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Development of an animal model to study the potential neurotoxic effects associated with welding fume inhalation

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Abstract

Serious questions have been raised regarding a possible causal association between neurological effects in welders and the presence of manganese in welding fume. An experimental model is needed that could examine the potential neurotoxic effect of manganese after pulmonary exposure to welding fume. The National Institute for Occupational Safety and Health (NIOSH) has recently finished construction of a completely automated, computer controlled welding fume generation and inhalation exposure system for laboratory animals. The system is comprised of a programmable six-axis robotic welding arm and a water-cooled arc welding torch. A flexible trunk has been attached to the robotic arm of the welder and is used to collect and transport fume from the vicinity of the arc to the animal exposure chamber. Preliminary fume characterization studies have indicated that particle morphology, size, and chemical composition were comparable to welding fume generated in the workplace. Animal inhalation studies are currently underway. With the development of this novel system, an animal model has been established using controlled welding exposures to investigate the possible mechanisms by which welding fume may affect the central nervous system.
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Keywords: Welding; Inhalation; Glial fibrillary acidic protein

1. Welding exposure

Welding is a common industrial process used to join metals. Welders can be exposed to a number of hazards that include radiation, heat, noise, vibration, and electricity. Respirable particles and gases also are formed in the welding environment and may cause adverse health effects after inhalation. Welding fume is composed of complex metal oxides. The primary component of fume generated during the welding of steel is iron oxide. Other metals that are commonly found in welding fume may include manganese, chromium, nickel, silicon, and copper. Welders also are exposed to gases, such as ozone, carbon monoxide, and nitrogen oxides, that may affect their health.

Millions of workers worldwide are exposed to welding aerosols on a daily basis. Nearly 350,000 workers are classified as full-time welders in the United States (Bureau of Labor Statistics, 2004). Hundreds of studies have been performed over the past 30 years that have evaluated the health effects associated with exposures during welding (Antonini, 2003a). A large majority of the studies have focused on the respiratory effects of welding fume. Bronchitis, metal fume fever, transient lung function changes, siderosis, and cancer have all been reported in welders (Antonini et al., 2003b). Less information exists regarding the non-pulmonary effects associated with welding, specifically any potential neurological effects (Antonini et al., 2006a). It has been suggested that welding may accelerate the onset of idiopathic Parkinson's disease (Racette et al., 2001). One recent study indicates that welders are at an increased risk for the development of neurodegenerative diseases (Park et al., 2005), whereas in another study, welding was observed to not be associated with an increased risk of parkinsonism (Goldman et al., 2005).

The health effects of welders can be difficult to study because of variations in workplace setting and exposure to diverse aerosols generated from different processes. Welders may work in a number of settings that include open, well-ventilated spaces (e.g., outdoors on a construction site) or confined, poorly ventilated spaces (e.g., ship hull, building crawl space, pipeline). Arc welding processes can be quite

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complex. It is believed that there are at least 80 different types of welding processes—each with its own potential safety and health hazard.

Three common welding processes that are associated with elevated fume exposures include shielded metal arc welding (SMAW), flux-cored arc welding (FCAW), and gas metal arc welding (GMAW). During GMAW, shielding gases are continually blown over the arc to protect the formed weld from weakening caused by oxidation. During SMAW and FCAW, fluxing compounds are incorporated into the electrode and create a shielding environment to protect the weld as the electrode is consumed in the process. The fluxing agents used in SMAW and FCAW can contribute to the inhalation exposure of welders, and fumes formed during these processes have been observed to be both chemically and physically more complex than fumes formed from GMAW processes (Jenkins and Eagar, 2005; Zimmer and Biswas, 2001).

2. Potential neurological effects among welders

Questions have been raised regarding a possible causal association between neurological effects in welders and the presence of manganese in welding fume. Manganese is an essential element in the welding of steel and is present in varying concentration in welding consumables. Manganese increases hardness and strength, improves metallurgical properties, and acts as a deoxidizing agent to remove iron oxide from the weld pool to form a stable weld (Harris, 2002). The majority of all welding (~90%) is performed using mild or carbon and low alloy steels (Beckett, 1996). The most common mild steel electrodes are composed of mostly iron with small amounts of manganese, usually <5% per total metal present. However, some hard-surfacing applications that are commonly used by the railroad industry require welding electrodes that contain between 15 and 20% manganese.

Inhalation of pure manganese in high doses has been shown to cause neurological effects in exposed workers. However, manganese is not present as a pure element in welding fume. It is complexed with other metals and may not produce the same health effects as pure manganese. Depending on the welding process and the composition of the welding electrode, manganese may be present in different oxidation states and have different solubility properties. These differences may affect the biological responses to manganese after the inhalation of welding fumes. X-ray diffraction and X-ray photoelectron spectroscopy have shown that manganese in steel welding fume exist as part of a spinel phase (e.g., predominantly iron mixed with manganese) with valence states of 2^+ and 3^+ (Jenkins and Eagar, 2005; Minni et al., 1984; Voitkevich, 1995).

It was observed that the welding process can influence the chemical nature of manganese in the fume (Jenkins and Eagar, 2005; Voitkevich, 1995). The core of the particles generated during GMAW by mild steel electrodes is comprised of a complex of iron and manganese in the forms of Fe_3O_4 and $MnFe_2O_4$. The distribution of iron and manganese is more complex when using welding consumables that use fluorine as a fluxing agent. Soluble forms of iron and manganese are complexed with fluorine (K_3FeF_6, FeF_3, MnF_2, and MnF_3) and concentrated on the particle surfaces, while more insoluble oxide compounds of iron and manganese are concentrated in the particle core in the form of Fe_3O_4 and $MnFe_2O_4$.

Several cases of manganese overexposure or ''manganism'' in welders have been reported in the literature (Discalzi et al., 2000; Franek, 1994; Josephs et al., 2005; Kim et al., 1999; Nelson et al., 1993; Sadek et al., 2003). Manganism is a neurological syndrome characterized by central nervous system abnormalities and neuropsychiatric disturbances and is frequently said to be similar to Parkinson's disease. Both conditions are characterized by generalized bradykinesia and widespread rigidity, but are differentiated based on their clinical, pharmacological, imaging, and pathological features. Manganese preferentially damages different areas of the brain from those affected in Parkinson's disease (Calne et al., 1994; Olanow, 2004). Parkinson's disease is associated with the loss of dopaminergic neurons within the substantia nigra (which is spared in manganism), whereas a degeneration of GABAminergic neurons within the globus pallidus in pathways postsynaptic to the nigrostriatal system is observed in manganism.

In most case reports of manganism in welders, the airborne manganese levels were quite high as the affected workers worked in poorly ventilated areas (Josephs et al., 2005; Sadek et al., 2003) or used welding rods high in manganese content (Franek, 1994; Nelson et al., 1993). A positive brain magnetic resonance imaging (MRI) T1 hyperintensity signal in the globus pallidus, indicative of manganese accumulation, has been observed in several affected welders (Discalzi et al., 2000; Kim et al., 1999; Nelson et al., 1993; Sadek et al., 2003). In a recent study, Josephs et al. (2005) observed combinations of parkinsonism syndromes, mild cognitive impairment, multifocal myoclonus, and vestibular-auditory dysfunction in welders who had worked in poorly ventilated areas without proper respiratory protection and had evidence of manganese accumulation in the brain as assessed by MRI.

A number of studies have reported decrements in neuropsychiatric and neurobehavioral effects in welders (Bowler et al., 2003; Chandra et al., 1981; Sinczuk-Walczak et al., 2001; Sjogren et al., 1990, 1996; Wang et al., 1989). However, the clinical significance of the neurological symptoms of welders observed in the studies is unknown and studies investigating the progression of these symptoms have not been reported. In addition, these studies are limited due to incomplete information on workplace exposure to manganese and other neurotoxicants, such as carbon monoxide, solvents, and metals (e.g., iron).

Only limited epidemiology addressing the association of welding fume exposure and the development of neurological disease exist. To date, no large scale, well-controlled study addressing this issue with complete and accurate exposure data has been performed. In a study of over 1400 welders in Alabama, Racette et al. (2005) concluded that the estimated prevalence of parkinsonism was higher in the welders compared to the general population of male residents from a county in Mississippi.

Limitations of the study included the welders tested were referred by attorneys and involved in litigation, the diagnosis of parkinsonism was performed by video tape analysis, and no comparable control group was used. In a Danish study, Fryzek et al. (2005) conducted a retrospective cohort study that evaluated the hospitalization rates of over 8000 workers who had been exposed to welding fumes for neurodegenerative disorders. The investigators observed no difference in the hospitalization rates of parkinsonism and other neurological conditions between the Danish welders and the general population of Denmark. Unfortunately, no information on the level of exposure to manganese and other metals, the method of welding, or the ventilation procedures used was included in the study. Another drawback was that shipyard welders, a group known to have high welding fume exposure due to work in confined spaces, were excluded from the cohort (that initially assessed the pulmonary effects of welding) due to potential confounding exposure to asbestos.

3. NIOSH welding fume generator and inhalation exposure system

The development of animal models and the ability to control the exposure would be useful in evaluating the potential effect of welding fume exposure on the central nervous system. Only a few welding fume inhalation exposure systems have been developed because of the complexity of the welding process and the need to continually generate welding fume at a constant concentration over extended periods. Previous welding fume inhalation systems for laboratory animals have needed experienced welders to operate the system, and the treatments were single exposures only at very high concentrations (400–1178 mg/m^3) for short periods of time (2–6 h) (Coate, 1985; Hicks et al., 1984).

During welding, the electrode or rod is consumed and the base metal piece becomes covered with the newly formed weld. Thus, the base metal piece needs to be continually changed and replaced, disrupting the exposure period and requiring numerous laboratory technicians to operate the system. The generation of constant welding fume for extended periods of time at reasonable fume concentrations for animal exposure has proven to be quite challenging. A completely automated system is needed that would perform welding operations and replace materials as they are consumed during the process. With such a system, ease of operation, exposure reproducibility, study of dose–response relationships, and comparison of toxicity of different welding fumes may be more advantageous.

Yu et al. (2000, 2001) developed a semi-automated welding fume generation system for exposing animals to fumes from SMAW processes. A circular base metal plate was slowly rotated, while at the same time a second pulley system advanced a welding rod toward the plate until an arc was formed. Welding fume was generated as the plate rotated and the welding rod slowly advanced. The procedure was repeated continually at 5 min cycles to process each rod—3 min to move it forward and be consumed to generate fumes, and 2 min for the holder to be returned and another rod installed. Thus, the exposure was disrupted every few min to replace the welding rod. This system may be appropriate for SMAW processes where the changing of the electrodes may simulate workplace practices, but it may not model exposure to fumes from more automated GMAW and FCAW processes.

It was our objective to construct an automated fume generation and inhalation exposure system for laboratory animals that would simulate real workplace exposures and would allow for continuous welding for extended periods of time without disruption (Fig. 1). The NIOSH welding fume generation system utilizes a programmable six-axis robotic arm

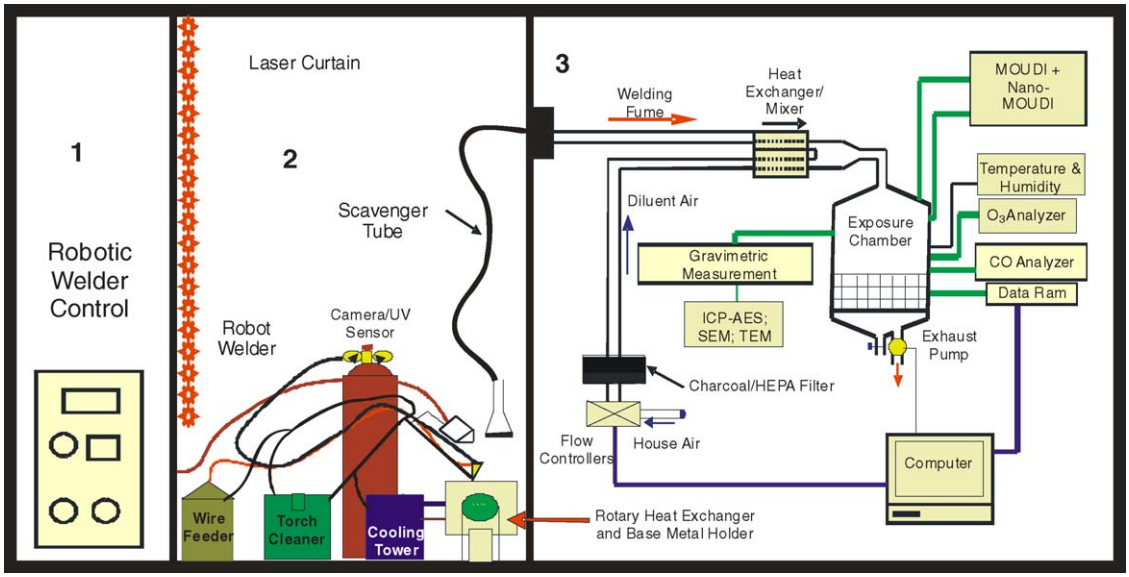

Fig. 1. Diagram of the NIOSH welding fume generation system including: (1) enclosed control room that contains the welding power source and controller; (2) robotic welding fume generator that contains the six-axis robotic arm, wire feeder, torch cleaner, coolers, and base metal holder; (3) animal exposure chamber with fume and gas characterization devices. Abbreviations—ICP-AES: inductively coupled plasma-atomic emission spectroscopy; SEM: scanning electron microscopy; TEM: transmission electron microscopy; Ram: real-time aerosol monitor; MOUDI: Micro-Orifice Uniform Deposit Impactor.

and a wire feeder that automatically supplies the wire to the torch at a programmed rate (Fig. 2). To avoid interruption of the exposure, a headstock was designed that holds and rotates a metal plate holder in different programmed positions. The metal plate holder has four sides and holds three metal plates per side. As one base metal plate becomes covered, the robotic welding arm can be programmed to move to the next metal piece. By having 12 base metal pieces, an adequate amount of surface area is provided to continually maintain welding for extended periods of time.

In a pilot study to assess the capability of the system, GMAW was performed using a stainless steel electrode (Blue Max E308LSi wire, Lincoln Electric, Cleveland, OH) for 3 h/day for 3 consecutive days at a desired fume concentration of 40 mg/m^3 (Antonini et al., 2006b). Chamber temperature, humidity, and fume concentration remained mostly constant during the 3 h of welding over the 3-day period. Fume concentrations were determined every 30 min during 3 h of operation for each day and were observed to be near the desired chamber concentration. Mean chamber fume concentration and standard deviation for the 3-day period were 38.4 ± 6.7 mg/m^3. Mean temperature and percent relative humidity and standard deviations for the 3-day period were 20.9 ± 1.4 °C and 37.7 ± 2.7%, respectively.

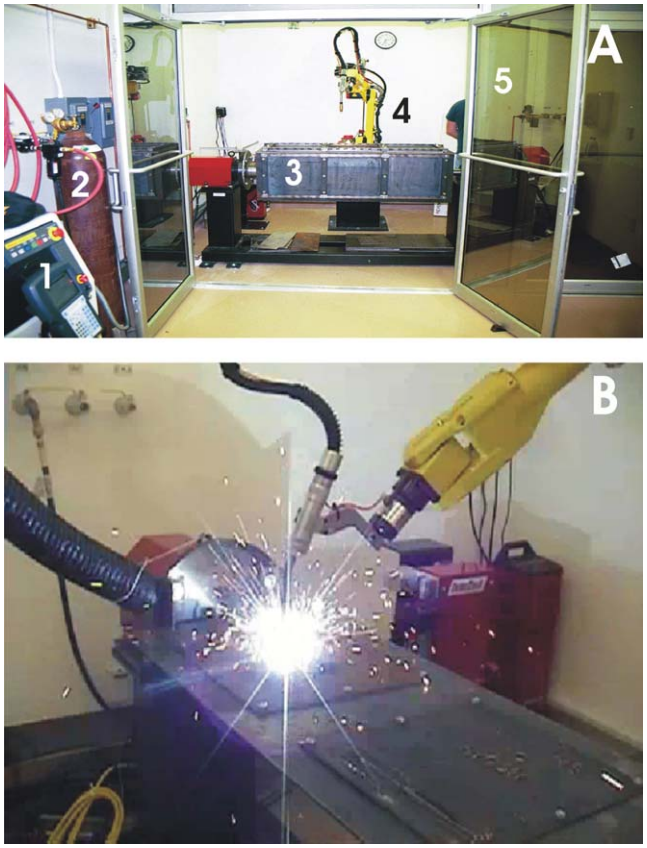

Fig. 2. (A) Photograph of the robotic welding fume generation system. The system includes a (1) Lincoln Electric (Cleveland, OH) Power Wave 455 power source and programmer; (2) shielding gas tank containing 95% Ar and 5% CO_2; (3) heat exchanger base metal plate holder and programmable headstock; (4) Lincoln Electric 100 Bi six-axis robotic arm; (5) aluminum airtight divider with UV-protective doors. (B) Photograph of electric arc generated during welding with the automated robotic welder.

The aerosols generated by the NIOSH robotic welder were characterized to determine if they were comparable to welding fume studied in the workplace. To determine which metals were present, inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was performed after collecting particle samples onto filters. The stainless steel welding particles were composed of (by wt.% of the metals measured): Fe (57%), Cr (20%), Mn (14%), Ni (9%), and Cu (0.2%). Trace amounts of Si, Al, and V also were present.

Particle size distribution was determined using a combination of Micro-Orifice Uniform Deposit Impactor (MOUDI) and Nano-MOUDI samplers. The size distribution of the aerosol generated by the NIOSH robotic welder during GMAW was trimodal with a mass median aerodynamic diameter (MMAD) of 0.24 μm. As observed by scanning and transmission electron microscopy (Fig. 3), most of the particles generated by the robotic welder during GMAW were aggregations of chain-like

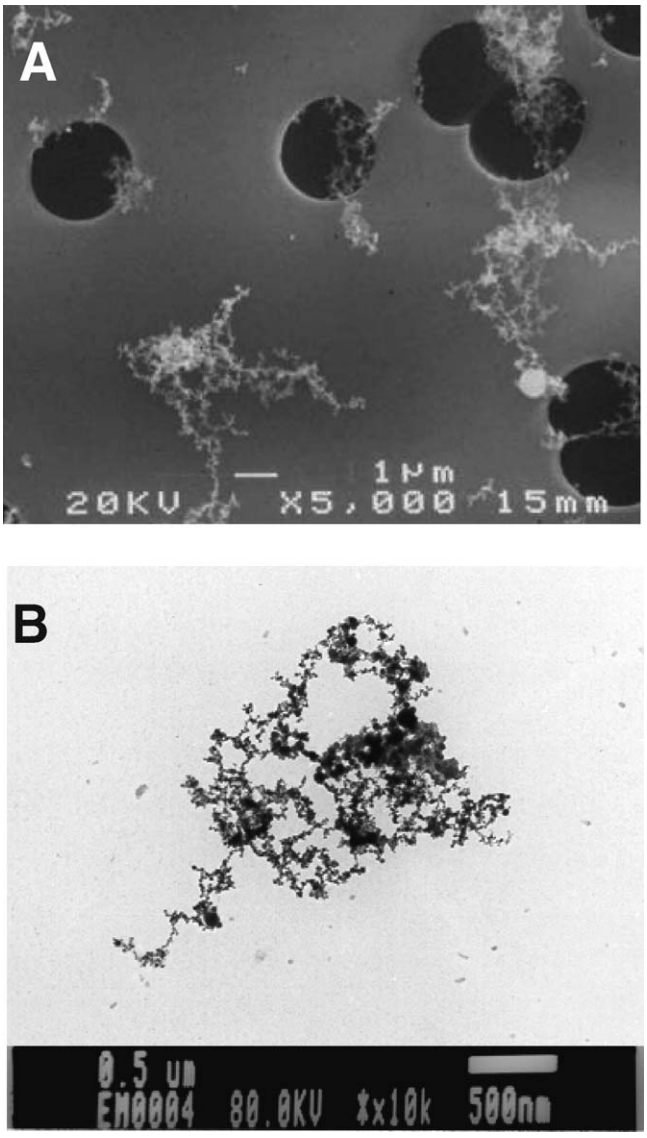

Fig. 3. (A) Representative scanning electron micrograph and (B) transmission electron micrograph of stainless steel gas metal arc welding fume generated by the NIOSH robotic welder.

agglomerates of nanometer-sized primary particles that were similar in appearance to what has been seen in previous studies (Jenkins et al., 2005; Zimmer and Biswas, 2001). Three modes of particle sizes also were clearly described by Zimmer and Biswas (2001) during GMAW: a nucleation mode (~0.01–0.10 μm) of individual primary particles, an accumulation mode (0.10–1.0 μm) of agglomerated and coalesced particles formed from the nucleation mode, and a coarse mode (~1–20 μm) of unagglomerated particles. The MMAD of the GMAW aerosol generated by the NIOSH robotic welder was comparable to what has been determined by other laboratories (Hewett, 1995; Zimmer and Biswas, 2001). Studies using the NIOSH robotic welder are ongoing in assessing the generation of welding fume using different types of electrodes (e.g., mild steel) and different processes (e.g., FCAW).

4. Animal model to study the neurotoxicity of welding fume

One advantage of using an animal model is the ability to measure the accumulation of different metals in the target organs of toxicity after welding fume exposure. Blood, lung tissue, and tissue from discrete brain regions can be collected from exposed animals at sacrifice to determine metal levels. Manganese concentrations in different brain regions (striatum, cerebellum, brain stem, cortex) have been easily measured and observed to be much higher in manganese-treated animals compared to untreated animals (Dorman et al., 2002; Gianutsos et al., 1997; Lai et al., 1999; Roels et al., 1997; Vitarella et al., 2000). In addition, MRI studies may be performed in exposed laboratory animals to assess manganese deposition in the brain. Chaki et al. (2000) observed a positive correlation between manganese blood content and T1-weighted MRI hyperintensity signals in selected brain regions of rats overexposed to manganese.

Methodology for assessing the neurotoxic effects of individual agents or mixtures has been developed and implemented at NIOSH. The approach used takes advantage of a universal cellular reaction to damage of the central nervous system: astrocytic hypertrophy. Astrocytic hypertrophy occurs at sites of damage throughout the central nervous system regardless of etiological basis of the damage or the specific cellular target involved. The hallmark of this response, often termed "reactive gliosis," is the enhanced expression of the major intermediate filament protein of astrocytes, glial fibrillary acidic protein (GFAP). Thus, an increase in the concentration of GFAP may serve as a potential biochemical indicator of neurotoxicity.

To validate the use of GFAP as a biomarker of neurotoxicity, prototype neurotoxicants have been administered to experimental animals and the effects of these agents on the tissue content of GFAP have been determined by immunoassay (O'Callaghan, 1991, 2002). Assays of GFAP were found to reveal dose-, time- and region-dependent patterns of neurotoxicity at toxicant dosages below those that cause light microscopic evidence of cell loss or damage (representative data shown in Fig. 4) (Norton et al., 1992; O'Callaghan, 1993; O'Callaghan and Sriram, 2005; Sriram and O'Callaghan,

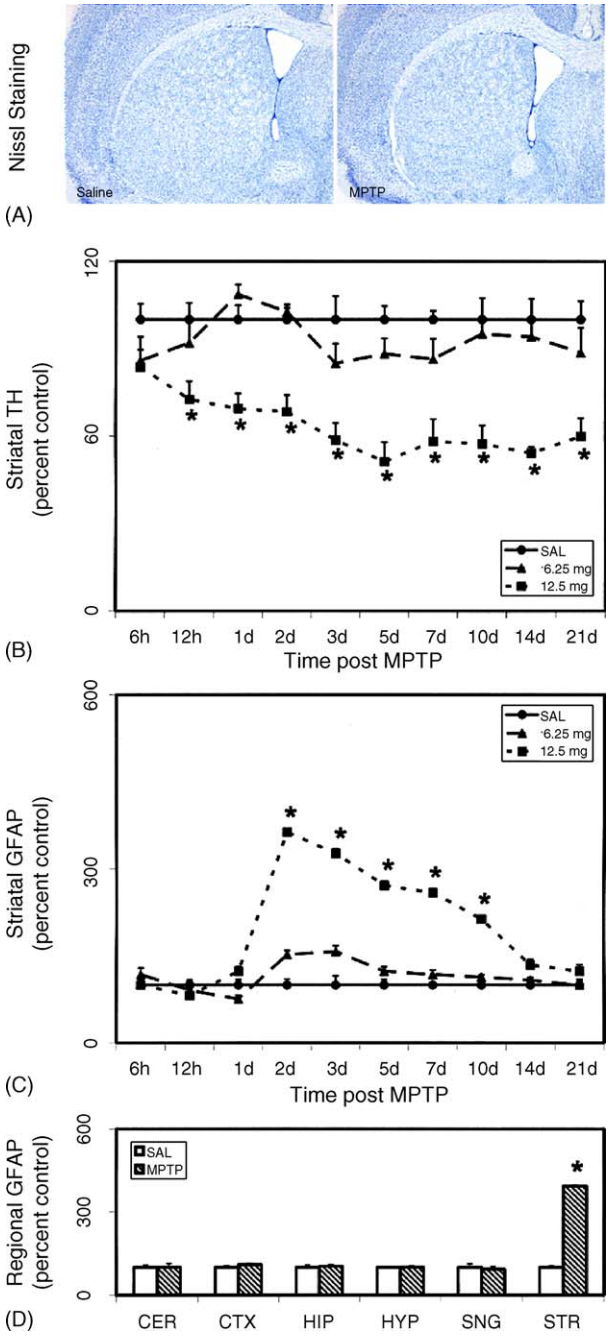

Fig. 4. Enhanced expression of GFAP reveals dose-, time- and region-dependent patterns of 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP)-induced neurotoxicity at dosages below those that cause light microscopic evidence of cell loss or damage. MPTP was subcutaneously administered to C57Bl6/J female mice. At the indicated dosages, damage is limited to destruction of dopaminergic nerve terminals as evidenced by dose- and time-dependent decrements in the levels of tyrosine hydroxylase (B), a marker in striatum of dopaminergic nerve terminal density. Nissl staining (thionine) does not reveal nerve terminal damage, therefore, staining of sections of striatum (A) fails to reveal underlying damage due to MPTP. The loss of tyrosine hydroxylase due to MPTP is followed by a marked dose- and time-dependent increase in striatal GFAP (C). Like the damage caused by MPTP with this dosage regimen, enhanced expression of GFAP is limited to striatum (12.5 mg/kg dose of MPTP at 72-h post-dosing) (D). It is important to note that MPTP was used as a model neurotoxicant to set up the GFAP assay, and the response of the brain to manganese may be different from those of MPTP. Abbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine; CER, cerebellum; CTX, cortex; HIP, hippocampus; HYP, hypothalamus; SNG, substantia nigra; STR, striatum. Adapted from O'Callaghan et al. (1990) and Sriram et al. (2004).

2005). Moreover, the temporal and regional increments in GFAP correspond to the temporal and regional patterns of neuronal damage, as revealed by sensitive silver stains (Balaban et al., 1988). These findings indicate that assays of GFAP represent a sensitive, simple and quantitative approach for evaluation of nervous system damage (Norton et al., 1992; O'Callaghan, 1993). Thus, the GFAP ELISA, combined with sensitive silver degeneration staining technology can be used to detect and quantify sites of damage throughout the central nervous system (O'Callaghan and Jensen, 1992).

Because dopaminergic systems have been implicated as targets of manganese exposure, assessment of dopaminergic neurotoxicity should be evaluated following welding fume exposure. Previously we have shown that known dopaminergic neurotoxicants cause loss of dopamine and tyrosine hydroxylase, effects consistent with a loss of dopaminergic nerve terminals. Silver degeneration staining of nerve terminal debris and a robust increase in GFAP levels and immunostaining are associated with this evidence of dopaminergic neurotoxicity. Taken together our previous findings demonstrate that GFAP assays can be used as a sensitive approach to identify and quantify sites of damage in the central nervous system regardless of the targets affected or the source of the damage. With respect to damage to dopaminergic systems, GFAP analysis can be combined with analysis of dopaminergic cell markers to verify dopaminergic neurotoxicity of welding fume.

5. Conclusions

A causal association between neurological effects and the presence of manganese in welding fume has yet to be established. With the development of the NIOSH welding fume generator and inhalation system, subchronic (~3 months) and chronic (~20 months) welding fume toxicology studies will be conducted. Animals will be exposed for set periods of time to known concentrations of welding fume generated using different welding materials. The potential neurotoxic effects of mild steel welding fume (low in manganese content) will be compared with fume generated from electrodes used during hard-surfacing welding (high in manganese content).

With the ability to control the exposure in an animal model, a number of concerns relating to the development of neurotoxicity and welding fume exposure may be addressed. Most of the case reports of manganese overexposure in welders are limited to exposure to very high levels of welding fumes, where welding has taken place in poorly ventilated areas. It needs to be determined whether or not exposure to long-term, low levels of manganese in welding fume can lead to neurotoxicity. Dose–response and time-course relationships regarding the potential development of neurotoxicity after welding fume inhalation can be established using the animal model. This would provide important information for risk assessment in developing safe or hazardous exposure limits for welding fume. Questions also remain as to whether or not the manganese can dissociate from its complex with iron in welding fume and become bioavailable to be transported out of the respiratory system. Toxicology studies would provide mechanistic data that would possibly elucidate the processes by which metals inhaled into the lungs after welding fume exposure may or may not translocate to other organs, such as the central nervous system. Questions exist as to whether the risk of neurotoxicity is dependent on the welding process or industry, where fume concentrations may be potentially higher or more hazardous. In vivo animal studies would allow for toxicological comparisons of welding fume generated from different processes that are used in specific industries.

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DISCLAIMER

The findings and conclusions of this manuscript have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

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