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From the Institute of Anatomy B, University of Aarhus, DK-8000 Aarhus C, Denmark

Hypoactivity in Silver Exposed Mice

By

Jørgen Rungby and Gorm Danscher

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Abstract: The functional implications of the presence of silver in the central nervous system are unknown. Since silver is present in the environment and since systemic silver poisoning leads to intraneuronal accumulations of the metal we have evaluated the possible effects of silver on the open field behaviour of mice. Argyric mice have been compared with controls in three experiments, one including long term administration of 0.015% silver nitrate in the drinking water and two in which the reactions of male and female mice to shock doses of silver lactate have been studied. In all experiments the silver treated mice were hypoactive. We suggest that this hypoactivity is due to an influence of silver upon the functional status of the CNS.

Key-words: Silver exposure – mice – open field behaviour – hypoactivity.

Silver is widely used in industry, medicine and dentistry. Absorption occurs by inhalation, ingestion or through broken skin (Fowler *et al.* 1979). Few reports have dealt with the effects of silver on the central nervous system. Cases have, however, been presented in which argyric patients have exhibited a diversity of symptoms: paralysis, convulsions, ataxia, disturbances of coordination and frontally located EEG changes (Dreisbach 1974; Franken & Langhof 1964; Reinhardt *et al.* 1971; Rosenmann *et al.* 1979; Aaseth *et al.* 1981). Silver penetrates the blood-brain barrier resulting in the deposition of the metal in vessel walls, neurones and glia throughout the brain. The maximum intraneuronal load is found in lysosomes of motor nuclei of the spinal cord and brain stem, in the deep cerebellar nuclei and in the nucleus ruber (fig. 1) (Rungby & Danscher 1983a & b). These findings suggest that a closer evaluation of possible harmful effects of silver on the CNS is needed.

Behavioural screening tests are sensitive tools in the assessment of possible pathological effects of environmental agents to the nervous system because of their ability to detect functional abnormalities before morphological or other distinct neuropathological changes can be observed (Tilson *et al.* 1980). Examination of spontaneous motor activities of intoxicated animals in open field cages is a test frequently used to evaluate effects of agents on the motor system (Mitchell *et al.* 1982; Tilson *et al.* 1980). Certain heavy metals, especially lead and mercury, can influence the open field behaviour of animals exposed either in utero or in adulthood (Alfano & Petit 1981; Booze *et al.* 1983; Driscoll & Stegner 1976; Driscoll & Stegner 1978; Evans *et al.* 1975; Silbergeld & Goldberg 1973; Sobotka & Cook 1974; Spyker *et al.* 1982; Tilson *et al.* 1980). Hyperactivity has been the most frequent finding although hypoactivity has been found after exposure of rats to lead pre- and postnatally (Booze *et al.* 1983; Driscoll & Stegner 1976) and after exposure of mouse pups to triethyl tin (Harry & Tilson 1981).

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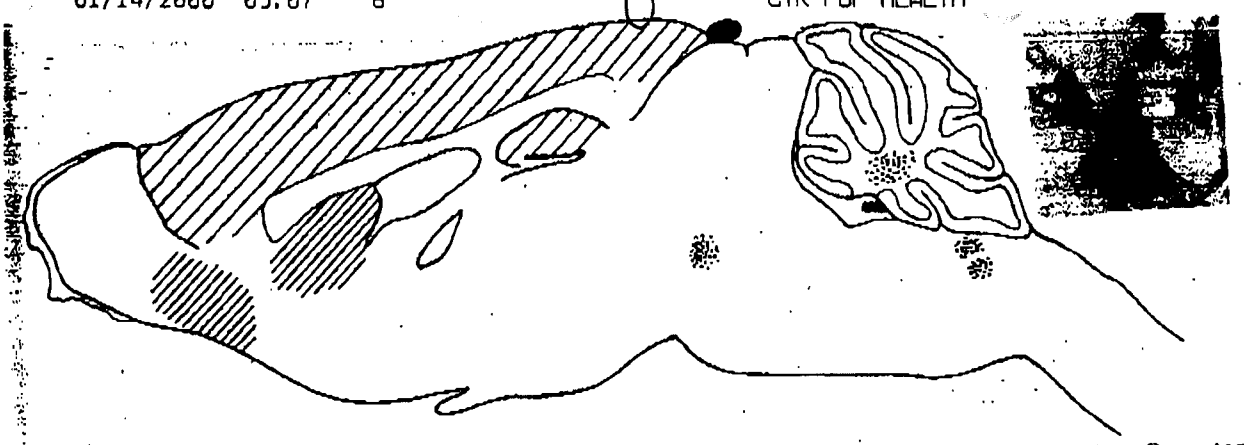

Fig. 1. Parasaggital section of a mouse brain showing areas containing large amounts of silver (dotted), motor nuclei, cerebellar nuclei and the nucleus ruber. Hatched areas, the cortex, basal ganglia and the anterior olfactory nucleus hold smaller amounts. Light areas e.g. sensory nuclei, pontine nuclei and diencephalic structures are practically void of silver. The choroid plexus and the pineal gland (black) which are both structures outside the blood-brain barrier contain very large amounts of silver. Inset: Cerebellar relay neurone with cytoplasmic silver deposits amplified by physical development (23), mouse injected with 1 mg of silver lactate (magnification × 400).

Materials and Methods

In the present study the open field behaviour of silver intoxicated mice has been examined in 3 experiments. One hundred mice of the NMRI strain (sixty days old at the day of the first exposure to silver) were used for the experiments. The animals were kept under normal laboratory conditions with free access to food and water, in a 12/12 hour light-dark cycle. Control animals were in all aspects – apart from silver treatment – treated like the intoxicated ones. Initially 20 male mice received two intraperitoneal injections totalling 1 mg of silver lactate (1 mg/ml distilled water) on successive days. Twenty male controls received two injections totalling 1 ml of saline.

Ten days after the last injection the animals were tested individually in an open field cage. The test was performed for 4 min. late in the day cycle, in this first experiment on 3 successive days and in the following 2 experiments on 4 successive days. The cage was a white 75 × 75 cm box with 25 cm high walls divided into 25 square fields and with a constant light intensity. Activity was blindly measured as the number of fields entered plus the total number of times the animal reared, leaned, washed, defaecated or urinated per min. Between testings, the cage was wiped with a damp sponge to remove urine and faeces. Prior to the first testing, the animals were weighed.

In the second experiment 20 female mice had their drinking water replaced by a 0.015% silver nitrate solution to which they had free access for 125 days (estimated 1/5 of the average life span) after which they were given normal drinking water again. The liquid intake of the silver treated group was decreased on the initial 3 days of exposure whereafter it did not differ from that of the control group. The average silver nitrate intake during the 125 days was 0.09 mg per mouse. Twenty non-exposed female controls were kept on the usual diet during the same period. Ten days after the termination of silver exposure the mice were tested as described above. For the last experiment 10 female mice received intraperitoneal injections of silver lactate (2 × 0.5 ml, 1 mg/ml on successive days) and 10 female controls received two intraperitoneal injections totalling 1 ml of saline. Testing was initiated 10 days after the last injection. One female mouse injected with silver died prior to the testing.

Differences in activity levels and weights were evaluated by non-parametric statistics (Mann-Whitney test).

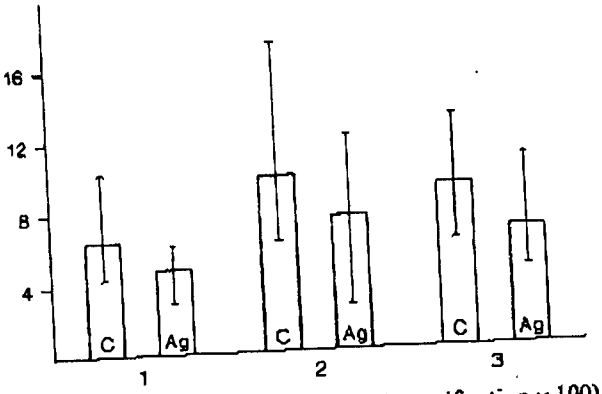

Fig. 2. Mean total acticity counts (magnification × 100) of controls (c) and silver treated mice (Ag). Thin lines indicate highest and lowest count in the group. 1. Male mice injected intraperitoneally; 2. Female mice exposed through the drinking water; 3. Female mice injected intraperitoneally.

Results

Silver treated animals were hypoactive (compared to the controls) in all experiments. P values are listed in table 1. The total activity levels are schematically shown in fig. 2. In the experiment involving long-term administration of silver nitrate in the drinking water to females, activity was significantly lowered throughout the test period (days 1–4). This was true also for the experiment involving male mice (days 1–3). The intraperitoneally injected females (an experiment involving fewer animals than the others) were only insignificantly hypoactive on days 1–3, but the hypoactive was significant on day 4 and when the total activity levels (day 1–4) were compared. The weight of the animals did not differ significantly in any of the experiments ($P > 0.05$) and the overt appearance of all animals seemed unaffected.

Discussion

The mechanisms underlying the lowered activity levels cannot be evaluated in these experiments because the animals were tested non-specifically for horizontal and vertical motor activities as well as for motor functions controlled by the autonomic nervous system. The observed hypoactivity could be due to a direct effect of silver on motor systems but it might just as well arise from altered reactions to internal or external stimuli due to changed sensory or integrative functions.

Lower motor neurones of the brainstem and spinal cord are, along with cerebellar nuclei and the nucleus ruber, primary sites of silver deposition (fig. 1). However, smaller amounts of intracellular silver are present throughout the CNS and the presence of silver in walls of cerebral blood vessels should not be ignored as large amounts of silver are stored in vascular basement membranes (Rungby & Danscher 1983a). These characteristics, along with an ultrastructural localization in lysosomes, are shared by silver and the known neurotoxic agent mercury (Danscher & Møller-Madsen, unpublished results). However, at present the site of action of silver cannot be determined from the anatomical distribution in the CNS.

Hypoactivity due to a shock dose of silver was present in both male and female mice. For practical reasons the effects of long term administration of low, subacute doses of silver were only tested in female animals. However, differences in activity levels in female animals compared to males are of minimal importance to the overall effects tested here in a controlled experiment (Reiter & Mac-

Table 1.

Values of P obtained by comparing the total activity levels of silver treated mice and controls (Mann-Whitney rank sum test). In all experiments the silver treated mice were hypoactive.

Mode of silver administration \ Test day	1	2	3	4	Total
Intraperitoneal injection of 2×0.5 mg silver lactate. Males N=40.	$0.025 < P < 0.05$	$0.01 < P < 0.05$	$0.01 < P < 0.025$	–	Σ 1–3 $0.001 < P < 0.01$
Intraperitoneal injection of 2×0.5 mg silver lactate. Females N=19.	$P > 0.05$	$P = 0.05$	$P \approx 0.05$	$0.01 < P < 0.025$	Σ 1–4 $0.01 < P < 0.025$
0.015% silver nitrate in the drinking water for 125 days. Females N=40.	$0.01 < P < 0.025$	$0.025 < P < 0.05$	$0.025 < P < 0.05$	$0.001 < P < 0.01$	Σ 1–4 $0.001 < P < 0.01$

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Phail 1982). The silver treated mice showed no signs of illness at the time of testing and their average weight had not decreased as a result of the silver intoxication in any of the experiments. This suggests that the observed hypoactivity was not due to an actue state of disease. Additional effects arising from the anions of the soluble silver salts are expected to have no large influence on the results ten days after the exposure due to their fast metabolic degradation and excretion by natural pathways.

It has been suggested that a common feature to the functions tested in these experiments is the emotional stability of the animals (Royce 1977), hypoactivity might accordingly result from an increased responsiveness to the novelty of the open field test environment (Silbergeld & Goldberg 1973), but effects of silver on e.g. endocrine organs (Danscher 1981; Thorlacius-Ussing & Rungby 1984) cannot be excluded as factors contributing to the hypoactivity. These crude observations need further investigations of the parameters involved since the significance in the results was achieved by examining overall motor activities, but they suggest that accumulations of silver in CNS might influence the function of the mammalian brain.

References

Aaseth, J., A. Olsen, J. Halse & T. Hovig: Argyria – tissue deposition of silver as selenide. *Scand. J. Clin. Lab. Invest.* 1981, **41**, 247–251.

Alfano, D. P. & T. L. Petit: Behavioral effects of postnatal lead exposure: Possible relationship to hippocampal dysfunction. *Behav. Neur. Biol.* 1981, **32**, 319–333.

Booze, R. M., C. F. Mactutus, Z. Annau & H. H. Tilson: Neonantal triethyl lead neurotoxicity in rat pups: Initial behavioral observations and quantification. *Neurobehav. Toxicol. Teratol.* 1983, **5**, 367–375.

Danscher, G.: Light and electron microscopic localization of silver in biological tissue. *Histochemistry* 1981, **71**, 177–186.

Dreisbach, R. H.: *Handbook of poisoning*. 8th ed. Lange, Los Altos, California, 1974.

Driscoll, J. W. & S. E. Stegner: Behavioral effects of chronic lead ingestion on laboratory rats. *Pharmacol. Biochem. Behav.* 1976, **4**, 411–417.

Driscoll, J. W. & S. E. Stegner: Lead-produced changes in the relative rate of open field activity of laboratory rats. *Pharmacol. Biochem. Behav.* 1978, **8**, 741–747.

Evans, H. L., V. G. Laties & B. Weiss: Behavioral effects of mercury and methylmercury. *Fed. Proc.* 1975, **34**, 1858.

Fowler, B. A. & G. F. Nordberg: Silver. In: *Handbook on the toxicology of metals*. Eds.: L. Friberg, G. F. Nordberg & V. B. Vouk Elsevier, Amsterdam, 1979, pp. 579–586.

Franken, E. & H. Langhof: Argyrosis universalis durch Targesin-Rollkuren. *Med. Klin.* 1964, **27**, 1094–1096.

Harry, G. J. & H. A. Tilson: The effects of postpartum exposure to triethyltin on the neurobehavioral functioning of rats. *Neurotoxicol.* 1981, **2**, 283–296.

Mitchell, C. L., H. A. Tilson & P. A. Cabe: Screening for neurobehavioral toxicity: Factors to consider. In: *Nervous system toxicology*. Ed.: C. L. Mitchel. Raven, New York, 1982, pp. 237–245.

Reinhardt, G., M. Geldmacher v. Mallinckrodt, H. Kittel & O. Opitz: Akute tödliche Vergiftung mit Silbernitrat als folge eines Abtreibungsversuches. *Arch. Kriminol.* 1971, **148**, 69–78.

Reiter, L. W. & R. C. MacPhail: Factors influencing motor activity measurements in neurotoxicology. In: *Nervous system toxicology*. Ed.: C. L. Mitchel Raven, New York, 1982, pp. 45–65.

Rosemann, K. D., A. Moss & S. Kon: Clinical implications of exposure to silver nitrate and silver oxide. *J. Occup. Med.* 1979, **21**, 430–435.

Royce, J. R.: On the construct validity of open-field measures. *Psychol. Bull.* 1977, **84**, 1098–1106.

Rungby, J. & G. Danscher: Localization of exogenous silver in brain and spinal cord of silver exposed rats. *Acta neuropathol.* 1983a, **60**, 92–98.

Rungby, J. & G. Danscher: Neuronal accumulation of silver in brains of progeny from argyric rats. *Acta neuropathol.* 1983b, **61**, 258–262.

Silbergeld, E. K. & A. M. Goldberg: A lead-induced behavioral disorder. *Life Sci.* 1973, **13**, 1275–1283.

Silbergeld, E. K. & A. M. Goldberg: Lead-induced behavioral dysfunction: An animal model of hyperactivity. *Exp. Neurol.* 1974, **42**, 146–157.

Sobotka, T. J. & M. P. Cook: Postnatal lead acetate exposure in rats: Possible relationship to minimal brain dysfunction. *Amer. J. Ment. Def.* 1974, **79**, 5–9.

Spyker, J. M., S. B. Sparber & A. M. Goldberg: Subtle consequences of methylmercury exposure: Behavioral deviations in offspring of treated mothers. *Science* 1972, **177**, 621–623.

Thorlacius-Ussing, O. & J. Rungby: Ultrastructural localization of exogenous silver in the anterior pituitary gland of the rat. *Exp. Mol. Path.* 1984, in press.

Tilson, H. A., P. A. Cabe & T. A. Burne: Behavioral procedures for the assessment of neurotoxicity. In: *Experimental and clinical neurotoxicology*. Eds.: P. S. Spencer & H. H. Schaumburg. Williams & Wilkins, London, 1980, pp. 758–766.