Pharmacology, Biochemistry and Behavior 91 (2009) 517–525

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Behavioral evaluation of rats following low-level inhalation exposure to sarin ☆

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ARTICLE INFO

Article history:
Received 26 June 2008
Received in revised form 8 September 2008
Accepted 11 September 2008
Available online 18 September 2008

Keywords:
Nerve agent
Spatial memory
Operant behavior
Radial maze
Cognitive deficit

ABSTRACT

We evaluated the effects, in rats, of single and multiple low-level inhalation exposures to sarin. Rats were trained on a variable-interval, 56 s (VI56) schedule of food reinforcement and then exposed to sarin vapor (1.7–4.0 mg/m^3×60 min) or air control. The exposures did not produce clinical signs of toxicity other than miosis. Subsequently, performance on the VI56 and acquisition of a radial-arm maze spatial memory task was evaluated over approximately 11 weeks. Single exposures did not affect performance on the VI56 and had little effect on acquisition of the radial-arm maze task. Multiple exposures (4.0 mg/m^3×60 min/day×3) disrupted performance on the VI56 schedule during the initial post-exposure sessions. The disruption, however, resolved after several days. Multiple exposures also produced a deficit on the radial-arm maze task in that sarin-exposed rats tended to take it longer to complete the maze and to make more errors. The deficit, however, resolved during the first three weeks of acquisition. These results demonstrate that in rats, inhalation exposure to sarin at levels below those causing overt signs of clinical toxicity can produce cognitive and performance deficits. Furthermore, the observed deficits do not appear to be persistent.

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The near-term effects of high-dose exposure to sarin, an organophosphorus chemical warfare agent (CWA), are relatively well documented (e.g., Somani, 1992). Sarin rapidly binds to acetylcholinesterase (AChE) and the resulting increase in cholinergic activity is generally believed to be the mechanism causing a cascade of detrimental effects. The clinical sequelae following sufficient exposure to CWA can include convulsions, salivation, nystagmus, tremors, muscle fasciculation, confusion and anxiety (Holstege et al., 1997). Near- and far-term effects of low-level exposure to CWA have not been investigated to the same extent, but have recently been of much interest (e.g., Somani and Romano, 2001). An integral aspect of the interest in low-level exposure to CWA is the possibility that an exposure that does not produce immediate and overt clinical signs of toxicity could subsequently produce performance and cognitive deficits at a later time. Additionally, it is of interest to determine the detrimental liability, with regard to recovery, from exposures that initially produce relatively mild signs of toxicity (e.g. non-convulsive doses).

A significant amount of data concerning the persistence of CWA-induced cognitive and performance deficits has come from the study of survivors of the sarin attacks in the subways of Matsumoto and Tokyo in 1994 and 1995, respectively. For example, high-dose sarin exposure, producing severe signs of toxicity including convulsions, was associated with impaired short-term memory within the days following exposure and was present when tested six months following the incident (Hatta et al., 1996). Yokoyama et al. (1998) report the persistence of neurobehavioral deficits in victims when tested 6–8 months following exposure. Nishiwaki et al. (2001) presents data suggesting a decline in memory function in victims tested as long as three years or later after exposure.

While many studies have investigated the near-term effects of high-dose exposures to sarin, fewer laboratory studies have focused on the effects of low-level exposures. In this regard, a low-level exposure is generally considered to be a level below that causing overt signs of clinical toxicity such as convulsions. Kassa et al. (2001a) found that the immune function and gait of rats was affected three months following repeated asymptomatic concentrations or single non-convulsive symptomatic concentrations of sarin. Henderson et al. (2001) found that repeated inhalation exposure to asymptomatic levels of sarin had little effect on motor activity or temperature regulation in rats, but suppressed immune response and altered cholinergic receptor subtype (M1) populations, when evaluated 30 days later. Several studies have specifically investigated the behavioral effects of low-level exposure to sarin. Marmosets (Pearce et al., 1999) were administered a single dose of sarin and evaluated for changes in EEG and cognitive functions using a complex visual discrimination test. No significant changes were found during the course of up to 15 months of post-exposure testing. Kassa et al. (2001b) evaluated the effects of single inhalation exposures to sarin that produced some signs of toxicity but not convulsions, and repeated (3 times) asymptomatic

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0091-3057/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.pbb.2008.09.006

inhalation exposures to sarin. Using performance on a previously learned, aversively motivated, Y-maze escape task, they found that rats showed an increase in escape latency for three weeks following exposure. In contrast, the same levels of sarin exposure did not affect performance on an appetitively-motivated T-maze task during testing that occurred 1–5 weeks after exposure (Kassa et al., 2001c). Using a functional observation battery with rats, Kassa et al. (2001d) found that, single symptomatic non-convulsive, and repeated (3 times) asymptomatic, inhalation exposures to sarin produce changes in several behavioral measures (e.g. gait, mobility, stereotypy, grip strength) when tested three and six months following exposure.

We further investigated the behavioral effects of low-level sarin inhalation exposure in rats. We chose to evaluate inhalation exposures of 1.7–4.0 mg/m^3×60 min. The lowest level was chosen because, it is above the smallest concentration we have found in previous studies to reliably produce temporary miosis, most likely as a local effect of the vapors, but not found to produce other clinical signs of toxicity. The highest level was chosen because, while not necessarily asymptomatic, it was substantially below the LC50 of 7.75 mg/m^3 established earlier for a 60 min exposure in male Sprague–Dawley rats (Mioduszewski et al., 2002). Thus, the exposure range used corresponded to 0.23–0.53 LC50. In addition to single exposures, we also evaluated multiple (one exposure per day for three consecutive days) exposures at the highest concentration.

We were particularly interested in evaluating behavior of a previously learned task, as has been the procedure of several other studies (e.g., Pearce et al., 1999; Kassa et al., 2001c), but also in evaluating the post-exposure acquisition and maintenance of a cognitive task. Thus, rats were trained on an appetitively-motivated operant conditioning task (VI56 schedule of reinforcement) before sarin exposures. Operant schedules, such as a VI56 have been previously shown to be sensitive to disruption by a variety of pharmacological agents, including cholinergic compounds (e.g., Genovese, 1990; Genovese and Doctor, 1997; Genovese et al., 1996, 1990). Following exposure, testing on the VI56 task was continued and the same subjects acquired performance on a radial-arm maze task. The radial-arm maze task is an established spatial memory task that has also been used to evaluate a variety of pharmacological agents, including cholinesterase inhibitors (see Olton, 1987; Walsh and Chrobak, 1987). In order to maximize the detection of short-term and moderately long-term effects, performance was evaluated during 55 sessions on the VI56 and on the radial-arm maze during approximately 11 weeks following exposure.

1. Methods

1.1. Animals

This study was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. All procedures were reviewed and approved by the Institutes' Animal Care and Use Committees, and performed in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Adult male Sprague–Dawley rats (Charles River, Wilmington, MA) were used. Rats were individually housed in a temperature-controlled environment under a 12L: 12D cycle (lights on at 06:00 h) and water was always available in the home cages. Body weights were maintained at approximately 320 g by food administered during experimental sessions and supplemental feedings (PMI Nutrition International, St. Louis, MO) occurring several hours after experimental sessions.

1.2. Behavioral apparatuses

Schedule-controlled behavior sessions were conducted in ten standard rodent operant conditioning chambers (model # E-10-10 or equivalent, Coulbourn Instruments, Lehigh Valley, PA), housed in ventilated, light- and sound-attenuating cubicles. Each chamber contained two response levers and a food trough that could be illuminated and was attached to a food dispenser capable of delivering 45 mg food pellets (Bio-Serv, Frenchtown, NJ). Each chamber also contained a houselight mounted near the top of the front panel and stimulus lights mounted above each of the response levers. A response was considered to occur when either lever was pressed with a downward force of at least 0.3 N. Experimental events were controlled and monitored by a microcomputer using the Med-PC (Med Associates, St. Albans, VT) hardware and software control system. Data files generated by the Med-PC software were subsequently analyzed with custom programs.

Radial-arm maze sessions were conducted using an eight-arm commercially available radial maze (Coulbourn Instruments, Allentown, PA) measuring 137.2 cm in diameter. The center of the maze was a plastic octagon hub measuring 26.67 cm across, with a Plexiglas lid and wire grid floor. A Plexiglas arm (37 cm (*L*), 7.6 cm (*W*), 12.7 cm (*H*)) with a wire mesh floor was attached to each of the eight sides of the hub. The entrance to each arm contained a motorized guillotine door allowing access to and from the hub. Each arm's runway contained two floor-mounted switches (approximately 8 cm and 29 cm from the hub) which were depressed by the weight of the rat when present in the proximal and distal portion of the runway, respectively. The terminal portion of each arm contained a food trough outfitted with a photo-emitter/detector unit which could detect access by the rats. The terminal portion also contained a pellet dispenser for delivering 45 mg food pellets (Bioserv, Frenchtown, NJ). Experiments were controlled and monitored using a hardware interface (Coulbourn Instruments, Model L91-16S) and a microcomputer using the L2T2S software control system (Coulbourn Instruments). Data files generated by the L2T2S software were subsequently analyzed with custom programs.

1.3. Behavioral procedures

Rats were first trained on a VI56 schedule of food reinforcement. Following training, they were exposed, via whole body inhalation, to either air or sarin vapor. After exposure, sessions on the operant schedule task continued, and acquisition of a radial-arm maze task began. During the post-exposure evaluation days, when both types of sessions were conducted, maze sessions were run first and were generally separated from the VI56 session by at least 30 min.

All rats were initially trained to lever press for food pellets under a continuous schedule of reinforcement. Although two levers were present in each chamber, only one lever produced food reinforcement. During this condition, a single response on the active lever produced delivery of a food pellet. An equal number of boxes were designated with the active lever on the left and the right. When lever pressing was maintained by food presentation, all rats were trained to lever press under a variable-interval, 56-second, schedule of food reinforcement (VI 56). The schedule specifies that the first lever press, following an interval that averages 56 s, will produce food reinforcement (i.e., a single food pellet). Interval values for the schedule are chosen pseudorandomly, without replacement, from normal distributions generated using the procedure of Fleshler and Hoffman (1962). The range of interval durations was 2.44–198.23 s. The houselight and the stimulus lights above both levers were illuminated during the session. Sessions were 30 min in duration and were conducted at approximately the same time, Monday–Friday.

When responding under the schedule of reinforcement was stable (as judged by inspection of the daily response rates and cumulative response records), rats were assigned to a treatment group. In all cases, at least 60 training sessions were conducted before responding was judged to be stable. Groups were balanced on the basis of rate of responding. Rats were then transported to a different facility for sarin or air control exposure. Following exposures, 55 sessions with the VI56 task and, on the same days, 55 sessions with the radial-arm maze task were conducted.

For the radial-arm maze task, rats learned to navigate an eight-arm maze that contained four baited arms. That is, a single food pellet was available upon a nose-poke into the food trough at the terminal portion of four arms. Each rat was randomly assigned a maze configuration of four baited arms from 37 possible configurations that excluded more than two consecutive baited arms. Thus, the same configuration of baited arms was used for a particular rat for each of the sessions, but different configurations could be used for different rats. A session began by placing the rat in the center hub compartment and raising the doors to the eight arms a few seconds later. The rat was then free to explore the maze to obtain the food rewards available from the four baited arms. An arm was considered to be chosen when a nose-poke into the food trough at the terminal portion of the arm was detected. In order to avoid inaccurately counting multiple nose-pokes as arm entries, subsequent entries into an arm were not recorded unless the rat was detected to have exited the current arm (by a switch closure for the arm area near the hub of the maze) and at least 5 s had elapsed from the previous nose-poke. The session was terminated when a rat had obtained all four food rewards or 15 min had elapsed. If a rat did not complete the maze within 15 min, a completion time of 15 min was assigned and errors were not analyzed. Failures to complete the maze, however, were infrequent and only occurred in some rats, and only during the initial few sessions on the maze. No familiarity training with the maze was conducted prior to the first session.

1.4. Inhalation exposure procedures

Isopropyl methylphosphonofluoridate (sarin or GB) was used for all vapor exposures in this study. Chemical agent standard analytical reagent material (CASARM)-grade sarin (lot # GB-U-6814-CTF-N (GB2035)) was verified as 98.3±0.48 wt.% pure as determined by quantitative NMR 31P (Brickhouse et al., 1997) and stored in sealed ampoules containing nitrogen. Ampoules were opened as needed to prepare external standards or to be used as neat agent for vapor dissemination. All external standards for sarin vapor quantification were prepared on a daily basis. Triethylphosphate (99.9% purity) (Aldrich Chemicals, Milwaukee, WI) was used as the internal standard for the sarin purity assay.

Rat whole body inhalation exposures to sarin vapor were conducted by the Operational Toxicology Team, Research and Technology Directorate at the US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, Maryland. Whole body exposures were conducted in a 750 L dynamic airflow chamber. The Rochester style chamber was constructed of stainless steel with Plexiglas windows on each of its six sides. The interior of the exposure chamber was maintained under negative pressure (0.635 mm H_2O), which was monitored with a calibrated magnehelix (Dwyer, Michigan City, IN). A thermoanemometer (Model 8565, Alnor, Skokie, IL) was used to monitor chamber airflow at the chamber outlet. Other physical parameters monitored during exposure included chamber room temperature and relative humidity.

Test rats were exposed to a fixed concentration of sarin vapor while control rats were exposed to air-only in a separate "clean" chamber identical in construction to the agent chamber. Both test and control rats were exposed for a 60 min duration in stainless steel compartmentalized cages (50.8 cm w×35.6 l×10.2 h) with each rat in a separate compartment. For the multiple exposure treatment, rats were exposed three times (sarin or air control), one exposure per day for three consecutive days, using the same general procedures as with single exposure treatment.

Sarin vapor was generated by two methods, depending upon the concentration required. For vapor concentrations >2 mg/m^3, the vapor generation system consisted of a gas-tight syringe (Hamilton, Reno, NV), variable-rate syringe drive (Model 22, Harvard Apparatus Inc., South Natick, MA), and other vaporization equipment (Anthony et al., 2004; Mioduszewski et al., 2002). For vapor concentrations <2 mg/m^3 saturated sarin vapor streams were generated by directing nitrogen carrier gas through the inlet of a glass vessel containing liquid sarin. The glass vessel (saturator cell) consisted of a 100-mm long, 25-mm o.d. cylindrical glass tube with two vertical 7-mm o.d. tubes connected at each end (inlet and outlet tubes). The cylindrical tube of the saturator cell contained a hollow ceramic cylinder that served to increase the contact area between the liquid sarin and the nitrogen. The saturator cell was fabricated to allow nitrogen to make three passes along the surface of the wetted ceramic cylinder before exiting the outlet arm of the saturator cell. The saturator cell body was immersed in a constant temperature bath so that a combination of nitrogen flow and temperature could regulate the amount of sarin vapor going into the inhalation chamber. The entire apparatus was contained within a stainless steel box mounted at the top of the inhalation chamber.

Two sampling methods were used to monitor and analyze the sarin vapor concentration in the exposure chamber. The first method was a quantitative technique using solid sorbent tubes (Tenax/Haysep) to trap sarin vapor, followed by thermal desorption and gas chromatographic (GC) analysis (HP Model 6890, Agilent Technology, Baltimore, MD). The second method was a continuous monitoring technique using a phosphorus monitor (HYFED, Model PH262, Columbia Scientific, Austin, TX) (Anthony et al., 2004; Mioduszewski et al., 2002).

1.5. Blood assay

Blood was sampled 24 h before and 30 min after the end of single sarin exposures. Samples were collected from the tail vein into glass tubes containing ethylene diamino tetraacetic acid (EDTA). Assays of red blood cell (RBC) acetylcholinesterase (AChE), and plasma butyrylcholinesterase (BChE) and carboxylesterase (CaE) were conducted using a modification of the Ellman reference method (Ellman et al., 1961) and procedures described by Chandra et al. (1997). Plasma and RBC fractions were also assayed for sarin using mass a fluoride regeneration procedure and spectrometry (GC/MS) (see Jakubowski et al., 2004 for procedure details). This procedure quantifies reactivated sarin in blood and has been used previously to evaluate CWA exposures (Adams et al., 2004; Jakubowski et al., 2004, van der Schans et al., 2004).

1.6. Data analysis

When a response (i.e., lever press) occurred during the VI56, the elapsed time within the session was recorded. From these data, the total number of responses and the rate of responding (responses per min) were calculated for each rat for the "active" lever (i.e., the lever producing food reward) and the inactive lever. Once performance on the VI56 stabilized, responding on the inactive lever was always very slow, made up a small proportion of the total responses (typically less than one%), and did not change systematically throughout the experiment. Therefore, these data were not analyzed further. Response rates on the active lever from the 10 sessions before inhalation exposure (baseline control) were averaged and response rate data from subsequent sessions were converted to a percentage of the average values obtained during baseline sessions, for each rat (i.e., percent of control). Fifty-five sessions on the VI56 task were conducted with rats following exposure. Response rate measures from these sessions were averaged into eleven blocks of five consecutive sessions for analysis. Since performance on the VI56 schedule was established prior to inhalation exposure, the first five post-exposure sessions were analyzed separately to maximize the detection of short-term, exposure-induced, performance changes.

For the radial-arm maze task, completion time and the number of errors were collected for each session. Completion time was defined as

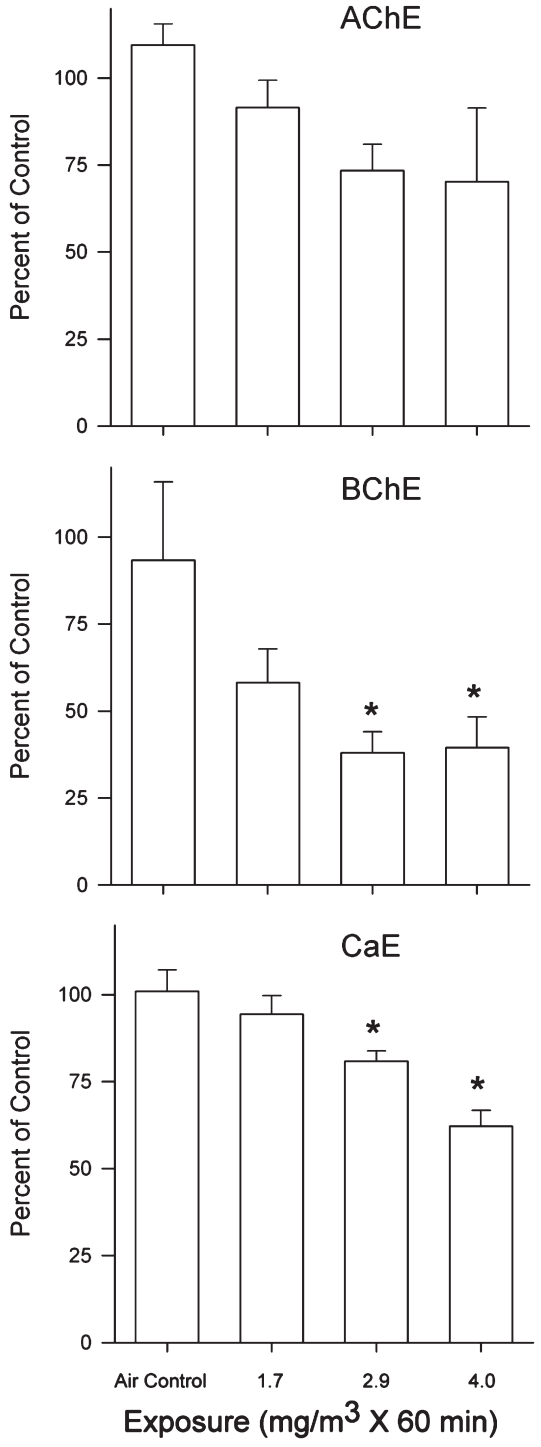

Fig. 1. Inhibition of acetylcholinesterase (top), butyrylcholinesterase (middle) and carboxylesterase (bottom) activity, as a percentage of baseline values, in rats after inhalation exposure to sarin or air control. Each bar represents the mean (±SEM) from six rats. Blood was sampled 24 h before exposure (baseline) and approximately 30 min after the end of the exposure. Asterisks denote values significantly different than air control (Dunnett's *t*, *ps*<.05).

the elapsed time between the start of the session and a nose-poke into the fourth baited arm. Two types of errors were measured. Reference memory errors occurred whenever a rat chose an arm that was not one of the baited arms. Working memory errors occurred whenever a rat chose a baited arm that had already been chosen during that session. Fifty-five sessions on the radial-arm maze task were conducted with rats following exposure. Dependent measures from these sessions were averaged into eleven blocks of five consecutive sessions for analysis.

Inferential statistics were calculated using the SAS (Cary, NC) statistical software package. Between-group effects were assessed with ANOVA. Multiple contrasts comparing sarin-exposed groups with air control groups were evaluated with two-tailed Dunnett's *t*-tests. For within-group effects, tests of sphericity were first made whenever sufficient degrees of freedom existed. In all cases when this was done, sphericity assumptions could not be accepted and a MANOVA was used. When degrees of freedom did not allow for tests of sphericity and multivariate analyses, repeated measures ANOVA was used with probability values corrected using Greenhouse–Geisser Epsilon. The criterion for statistical significance was set at $p<.05$.

2. Results

2.1. Single sarin exposures

All rats were observed during the exposure and within 30 min following inhalation exposures for the presence of the following signs of organophosphorus toxicity: miosis, ataxia, tremors, subconvulsive jerks, convulsions, salivation, straub tail, exophthalmos, gasping, collapse and prostration. Of these signs, only miosis was observed and

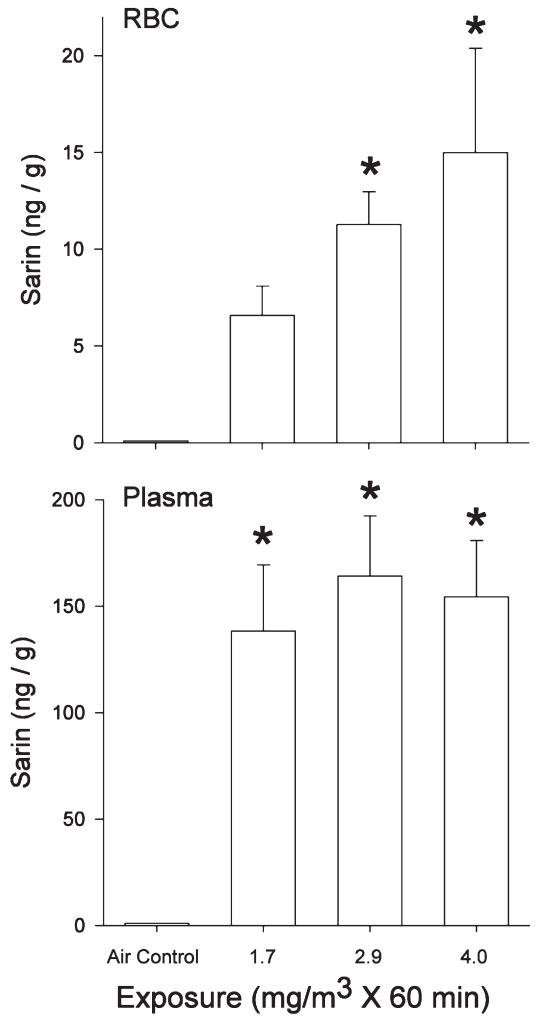

Fig. 2. Regenerated sarin in RBC (top) and plasma (bottom) in rats after inhalation exposure to sarin or air control. Each bar represents the mean (±SEM) from six rats. Blood was sampled at approximately 30 min after the end of the exposure. Asterisks denote values significantly different than air control (Dunnett's *t*, *ps*<.05).

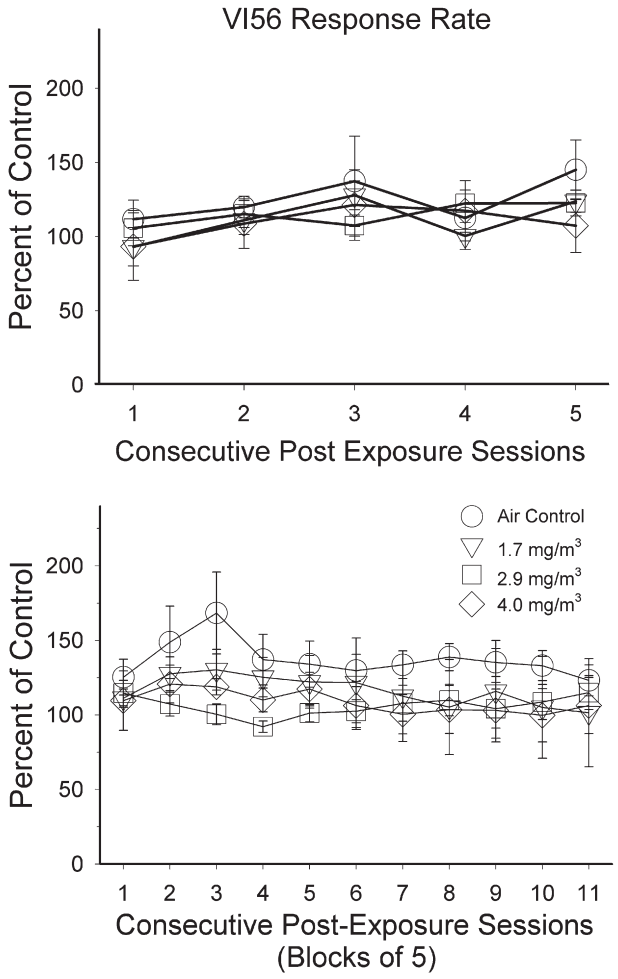

Fig. 3. Average rates of responding under the VI56 sec schedule of reinforcement in rats during the first five consecutive sessions (top) and during 55 consecutive sessions (in blocks of 5 consecutive sessions) (bottom) following a single inhalation exposure to sarin or air control. Response rate is expressed as a percentage of the average response rate during the 10 sessions preceding exposure (i.e., baseline). Post-exposure sessions began approximately 48 h after exposure. Each point represents the mean (±SEM) from six rats.

only in sarin-exposed rats. Although the time-course was not specifically quantified, miosis resolved within 48 h. All rats were maintained at target body weight level (320 g) with normal food supplementation procedures.

Single exposures to sarin (1.7 mg/m^3–4.0 mg/m^3×60 min) produced dose-dependent inhibition of AChE, BChE and CaE activity (see Fig. 1). Inhibition was greatest for BChE and was greater than 50% at the higher doses. AChE inhibition was comparatively moderate with the greatest reduction in activity at approximately 25%, relative to pre-exposure values. CaE activity was also decreased by sarin. When compared to air control, the difference in BChE and CaE activity following 2.9 mg/m^3 and 4.0 mg/m^3 treatments was statistically significant ($p<.05$).

Single exposures to sarin also produced dose-dependent regenerated sarin in blood (Fig. 2). In this respect, approximately ten-fold more sarin was regenerated in plasma than in RBC. When compared to air control, the difference in regenerated sarin was significant in RBC following 2.9 mg/m^3 and 4.0 mg/m^3 (Fig. 2, top) and in plasma for all three exposure levels (Fig. 2, bottom) ($ps<.05$).

Performance maintained by the VI56 schedule was acquired by all rats and produced relatively stable responding. Baseline measures of responding on the active lever, defined as the average of the last 10 sessions conducted before exposure, for the four groups ($n=6$ each) were as follows (mean±SEM responses per minute): Air Control=48.23±19.0, 1.7 mg/m^3=45.3±13.1, 2.9 mg/m^3=61.2±28.7, 4.0 mg/m^3=61.0±12.9. Single exposures to sarin did not affect responding during the first five sessions, beginning approximately 48 h following exposure (Fig. 3, top). ANOVA revealed no significant main effect for dose ($F(3, 20)=0.75$, $p>.05$) and none of the contrasts comparing exposed rats to air controls were significant ($ps>.05$). Further, MANOVA did not reveal a significant effect for consecutive sessions ($F(4, 17)=2.75$, $p>.05$) or for the consecutive sessions by dose interaction ($F(12, 45)=1.28$, $p>.05$).

Although some differences from baseline were observed, sarin exposures did not, in general, affect responding on the VI56 during 55 post-exposure sessions (Fig. 3, bottom). ANOVA revealed no significant effect for dose ($F(3, 20)=1.56$, $p>.05$). Multiple contrasts comparing each of the sarin-exposed groups to the air control, however, showed a difference for the 2.9 mg/m^3 treatment condition at blocks 3 and 4 ($ps<.05$) only. MANOVA showed no significant effect for consecutive blocks of sessions ($F(10, 11)=2.75$, $p>.05$) or for the dose by sessions interaction ($F(30, 33)=0.150$, $p>.05$).

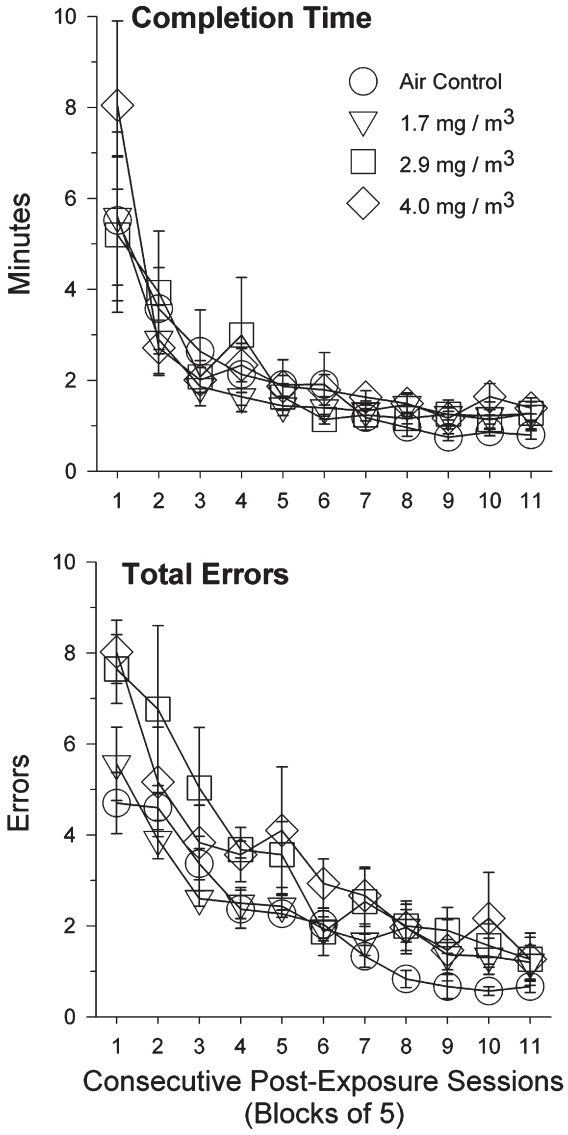

Fig. 4. Average completion times (top) and errors (bottom) on the radial-arm maze task in rats during 55 sessions (in blocks of 5 consecutive sessions) following a single inhalation exposure to sarin or air control. Sessions began approximately 48 h after exposure and rats had no previous experience with the maze. Each point represents the mean (±SEM) from six rats.

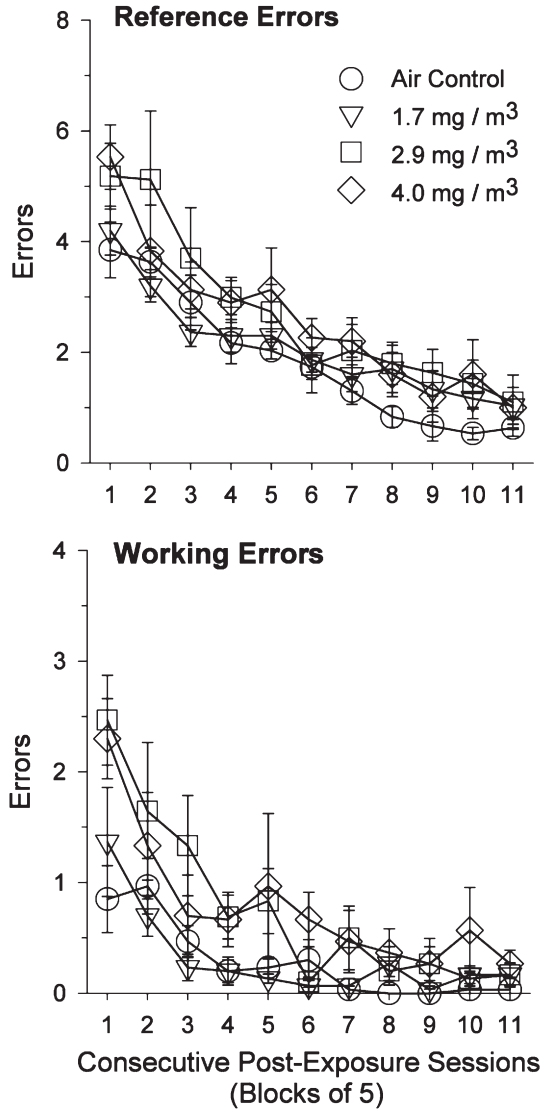

Fig. 5. Average number of reference memory errors (top) and working memory errors (bottom) on the radial-arm maze task in rats during 55 sessions (in blocks of 5 consecutive sessions) following a single inhalation exposure to sarin or air control. Sessions began approximately 48 h after exposure and rats had no previous experience with the maze. Each point represents the mean (±SEM) from six rats.

Sessions on the radial-arm maze task began approximately 48 h following exposure. The amount of time taken to complete the task (i.e., obtain all four food pellets from the baited arms) decreased systematically as a function of consecutive sessions (Fig. 4, top). That is, with experience, all rats learned to navigate the maze relatively quickly. After the fourth training block had occurred (i.e., 20 sessions) the average time to complete the maze was less than 2 min for all groups. MANOVA revealed a significant effect for consecutive sessions ($F(10,11)=3.19$, $p<.05$). In this regard, however, no significant differences were observed between the different treatment groups as evidenced by a lack of significant main effects for dose (ANOVA, $F(3,20)=0.24$, $p>.05$) and by the lack of a significant sessions by dose interaction (MANOVA, $F(30,33)=1.05$, $p>.05$). Further, none of the multiple contrasts comparing each of the sarin-exposed treatment groups with the air control were statistically significant ($ps>.05$).

Fig. 4 (bottom) shows the number of total errors (working memory errors + reference memory errors) during the 11 blocks (55 sessions) of radial-arm maze sessions. All rats learned the maze as evidenced by a systematic decline in the number of errors made as a function of consecutive sessions. During the last block of testing (post-exposure sessions 51–55) rats in all treatment groups averaged less than two errors per session. In this regard, MANOVA showed a significant effect for consecutive blocks ($F(10,11)=37.94$, $p<.01$). In general, treatment groups showed very little difference in the rate or degree of errors made on the maze task. ANOVA revealed no significant difference for dose ($F(3,30)=2.48$, $p>.05$) and MANOVA revealed no significant blocks by dose interaction ($F(30,33)=1.29$, $p>.05$). Multiple contrasts comparing each sarin-exposed group to air control, at each block, however, showed a significant difference for the 2.9 mg/m^3 and 4.0 mg/m^3 groups, but only at block one ($p<.05$).

Reference memory errors and working memory errors during the 11 blocks of sessions following exposure are shown in Fig. 5. On average, rats made more reference memory errors (Fig. 5, top) than working memory errors (Fig. 5, bottom). Both types of errors decreased as a function of experience on the maze. MANOVA revealed a significant effect for consecutive blocks of session for both reference memory errors ($F(10,11)=31.35$, $p<.01$) and working memory errors ($F(10,11)=11.28$, $p<.01$). For reference memory errors, no significant difference for main effect of dose (ANOVA, $F(3, 20)=1.68$, $p>.05$) or significant session block by dose interaction (MANOVA, $F(30,33)=.96$,

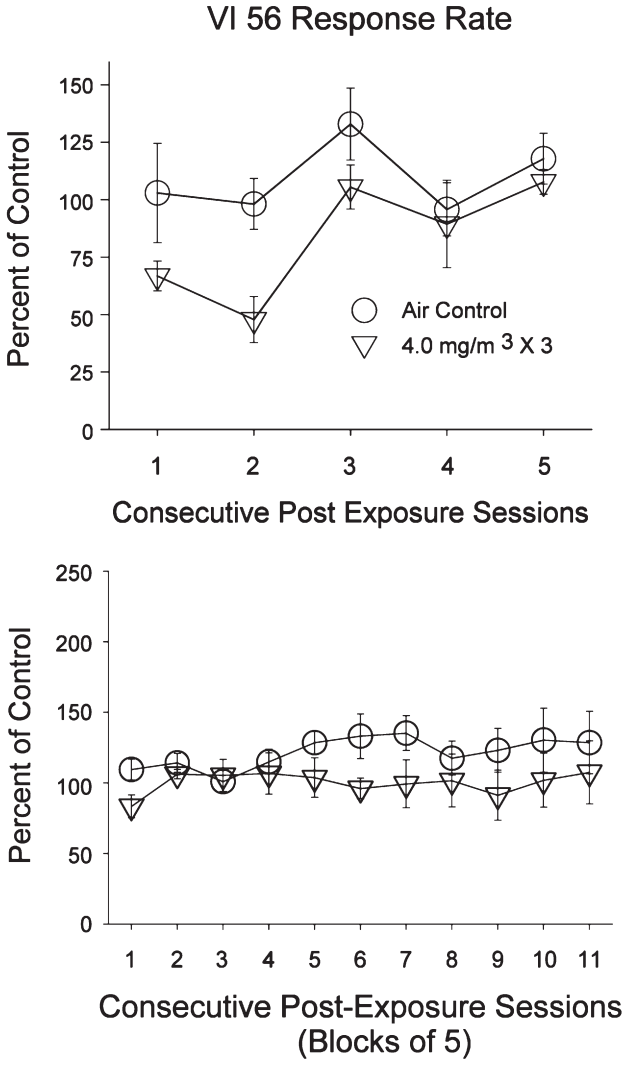

Fig. 6. Average rates of responding under the VI56 sec schedule of reinforcement in rats during the first five consecutive sessions (top) and during 55 consecutive sessions (in blocks of 5 consecutive sessions) (bottom) following three inhalation exposures to sarin or air control. Response rate is expressed as a percentage of the average response rate during the 10 sessions preceding exposure (i.e., baseline). Post-exposure sessions began approximately 48 h after the last exposure. Each point represents the mean (±SEM) from five rats.

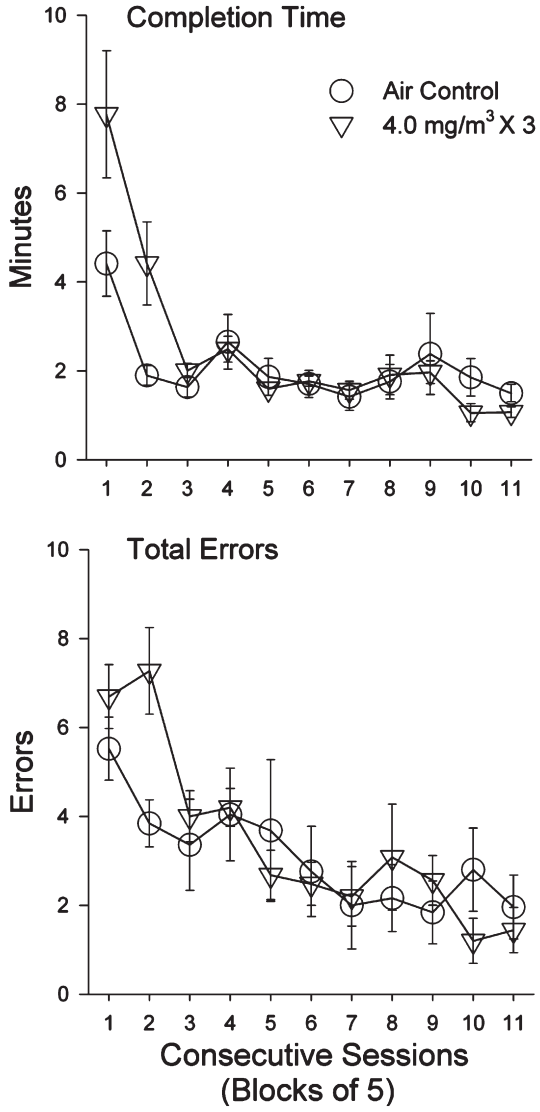

Fig. 7. Average completion times (top) and errors (bottom) on the radial-arm maze task in rats during 55 sessions (in blocks of 5 consecutive sessions) following three inhalation exposures to sarin or air control. Sessions began approximately 48 h after the last exposure and rats had no previous experience with the maze. Each point represents the mean (±SEM) from five rats.

$p>.05$) was found, showing that the treatment groups did not differ in this regard. Contrasts comparing treatment groups with air control at each of the 11 blocks of sessions, however, showed a significant difference for the 4.0 mg/m^3 treatment for block one only ($p<.05$). For working memory errors, the differences for main effect of dose approached significance (ANOVA, $F(3, 20)=2.98$, $p<.06$) as did the session block by dose interaction (MANOVA, $F(30,33)=1.70$, $p<.07$), suggesting a difference in the treatment groups. Contrasts comparing treatment groups with air control at each of the 11 blocks of sessions showed a significant difference for the 2.9 mg/m^3 treatment for block one only.

2.2. Multiple sarin exposures

Rats were observed within 30 min following each of the inhalation exposures for the presence of signs of organophosphorus toxicity as described for the single sarin exposures. As in the single exposures, temporary miosis was observed in sarin-exposed rats. All rats were maintained at the target body weight level (320 g) with normal food restriction procedures.

As with rats in the single inhalation exposure experiments, the VI56 schedule produced relatively stable responding and baseline measures of responding on the active lever, defined as the average of the last 10 sessions conducted before exposure, for the two groups ($n=5$ each) were as follows (mean±SEM responses per minute): Air Control=47.01±25.2, 4.0 mg/m^3×3=40.89±23.10. VI56 sessions resumed approximately 48 h after the third exposure to sarin or air control. Sarin decreased response rate, relative to pre-exposure baseline, during initial post-exposure sessions, while the air control treatment had no effect (Fig. 6, top). Analysis of the first five VI56 sessions following exposure revealed a significant difference between treatment groups ($F(1,8)=5.75$, $p<.05$) and multiple contrast tests revealed a significant difference between the two groups for session two ($p<.05$) . MANOVA revealed a significant difference for consecutive sessions ($F(4,5)=5.55$, $p<.05$) but not a significant session by treatment interaction ($F(4,5)=2.49$, $p>.05$).

Response rates were relatively unaffected by sarin during the subsequent 55 sessions. Analysis of the 11 blocks (of 5 sessions each) following exposure (Fig. 6, bottom) revealed no significant main

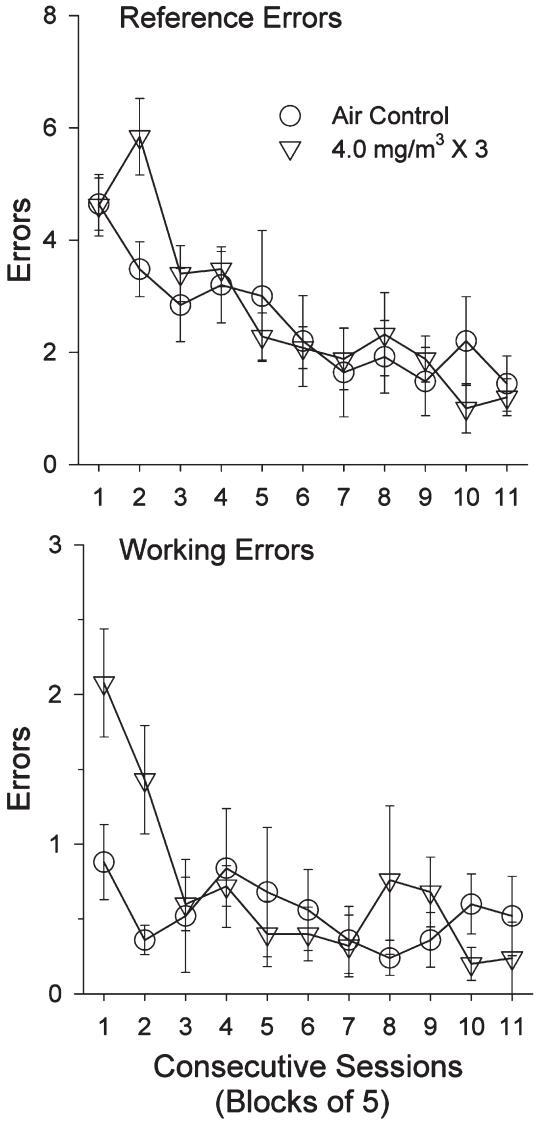

Fig. 8. Average number of reference memory errors (top) and working memory errors (bottom) on the radial-arm maze task in rats during 55 sessions (in blocks of 5 consecutive sessions) following three inhalation exposures to sarin or air control. Sessions began approximately 48 h after the last exposure and rats had no previous experience with the maze. Each point represents the mean (±SEM) from five rats.

effects between sarin-treated and air control-treated groups ($F(1,8)=1.73$, $p>.05$). Multiple contrasts, however, showed a significant difference between groups at block one ($p<.05$). Univariate repeated measures ANOVA showed no significant main effect for sessions ($F(10,80)=1.03$, $p>.05$) or the treatment by sessions interaction ($F(10,80)=0.97$, $p>.05$).

Sessions on the radial-arm maze task began approximately 48 h following the last inhalation exposure to sarin or air control. The amount of time taken to complete the maze task decreased systematically as a function of consecutive sessions (Fig. 7, top). As in the single exposure experiment, all rats learned to complete the maze in a relatively short time and ANOVA revealed a significant effect for consecutive sessions ($F(10,80)=25.63$, $p<.01$). Sarin-treated rats tended to take longer to complete the maze during the initial 10 sessions (blocks one and two). Although no significant effect for treatment was observed (ANOVA, $F(1,8)=0.58$, $p>.05$), a significant treatment by session interaction was seen (ANOVA, $F(10,80)=5.58$, $p<.01$). Additionally, multiple contrasts showed a significant difference between groups at block two (sessions 6–10) ($p<.05$).

Fig. 7 (bottom) shows the number of total errors during the 11 blocks (55 sessions) of radial-arm maze sessions for both groups. As in the single sarin exposure experiment, all rats learned the maze task as evidenced by a systematic decline in the number of errors made as a function of consecutive sessions. During the last block of testing (post-exposure sessions 51–55), rats in both groups averaged two or fewer errors per session. In this regard, ANOVA showed a significant effect for consecutive blocks ($F(10,80)=18.39$, $p<.01$). On average, the sarin-treated rats made more errors than the air control-treated rats during the initial 10 sessions (blocks one and two). Although no significant effect for treatment was observed (ANOVA, $F(1,8)=0.13$, $p>.05$), a significant treatment by session interaction was observed (ANOVA, $F(10,80)=3.73$, $p<.01$). Additionally, multiple contrasts showed a significant difference between groups at block two (sessions 6–10) ($p<.05$).

Reference memory errors and working memory errors during the 11 blocks of sessions following the last exposure are shown in Fig. 8. As in the single exposure experiments, on average, rats made more reference memory errors (Fig. 8, top) than working memory errors (Fig. 8, bottom). Both types of errors decreased as a function of experience on the maze. ANOVA revealed a significant effect for consecutive blocks of session for both reference memory errors ($F(10,80)=19.61$, $p<.01$) and working memory errors ($F(10,80)=6.83$, $p<.01$). Although no significant effect for treatment was observed for either reference memory errors ($F(1,8)=0.06$, $p>.05$) or working memory errors ($F(1,8)=0.33$, $p>.05$), sarin-treated rats tended to make more errors of both types, but only during the initial two blocks of sessions. The session by treatment interaction was significant for reference memory errors ($F(10,80)=2.85$, $p<.01$) and multiple contrasts comparing the two groups showed a significant difference at block two ($p<.05$). The session by treatment interaction for working memory errors was also found to be significant ($F(10,80)=4.73$, $p<.01$) and multiple contrasts revealed significant differences between groups at blocks one and two ($ps<.05$).

3. Discussion

Rats were exposed, via whole body inhalation, to low-level sarin vapor. All dosages were largely asymptomatic, producing temporary miosis as the only observable clinical effect. Single exposures (1.7–4.0 mg/m^3×60 min) produced dose-dependent decreases in blood esterase activity (AChE, BChE, CaE). Additionally, regenerated sarin (as an index of systemic dose) was detected in blood in a dose-dependent manner. On average, the largest single exposure produced a moderate degree of AChE inhibition (approximately 30%) and BChE and CaE were inhibited to a greater extent. Consistent with the latter finding is the detection of a greater amount of regenerated sarin in plasma (containing BChE and CaE) as compared to RBC (containing AChE) blood fractions. The largest single exposure dose was also administered daily for three consecutive days. The treatment, however, was also largely asymptomatic, producing only miosis as a clinical sign. Thus, the sarin exposure levels used in the present study are consistent with the general characterization of low-level doses.

Prior to sarin exposure, rats were trained on a VI56 schedule of food reinforcement. The schedule produced stable responding at a relatively constant rate throughout the sessions. Testing under the schedule resumed 48 h after exposures. None of the single exposures had any near-term effects on performance under the schedule as evidenced by a lack of a difference between performance during the first five post-exposure sessions and baseline. Performance of sarin-exposed rats during the first five sessions also did not differ from the air controls. The single sarin exposures did not have any long-term effects on performance under the VI56 as evidenced by no significant within-group effects for any of the single exposure treatments during the 55 post-exposure evaluation sessions. During two of the 11 blocks of testing, a small difference was observed between the 2.9 mg/m^3 sarin treatment and the air control. This effect, however, appears to be due to a small upward drift (relative to baseline) in response rate of the air control group rather than a deficit in the sarin-treated group.

Multiple sarin exposures (4.0 mg/m^3×3) clearly depressed responding under the VI56 during the initial post-exposure sessions. The deficit, however, completely resolved within the first week of testing and there was no evidence of the occurrence of a delayed deficit. That is, no differences, relative to baseline, or to performance of the air control group, were observed during blocks 2–11. It is notable that since testing resumed 48 h after exposure that a greater deficit may have been observed in these rats (and in the single exposure treatments) if testing began immediately after exposures.

All of the sarin-treated rats learned the radial-arm maze task to the same extent as air control-treated rats. That is, no differences existed on measures of accuracy (working and reference errors) or completion time between rats in the single exposure or multiple exposure sarin treatments and the respective air controls during the final weeks of testing. Thus, the levels of sarin exposure used in this study were insufficient to either prevent the acquisition of a spatial memory task or to degrade performance on the task once it was acquired. Multiple sarin exposures, and to a lesser extent, the larger single sarin exposures, however, did have some effects on performance on the radial-arm maze. As evidenced by statistically significant interactions of errors (total, reference memory and working memory) and sessions, and completion time and sessions, the acquisition functions for the multiple sarin exposure-treated rats and the air controls were not parallel. Further, comparisons between groups showed differences for all measures during the second (and first and second for working memory errors) week of testing. Therefore, rats in the multiple sarin exposure condition initially made more errors and took longer to complete the maze than the air control rats. In the case of single sarin exposures, only a few differences were observed between groups. In a general sense, however, the differences were similar to, but to a lesser extent, than those observed with the multiple exposures. That is, following 2.9 and 4.0 mg/m^3 sarin, rats tended to make more errors in the initial weeks of acquisition and the interaction of working memory errors and sessions approached significance.

In summary, we have demonstrated that low-level, generally asymptomatic, inhalation exposures to sarin can produce deficits in behavioral performance. It is notable that testing began 48 h after exposure and it is possible that greater performance deficits could have been observed if testing had been conducted during and immediately after the exposure. Under a VI56 schedule of food reinforcement, three daily exposures to 0.53 LC50 of sarin decreased responding for several sessions but otherwise did not affect performance over an approximately 11-week period of testing. The exposure also produced a deficit in the initial stages of acquisition of a radial-arm maze task. The deficit, however, was non-persistent, did not prevent acquisition of the task and did not affect performance on the task once it was acquired. No evidence for a delayed onset for the deficit was observed.

Our results are generally consistent with and extend those of Kassa et al. (2001b,c) that have reported short-term performance deficits using T-maze and Y-maze procedures in rats following low-level sarin exposure. Our results are also generally consistent with those obtained in our laboratory with cyclosarin (GF) and VX (Genovese et al., 2006; 2007). Since we found only short-term (i.e., non-persistent) performance deficits, our results are in contrast to other studies (Kassa et al., 2001a,d) that have demonstrated low-level sarin-induced deficits in gait, motor activity and other behavioral indices lasting three months and longer. If similar deficits occurred in the present study they were apparently not of a sufficient degree to affect long-term performance on the behavioral tasks used for assessment.

Acknowledgements

The authors thank Dr. E. Michael Jakubowski, Dr. Mary Lou Klotz, Angelika Larsen, Eva Derecskei, William Muse, James Manthei, Jacquelyn Scotto and David Burnett for assistance with the studies.

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