ORIGINAL ARTICLE

# LONG TERM EFFECTS OF LOW-LEVEL SARIN INHALATION EXPOSURE ON THE SPATIAL MEMORY OF RATS IN A T-MAZE

*Jiří Kassa, Marie Koupilová, Josef Vachek*

Purkyně Military Medical Academy in Hradec Králové: Department of Toxicology

*Summary:* 1. To study the influence of low-level sarin exposure on cognitive functions, male albino Wistar rats were exposed to three various low concentrations of sarin (LEVEL 1–3) for 60 minutes in the inhalation chamber. Testing of cognitive functions was carried out using the T-maze evaluating learning and spatial memory. The behavior of sarin-exposed rats in the T-maze was tested several times within five weeks following sarin inhalation exposure to look for any cognitive impairments. The alteration of cognition was evaluated by using a method studying memory elicitation in response to appetitive motivation in a multiple T-maze. 2. Statistically significant, short-term deficiency in the T-maze performance was observed in rats exposed to symptomatic (LEVEL 3) as well as clinically asymptomatic concentration (LEVEL 2) of sarin. The repeated exposure of rats to clinically asymptomatic dose of sarin (LEVEL 2R) did not change the effect of low-level sarin exposure on spatial memory compared to the single exposure to the same dose of sarin. 3. Thus, sarin is able to influence the cognitive functions (e.g. spatial memory) even at low doses that do not cause clinically manifested intoxication following the inhalation exposure. Nevetheless, the alteration of spatial memory lasts for a short time only, in contrast with the severe sarin poisoning.

***Key words:*** *Sarin; Low-level inhalation exposure; Spatial memory; T-maze; Rat*

## Introduction

The potential for the exposure to highly toxic organophosphorus compounds (OPs), called 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 – 12) or as an accident as a 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), excessive stimulation of postsynaptic cholinergic receptors and consequent signs of neurotoxicity. Signs of acute toxicity with extensive AChE inhibition include autonomic dysfunction (e.g. excessive salivation, lacrimation, urination and defecation), involuntary movements (e.g. tremor, fasciculation), respiratory dysfunction and other signs and symptoms (9, 19).

OP-induced cholinergic effects are usually manifested immediately following high-level exposure (9, 19), 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 (2). 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 of single or repeated low-level, asymptomatic exposure to OPs. The purpose of this study is to find out whether a nerve agent sarin might cause adverse effects on cognitive functions following the single or repeated low-level inhalation exposure in rats.

## Material and methods

Male albino Wistar rats weighing 180-200 g were purchased from VÚFB Konárovice (Czech Republic). They were kept in an air-conditioned room and allowed access to standard food and tap water ad libitum. The rats were divided into groups of ten. 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 in Hradec Králové (Czech Republic).

The rats were exposed to various low concentrations of sarin (obtained from Military Technical Institute, Zemianské Kostolany, Slovak Republic) for 60 minutes in the inhalation chamber. Three low concentrations of sarin were chosen:

- clinically nad laboratory asymptomatic concentration (0.8 μg/L) – 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 during one week) exposure (LEVEL 2R)
- non-convulsive symptomatic concentration (2.5 μg/L) – LEVEL 3

Cognitive functioning was tested using a T-maze, consisting of five segments, a starting and a goal compartment to evaluate learning, spatial memory and spatial orientation (6,7). The rats were trained, with the food reward, to run through the maze in less than 10 seconds without entering the side arm. The time necessary to reach the goal box was recorded. Before inhalation exposure to sarin, the rats were trained to reach the goal box as soon as possible by moving to the correct segment in the T-maze. It usually took 4-6 weeks of training to reach the criterion which was 80% or more correct behavior. The exposure started the day after the animals had reached this criterion. The spatial memory was tested 1 hour, 2 hours, 1 day and 1 week following the sarin inhalation exposure and then, once a week till the end of the fifth week following the exposure. The time of reaching the goal box by sarin-exposed rats was compared to the values obtained from the same rats immediately before sarin exposure and from control rats exposed to pure air instead of sarin.

Analysis of variance (ANOVA) with Bonfferoni's corrections for multiple comparisons was used for the determination of significant differences between experimental and control values (1). The differences were considered significant when $P < 0.05$.

## Results

While the rats exposed to LEVEL 1 of sarin did not show any significant changes in the rapidity of spatial discrimination in T-maze following their exposure in comparison with the control rats exposed to the pure air, the significant impairment of spatial memory of rats exposed to other low concentrations of sarin (LEVEL 2 and 3) was observed. The results of the influence of various sarin concentrations on the T-maze performance of rats following single inhalation exposure are shown in Figure 1. While a spatial memory of rats exposed to LEVEL 1 of sarin was not significantly influenced, rats exposed to LEVEL 2 and 3 of sarin showed a significant decrease in T-maze performance for a short time (till the first day following the exposure). Their latency time in the choice of the correct segment and reaching the goal box of the T-maze was extended. In addition, the effects of low-level sarin inhalation

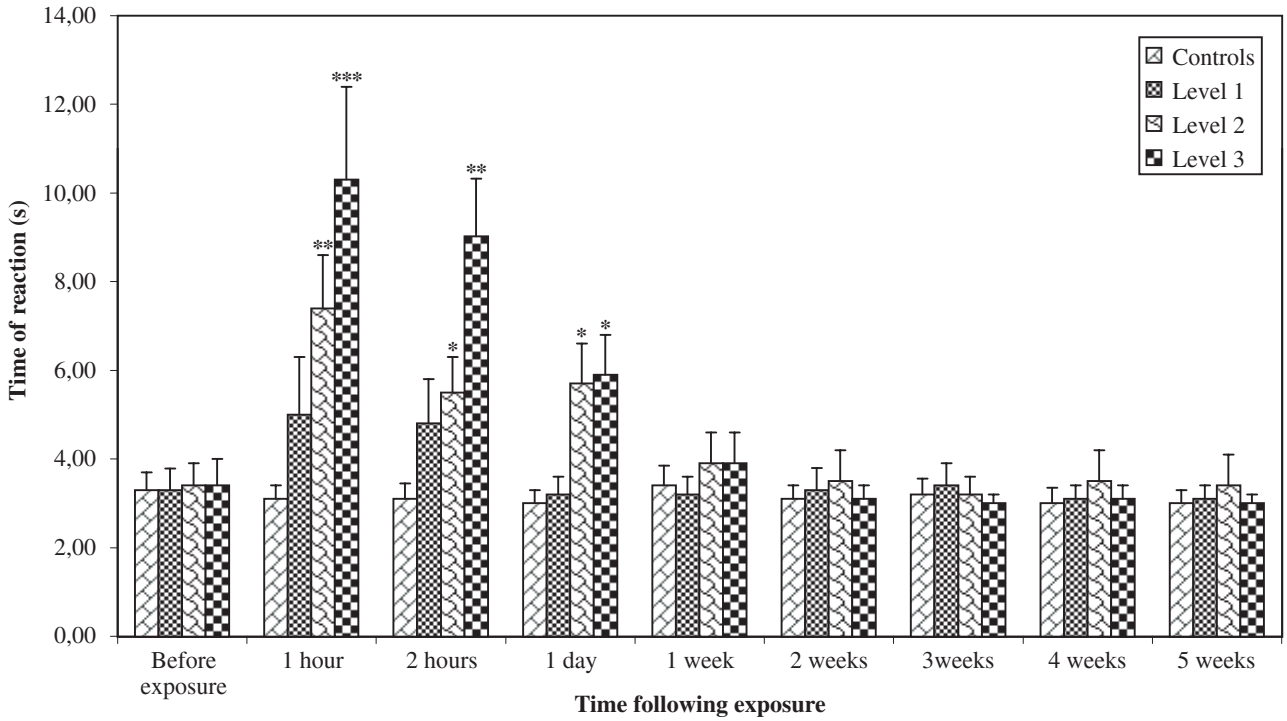


**Fig. 1:** The changes in the T-maze performance of rats singly exposed to sarin at LEVEL 1 - 3 in comparison with control rats. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

exposure was dose-dependent. When the rats were exposed to LEVEL 3 of sarin, their time of passage through the maze was more lengthened at 1 and 2 hours following the inhalation exposure compared to the rats exposed to LEVEL 2 of sarin (Fig. 1).

The results of T-maze performance of rats repeatedly exposed to LEVEL 2 of sarin are given in Figure 2. The repeated exposure of rats to clinically asymptomatic concentration of sarin (LEVEL 2) did not change the effect of low-level sarin exposure on spatial memory in comparison with the single exposure to the same dose of sarin (Fig. 2).

## Discussion

The exposure to high doses of OPs has been demonstrated to result in severe brain neuropathology that involves not only neuronal degeneration and necrosis of various brain regions (8,11,13) but also persistent severe alteration in behavior and cognitive functions, especially impairment of learning and memory (4,10,16). 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 (9,10). In addition, the adverse effects of OP compounds on cognitive functions, such as learning and memory, may persist for a relatively long time following the 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 (3,4,10). The chronic exposure to OP compounds can also result in specific long-term cognitive deficits even when signs and symptoms of excessive cholinergic activity are not present (14,15). Recently, the ability of a nerve agent sarin to cause subtle long-term neurobehavioral and neurophysiological effects in rats exposed to its low level without a significant inhibition of AChE activity and a clinically manifested alteration of cholinergic nervous system has been described (5).

Our data clearly demonstrate that sarin is also able to induce dose-dependent alteration of cognitive functions in the case of the inhalation exposure of rats to its low concentrations. The adverse effect of low-level sarin inhalation exposure was manifested in the time determining rate of orientation (latency time). Therefore, 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 (17,20). In addition, a current study attempts to show a temporal rela-

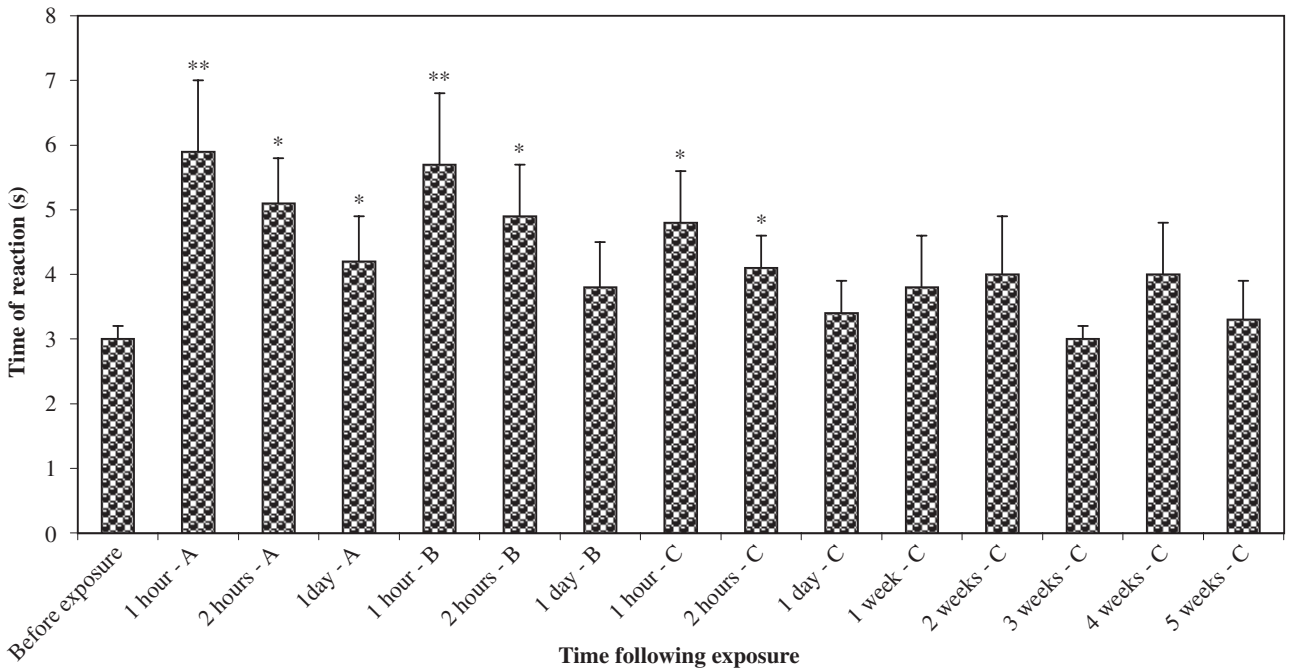


**Fig. 2:** The alteration of the T-maze performance in rats repeatedly exposed (A – the first exposure, B – the second exposure, C – the third exposure) to LEVEL 2 of sarin. Statistical significance - see Figure 1.

tionship 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 caused by asymptomatic exposure to an OP compound (18).

Although these findings are difficult to extrapolate directly to human low-level exposures to OPs, they indicate that short cognitive impairments without clinically manifested disturbance of central cholinergic nervous system could occur in humans following the inhalation exposure to clinically asymptomatic concentrations of sarin.

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***Doc. MUDr. Jiří Kassa, CSc.,***
***Purkyně Military Medical Academy,***
***P.O. Box 35/T, 500 01 Hradec Králové,***
***Czech Republic.***
***e-mail: kassa@pmfhk.cz***