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The influence of low-level sarin inhalation exposure on spatial memory in rats

Jiři Kassa*, M. Koupilová, J. Vachek

Purkyně Military Medical Academy, 500 01 Hradec Králové, Czech Republic

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Abstract

To study the influence of low-level sarin exposure on cognitive functions, the rats were exposed to three various low concentrations of sarin (Levels 1–3) for 60 min in the inhalation chamber. In addition, one group of rats was exposed to Level 2 of sarin repeatedly. Testing of cognitive functions was carried out using the Y-maze evaluating learning and spatial memory. The correct averse behavior of sarin-exposed rats in the Y-maze was tested several times within 6 weeks following sarin inhalation exposure to look for any cognitive impairments. The results were compared to the Y-maze performance of control rats exposed to pure air instead of sarin. While a subtle and short-term deficiency in the Y-maze performance was observed in rats exposed to the Levels 1 and 2 of sarin, the exposure to the Level 3 of sarin caused a significant decrease in the Y-maze performance for a relatively long time. Similar sarin-induced spatial memory impairments were demonstrated in rats exposed repeatedly to the Level 2. A decrease in the Y-maze performance was observed until the end of the third week following the last exposure to sarin. Thus, our findings confirm that both nonconvulsive symptomatic and clinically asymptomatic concentrations of sarin can cause relatively long-term memory impairments in sarin-poisoned rats when the rats are exposed to clinically asymptomatic sarin concentration repeatedly.

Keywords: Sarin; Low-level inhalation exposure; Spatial memory; Rat

1. Introduction

The potential for the exposure to highly toxic organophosphorus compounds (OPs), called as nerve agents, exists on the battlefield (e.g., Iran–Iraq war), as well as in a civilian sector as a threat by a terrorist group (e.g., Tokyo subway incident — Ohtomi et al., 1996) or as an accident as part of current demilitarization efforts. OPs elicit their toxic effects by irreversible inhibiting acetylcholinesterase (AChE, EC 3.1.1.7) in the central, as well as peripheral nervous system allowing accumulation of acetycholine (ACh) and excessive stimulation of postsynaptic cholinergic receptors. The overstimulation of central cholinergic system is followed by the activation of other neurotransmitter systems including glutamate receptors leading to the increase in extracellular levels of the excitatory amino acid glutamate, a major excitotoxin mediating central neurotoxicity of OPs (McDonough and Shih, 1997; Solberg and Belkin, 1997). Signs of acute toxicity with extensive AChE inhibition also include autonomic dysfunction (e.g., excessive salivation, lacrimation, urination and defecation), involuntary movements (e.g., tremor and fasciculation), respiratory dysfunction and other signs and symptoms (Marrs 1993; Taylor 1996).

OP-induced cholinergic effects are usually manifested immediately following high-level exposure (Marrs 1993; Taylor 1996), nevertheless, there are numerous studies in both humans and animals showing that survivors of high-level OP exposure can experience subtle but significant long-term neurological and neuropsychological outcomes that are detectable months or even years following the recovery from acute poisoning (Brown and Kelley, 1998). The rapid onset of signs and symptoms of poisoning following OP exposure can be explained in terms of ACh accumulation following AChE inhibition but no mechanism has been identified for the induction of long term effects. In addition, very little is known about possible neurological and neuropsychological effects including the impairments of cognitive functions following single or repeated low-level, asymptomatic exposures to OPs. The purpose of this study is to find out whether a nerve agent sarin might cause

* Tel.: +-420-49-525-1500; fax: +420-49-521-0094.
E-mail address: kassa@pmf.hk.cz (J. Kassa).

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long-term adverse effects on cognitive functions, especially spatial memory, following the single or repeated low-level inhalation exposure in rats.

2. Material

2.1. Subjects

Male albino Wistar rats weighing 180–200 g were purchased from VÚFB Konárovice (Czech Republic). They were kept in an air-conditioned room with light from 07:00 to 19:00 h at room temperature in cages (370 × 570 × 200 mm, 10 animals/cage) and allowed access to standard food and tap water ad libitum. The rats were divided in six groups of 10 animals/group including two control groups ($N = 10$). Handling of the experimental animals was done under supervision of the Ethics Committee of the Medical Faculty of Charles University and the Purkyně Military Medical Academy (Czech Republic).

2.2. Chemicals

Sarin was obtained from Zemianské Kostolany (Slovak Republic) and was 98.5% pure. All other chemicals of analytical grade were obtained commercially and used without further purification.

2.3. Procedure

The experimental rats were exposed to various low concentrations of sarin for 60 min in the inhalation chamber, while control rats were exposed to pure air for 60 min in the same inhalation chamber. The chamber is a box (300 × 400 × 250 mm) made from enamel metal with sarin applicator where the rats are placed in the cage (185 × 310 × 65 mm, 3 rats/cage) Three concentration of sarin were chosen:

- clinically and laboratory asymptomatic concentration (0.8 μg/l) with a nonsignificant inhibition of erythrocyte AChE by 10% — Level 1.
- clinically asymptomatic concentration with a significant inhibition of erythrocyte AChE by 30% (1.25 μg/l) — Level 2. This concentration was used for a single (Level 2) or repeated (three times, each other day) exposure (Level 2R). After the third exposure, a significant inhibition of erythrocyte AChE by 48% was measured.
- nonconvulsive symptomatic concentration with a significant inhibition of erythocyte AChE by 50% (2.5 μg/l) — Level 3.

The erythocyte AChE activity of sarin-exposed rats was measured in another experiment immediately following the inhalation exposure by the same laboratory using Ellman spectrophotometric method (Ellman et al., 1961). The development of sarin-induced AChE inhibition during the testing

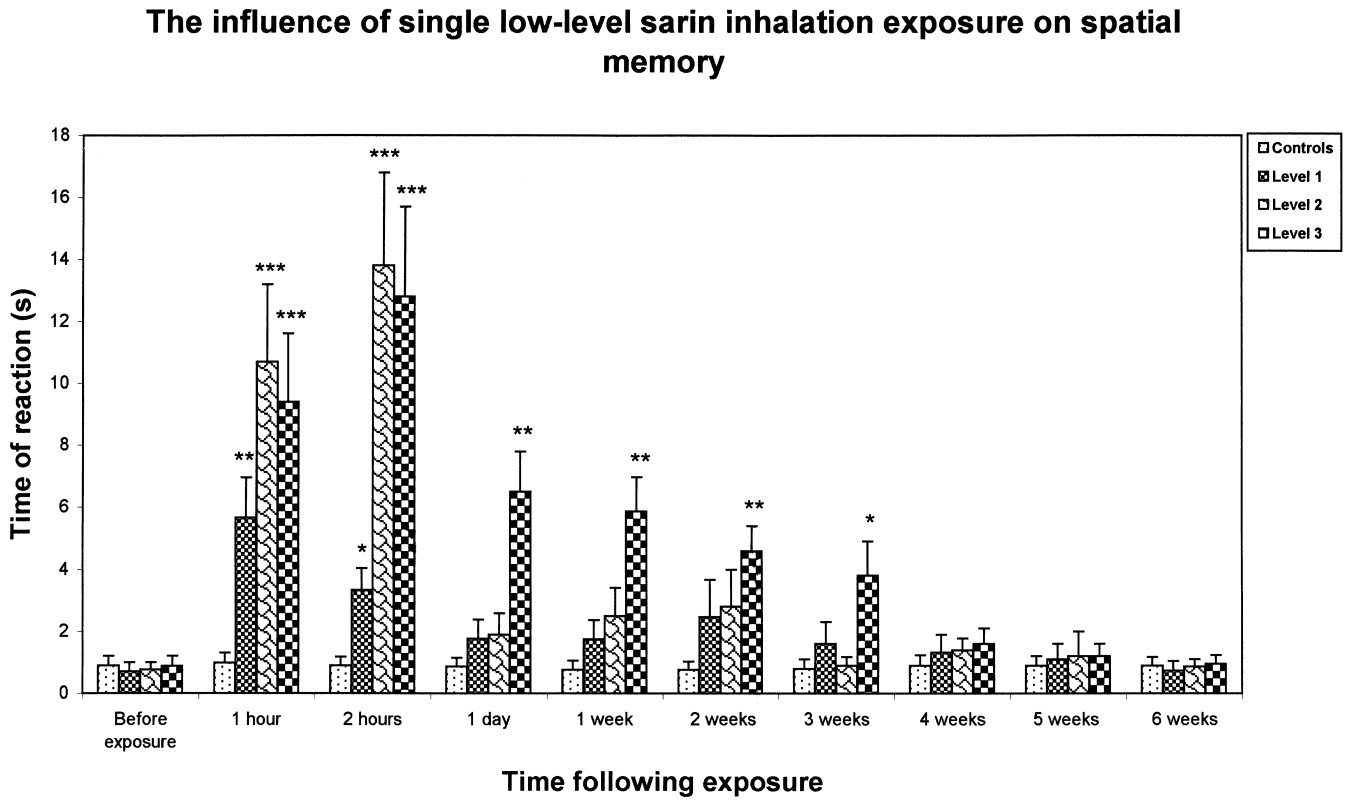

Fig. 1. The alteration of the Y-maze performance in rats singly exposed to Levels 1–3 of sarin. Statistical significance: * $P < .05$, ** $P < .01$, *** $P < .001$.

of cognitive functions did not follow. In the case of 60-min inhalation exposure in the same experimental conditions, the lethal concentration of sarin for rats was 4.48 μg/l and the convulsive concentration of sarin for rats was 3.25 μg/l.

Before starting the testing of cognitive functions, the rats were shortly monitored using a functional observatory battery (FOB), a noninvasive and relatively sensitive type of neurobehavioral examination of sensory, motor and automatic nervous functions (Frantík and Hornychová, 1995) to exclude any acute sarin-induced neurotoxic effects. Cognitive functioning was tested using a Y-maze with averse motivation by a strong electric footshock, evaluating learning and spatial memory (Koupilová et al., 1995). The Y-maze is a fully automated apparatus used for the study of behavior of laboratory rats. It is a plastic box consisting of a square start area (285 × 480 mm) separated by a plexiglass sliding door from two trapezoid, black and white arms-choice area (140 × 324 mm). The grid-floor in the start and choice area is electrifiable. The animals are placed at the start area and, after 48 s, electric footshocks (60 V, 50 Hz, duration 0.5 s) are applied at 5-s intervals. The rats try to avoid the shock by escaping to one of two arms. In the case of moving a rat to wrong (dark) arm, the rats fail to avoid further footshock. The animals were taught spatial discrimination with the preference of black or white arm in the Y-maze. The latency to enter the correct arm was measured and the number of wrong entries was counted. Before inhalation exposure to sarin, the rats were trained to avoid footshock by moving to correct (white) arm in the Y-maze. It usually takes 4 weeks of training to reach the criterion, which was 80% or more correct averse behavior (moving to the correct arm) within <1.5 s. During the training, 10 sessions (2 trials/session) per week lasting 4 min were realized. The exposure started the day after the animals had reached this criterion. The spatial memory was tested 1 h, 2 h, 1 day and 1 week following the sarin inhalation exposure, and then once a week until the end of the sixth week following the exposure. The latency time to enter the correct arm by sarin-exposed rats and the number of entry errors were compared to the values obtained from the control rats exposed to the pure air instead of sarin.

2.4. Data analysis

Analysis of variance (ANOVA) with Bonfferoni's corrections for multiple comparisons was used for the determination of significant differences between experimental and control values (Afifi and Azen, 1979). The difference were considered significant when $P<.05$.

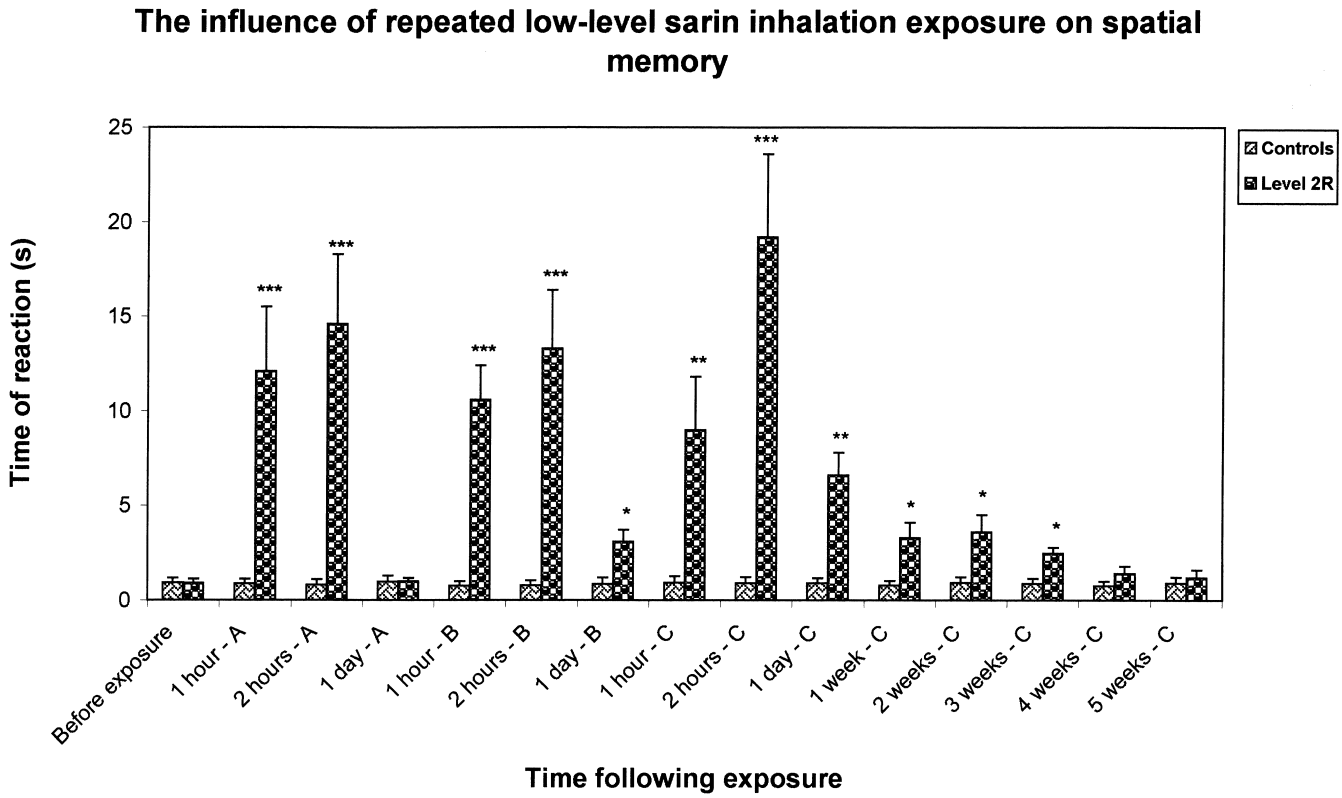

Fig. 2. The alteration of the Y-maze performance in rats repeatedly exposed (A — the first exposure, B — the second exposure, C — the third exposure) to Level 2 of sarin. Statistical significance — see Fig. 1.

3. Results

While control rats did not show any changes in the rapidity of spatial discrimination in Y-maze following their exposure to the pure air in comparison with the values obtained before the exposure, the significant increase in the reaction time of rats exposed to chosen low concentrations of sarin was observed ($P < .05$). The results of the influence of various sarin concentrations on the Y-maze performance of rats following the single inhalation exposure are shown in Fig. 1. While a spatial orientation of rats exposed to Level 1 or 2 of sarin was significantly influenced for a short time only (1 and 2 h following the exposure), rats exposed to Level 3 of sarin showed a decrease in Y-maze performance for a relatively long time (until the third week following the exposure) (Fig. 1). The effects of low-level sarin inhalation exposure were dose-dependent. When the rats were exposed to Level 2 of sarin, their impairment of spatial memory was as short as the impairment of spatial memory of rats exposed to Level 1, but the latency time in their choice of the correct arm was longer (Fig. 1). In contrast to the reaction time, the number of entry errors of experimental rats was not different from the number of entry errors of control rats exposed to pure air, regardless of the sarin concentration used for the inhalation exposure of experimental rats.

The results of Y-maze performance of rats repeatedly exposed to Level 2 of sarin compared to control values are given in Fig. 2. The first exposure to Level 2 brought only short-time increase in the reaction time of sarin-exposed rats, nevertheless, the second and the third exposures to the same sarin concentration caused a significantly higher and longer spatial memory impairments compared to rats singly exposed to Level 2 of sarin. The decrease in Y-maze performance of repeatedly exposed rats lasted until the end of the third week following the last exposure to sarin (Fig. 2). The number of entry errors of rats repeatedly exposed to Level 2 of sarin did not differ from control values.

4. Discussion

The exposure to high doses of OPs including nerve agents has been demonstrated to result in severe brain neuropathology that involves not only neuronal degeneration and necrosis of various brain regions (Lemercier et al., 1983; McLeod et al., 1982; Petras 1981), but also persistent severe alteration in behavior and cognitive incapacitation especially impairments of learning and memory (Bushnell et al., 1991; McDonald et al., 1988; Rafaelle et al., 1990). The most significant injury caused by OP poisoning is neuronal degeneration of the hippocampus that is associated with the spatial learning and memory. Therefore, impairment of cognitive functions, especially incapacitation of learning and memory, belongs to the most frequent central signs of acute OP poisoning (Marrs 1993; McDonald et al., 1988). In addition, the adverse effects of OP compounds on cognition functions, such as learning and memory, may persist for quite some time after termination of toxicant exposure. The results from several studies have demonstrated the presence of OP-induced learning impairments several days after the behavioral signs of OP toxicity have subsided (Buccafusco et al., 1990; Bushnell et al., 1991; McDonald et al., 1988). The chronic exposure to OP agents can also result in specific long-term cognitive deficits even when signs and symptoms of excessive cholinergic activity are not present (Prendergast et al., 1997; Prendergast et al., 1998).

Our data clearly demonstrate that sarin is also able to induce a subtle alteration of spatial orientation and spatial memory in the case of the exposure of rats to its low, clinically asymptomatic doses. Their memory impairments are not influenced by stress induced by the presence in the inhalation chamber because control rats did not show any impairments of the Y-maze performance although they were also exposed (to pure air) in the same inhalation chamber for 60 min as the experimental rats. In addition, the influence of other signs of sarin-induced neurotoxicity such as an alteration of pain sensitivity or locomotor function and a disruption of vision was excluded by monitoring the rats with the help of FOB before starting the Y-maze performance.

Thus, the significant clinically manifested AChE inhibition in the central nervous system leading to the neuronal degeneration of some brain regions including hippocampus, associated with the spatial learning and memory, is not necessary for the clinically manifested cognitive impairments. These findings correspond with earlier published data about neurological and neurophysiological outcomes detectable months or even years following recovery from acute OP poisoning (Savage et al., 1988; Yokoyama et al., 1998). It is very difficult to find the real reason for the memory impairments in the case of low-level sarin inhalation exposure. Recently, it has been demonstrated a temporal relationship between OP-induced impairment in performance of a spatial memory task and the protracted decrease in the expression of cholinergic receptors in specific brain regions (including hippocampus) following the asymptomatic exposure to OPs (Stone et al., 2000). It means that a decrease in the number of cholinergic receptors in hippocampus following the low-level exposure to OPs without significant AChE inhibition could cause the memory impairments. Moreover, other factors than the cholinergic nervous system could be involved in OP-induced alteration of cognitive functions. It has been reported that there are protein targets present in brain, which are known to be very sensitive to some anticholinesterase compounds including the nerve agents. They may represent a target for sarin-induced low-level effects. However, the function of these protein targets has not yet been elucidated (Ray 1998).

The repeated low-level sarin inhalation exposure made the cognitive impairments longer and higher compared to the single sarin exposure. The effect of repeated inhalation exposure to sarin could be caused by the accumulation of sarin in the exposed organism causing a higher inhibition of

AChE compared to singly exposed rats. The inhibition of erythrocyte AChE measured following the third exposure to sarin at Level 2 corresponds to the inhibition of erythrocyte AChE in rats exposed to sarin at Level 3. Thus, the repeated inhalation exposure to low asymptomatic level of sarin can cause an alteration of cognitive functions corresponding to the effect of higher symptomatic level of sarin. These findings are supported by recently published data about neurological and immunological effects of single or repeated low-level sarin inhalation exposure (Kassa et al., 2001).

Although these findings are difficult to extrapolate directly to human low-level exposures to OPs, they indicate that subtle spatial memory impairments without clinically manifested disturbance of central cholinergic nervous system could also occur in humans for a relatively long time (a few weeks) following the inhalation exposure to asymptomatic or nonconvulsive symptomatic levels of sarin, especially in the case of repeated low-level exposure.

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