Correlation of Brain Magnetic Resonance Imaging Changes with Pallidal Manganese Concentrations in Rhesus Monkeys Following Subchronic Manganese Inhalation

David C. Dorman,*† Melanie F. Struve,* Brian A. Wong,* Janice A. Dye,† and Ian D. Robertson‡

*CIIT Centers for Health Research, Research Triangle Park, North Carolina 27709-2137; †Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, ORD, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27709; and ‡Department of Biomedical Science, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606

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High-dose manganese exposure is associated with parkinsonism. Because manganese is paramagnetic, its relative distribution within the brain can be examined using magnetic resonance imaging (MRI). Herein, we present the first comprehensive study to use MRI, pallidal index (PI), and $T_1$ relaxation rate (R1) in concert with chemical analysis to establish a direct association between MRI changes and pallidal manganese concentration in rhesus monkeys following subchronic inhalation of manganese sulfate (MnSO₄). Monkeys exposed to MnSO₄ at $\geq 0.06$ mg Mn/m³ developed increased manganese concentrations in the globus pallidus, putamen, olfactory epithelium, olfactory bulb, and cerebellum. Manganese concentrations within the olfactory system of the MnSO₄-exposed monkeys demonstrated a decreasing rostral-caudal concentration gradient, a finding consistent with olfactory transport of inhaled manganese. Marked MRI signal hyper-intensities were seen within the olfactory bulb and the globus pallidus; however, comparable changes could not be discerned in the intervening tissue. The R1 and PI were correlated with the pallidal manganese concentration. However, increases in white matter manganese concentrations in MnSO₄-exposed monkeys confounded the PI measurement and may lead to underestimation of pallidal manganese accumulation. Our results indicate that the R1 can be used to estimate regional brain manganese concentrations and may be a reliable biomarker of occupational manganese exposure. To our knowledge, this study is the first to provide evidence of direct olfactory transport of an inhaled metal in a non-human primate. Pallidal delivery of manganese, however, likely arises primarily from systemic delivery and not directly from olfactory transport.

Key Words: Parkinson's disease; secondary; manganese poisoning; pharmacokinetics; inhalation exposure; Macaca mulatta; magnetic resonance imaging.

Couper’s 1837 report of five Scottish workers with paraplegia, muscle weakness, and staggered gait following high-dose exposure to manganese oxide dust is widely acknowledged as the first clinical description of manganese neurotoxicity. Manganese neurotoxicity is a form of parkinsonism because affected individuals develop generalized bradykinesia, widespread rigidity, and occasional resting tremor (Pal et al., 1999). Manganese neurotoxicity occurs in manganese miners and workers engaged in battery assembly, ferroalloy production, iron and steel foundry work, and welding (Pal et al., 1999). Epidemiological reports suggesting that welders are at an increased risk for the development of Parkinson’s disease (Gorell et al., 2004; Racette et al., 2001) have further fueled concerns about public health ramifications from exposure to this metal.

Increased manganese concentration in select regions of the brain is a critical step in the pathogenesis of manganese neurotoxicity. Transfer of manganese across the blood-brain barrier involves active and passive transport (Aschner et al., 2005). Once within the central nervous system (CNS), manganese can undergo additional translocation along nerve fibers. For example, manganese can be transported via efferent axons from the nucleus accumbens, caudate-putamen, and other brain nuclei with a high density of transferrin receptors into the ventral pallidum, globus pallidus, and substantia nigra (Lin et al., 2001). Excess accumulation of manganese within the basal ganglia induces degeneration predominantly within the globus pallidus and subthalamic nucleus, with less frequent injury occurring in the putamen and caudate nucleus (Pal et al., 1999).

Because manganese is paramagnetic, its distribution within the CNS can be visualized by nonenhanced magnetic resonance imaging (MRI). Newland and coworkers (1989) were among the first to use MRI to assess brain manganese...
deposition in nonhuman primates. Subsequent MRI studies in humans have documented that either increased manganese exposure or reduced hepatobiliary excretion of manganese can result in appreciable hyperintensities within the pallidum and other brain regions known to accumulate manganese (Ikeda et al., 2000; Kim, 2004; Krieger et al., 1995; Nagatomo et al., 1999; Newland et al., 1989; Rose et al., 1999; Sadek et al., 2003; Stewart et al., 2005). MRI has been used to support a diagnosis of manganese neurotoxicity in welders, individuals receiving total parenteral nutrition, and patients with hepatobiliary insufficiency (Kim, 2004; Nagatomo et al., 1999; Stewart et al., 2005). One means of quantifying the MRI signal hyperintensity is the pallidal index (PI), which reflects the relative signal intensity in the T1 MRI of the globus pallidus versus the adjacent subcortical frontal white matter (Krieger et al., 1995). Despite growing interest in the use of the PI as a biomarker of manganese exposure (Kim, 2004), there is little data available demonstrating whether changes in the PI in fact reflect actual brain manganese concentrations.

Studies documenting the presence of manganese in the olfactory bulb of rats, mice, and freshwater pike following intranasal instillation have raised concerns that manganese can undergo direct transport from the olfactory region of the nasal cavity to the brain, hence direct “nose-to-brain” transport (Tjälv and Henriksson, 1999). In rats, intranasal instillation of manganese has been shown to result in direct transport of manganese to the olfactory bulb and the telencephalon through translocation in secondary olfactory neurons (Tjälv and Henriksson, 1999). Once in the brain, manganese can continue to move across synaptic connections and along neural processes to sites distantly connected to the olfactory center in the brain (Tjälv and Henriksson, 1999). Our laboratory has demonstrated that in rats, inhaled manganese is absorbed by the olfactory epithelium and subsequently undergoes transport via the olfactory nerve to the olfactory bulb (Brenneman et al., 2000). Although direct nose-to-brain transport appears to be an important route by which inhaled manganese reaches the rodent brain, it remains to be determined as to whether similar processes may play a role in brain manganese deposition in nonhuman primates. Anatomical similarities between nonhuman primates and humans give such information important public health ramifications.

The following report presents the first comprehensive subchronic manganese inhalation study to use MRI to establish whether a direct association exists between MRI changes and pallidal manganese concentration in nonhuman primates. Importantly, this report also provides MRI evidence to suggest that direct nose-to-brain transport of manganese occurs in rhesus monkeys following manganese inhalation.

**MATERIALS AND METHODS**

**Animals.** This study was conducted under federal guidelines for the care and use of laboratory animals (NRC, 1996) and was approved by the CIIT Institutional Animal Care and Use Committee. Twenty, 17- to 22-month-old, naive male rhesus monkeys were purchased from Covance Research Products, Inc. (Alice, TX). Monkeys were approximately 20- to 24-months old at the start of the inhalation exposure. Monkeys were exposed 6 h/day, 5 days/week, for 13 weeks (65 exposure days). Monkeys were allocated as follows: air (n = 6) and 0.06 mg Mn/m³ (n = 6), 0.3 mg Mn/m³ (n = 4), and 1.5 mg Mn/m³ (n = 4). Additional details concerning these animals and their husbandry have been recently published (Dorman et al., 2005).

**Manganese exposures.** Manganese(II) sulfate monohydrate (MnSO₄·H₂O) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Four, 8-m³, stainless steel and glass inhalation exposure chambers were used. Methods describing chamber monitoring as well as generation and characterization of the MnSO₄ aerosol have been previously described (Dorman et al., 2004, 2005). Based upon optical particle sensor measurements, the overall average concentrations (± SD) for the MnSO₄ atmospheres were 0.19 ± 0.01, 0.97 ± 0.06, and 4.55 ± 0.33 mg/m³ for the target concentrations of 0.18, 0.92, and 4.62 mg MnSO₄/m³, respectively. The geometric mean diameter, geometric standard deviation (σg), and calculated mass median aerodynamic diameters (MMAD) of the MnSO₄ aerosols were determined to be 1.04 (σg = 1.51; MMAD = 1.73 μm), 1.07 (σg = 1.54; MMAD = 1.89 μm), and 1.12 μm (σg = 1.58; MMAD = 2.12 μm) for the target concentrations of 0.18, 0.92, and 4.62 mg MnSO₄/m³, respectively.

**Neuroimaging studies.** T₁-Weighted MRI studies of the head were performed near the end of the 13-week inhalation exposure (i.e., after 63–64 exposures and 3–4 days before the necropsies were performed). The following anesthesia protocol was used. Animals were fasted overnight and then tranquillized with ketamine (approximately 10–15 mg/kg, im, Fort Dodge Animal Health, Fort Dodge, IA). Animals were given atropine (approximately 0.05 mg/kg·s, Phoenix Scientific Inc., St Joseph, MO) to decrease bronchial secretions and prevent bradycardia. This was followed by induction of generalized anesthesia using an iv bolus of propofol (Gensia Sicor Pharmaceuticals, Inc., Irvine, CA) at an approximate dose of 1.25–2.5 mg/kg followed by slow iv propofol infusion at approximately 300 μg/kg/min (range 105–470 μg/kg/min) to maintain a light plane of anesthesia (Fowler et al., 2001). Heart rate and pulse oximetry were monitored throughout the procedure (Nonin Medical Inc, Plymouth, MN). Earplugs were placed in the ear canal to reduce auditory arousal during imaging.

MRI scans were done on a 1.5-T MRI scanner (GE Genesis Signa, General Electric Medical, Milwaukee, WI) with a standard Quadknee coil. The anesthetized animal’s head was packed into the coil using foam; however, coregistration using a coordinate system was not used. Axial images were obtained using a standard spin echo pulse sequence at different repetition times (TR). Shimming, receiver gain, radio frequency (RF) gains, and RF were calibrated at 4000 ms and kept unchanged for all subsequent TRs. Eight different TRs, 100, 200, 400, 600, 800, 1000, 2000, and 4000 ms, were used to cover the full T₁ recovery range of brain tissue. The acquisition parameters of the T₁-calculated images included echo time (TE) = 10 ms; slice thickness (ST) = 2 mm (contiguous); number of averages (NA) = 100 ms × 200, 200 ms × 3, 400 ms × 2, 600 ms, and all subsequent TRs × 1; field of view (FOV) = 120 mm; and matrix = 128 × 128, resulting in a cumulative imaging time of approximately 1 h per subject. Slice acquisition was done in an “interlaced” configuration to eliminate cross talk between slices. MRI data were interpolated to 256 × 256, and the images were processed at a DICOM workstation (Merge eFilm, Toronto, Canada). The standard spin echo sequences were used for generation of the T₁ values. Fast spin echo (FSE) images were only used to generate images of the entire brain for anatomy. Fast imaging was not used for T₁ calculation. Slices were interlaced to prevent cross talk. The signal intensities were not normalized between subjects as each animal acted as its own control in the generation of the T₁ values, given the imaging parameters. The images were evaluated, and the appropriate anatomy was identified by clinical evaluation. Four nonadjacent data points were selected from each anatomical region of interest from each side of the brain. Once the appropriate neuroanatomical structure was identified and voxels were selected, the coordinates of that voxel were propagated over the range of T₁-weighted sequences, and the signal intensity was recorded. Average pixel signal intensity was then
calculated for each TR (left vs. right). One animal had evidence of misregistration (movement), and the imaging study for that animal was repeated the following day. Otherwise, there was no evidence of scan-to-scan misregistration based on random checking of selected data points.

To calculate the left and right pallidal $T_1$ relaxation times, these data were fitted with a standard two-parameter single exponential decay function of the form $I = I_0[1 - \exp(-TR/T_1)]$, where $I_0$ is the initial signal intensity, TR is the repetition time, and $T_1$ is the relaxation time under investigation. The nonlinear fitting of the exponential data to this equation was done using a general nonlinear Levene-Marquardt algorithm (Microsoft Excel, Microsoft Corporation, Redmond, WA). The spin-lattice relaxation rate ($R_1$) was subsequently calculated from $1/T_1$.

An additional $T_2$-weighted FSE sequence was generated, TR = 450 ms, TE = 12 ms, ST = 2 mm, NA = 2, echo train length (ETL) = 4, FOV = 120 mm, matrix $256 \times 192$. Axial images were acquired, and four pixels were evaluated in each of the left and right globus pallidus and left and right subcortical white matter. From the averages of these data, the PI as described by Krieger and coworkers (1995) and Spahr et al. (1996) was generated for each subject. Total anesthesia time was generally less than 2 h.

**Necropsy procedures.** Necropsies were performed the day following the last inhalation exposure. Food was withheld overnight prior to necropsy. Monkeys were anesthetized with ketamine (20 mg/kg, im, Fort Dodge Animal Health), and blood was collected from a peripheral vein using plastic syringes (80–150 mg/kg, iv, Henry Schein Inc., Port Washington, NY) followed by exsanguination. Brains were removed and divided on the midsagittal plane with anatomical structures identified using a published atlas (Martin and Bowden, 2000). Representative samples of olfactory epithelium, pituitary gland, olfactory bulb, olfactory tract, olfactory cortex, caudate, putamen, globus pallidus, cerebellum, white matter, frontal cortex, and whole blood were collected for manganese analyses. Tissue manganese concentrations were determined by graphite furnace atomic absorption spectrometry using previously published methods (Dorman et al., 2004). Globus pallidus iron concentrations were determined by inductively coupled plasma mass spectrometry (West Coast Analytical Service Inc., Santa Fe Springs, CA).

**Statistics.** The data for quantitative, continuous variables were compared for the exposure and control groups by tests for homogeneity of variance (Levene’s test), ANOVA, and the Dunnett multiple comparison procedure for significant ANOVA. A natural log transformation of the data was performed if the Levene’s test was significant. Statistical analyses were performed using JMP Statistical Software (SAS Institute, Cary, NC). A probability value of $< 0.01$ was used for Levene’s test, while $< 0.05$ was used as the critical level of significance for all other statistical tests. Unless otherwise noted, data presented represent mean values ± SEMs.

### RESULTS

Subchronic exposure of rhesus monkeys to high airborne manganese concentrations was associated with increased blood, olfactory epithelial, and brain manganese concentrations (Table 1). Importantly, the magnitude of the increase in CNS manganese concentration was dependent upon the brain region and the MnSO$_4$ exposure concentration (Table 1). An approximate 1.7-, 2.7-, and 6-fold increase (vs. controls) in mean pallidal manganese concentration was observed following subchronic exposure to MnSO$_4$ at 0.06, 0.3, and 1.5 mg Mn/m$^3$, respectively. Markedly increased olfactory epithelial, olfactory bulb, and olfactory cortex manganese concentrations also occurred in monkeys exposed to MnSO$_4$ at all exposure levels tested. Increased pituitary gland and whole-blood manganese concentrations occurred in monkeys exposed to MnSO$_4$ at $\geq 0.3$ mg Mn/m$^3$. Increased frontal cortex manganese concentrations occurred only in monkeys from the highest exposure group, and unlike the globus pallidus, this tissue developed merely a twofold increase in manganese concentration (vs. controls). Subchronic manganese exposure did not affect pallidal iron concentrations. Mean pallidal iron concentrations were $73.7 \pm 11.4, 73.3 \pm 5.7, 71.6 \pm 9.8$, and $62.6 \pm 7.2 \mu$g/g following exposure to air or MnSO$_4$ at 0.06, 0.3, and 1.5 mg Mn/m$^3$, respectively.

### TABLE 1

<table>
<thead>
<tr>
<th>Novel MnSO$_4$ concentration (mg Mn/m$^3$)</th>
<th>Air</th>
<th>0.06</th>
<th>0.3</th>
<th>1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory epithelium*</td>
<td>0.42 ± 0.01</td>
<td>1.22 ± 0.15*</td>
<td>2.96 ± 0.46*</td>
<td>7.10 ± 2.01*</td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>0.31 ± 0.01</td>
<td>0.77 ± 0.04*</td>
<td>1.36 ± 0.15*</td>
<td>2.40 ± 0.18*</td>
</tr>
<tr>
<td>Olfactory tract</td>
<td>0.30 ± 0.06</td>
<td>0.43 ± 0.02</td>
<td>0.61 ± 0.05*</td>
<td>1.12 ± 0.08*</td>
</tr>
<tr>
<td>Olfactory cortex</td>
<td>0.19 ± 0.004</td>
<td>0.27 ± 0.02*</td>
<td>0.31 ± 0.01*</td>
<td>0.42 ± 0.01*</td>
</tr>
<tr>
<td>Globus pallidus*</td>
<td>0.48 ± 0.04</td>
<td>0.80 ± 0.04*</td>
<td>1.28 ± 0.15*</td>
<td>2.94 ± 0.23*</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.36 ± 0.01</td>
<td>0.58 ± 0.04*</td>
<td>0.75 ± 0.05*</td>
<td>1.81 ± 0.14*</td>
</tr>
<tr>
<td>Caudate</td>
<td>0.34 ± 0.02</td>
<td>0.47 ± 0.04</td>
<td>0.69 ± 0.03*</td>
<td>1.72 ± 0.10*</td>
</tr>
<tr>
<td>White matter</td>
<td>0.17 ± 0.01</td>
<td>0.25 ± 0.01*</td>
<td>0.39 ± 0.04*</td>
<td>0.87 ± 0.08*</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>0.25 ± 0.03</td>
<td>0.29 ± 0.02</td>
<td>0.29 ± 0.01</td>
<td>0.47 ± 0.02*</td>
</tr>
<tr>
<td>Cerebellum*</td>
<td>0.44 ± 0.01</td>
<td>0.62 ± 0.02*</td>
<td>0.70 ± 0.04*</td>
<td>1.10 ± 0.11*</td>
</tr>
<tr>
<td>Pituitary</td>
<td>0.84 ± 0.12</td>
<td>1.53 ± 0.25</td>
<td>2.43 ± 0.13*</td>
<td>6.19 ± 0.61*</td>
</tr>
<tr>
<td>Blood</td>
<td>0.010 ± 0.001</td>
<td>0.015 ± 0.002</td>
<td>0.022 ± 0.003*</td>
<td>0.026 ± 0.003*</td>
</tr>
</tbody>
</table>

*Significantly different from control, $p < 0.05$.

*Data analysis performed following natural log transformation.
Changes in manganese concentrations were in good accord with regional changes noted during brain MRI studies. Brain regions with increased manganese concentrations had increased MRI signal intensities that were symmetrical between the two hemispheres. For example, increased signal intensities in the olfactory bulb and globus pallidus were consistently observed in MnSO$_4$-exposed monkeys (Fig. 1). As the MnSO$_4$ exposure concentration increased, a more extensive pattern of $T_1$ signal hyperintensity could be discerned within different brain regions (Fig. 2). Mean pallidal $T_1$ relaxation times consistently shortened from 1008 ± 45, 879 ± 25, 761 ± 57, and 468 ± 43 ms following exposure to air or MnSO$_4$ at 0.06, 0.3, and 1.5 mg Mn/m$^3$, respectively. A significant decrease in pallidal $T_1$ relaxation time (vs. air-exposed controls) was noted following exposure to MnSO$_4$ at ≥ 0.06 mg Mn/m$^3$. Linear correlations between pallidal, white matter, and pituitary manganese concentrations and the $R_1$ relaxation rate were observed (Fig. 3). Mean $PI$ values were 1.04 ± 0.02, 1.08 ± 0.03, 1.13 ± 0.05, and 1.29 ± 0.02 following exposure to air or MnSO$_4$ at 0.06, 0.3, and 1.5 mg Mn/m$^3$, respectively. A significant increase in $PI$ (vs. air-exposed controls) was noted following exposure to MnSO$_4$ at 1.5 mg Mn/m$^3$. We found statistically significant linear relationships between both the pallidal and whole-blood manganese concentrations and the $PI$ (Fig. 4) and between whole-blood manganese concentration and pallidal manganese concentration ($r^2 = 0.50$) (Fig. 5).

**DISCUSSION**

There is overwhelming evidence that the human striatum, globus pallidus, and substantia nigra show preferential increases in manganese concentrations and are also believed to be the primary target sites for manganese neurotoxicity (Pal et al., 1999). Previous investigations have shown that manganese-exposed monkeys also develop elevated manganese concentrations at these target sites (Eriksson et al., 1992; Newland et al., 1989; Olanow et al., 1996). Although the target sites for manganese neurotoxicity are well known, the processes by which certain manganese exposure conditions lead to increased brain manganese concentrations at these susceptible sites are not as well established. This study was designed to resolve some of these uncertainties. We observed that monkeys exposed to the lowest MnSO$_4$ exposure concentration used in our study (0.06 mg Mn/m$^3$) developed increased globus pallidus and putamen manganese concentrations. Somewhat unexpectedly, these same animals developed increased cerebellar and white matter manganese concentrations. Increased manganese concentrations in the caudate and pituitary gland emerged following subchronic exposure to MnSO$_4$ at 0.3 mg Mn/m$^3$. Increased frontal cortex manganese concentrations occurred following subchronic exposure to MnSO$_4$ at 1.5 mg Mn/m$^3$. Brain MRI evaluations of animals exposed to MnSO$_4$ at 0.3 mg Mn/m$^3$ revealed basal ganglia signal hyperintensities consistent with published reports in monkeys (Eriksson et al., 1992; Newland et al., 1989; Olanow et al., 1996) and humans (Pal et al., 1999) with excessive manganese exposure. The highest MnSO$_4$ exposure (1.5 mg Mn/m$^3$) led to a more extensive manganese distribution pattern that could be visualized by MRI. For example, we observed increased manganese concentrations extending into the frontal cortex in monkeys exposed to the highest MnSO$_4$ concentration. The changes noted on MRI correlated well with subsequent manganese tissue analysis.

This study confirmed that the increase in tissue manganese concentration varies among different brain regions. A greater than five- to sixfold increase (vs. controls) in mean tissue manganese concentration was observed in the globus pallidus, putamen, caudate, and white matter of monkeys exposed to MnSO$_4$ at 1.5 mg Mn/m$^3$. In contrast, manganese concentrations in the frontal cortex and cerebellum, two brain regions thought not to be associated with manganese neurotoxicity (Pal et al., 1999), had less than a threefold increase (vs. controls). An approximately sevenfold increase (vs. controls) in pituitary gland manganese concentration was observed in the most heavily exposed monkeys. These results suggest that like the globus pallidus, the pituitary gland also preferentially accumulates manganese. Brain MRI studies conducted by Newland et al. (1989) showed a similar distribution pattern in a cynomolgus monkey exposed to a manganese chloride aerosol, consistent with our findings.

Our study also considered whether inhaled manganese could undergo direct olfactory transport. Resolving this question is critical because the olfactory system is in direct contact with the external environment. Our previous studies showed that the olfactory system can provide a pathway by which inhaled manganese is translocated to the rodent brain (Brenneman et al., 2000). This present study further demonstrates that MnSO$_4$-exposed monkeys develop markedly (7- to 15-fold)
increased olfactory epithelial and olfactory bulb manganese concentrations. Interestingly, olfactory bulb manganese concentrations were comparable to those seen in the globus pallidus. Absolute manganese concentrations in the MnSO₄-exposed monkeys demonstrated a decreasing peripheral-central concentration gradient within the olfactory system, i.e., olfactory epithelium > olfactory bulb > olfactory tract > olfactory cortex. The increase in olfactory bulb manganese concentration seen in the MnSO₄-exposed monkeys is qualitatively similar to that seen in young adult male rats exposed subchronically to comparable MnSO₄ exposure concentrations (Dorman et al., 2004). When considered collectively, the data are consistent with direct olfactory transport of inhaled manganese. To our knowledge, this is the first evidence of direct nose-to-brain transport of an inhaled material in a non-human primate.

Neuronal connections between the rhesus monkey olfactory bulb and olfactory cortex have been examined using anterograde and retrograde axonal tracers. Anterograde tracers placed into the monkey olfactory bulb labeled axons in the anterior olfactory nucleus, piriform cortex, ventral tenia tecta, olfactory tubercle, anterior cortical nucleus of the amygdala, periamygdaloid cortex, and olfactory division of the entorhinal cortex (Carmichael et al., 1994; Insausti et al., 2002). Retrograde tracers injected into the olfactory bulb labeled cells in the nucleus of the diagonal band and the majority of the primary olfactory cortical area. The entorhinal cortex can also give rise to projections that terminate within the subiculum, hippocampus, and dentate gyrus (Witter and Amaral, 1991). To our knowledge, significant neuronal connections between the monkey olfactory bulb and globus pallidus do not exist. Cross et al. (2004) used MRI to evaluate nose-to-brain transport of manganese in rats following intranasal instillation of manganese chloride. These investigators showed that manganese-enhanced MRI results were qualitatively similar to those obtained using more classical nerve tract-tracing methods. The Cross et al. (2004) study as well as those conducted in our laboratory (Brenneman et al., 2000) showed that manganese does not undergo transport to the rat striatum or other more distal brain structures directly from the nasal cavity. Brain MRI studies of our MnSO₄-exposed monkeys demonstrated marked signal hyperintensities within the olfactory bulb and the globus pallidus. The resolution of the MRI used in this study does not allow resolution of individual nerve tracts. However, comparable signal hyperintensities could not be visualized in the intervening brain parenchyma, and thus our study failed to provide evidence that direct translocation of manganese from the olfactory bulb to the globus pallidus.
occurred in the manganese-exposed monkeys. This finding is consistent with neuroanatomical studies that failed to demonstrate direct anterograde projections from the macaque olfactory bulb to the globus pallidus (Carmichael et al., 1994).

Another important finding of our study is that MRI can be used as a biomarker of ongoing subchronic manganese exposure. Two MRI measurements, namely the PI and the $T_1$ relaxation rate, were used to characterize manganese accumulation within the globus pallidus, pituitary, and white matter. Previous reports in rats indicate that significant correlations between the concentration of manganese in the globus pallidus and the PI are possible (Gallez et al., 2001). Our study further demonstrates that pallidal manganese concentrations in nonhuman primates are also linearly correlated with the PI. The primary advantage of using the PI to estimate regional brain manganese content is that this procedure is straightforward and can be performed relatively quickly using routine $T_1$ MRI scans. However, it should be noted that calculation of the PI presumes that white matter manganese concentrations are unaffected. Our data demonstrate that this is not necessarily the case since white matter manganese concentrations increased in all exposure groups following subchronic MnSO$_4$ inhalation.

We believe that our data may provide a rational basis for beginning to understand the relationship between PI and pallidal manganese concentration. Although there is little data evaluating whether an increased PI in humans is associated with elevated pallidal manganese concentrations, our data appear to be in relatively good concordance with the published clinical literature. For example, patients with hepatic cirrhosis demonstrate increased pallidal manganese accumulation and signal hyperintensity on MRI (Hauser et al., 1996; Layrargues et al., 1998; Maeda et al., 1997; Spahr et al., 1996). Spahr et al. (1996) reported that PI values in 62 normal and 57 cirrhotic patients were $99.1 \pm 1.8$ and $114 \pm 17.4$, respectively. A PI value of $\geq 114$ was seen in six of our MnSO$_4$-exposed monkeys. These animals had pallidal manganese concentrations ranging from 0.97 to 3.38 $\mu$g Mn/g wet weight, representing a two- to sevenfold increase in pallidal manganese concentration (vs. controls). By comparison, autopsy specimens from cirrhotic patients who died in hepatic coma also revealed a two- to sevenfold increase in pallidal manganese concentration (Layrargues et al., 1998; Maeda et al., 1997). Hence, the relative increase in pallidal manganese levels observed in our monkeys with the highest PI values are remarkably similar to those reported in people with advanced liver cirrhosis. Maeda and coworkers (1997) reported pallidal manganese concentrations in one normal human of 0.42 $\mu$g Mn/g wet weight compared to three individuals with hepatic cirrhosis (2.6–3.5 $\mu$g Mn/g wet weight). Lucchini and co-workers (2000) reported that PI values in workers with occupational exposure to manganese were lower than those occurring in patients with significant hepatic cirrhosis. The median PI value reported in seven manganese-exposed workers with neurobehavioral impairment was $107.7 \pm 7.7$ (Lucchini et al., 2000); however, very similar PI values ($107.7 \pm 6.5$) have also been reported in 89 asymptomatic manganese-exposed workers (Kim et al., 1999). To put this in perspective with our study, the mean pallidal manganese concentrations in monkeys with a PI of 105–110 was $0.86 \pm 0.31$ $\mu$g Mn/g wet weight. This manganese concentration represents an approximate

![FIG. 3. Statistically significant linear relationships were found between white matter (top), globus pallidus (middle), and pituitary (bottom) manganese concentrations and the relaxation rate ($R_1$). MnSO$_4$ exposure concentrations are provided in mg Mn/m$^3$. Solid symbols display mean values (± SEMs). Asterisk indicates that brain manganese concentration is increased relative to air-exposed controls ($p < 0.05$) while double dagger indicates that both brain manganese concentration and relaxation rate are increased relative to air-exposed controls ($p < 0.05$).]
80% increase in pallidal manganese concentration (vs. control monkeys).

Several investigators have attempted to correlate blood manganese concentrations with the PI, with mixed results. Positive correlations between blood manganese concentration and the PI have been reported in welders (Kim et al., 1999) and patients with liver cirrhosis (Choi et al., 2005; Park et al., 2003). In contrast, patients with iron deficiency anemia typically have a normal-appearing globus pallidus MRI signal intensity despite elevated blood manganese concentrations (Kim et al., 2005). We observed linear relationships between whole-blood manganese concentration and either the PI or the pallidal manganese concentration. The strengths of these correlations were weaker (i.e., \( r^2 \) values from 0.45 to 0.50) than those seen between pallidal manganese concentration and the PI, suggesting that blood manganese concentration is a less robust predictor of pallidal manganese concentration.

Importantly, we also observed a linear relationship between pallidal manganese concentration and the \( T_1 \) relaxation rate. In fact, the strength of the linear association between \( R_1 \) and the pallidal manganese concentration was somewhat greater than that seen between the pallidal manganese concentration and PI. Unlike the PI, there was little overlap between pallidal \( T_1 \) relaxation rates measured among different MnSO\(_4\) exposure groups. The major disadvantage of using the relaxivity of manganese (\( R_1 \)) is that the time required to acquire the \( T_1 \) relaxation time results in prolonged imaging sessions of one or more hours. Although the \( R_1 \) appears to be a more accurate way to estimate pallidal manganese concentrations than the PI, this measure may still not be suitable for detecting small changes (e.g., < 50% increase) in pallidal manganese concentration. There are conflicting reports as to whether age-related changes in brain iron concentrations (as estimated by MRI) and MRI signal intensity change during aging in people (Martin et al., 1998; Schenker et al., 1993). Such changes may influence the measurement of either the PI or the \( R_1 \). In our study, globus pallidus iron concentrations were unaffected by MnSO\(_4\) inhalation, and thus it is unlikely that the measurements of either the PI or the \( R_1 \) in the present study were confounded by alterations in pallidal iron concentration.

Our lowest exposure MnSO\(_4\) concentration (0.06 mg Mn/m\(^3\)) is below the current 8-h threshold limit value for inhaled manganese of 0.2 mg Mn/m\(^3\) that has been established by the American Conference of Governmental Industrial Hygienists. Previous rodent studies have shown that brain delivery of manganese is favored following inhalation of the more soluble sulfate form of manganese (Dorman et al., 2004). Thus, comparable exposure (on a mg Mn/m\(^3\) basis) to mixed manganese oxides associated with welding and other metal-working occupations should be less likely to induce increased brain manganese concentrations. The present data from exposed monkeys are also relevant for environmental exposures to manganese. In particular, modern automobiles that use the gasoline fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT) emit manganese primarily in the phosphate and sulfate forms with smaller amounts of manganese oxides also being discharged (Aschner et al., 2005). The lowest exposure concentration used in this study

![FIG. 4. Statistically significant linear relationships were found between globus pallidus (left) and whole-blood (right) manganese concentrations and the PI. MnSO\(_4\) exposure concentrations are provided in mg Mn/m\(^3\). Solid symbols display mean values (± SEMs). Asterisk indicates that tissue manganese concentration is increased relative to air-exposed controls (\( p < 0.05 \)) while double dagger indicates that both brain manganese concentration and PI are increased relative to air-exposed controls (\( p < 0.05 \)).](https://academic.oup.com/toxsci/article-abstract/92/1/219/1642998)

![FIG. 5. Statistically significant linear relationships were found between globus pallidus manganese concentration and whole-blood manganese concentration. MnSO\(_4\) exposure concentrations are provided in mg Mn/m\(^3\). Solid symbols display mean values (± SEMs). Asterisk indicates that only the pallidal manganese concentration is increased relative to air-exposed controls (\( p < 0.05 \)) while double dagger indicates that both pallidal and blood manganese concentrations are increased relative to air-exposed controls (\( p < 0.05 \)).](https://academic.oup.com/toxsci/article-abstract/92/1/219/1642998)
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REFERENCES


