTP	-1.091±0.087 ^a	-0.999±0.063 ^a	-0.666±0.133 ^a	-0.86 ^{ns}	-2.67 ^{ns}	-2.26 ^{ns}
	Aged	-0.450±0.168 ^{a,b}	0.195±0.119 ^b	-0.106±0.295	-3.13 ^{**}	-1.01 ^{ns}	0.96 ^{ns}
Putamen	Middle-Aged	0.197±0.071	0.466±0.071	-0.075±0.044	-2.68 ^{ns}	3.26 ^{**}	6.48 ^{***}
	MPTP	-1.130±0.114 ^a	-0.822±0.043 ^a	-2.939±0.297 ^a	-2.53 ^{ns}	5.69 ^{***}	7.05 ^{***}
Global pallidus externa (GPe)	Aged	-0.672±0.302 ^a	-1.103±0.195 ^a	-0.256±0.392 ^b	1.20 ^{ns}	-0.84 ^{ns}	-1.93 ^{ns}
	Middle-Aged	0.385±0.103	0.198±0.266	0.224±0.090	0.66 ^{ns}	1.18 ^{ns}	-0.09 ^{ns}
	MPTP	-0.249±0.196 ^a	-0.126±0.061	0.005±0.128	-0.60 ^{ns}	-1.13 ^{ns}	0.85 ^{ns}
Global pallidus interna (GPi)	Aged	-2.375±0.598 ^{a,b}	-1.488±0.242 ^{a,b}	-2.453±0.496 ^{a,b}	-1.38 ^{ns}	0.10 ^{ns}	1.75 ^{ns}
	Middle-Aged	0.063±0.144	0.013±0.126	0.689±0.145	0.26 ^{ns}	-3.06 ^{**}	-3.52 ^{**}
	MPTP	-1.356±0.248 ^a	1.101 ±0.215 ^a	-2.840±0.535 ^a	-7.49 ^{***}	2.52 ^{ns}	8.84 ^{***}
Substantia nigra (SN)	Aged	-0.808±0.677	0.836±0.495	-2.035±1.183	-1.96 ^{ns}	0.90 ^{ns}	2.24 ^{ns}
	Middle-Aged	0.099±0.024	0.156±0.059	-0.391±0.138	-0.89 ^{ns}	3.50 ^{**}	3.64 ^{***}
	MPTP	-0.136±0.062 ^a	0.483±0.076 ^a	-1.483±0.172 ^a	-6.31 ^{***}	7.37 ^{***}	10.4 ^{***}
	Aged	-1.087±0.466	-0.929±0.264 ^{a,b}	-2.439±0.483 ^a	-0.30 ^{ns}	2.01 ^{ns}	2.74 ^{**}

Note: The means ± STD of the R_2^* in the five basal ganglia regions examined are shown in responses to systemic administration APO and subsequent administration of either SCH23390 (SCH) or Raclopride (RAC). Mean and STD values are estimated from time series data averaged across animals and repeated scans within each group and using the last 20 time points (5–15 minutes post drug). P-values are determined from z-scores based on a t-distribution with 38 degrees of freedom. Differences in responses to dopaminergic stimulation between drugs or between groups are considered significant (for P<0.01);

^a P<0.01 compared with normal middle-aged controls;

^b P<0.01 compared with MPTP; ns, not significant;

** P<0.01;

P<0.0001

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