

# Animal Models of Manganese's Neurotoxicity

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**Abstract:** M. CHRISTOPHER NEWLAND. Animal Models of Manganese's Neurotoxicity. *Neurotoxicology* 20(2-3):415-432, 1999. Manganese's neurotoxicity continues to present a puzzling array of differences across individuals and across published reports in the profile of effects seen in humans and nonhuman species, but some of the sources of individual variability are becoming clear from studies of animals. The kinetics of manganese is a critical component of any assessment of risk associated with exposure. After inhalation, the uptake of manganese into and elimination from the central nervous system are slow and some manganese remains in the nervous system a year after inhalation. Comparison with other parenteral routes suggests that manganese depots in lung prolongs exposure even after environmental exposure has ended. Manganese's neurotoxicity is associated with its appearance in basal ganglia structures, especially the globus pallidus. Manganese also appears in the pituitary gland but the functional consequences of this are not well understood. Other critical components in characterizing manganese's neurotoxicity appear to be the behavioral endpoints used, the species studied, and the exposure rate. Overt neurological signs and excitability are associated with high exposure rates and the appearance of manganese throughout basal ganglia and basal forebrain regions. More focused behavioral endpoints are required to detect the subtle signs associated with slow exposure rates low exposure levels, but when such designs are used the effect is unequivocal. At lower exposure levels, doses of 5 mg/kg and greater, deficits in a task in which a monkey executed a rowing type motion against a spring approximating its body weight were clearly related to manganese exposure while other traditional measures of response patterns under schedules of reinforcement remained intact. Excitability and other signs of emotionality have not been reported at low exposure rates. In rodents, manganese accumulation and alterations in the function or concentration of neurotransmitters have been reported. Investigations of behavioral effects in these species, which usually involved locomotor activity, have resulted in less consistent results. Manganese produces a constellation of neurotoxic signs whose appearance and detection are influenced by dose and exposure rate. Despite investigations of manganese's neurotoxicity in animals over a wide range of exposure levels, a NOAEL has not been identified. © 1998 Intox Press, Inc.

**Key Words:** Manganese, Neurotoxicity, Magnetic Resonance Imaging, Schedule-Controlled Behavior, Kinetics, Animal Models, Primates, Rats

## INTRODUCTION

Manganese's neurotoxicity and its connection with the inhalation of manganese oxides were recognized more than 150 years ago on the basis of overt, ineluctable signs including distorted postures and severe gait disorders (Couper, 1837). Similar signs sparked concern over the next 100 years about miners exposed to very high concentrations of manganese (Cotzias, 1958; Edsall *et al.*, 1919; Rodier, 1955). Rodier's (Rodier, 1955) and Cotzias'

(Cotzias 1958) detailed reviews laid a solid foundation for hygienic interventions aimed at reducing exposure and anticipated contemporary discussions of the public health consequences of chronic exposure to lower levels.

Rodier and Cotzias argued that manganism involves two domains of effects, emotional and motor, that thread through an apparently progressive syndrome. The early phase was said to be dominated by emotional lability, mania, disturbances of sleep and eating, sexual disturbances, the commission of "stupid crimes" (Rodier,

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Submitted: February 9, 1998. Accepted: August 5, 1998.

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1955) but few, and subtle, motor effects. A later phase, which Rodier called the "established" phase, was dominated by motor sequelae, illustrated in gruesome photographs of severe dystonias and gait disorders in affected miners. The signs and symptoms of the early phase were difficult to associate with the motor effects of the established phase, although the latter was sometimes said to contain some emotional characteristics present in the early phase. Rodier and, later, others attributed the motor effects to basal ganglia function and explicitly ruled out involvement of other possible motor systems, such as cerebellum or cortex (Barbeau, 1984; Barbeau *et al.*, 1976; Calne *et al.*, 1994; Cotzias, 1958; Donaldson and Barbeau, 1985; Mena *et al.*, 1969; Rodier, 1955).

Another observation made by Rodier that is pertinent here is that of individual susceptibility, a topic that received considerable discussion under that heading in his paper. He noted that all cases were seen in those exposed in underground mines to high levels of new dust of manganese; by his estimation the concentrations were on the order of hundreds of  $\text{mg}/\text{m}^3$ , of which many particles were less than  $1 \mu$ . Not all miners experiencing these conditions were affected and, of those that were, the latency to the onset of illness appears to have varied considerably. Rodier considered individual differences

in the metabolic handling of manganese, exposure, age, and other factors in attempting to explain these differences.

A particularly interesting account of these individual differences can be found in Rodier's reconstruction of the employment history of the affected people, illustrated in Fig. 1. Even with exposure to high levels of dust, the median duration to the onset of illness was about one year, and 2% of the miners did not show signs until after 10 years of employment. Note that this 2% comes from a population of young to middle-aged men, healthy enough to work in a mine and exposed to a large dose of manganese. That 2% is an important group and a significant size if viewed from a public health perspective, where large, diverse populations exposed to lower levels are the focus. It is one in which the link between exposure and effects will be difficult to ascertain. Even if it can be identified, such a group would present difficulties in determining a dose effect relationship because it will not be clear whether their effects are due to the total accumulated exposure or to delayed effects of early exposure. The silent damage that this delayed onset represents, if it results from exposures to lower levels in the ambient air, would appear in advanced years and would be difficult to link to exposure.

**Time Between Employment and Onset of Illness**  
(from Rodier, 1955)

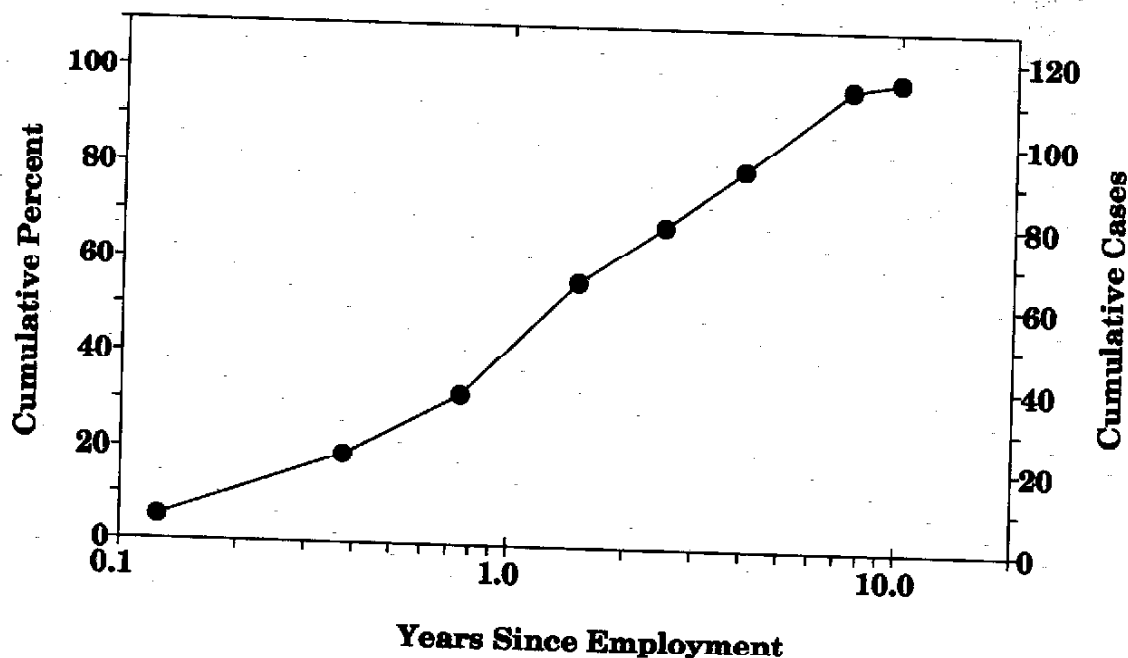


FIG. 1. Latency to the onset of illness as reported among miners examined by Rodier. Note the log scale on the horizontal axis.

There is considerable variability across studies and even across individuals within the same study in the profile of neurotoxic effects associated with manganese. The present review represents an attempt to isolate some of the important sources of variability by focusing on kinetics, dose, exposure rate, and the endpoints chosen for examination. The literature on human exposures, which includes extremely high levels of manganese in mines and other forms of occupational exposure to lower levels, describes considerable individual susceptibility and, among those experiencing effects, wide variability in the latency to their onset. To gain a clearer picture of these different variables we must turn to the animal literature, where experimental control is much better. The animal literature also contains order-of-magnitude differences in the dose at which effects appear and discrepancies in the specific symptoms manifested. Many of these differences appear to be related to the temporal properties of exposure and to the endpoints chosen for examination.

### THE ROLE OF ANIMAL STUDIES

Attempts to extend observations derived from people exposed to the high levels seen in mines to people experiencing lower exposure levels in occupational settings or ambient air have presented a puzzling array of differences in the profile of effects reported. Epidemiological or field studies conducted with human populations are advantageous because they are conducted on the population of concern, but they also carry a number of disadvantages with them, even when the testing protocols are sophisticated. Perhaps the most serious is that these studies are correlational in nature (Gullion and Eckerman, 1986; Needleman *et al.*, 1979) so, except under unusual circumstances, cause-effect and dose-effect relationships cannot be identified unequivocally. Experimental control over exposure is required to produce clear relationships between dose and effect.

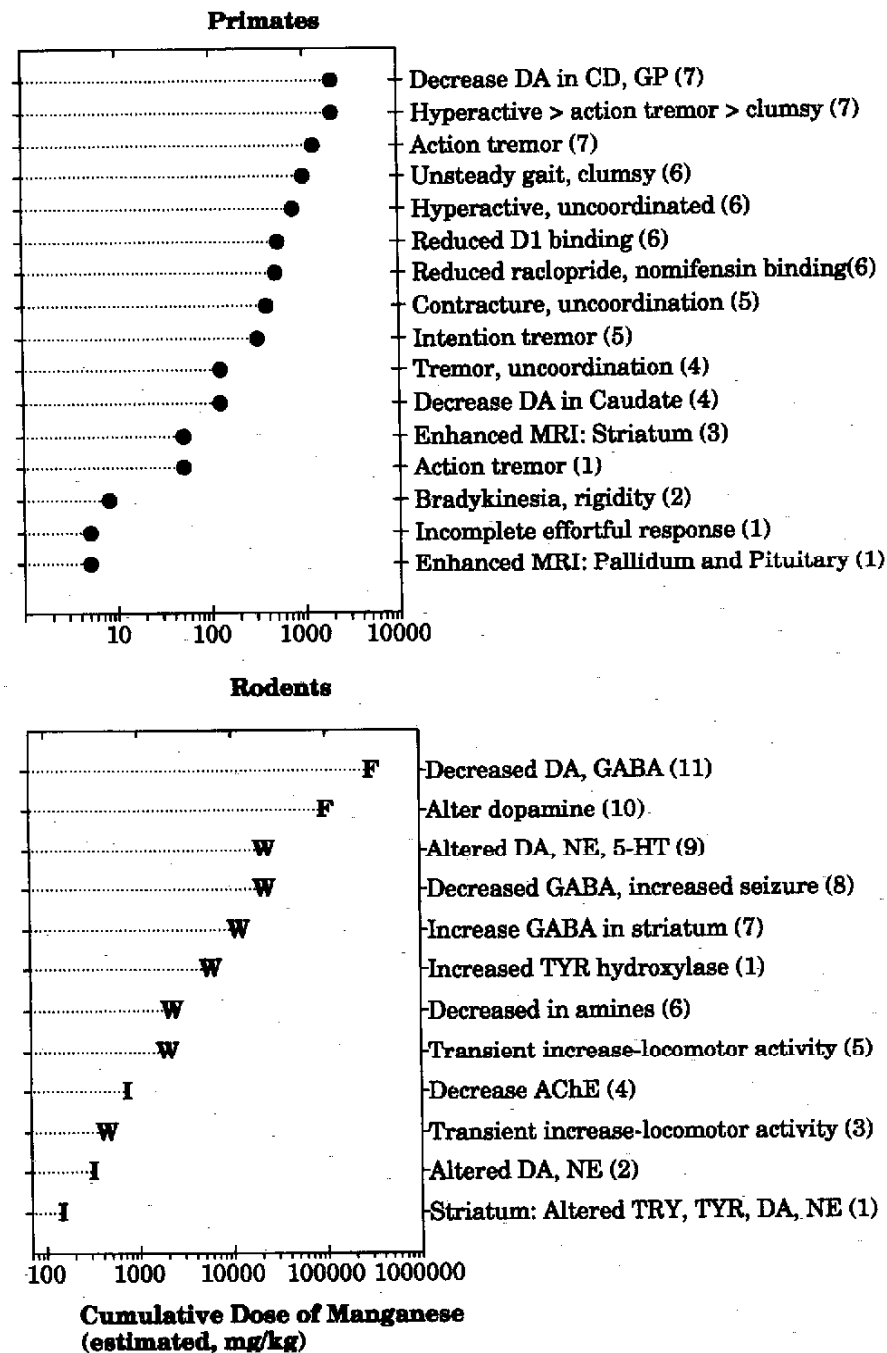
If experience with other known neurotoxicants is a guide, and it should be, then a risk assessment that integrates human and animal studies, including the behavior of intact animals, is an especially powerful tool in support of the public health. The establishment of exposure thresholds for methylmercury (Gilbert and Grant-Webster, 1995; Rice, 1996) and lead (Cory-Slechta, 1990; Davis *et al.*, 1990; Rice 1990) received strong support from the similarity between human and nonhuman species in dose-effect relationships and in the spectra of

effects reported. Once an appropriate biomarker of exposure was identified a close correspondence across species was noted in the exposure levels at which toxic effects appeared. Moreover, these two metals exert very different constellations of effects in humans, differences that are reflected in nonhuman species, too. The ability to differentiate neurotoxicants and identify dose-effect relationships is a necessary condition for comprehensive characterization and the identification of neural and behavioral mechanisms of neurotoxicity.

Laboratory studies with animals must be drawn upon to identify mechanisms of action but they can be used for far more. Risk assessments based on animal studies, such as those that have been undertaken with methylmercury (Gilbert and Grant-Webster, 1995; Rice, 1996), lead (Davis *et al.*, 1990), and organic solvents (Glowa and MacPhail, 1995) produce results similar to those derived from human studies for those compounds for which such sets of data are available. These, too, support the use of nonhuman species in identifying tolerable exposure levels for neurotoxicants. Results from laboratory studies also can be used to examine critically the absence of effects in human studies. For example, as will be shown below, the target tissues for manganese include the basal ganglia and pituitary gland, while cortex and sensory organs show relatively little affinity for this metal. Accordingly, the absence of an effect in an epidemiological study can be interpreted only if pituitary or basal ganglia function is examined directly. Human studies that rely on relatively insensitive screening measures should be interpreted cautiously if refined measures of function known to be affected by manganese are absent. Studies with nonhuman primates, and even some of the rodent studies are clear in showing that subtle features of behavior are affected in ways that are understandable in view of manganese's neural effects and in animals that show no gross impairment.

Animal studies can also help resolve ambiguities in threshold estimates or perhaps determine if there is a threshold. Estimates from epidemiological studies depend critically on the statistical model employed and the assumptions embedded in them. These differences, which can range over several orders-of-magnitude (Cox *et al.*, 1995; Crump *et al.*, 1995) can be resolved by a review of the animal literature, especially when data are available on subtle effects of low-level exposure, interactions among dose, route, duration and spacing of dose, or nutritional status. As will be seen below, the dose, the duration of exposure and, especially, the rate of exposure all influence the expression of manganese's neurotoxicity.

## Manganese Neurotoxicity in Animals



**FIG. 2.** A summary of the cumulative doses at which different effects of manganese appears in various published studies using nonhuman primates (top) or rodents (bottom). Different effects are shown on the vertical axes and the dose at which they appear on the horizontal axes. Note the logarithmic scales on the horizontal axes, and the differences in range between the top and bottom panels. The route of administration was by injection for all primate studies in the top panel. The route of administration was by injection (labeled with an I), drinking water (W) or food (F) for the rodent studies in the bottom. Numbers to the right of each endpoint refer to the relevant study as follows. Primate studies (top panel): (1) Newland and Weiss, 1992; (2) Olanow *et al.*, 1996; (3) Newland *et al.*, 1989; (4) Neff *et al.*, 1969; (5) Suzuki *et al.*, 1975; (6) Eriksson *et al.*, 1992; (7) Eriksson *et al.*, 1987b. Rodent studies (bottom panel): (1) Bonilla, 1980; (2) Autissier *et al.*, 1982; (3) Bonilla, 1984; (4) Sitaramayya *et al.*, 1974; (5) Nachtman *et al.*, 1986; (6) Bonilla and Prasad, 1984; (7) Bonilla, 1978; (8) Ali *et al.*, 1983; (9) Ali *et al.*, 1985; (10) Komura and Sakamoto, 1992; (11) Gianutsos and Murray, 1982.

## ANIMAL MODELS OF MANGANESE NEUROTOXICITY

Animal studies of manganese's neurotoxicity have been conducted with primate and rodent species. Much of the rodent work has emphasized neurochemical effects of manganese and neurological effects have not often been reported. This may reflect the large role that the basal ganglia play in manganese's neurotoxicity and difficulties in characterizing basal ganglia damage, such as is produced by manganese or MPTP, in nonprimate species. Nevertheless, it is possible to identify motor effects of neuroleptics, which also act on the basal ganglia, in rodents; painstaking studies of force differentiation or of the mechanics of licking have been used to characterize neuroleptics' apparent basal ganglia effects in rats (Fowler, 1987; Fowler and Mortell, 1992).

The appearance of manganese's neurotoxicity depends upon the cumulative dose of manganese, the rate at which doses are administered, exposure route, and the endpoints selected for examination. In this section the variability in doses at which signs appear is described; two important contributors to this variability, exposure rate and neurobehavioral endpoint, are examined in later sections.

Overt signs detectable with a neurological examination dominate the profile seen after high levels of manganese exposure, but more subtle signs, detectable only with specialized tests, appear at much lower levels. Fig. 2 summarizes the doses at which certain important effects of manganese appear as reported in several investigations (Eriksson *et al.*, 1987; Eriksson *et al.*, 1987b; Neff *et al.*, 1969; Newland and Weiss, 1992; Olanow *et al.*, 1996; Suzuki *et al.*, 1975) using different manganese compounds, species, and exposure regimens. In an attempt to provide a common metric, exposure is represented as cumulative dose of manganese in mg of Mn/kg body mass that had been administered when the sign appeared (manganese mass was isolated from the compound containing it). This measure was described in the study or estimated according to the rate at which dosing was provided and the time that the sign was first described.

The left most point, over 5 mg/kg, in the top panel comes from a study of effortful responding in cebus monkeys described in some detail below. Action tremor appeared in some of those monkeys at cumulative doses of about 50 mg/kg. In other studies, tremor and contracture appeared at cumulative doses ranging from about 200 to 2000 mg/kg (Eriksson *et al.*, 1987b; Eriksson *et al.*, 1992; Suzuki *et al.*, 1975). Alterations in biogenic amines were determined at the end of the study when nonhuman primates are used, so the doses reported

certainly overestimate the dose at which these alterations would first appear (Eriksson *et al.*, 1987b). Such alterations have been reported in monkeys showing no visible signs (Bird *et al.*, 1984), but that study could not be listed in Fig. 2 here because exposure was by inhalation and a cumulative dose could not be determined.

As reported from the careful descriptions of the effects of manganese oxide (Eriksson *et al.*, 1987b; Suzuki *et al.*, 1975) the nonhuman primate closely resembles the human in the expression of manganism: dystonic posture, gait disorders, action tremor, hyperactivity and emotional displays are all seen at the high exposure levels in those studies. Studies using rodents as subjects have generally examined a different set of endpoints and Fig. 2 represents some of these studies. The most common effect is that of alterations of biogenic amines, especially in striatum. No neurological signs are reported here but there are sporadic indications of alterations in locomotor activity. The I's show studies using injected manganese and in these studies the cumulative dose ranges from about 50 to 350. With manganese in drinking water (W's) the cumulative doses at which these effects appear are on the order of 1000 to 5000 mg/kg with one study at 27,000 mg/kg. The concentrations represented on that figure range from 0.1 mg/ml for 240 days (Nachtmann *et al.*, 1986) to 10 mg/ml for 60 days or 3 mg/ml for 90 days (Ali *et al.*, 1985; Ali *et al.*, 1983). Only one feeding study is represented (labeled with an F) and that is with cumulative doses of 7000 to 25000 mg/kg (Komura and Sakamoto, 1992). Another study identified body mass decreases at 14,000 to 18,000 mg/kg in rats (Rehnbart *et al.*, 1982).

Fig. 2 reveals a range of estimated doses spanning 3 orders of magnitude over which effects were reported in nonhuman primates, and similarly for rodents. This variability across studies is in addition to the considerable individual variability that appears within the primate studies when conditions are more homogeneous (Cotzias, 1958; 1958 Cotzias *et al.*, 1968; Eriksson *et al.*, 1987b; Newland and Weiss, 1992; Rodier, 1955).

Some sources of variability can be identified. The route of administration is, naturally, an important one. Effects appear at higher cumulative doses through oral routes, although it is interesting that the effects appear at all. As much as 97% of dietary manganese can be eliminated but this regulation can be overwhelmed if concentrations are excessive (Ballatori *et al.*, 1987; Papavasiliou *et al.*, 1966). When this happens, the effects of manganese in rodents consuming manganese in drinking water are similar to those seen with injection, as seen in Fig 2.

The species is an important source of variability on some measures, but not all. Alterations in biogenic amines

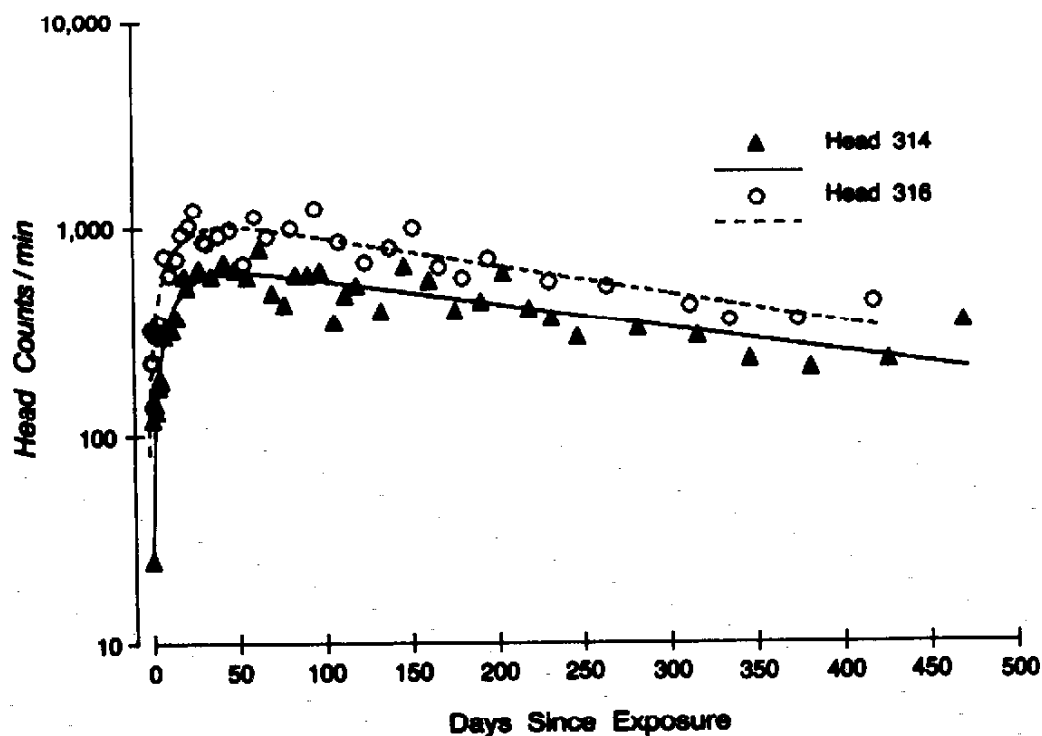


FIG. 3. Head radioactivity in two macaques after acute inhalation trace levels of  $^{54}\text{Mn}$  (from Newland *et al.*, 1987, with permission).

were reported after cumulative doses of 100 to 1000 mg/kg in nonhuman primates (which may be an overestimation) and less than 100 mg/kg in rodents. An important difference between rodents and primates is that the overt signs of basal ganglia disorders are easier to detect in primates, and are unlikely to appear in measures of locomotor activity. We have to await more precise studies of rodents before making firm conclusions about behavioral toxicity in rodent species.

The manganese literature is not as well developed as that of methylmercury or lead, but it is sufficiently mature that manganese can be identified as neurotoxicant with a spectrum of effects that distinguishes it clearly from other known heavy metals and, now, from Parkinson's disease (Barbeau, 1984), which it was once linked (Cotzias, 1958). Among the important components in the assessment of manganese's risk are the route of administration, dose, exposure protocol and especially the rate of exposure, and the endpoints examined. These components will be examined in that order.

#### KINETICS AND ROUTE OF ADMINISTRATION

The kinetics describing the flow of manganese can be separated into four components: uptake from the route

of exposure, distribution to target organs, storage in those organs, and elimination from them. The kinetics of uptake is critically dependent upon the route of administration. The kinetics of distribution indicates that with some routes of exposure, notably inhalation, depots of manganese form that may prolong exposure to other tissues by releasing manganese slowly. The long-term kinetics of elimination may be independent of the route of exposure, but this has not yet been conclusively shown.

The uptake and elimination of manganese in the brain were studied after chronic subcutaneous administration and inhalation (Newland *et al.*, 1987). To accomplish subcutaneous exposure,  $^{54}\text{Mn}$  was infused at a constant rate for 50 days using osmotic minipumps and radioactivity was measured in the head for up to a year. Radioactivity from the head rose at a constant rate while the pumps were installed and infusing manganese at a constant rate. When the pumps were removed, manganese in the head declined initially with a  $T_{1/2}$  of 3.9 days and then at a slower rate, with a  $T_{1/2}$  of 53 days over the course of the next 150 days. The latter  $T_{1/2}$  value closely replicated the value of 54 days observed in human miners after *i.v.* administration of manganese chloride (Cotzias *et al.*, 1968).

Fecal levels of radioactivity remained constant while the pump was in place indicating that the rate of

elimination did not change over the course of exposure, an observation consistent with feces being the primary route of manganese elimination (Lamirande and Plaa, 1979; Leach and Lilburn, 1978; Papavasiliou *et al.*, 1966). After exposure ended, fecal levels dropped by two orders of magnitude over about 20 days, a period during which head radioactivity also began to decline, albeit much more slowly. In that experiment, fecal levels of  $^{54}\text{Mn}$  were an excellent measure of contemporary exposure because the radioactivity permitted the separation of parenterally administered manganese from dietary sources. In general, however, fecal levels are a problematic biomarker because they reflect manganese that is not absorbed and because dietary intakes can vary over a wide range, even in commercially available lab-animal diets.

The elimination of manganese from the brain after inhalation was about 4 times slower than after subcutaneous inhalation. Fig. 3 shows the disappearance of manganese from the head, and presumably the brain, of two monkeys after a single episode of inhaling trace levels of  $^{54}\text{Mn}$ . Manganese levels in the brain did not peak for 40 days (the rising component had a  $T_{1/2}$  of 10 days) even though exposure lasted only a portion of one day. Elimination occurred with a  $T_{1/2}$  of 223-267 days. Manganese was detected in the chest, presumably reflecting manganese in the lungs, for 500 days after exposure. Three exponential terms were required to describe manganese elimination from the chest; the slowest term had a half-time of 94 to 187 days. Fecal levels fell rapidly and at different rates than head manganese during this period. Nevertheless, fecal activity could be detected, just barely, for up to 250 days after exposure ended.

The different rates of elimination from the brain following subcutaneous and inhalation routes could be accounted for by several differences in the exposure protocols. A plausible account is that the chest stored manganese and that these depots continued to supply the brain long after exposure terminated. This would explain the continued uptake for 40 days after exposure ended and the slow elimination phase. Kinetic modeling suggested that the lung could have served this function, but that lung deposits could not explain completely the slow elimination from the head. Other depots might be present or, alternatively, the kinetics of elimination could be dose-dependent (Wieczorek and Oberdorster, 1989) since a frankly neurotoxic dose was administered subcutaneously but only trace levels, more representative of what might be inhaled in the ambient air, were used in the inhalation study.

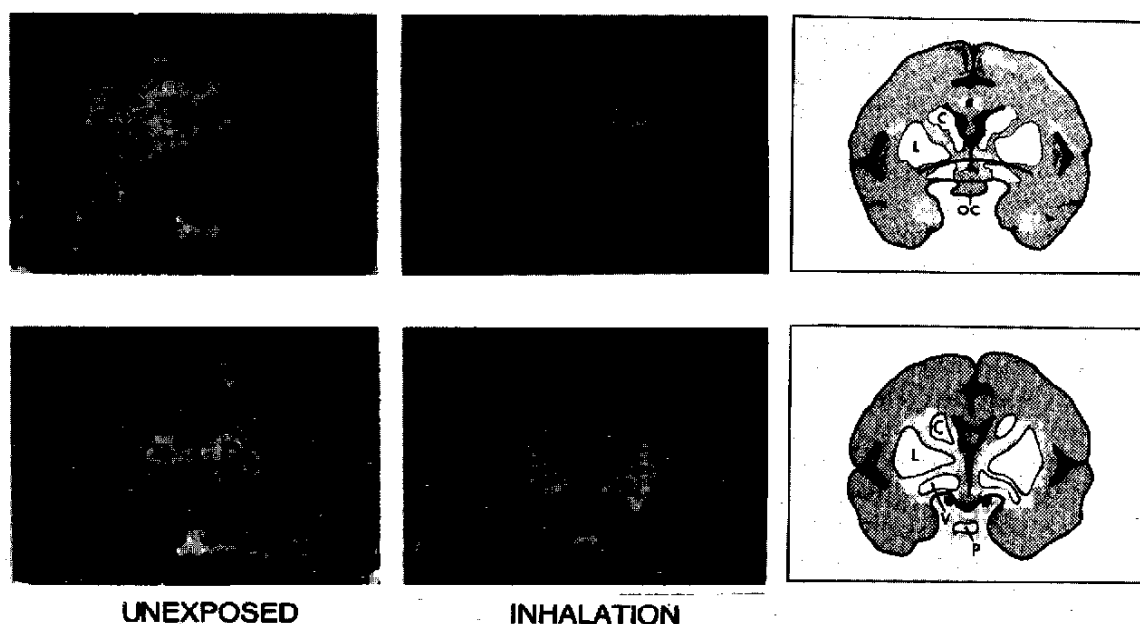
A pattern of asymmetric uptake and elimination describes exposure to both manganese oxide and chloride

in mice. Gianutsos and colleagues examined manganese in the brain and blood after administration of manganese oxide or manganese chloride (Gianutsos *et al.*, 1985). Manganese in the blood and brain was followed for 7 days after injection of manganese. Regardless of the form, the manganese levels in the blood peaked within hours of exposure and declined quickly. Manganese levels in the brain increased with a day of exposure and showed no decline over 7 days.

### IDENTIFICATION OF TARGET AREAS WITH MAGNETIC RESONANCE IMAGING

The identification of a readily available biomarker of manganese levels in the central nervous system is not a straightforward task. Conventional markers such as blood, urine, or fecal concentrations do not represent manganese in the target tissues because of the heterogeneity in distribution and in kinetics and the asymmetry in the uptake and elimination of manganese from the nervous system (Aschner and Aschner, 1991). Manganese enters some tissue, including the nervous system, within hours to days of exposure but remains for months and maybe years, but manganese levels in the blood have a half-time of elimination on the order of seconds to minutes (Cotzias *et al.*, 1968). Simple intake from all sources is not sufficiently discriminating since manganese intake from the diet is regulated by the entero-hepatic circulation levels. Fecal levels reflect dietary intake, and primarily the lack of uptake from the diet since about 97% of dietary manganese is eliminated in the adult (Ballitori *et al.*, 1987; Papavasiliou *et al.*, 1966). Exposure to small levels of manganese through parental routes, especially inhalation, could have important health effects but would be undetectable in, say, feces because of the large and highly variable amount of manganese present, and essential, in the diet. An ideal biomarker will reflect manganese burden in the central nervous system regardless of route of exposure. Magnetic resonance imaging accomplishes this task noninvasively.

Manganese is a paramagnetic metal, meaning that it has an unpaired electron in the outer shell and that it can be detected with magnetic resonance imaging. The presence of paramagnetic metals in tissue influences the sensitivity of protons (hydrogen nuclei), in a concentration dependent fashion, to changes in the polarity of magnetic fields (Elster, 1986; Kay and Mattison, 1986; Runge *et al.*, 1984; Schuhmacher *et al.*, 1985). After being perturbed by a strong magnetic field, protons return to their original state faster if paramagnetic elements are nearby, and the more that are



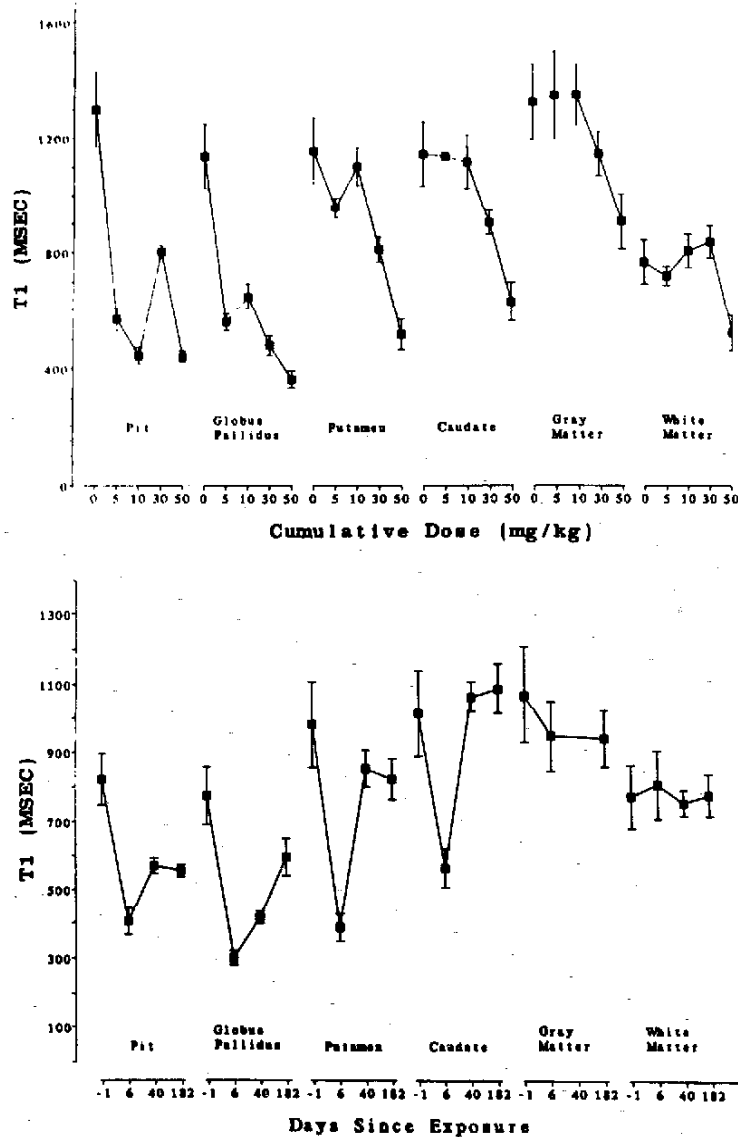
**FIG. 4.** Coronal sections of the brain at about the level of the optic chiasm (OC, top) and the pituitary gland (P, bottom) of a macaque before manganese administration (left) and of a different macaque after inhaling a  $MnCl_2$  for about 5 months (middle). A sketch showing some neuroanatomical markers is on the right. The images were taken with a TR:TE of 450ms/15ms. The lentiform nuclei (L) comprising putamen and globus pallidus, caudate (C), pituitary gland (P), and basal forebrain structures probably including ventral pallidum (V) are visible in this image (from Newland *et al.*, 1989, with permission).

present the faster it returns to its original states. This property, measured with the spin-lattice relaxation time (denoted T1), permits the application of magnetic resonance imaging (MRI) to contrast tissues containing high concentrations of manganese with those that do not (Kay and Mattison, 1986; Newland *et al.*, 1989).

Manganese's paramagnetism can be exploited to visualize the accumulation of manganese in selective tissue in the nervous system (Eriksson *et al.*, 1992; Newland *et al.*, 1989; Newland and Weiss, 1992; Shinotoh *et al.*, 1995). Since MRI is noninvasive, the dynamics of manganese can be tracked repeatedly in the same subject, *in vivo*, a single-subject experiment design with exceptional experimental power. Fig. 4 (Newland *et al.*, 1989) shows images in a plane that includes the pituitary gland and most components of the basal ganglia: the globus pallidus, putamen, and caudate. The structure at the base of the brain, labeled V, was identified as ventral pallidum, but the extensiveness of the signal change suggests that other basal regions might also be included (Olanow *et al.*, 1996; Shinotoh *et al.*, 1995), regions that have been associated with emotional disorders, including schizophrenia (Heimer *et al.*, 1991). The images on the left are featureless at these parameters but after manganese inhalation these regions become highlighted, or brighter.

These regions correspond to those identified in terminal studies to contain manganese (Dastur *et al.*, 1971; Suzuki *et al.*, 1975).

Brightness in the image is linked to TR, the repetition rate of the magnetic pulse used to generate the image, and T1, which reflects changes with the concentration of paramagnetic materials in a proton's environment, according to an exponential relationship in which T1 is a parameter (Elster, 1986; Kay and Mattison, 1986; Runge *et al.*, 1984; Schuhmacher *et al.*, 1985); see Newland *et al.*, 1989 for details. In our experiments, this was accomplished by 1) identifying a plane that included the target structures containing manganese and other regions that did not accumulate it; 2) taking images (five were required, requiring about one hour) in that plane using different TRs; 3) identifying the coordinates of the relevant structures in all images; 4) estimating the intensity of the image by counting the number of pixels lit in the charted area and 5) using the expected mathematical relationship between brightness and TRs to estimate the value of T1. We did this in the plane containing the caudate, putamen, globus pallidus, gray matter near the cortex, and white matter, either subcortical or, sometimes, the corpus callosum, and pituitary gland. The result is a region-specific account of the kinetics of manganese-induced



**FIG. 5.** T1 in five brain regions and the pituitary gland after i.v. administration of manganese chloride. The top panel, representing CM 846, is presented as a cumulative dose-effect curve. The first point in each group was taken before manganese exposure, the second point was taken 6 days after 5 mg/kg, the third point one day after 5 mg/kg (and 27 days after the second point), the fourth point 22 days later and the final point 13 days after 10 mg/kg and after a cumulative dose of 50 mg/kg. T1's were also calculated on day 333, 157 days after the last administration of manganese and they were within normal ranges (not shown). The bottom panel shows the rate at which T1 returns to control values in a second cebus monkey after a cumulative dose of 30- mg/kg administered over the course of 6 weeks. Error bars indicate the standard error of the least-squares estimate of T1. (From Newland *et al.*, 1989, with permission).

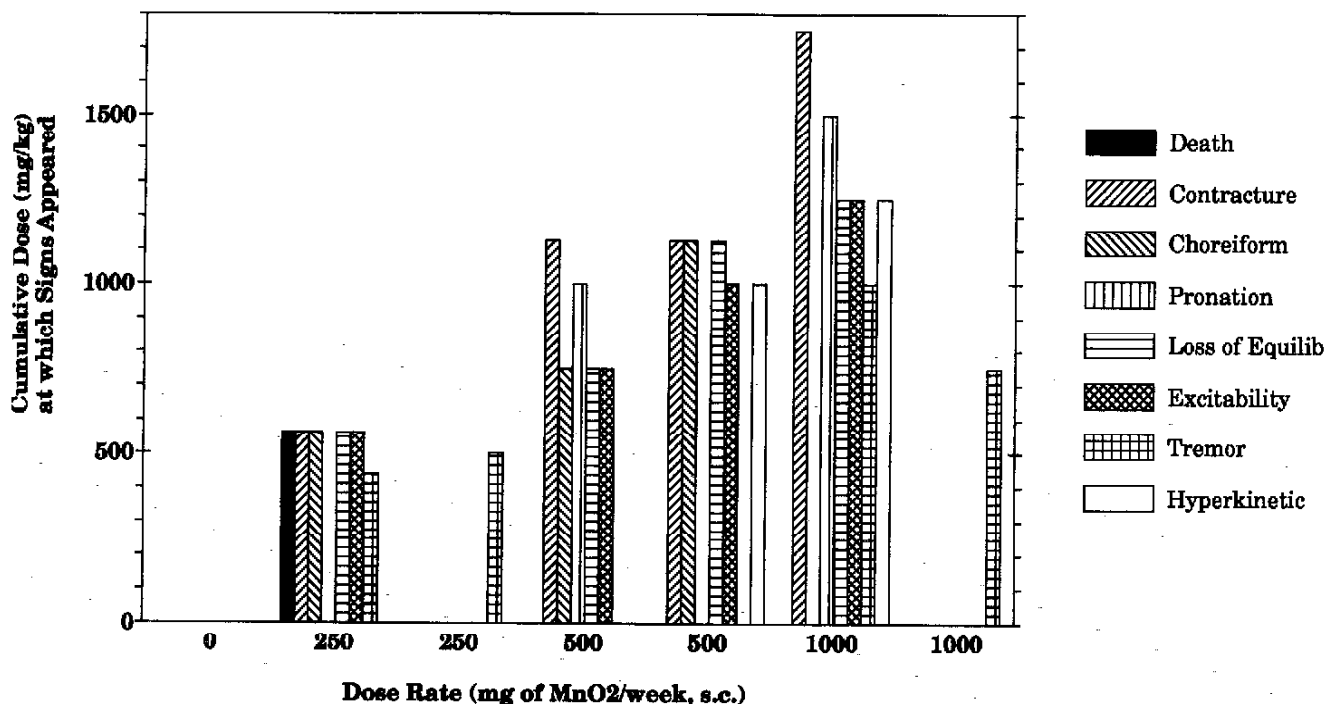
signal changes through important structures in the central nervous system.

Fig. 5 shows estimates of T1 taken from those five different regions from a single monkey at different times in an exposure regimen. Under control conditions, prior to manganese exposure, T1 in pituitary, basal ganglia, and gray matter were all close to 1100 msec at the 2 Tesla field strength used. Incidentally, the close similarity in T1

in these regions is the reason that they are difficult to distinguish with T1 weighted MRIs. The T1 for white matter, however, was about 700 msec, in sharp contrast to the other regions.

To obtain the data in the top panel, manganese was administered in 5 mg/kg boli, i.v., with at least two weeks separating administrations. Behavioral data from this monkey will be described below. T1 shortened from 1100

**Role of Manganese Dosing Rate on the Estimated LOAEL**  
(from Suzuki et al., 1975)



**FIG. 6.** The cumulative dose of manganese at which signs of exposure first emerged, as estimated from Suzuki *et al.* (1975). The horizontal axis shows exposure rate in mg of MnO<sub>2</sub>/week and the vertical axis the cumulative dose in mg/kg at which a sign appeared. Individual bars indicate the particular sign reported. Each cluster of bars represents a single animal. In the original paper, dosing rate was reported as mg/week. The dose at which signs appeared has been converted to mg/kg/week (based on a body mass of 4 kg), which is the reason that differences in the doses at which signs appear shown on the vertical axis are not even multiples of the dosing rate shown on the horizontal axis. The lower the dosing rate the lower the dose at which signs appeared. At these exposure levels there was no specific relationship between the type of sign and the dosing rate.

msec to about 550 msec in the globus pallidus and pituitary after the first administration of 5 mg/kg, but T1 in other regions was unaffected at this dose. T1 in putamen and caudate declined at a cumulative dose of 30 mg/kg. It was only after higher cumulative doses, or more elapsed time, that manganese began to appear in caudate and putamen. Cortical gray matter declined only at the higher two doses and white matter only at the highest dose.

A return of T1 to control levels after exposure is terminated in a different monkey is shown in the bottom panel. The values of T1 for white and gray matter during control conditions were similar to those seen in the top panel. After a cumulative dose of 30 mg/kg of manganese no decline in T1 appeared in white matter or cortical gray matter. The pituitary and globus pallidus showed large declines in T1 at this cumulative dose of manganese. T1s returned to control levels within 40 days after exposure ended in the caudate and putamen but T1 in the pituitary and globus pallidus remained shortened for the duration of this experiment, 182 days.

The decreases in T1 were related to manganese administration and may represent manganese accumulation in the areas of the nervous system imaged. Manganese shortens T1, as described above. Investigators using different species, exposure protocols, and experimental tactics have reported that manganese accumulation and elimination from various CNS regions is heterogeneous and elimination is slow from some, including globus pallidus and pituitary (Dastur *et al.*, 1971; Eriksson *et al.*, 1987b; Suzuki *et al.*, 1975). This ability to replicate across a range of conditions is powerful evidence that the globus pallidus and the pituitary gland have a special affinity for manganese.

While Newland *et al.* linked alterations in T1 with manganese exposure in regions of the nervous system shown to retain manganese for an extended period, they did not determine the manganese content of these regions. Olanow *et al.*, using an exposure protocol similar to Newland *et al.*'s, were unable to find manganese in similar regions of the rhesus monkey's brain that had shown

dramatic signal changes under MRI, but they did find iron, which would affect the MRI signal similarly to manganese, and aluminum deposits. While that report is difficult to reconcile at present with other reports of manganese's appearance in globus pallidus (Dastur *et al.*, 1971; Dastur *et al.*, 1969; Suzuki *et al.*, 1975), Olanow *et al.*'s report of high levels of aluminum is especially interesting in light of Gajdusek's identification of an association between high levels of manganese and aluminum in an ALS/PD syndrome in West Pacific Islanders and monkeys (Garruto *et al.*, 1989; Yoshimasu *et al.*, 1980; Yoshimasu *et al.*, 1982). The report of elevated iron could link iron to manganese's neurotoxicity. These two metals accumulate in the same regions of the nervous system (Scheuhammer and Cherian, 1981; Scheuhammer and Cherian, 1982). Hallervorden-Spatz syndrome, in which regulation of iron content of neural tissue is impaired, produces a Parkinsonian syndrome without dementia, and an MRI pattern very similar to that seen with manganese exposure (Drayer *et al.*, 1986; Rutledge *et al.*, 1987; Sethi *et al.*, 1988).

The globus pallidus may be a source of manganese's neurotoxicity. After exposure to very high levels of manganese, globus pallidus showed not only a particularly high concentration of manganese but also sizeable reductions in dopamine and serotonin function and severe loss of neurons and astrogliosis (Eriksson *et al.*, 1987b). These effects appeared consistently in pallidum and less consistently in other regions of the basal ganglia: striatum, putamen, and substantia nigra. As shown below, the appearance of manganese in the globus pallidus can be linked to subtle effects of manganese with its early appearance in the nervous system.

### EXPOSURE RATE

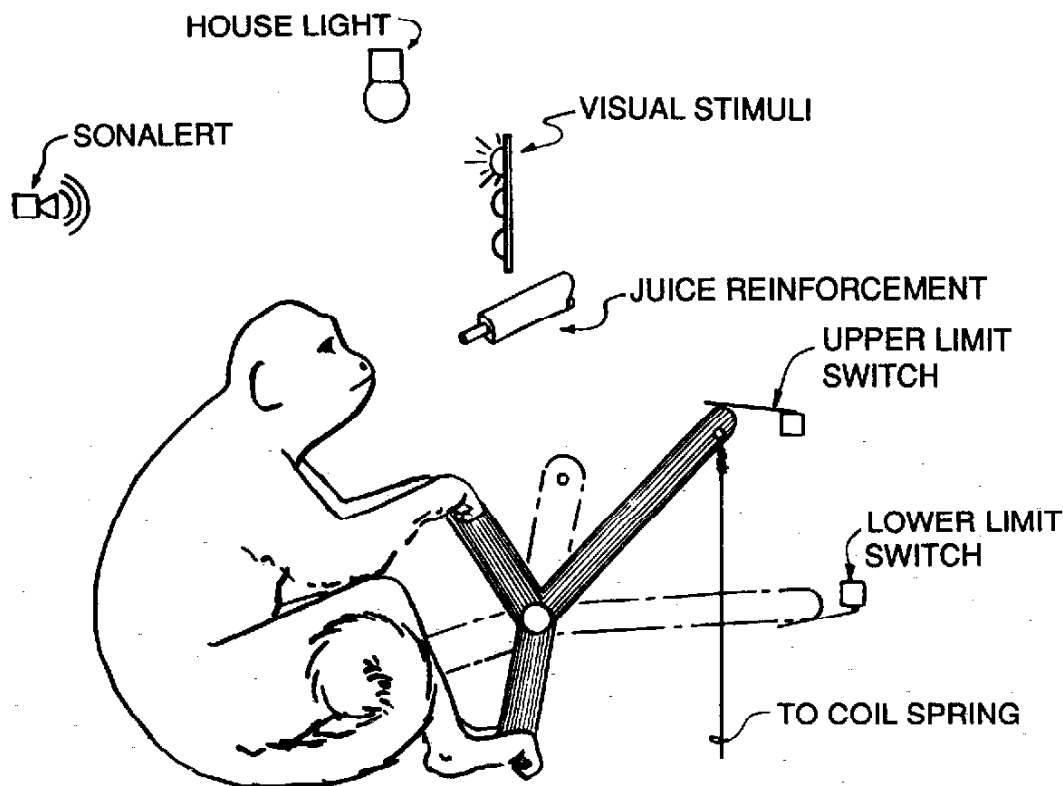
In order to identify toxic effects of low levels of exposure it is necessary, of course, to administer low levels of the toxicant. With manganese this is more difficult than it sounds because of the cumulative nature of its toxicity and the long latency that lies between dose and effect. Effects that appear after 8 weeks of 100 mg/kg/wk, could represent an eight week delayed effect of the first 100 mg/kg or the effects of a cumulative dose of 800 mg/kg. This is a fundamentally important distinction to make when attempting to identify a low level effect, but one that can be impossible to form with conventional chronic exposure procedures. It is a distinction that should yield caution in interpreting some of the high-dose effects reported in primates and illustrated in Fig. 2. The studies of Eriksson *et al.* and Suzuki *et al.* for example (Eriksson *et al.*, 1987b; Suzuki *et al.*, 1975), used a high dosing rate over a short

period of time so it is difficult to determine whether the neurological signs reported represent the cumulative dose experienced when the signs appeared, or a latent effect of, say, the first dose.

Suzuki and colleagues (Suzuki *et al.*, 1975) examined the role of exposure rate by administering manganese dioxide *s.c.* at rates of 250, 500, or 1000 mg/kg/week to macaques. Regardless of exposure regimen, the same constellation of signs appeared but they appeared at lower cumulative doses when the exposure rate was slower (Fig. 6). No signs appeared in the unexposed monkey. A monkey receiving 250 mg Mn/week began to show signs after about 9 weeks, a cumulative dose of 1845 mg or, assuming a body mass of 4 kg, a cumulative dose of 562 mg/kg. The monkeys experiencing higher exposure rates showed signs at higher cumulative doses, although they appeared sooner. The only death was at the lowest exposure rate but otherwise the number of signs was indistinguishable across exposure regimens. One interpretation of this study is that the signs that seemed to appear at the higher cumulative doses had actually been unleashed by the earliest manganese administrations; low-dose effects were masked by the high exposure rates. This analysis would also account for why Newland *et al.* reported action tremor and Olanow reported other neurological signs at low doses; those studies used a low exposure rate and the tremor did not appear until months after exposures began.

The rate of exposure may also determine what classes of effects appear. Studies reporting on low exposure rates in human occupational settings (Huang *et al.*, 1989; Iregren, 1990; Roels *et al.*, 1983; Wang *et al.*, 1989) or nonhuman primates with experimentally controlled exposures (Bird *et al.*, 1984; Newland and Weiss, 1992; Olanow *et al.*, 1996) did not contain reports of emotional displays or hyperactivity so often reported after high rates of exposure, even when other neurological signs were detected. This raises some question about contentions that manganese represents a cascade of events beginning with emotional/regulatory disorders and followed by severe motor disturbances. Plausibly, the florid emotional disturbances described in the miners and in some studies of nonhuman primates are due to the very high exposure rates seen in those studies.

The consequences of manganese's accumulation in the pituitary gland are not clear. It may be tempting to relate the mania, eating, and sleep disorders often associated with manganese exposure to pituitary function in view of the early appearance of manganese in that gland (Dastur *et al.*, 1971; Newland *et al.*, 1989) and its role in these functions (Cohen and Cohen, 1981; Fehm *et al.*, 1993; Friess *et al.*, 1995; Kiriike *et al.*, 1988; Krause and



**FIG. 7.** The device used to examine effortful responses. The monkey perched on the device and pulled with its arms and pushed with its feet, executing a rowing-type movement, against a 40 Newton (4 kg) spring through a displacement of 10 cm under a fixed-ratio or a fixed-interval schedule of reinforcement. Limit switches detected the extremes of movement. A juice reinforcer was delivered through the indicated dispenser. Lights indicated which schedule was in effect

Dubocovich, 1990; Muller and Boning, 1988; Van Cauter, 1990). Alternatively, these could suggest a relationship to manganese accumulation in the basal forebrain (Heimer *et al.*, 1991). In either case, the absence of such behavioral signs in nonhuman primates at doses that result in accumulation of manganese in the relevant areas, and the absence of effects on studies of low-level exposure to people, suggest that any links that may exist are not straightforward. The absence of such emotional displays at low exposure rates that result cause neurological signs presents a challenge to hypotheses that posit a manganese cascade beginning with emotional displays and ending with irreversible neurological signs.

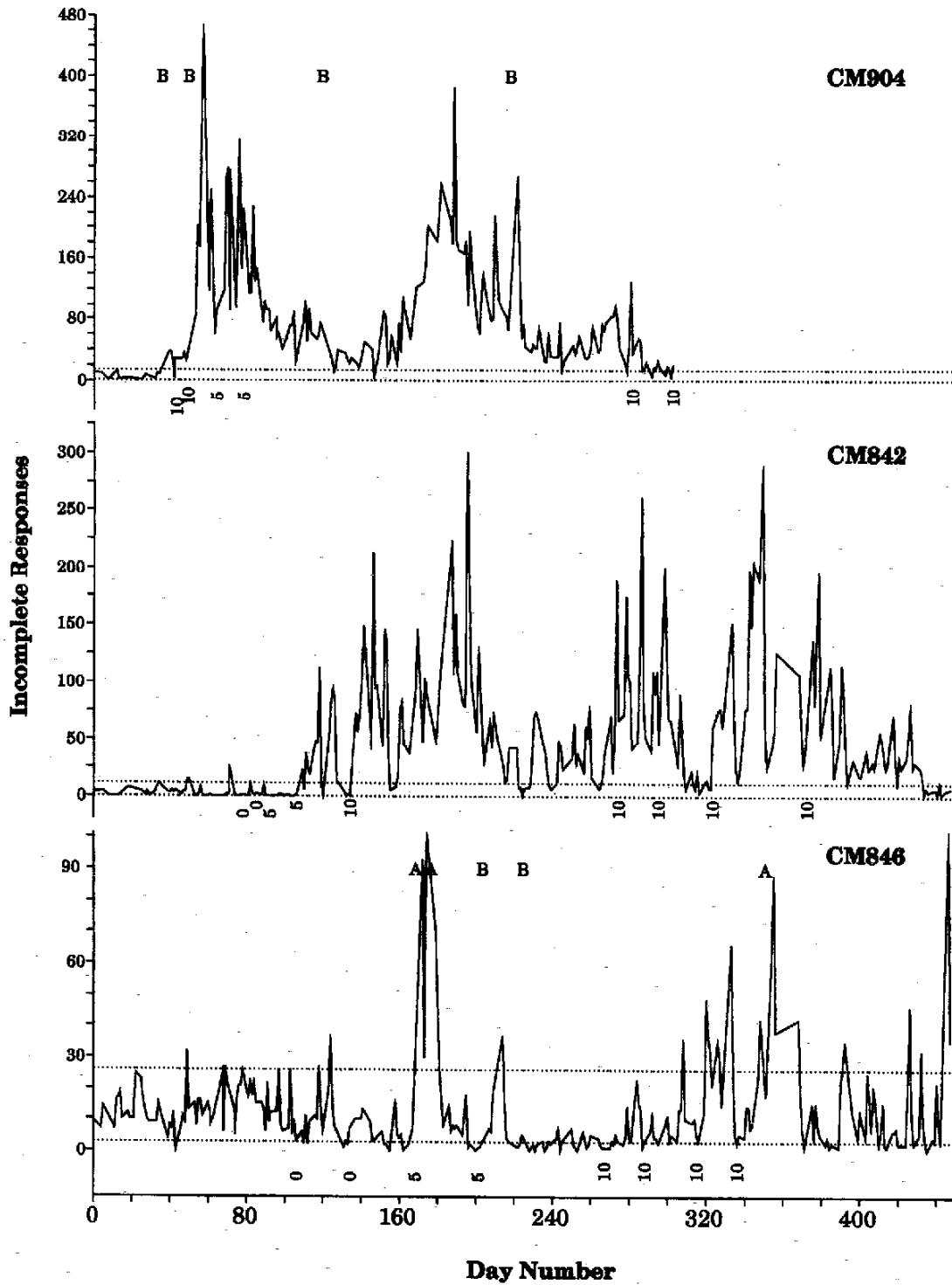
A retrospective study in which California prison inmates were reported to have higher levels of manganese in their hair than controls taken from the community (Gottschalk *et al.*, 1991) may appear to disconfirm this suggestion about exposure rate, but the study presents several interpretative difficulties and should be viewed with considerable caution. The controls and cases were samples of convenience and were not matched on important variables, including hair pigmentation, a very

important dimension because manganese is attracted to melanin (Lyden *et al.*, 1984). In the study reported by Gottschalk, hair levels of manganese were reported as group averages, and higher levels were reported in groups that also had a higher proportion of blacks or Hispanics, suggesting a confound with hair pigmentation. Hair has not been validated as a biomarker of manganese exposure.

### LOW DOSE EFFECTS ON EFFORTFUL RESPONDING

The neurobehavioral endpoints chosen for study in animal experiments also contribute to the broad range of doses at which signs appear. Neurological signs such as dystonic postures, contracture, or gait disorders appear at high rates of exposure in manganese miners and in nonhuman primates. Subtle signs that might appear at lower rates or levels of exposure in occupational settings (Iregren, 1990; Roels *et al.*, 1987; Roels *et al.*, 1985) or animal studies require more refined techniques.

**Incomplete Responses During the Fixed-Ratio Component**



**FIG. 8.** The number of responses that failed to meet the displacement criterion under the fixed ratio schedule of reinforcement of effortful responses before and after manganese for three monkeys. The counting of sessions began on the same day for each monkey so a vertical line down all three figures would indicate the same day. A 0, 5, or 10 represents the administration of 0 (sham), 5, or 10 mg/kg of manganese. The rises in incomplete responses for CM 904 beginning at about sessions 180 and 260 corresponded to the appearance of action tremor in, respectively, the legs and arms.

In one series of experiments designed to examine the execution of an effortful motor response, cebus monkeys were trained to execute a rowing-type movement under a Multiple Fixed-Ratio Fixed-Interval schedule of reinforcement (Newland and Weiss, 1990; Newland and Weiss, 1992). The monkeys perched on a response device (Fig. 7) and simultaneously pulled with their arms and pushed with their feet against a spring that resisted movement with a force approximating the animals' body weight through a displacement of 10 cm. A movement that did not span the required displacement was termed an "incomplete response." In this experiment, conventional measures of response rate were insensitive to manganese exposure under both schedules. Two measures of motor function, however, response duration as reported in Newland (1994) and the number of incomplete responses, were extremely sensitive to exposure.

To see the effect in a single monkey examine CM 904, the monkey that received manganese first, in the top panel of Fig. 8. Prior to the first exposure of manganese the number of incomplete responses hovered at around 10 to 20 per session under the fixed-ratio schedule, a level that had been maintained for many months preceding the leftmost point in this figure. No change was noted in the few days following the first administration of 5 mg Mn/kg, *i.v.*, but after the second administration the number of incomplete responses rose over the course of the next few weeks to a peak value of 450 per session, a 20-fold increase. Over the course of the next few months this value fell but never to the low levels seen in the baseline sessions. This general pattern of effects was replicated in the two other monkeys. In that study, the lowest dose used, 5 mg/kg, produced sizeable increases in incomplete responses (bottom panel, Fig. 8) and 10 mg/kg produced irreversible changes.

The exposure regimen used in that experiment adapts an experimental design called a multiple baseline because there is a separate baseline for each animal, the baselines are perturbed at different times, and comparisons can be made against an animal's own baseline or across animals performing on the same day but with a different level of treatment (Johnston and Pennypacker, 1980). This powerful design permits secure conclusions to be drawn from a small number of subjects. Multiple control conditions support conclusions that the change in incomplete responses is attributable to manganese. First, a stable baseline was established before manganese was administered; this baseline extends for months before the leftmost points on the figure. Second, the two other monkeys shown in Fig. 8 were examined in the same apparatus on the same day as the exposed monkeys. The numbering of session days began on the

same day for each subject, so a vertical line down the whole figure refers to the same day. Extraneous factors such as alterations in the colony or apparatus problems that might influence behavior can be ruled out if only one monkey shows an effect. An additional strength is the staggering of dosing. Monkey 842 did not begin receiving manganese until weeks after 904, and monkey 846's dosing began even later. Finally, a vehicle control was used, in which all dosing conditions were applied except that the injection did not contain manganese. The experimental controls in place distinguish studies such as the one illustrated in Fig. 8 from case studies. The three monkeys shown in Fig. 8 can be considered an experiment and two replications, with the same pattern of effects appearing in all three monkeys.

Manganese's effects were specific to a vigorous, high-rate response pattern. Manganese's disruption of the execution of this effortful response appeared under the fixed-ratio schedule, a schedule of reinforcement in which 20 (or 10 for one subject) were required for a juice-reinforcer. This schedule maintained a high, vigorous rate of responding characterized by short response durations and interresponse times (Newland and Weiss, 1990). The same effortful response was also maintained under a fixed-interval schedule, in which only one response after 90 seconds was required. The fixed-interval schedule maintained a lower rate and less energetic pattern characterized by longer response durations and interresponse times (Newland, 1995; Newland and Weiss, 1990). This schedule was much less sensitive to manganese, and the behavior changes that appeared were erratic.

The increased number of incomplete responses appeared in the absence of other disruptions in behavior. Overall response rate and even schedule-typical patterns of responding under the FR and FI schedules remained intact after manganese exposure. This contrasts with the sensitivity of response rate or pattern commonly seen in schedule-controlled operant behavior to other neurotoxicants. This insensitivity of response rate and pattern is also remarkable in view of the vigor with which the fixed-ratio responding occurred. The monkeys continued to respond under the fixed-ratio schedule at very high rates, with the same temporal pattern in interresponse times even in the face of an enormous increase in response failures.

Disruptions in effortful responding were associated with enhanced signal in the globus pallidus, but not the striatum, as detected in magnetic resonance images. Action tremor, detected when the monkey reached for marshmallow treats, was detected in these monkeys at cumulative doses of about 50 mg/kg, but resting tremor was never seen. At no point did these monkeys show

emotional disturbances, hyperactivity (based on visual observations and daily handling), or aggressive displays. The monkeys were handled every day and the only difficulties noted were sluggishness and constipation for a few days after manganese exposure.

## CONCLUSION

From the animal literature it is clear that manganese is a cumulative neurotoxicant and that its toxicity is influenced by route of exposure, dose, and rate of dosing. The ability to detect effects of low levels of exposure is also dependent upon the endpoint chosen for study. Observations and screening techniques such as locomotor activity tend to be insensitive, but refined experimental techniques, neurochemical endpoints, and highly structured observations have identified effects at low exposure levels. The primary routes of toxic exposure in humans are parenteral, usually inhalation, and here the exposure levels of concern can be quite low because the respiratory system does not regulate input and manganese can accumulate. Higher levels in diet can be tolerated because input is regulated, but these can also be sufficiently high that they overwhelm enterohepatic control. Similarly with animal species, the parenteral routes of exposure, usually injection, identify effects at much lower levels than seen with oral routes.

Manganese presents an interesting case because it is an essential trace element over a wide range of exposure levels when taken orally. Adverse behavioral effects of manganese deficiency and excess can be identified so the dose-effect curve for this element might be viewed as U-shaped: both low doses and high doses are problematic, but with different spectra of effects identified. This should not be interpreted as asserting that any behavior change is neurotoxic. A behavioral effect associated with a change from manganese deficiency to adequate levels might be viewed as pharmacological, or nutritional. The effects of excess manganese exposure reviewed here represent a change from nutritional to toxic levels and clearly imply impairment because: 1) exposed animals and controls were maintained under generally healthy conditions with an adequate diet, including adequate dietary levels of manganese, and differences could be related to manganese administration; 2) the effects reported here represent a coherent constellation linking behavior and other nervous system functions; and 3) where behavior has been studied it reflects an impairment in the execution of normal, usually motor, acts.

Delayed and cumulative toxicity observed with human and nonhuman species can be related to the rate

at which this metal enters the central nervous system and the pituitary gland, which is faster than the rate at which it disappears. The more subtle effects of manganese can be linked to its accumulation in the globus pallidus, or at least to disruption in MRI signals from this region after manganese exposure. Other areas of the basal ganglia and, possibly, basal forebrain, accumulate manganese at higher levels of exposure and these, especially the substantia nigra have been, and remain, possible sites of action.

Accumulation in the central nervous system, can occur over the course of years because of the asymmetry in transport across the blood-brain barrier. The detection of these levels, *i.e.*, the identification of a biomarker, is not easy. Low blood concentrations of manganese cannot be a guarantee of the absence of significant nervous system levels of manganese. Fecal levels primarily reflect manganese not absorbed from the diet so will not be a marker of body burden. Blood, feces, and urine do not reflect the concentrations of manganese in the central nervous system and hair has not been validated as a biomarker.

Alterations in the execution of a vigorous, effortful response occurred concurrently with altered MRI signal in the globus pallidus. The behavioral effect was specific: less vigorous behavior and even the temporal pattern of schedule-controlled behavior, a sensitive indicator of other neurotoxicants, were unaffected even at higher levels of exposure. This conclusion is based on a powerful of experimental design in which in the behavior of the same subjects on the same days was examined over the course of a year after initial exposure.

Despite the presence of exposure protocols spanning a huge range of doses, a No-Observed-Adverse-Effect level (NOEL) has not been identified with manganese in animal studies. Cumulative doses on the order of hundreds to thousands of mg/kg produce, in animals and humans, overt neurological signs. A single dose of 5 mg/kg, however, produced clear and dramatic deficits in executing a vigorous, effortful movement in a monkey and at 10 mg/kg these effects were irreversible.

## ACKNOWLEDGEMENTS

Supported by RO1 ES06466 from the National Institute of Environmental Health Sciences.

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