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Effect of Soman Exposure on the Acquisition of an Operant Alternation Task[1,2]

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MODROW, H. E. AND N. K. JAAX. *Effect of soman exposure on the acquisition of an operant alternation task.* PHARMACOL BIOCHEM BEHAV 32(1) 49–53, 1989.—Following recovery from soman administration, rats were trained on an operant alternation task with a time-out between response periods. As animals became proficient at the task, both the operant requirements and the length of time-out periods were gradually raised to Fixed Ratio 20 with a 20-second Intertrial Interval. Training sessions continued until animals attained criterion or 100 training sessions had been given. Soman produced a dose-related lethality and signs of cholinergic hyperstimulation. Although all saline controls and 90% of animals receiving 75 μg/kg soman attained terminal performance, only one-third of the animals given either 85 or 95 μg/kg soman were able to learn this task. Sessions to attain criterion performance produced similar dose-dependent results: All saline animals attained criterion, while only 60%, 33% and 33% of the animals given 75, 85 or 95 μg/kg soman respectively reached criterion. Additionally, both 85 and 95 μg/kg soman produced severe neural lesions, including cortical atrophy and ventricular dilation.

Soman Alternation task Rats Organophosphonates Operant behavior

ANIMALS exposed to various doses of the toxic organophosphonate compound soman have been shown to demonstrate neural axonal degeneration (11). Other research (6,8) has identified several sites of CNS neural damage. These sites include the hippocampus, neocortex, claustrum, entorhinal cortex, septal nucleus and thalamus (6). In Lemercier's study (6), all animals demonstrating soman-induced convulsions also exhibited damage to the hippocampus at necropsy. A majority of these animals also exhibited neural lesions in other areas of the limbic system. However, recent preliminary evidence indicates the soman-induced lesions are not identical to other convulsant-induced lesions. Rats given soman displayed more widespread and severe lesions than those administered the convulsant, metrazol, who displayed only minimal scatter necrosis (1).

Rats surviving a convulsion producing dose of soman display a wide variety of behavioral anomalies (7,12), many reminiscent of those produced by experimental lesions of the hippocampus or septal nucleus (2,14). For example, after recovery from a convulsion-producing dose of soman, rats display a hyperactivity to both tactile and auditory stimuli (9,12), similar to that seen after electrolytic lesions of either the hippocampus or septal nucleus (4). However, the sensitivity to auditory stimuli does not dissipate over time as commonly occurs after septal lesions (3).

Recent work by McDonough *et al.* (7) has shown that soman-exposed rats also experience difficulty in accurate performance when performing an operant task utilizing a differential reinforcement for low rates of responding (DRL). Although soman-exposed animals are capable of learning to respond to an operant lever, they are incapable of withholding responses as is required by the DRL task. This work suggests that the behavioral deficits seen in animals surviving a convulsion producing exposure to soman, may be due, in part, to damage to the limbic system, specifically the hippocampus and septal nucleus.

While this study was not specifically designed to test that hypothesis, it was designed such that behavioral deficits could be correlated to overall neural damage. In the present study, rats were required to alternate between two levers in an operant chamber. Completion of a FR20 on the correct

[1]The experiments reported here were conducted according to "The Guide for the Care and Use of Laboratory Animals" (1985), as prepared by the Committee on the Care and Use of Laboratory Animals, National Research Council, NIH Publication No. 80-23.

[2]The opinions or assertations contained herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

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lever resulted in delivery of a reinforcement following by a 20-second time-out period. When the lights came back on, the cue light over the other lever was illuminated. The rat was required to complete a FR20 schedule on this lever to receive an additional reinforcement. If the behavioral deficits seen in soman-treated rats were due, at least in part, to the diffuse limbic system damage observed at necropsy (8), then these rats would also be expected to demonstrate the same decrements. Further, the study was designed to determine whether a dose effect relationship could be quantified between the behavioral decrements and soman dose.

METHOD

Subjects

The subjects in this study consisted of 70 adult male Sprague-Dawley rats (Charles River Inc.) weighing between 300 and 350 g at the start of the study. All rats were individually housed in stainless steel rack cages with ad lib access to water. Prior to soman exposure, all animals were given ad lib access to food. During the study each rat was maintained at approximately 85% of its free-feeding body weight, using a restricted feeding schedule. The daily food ration was given approximately 2 hr after the experimental session each day. The animals were maintained on a 12 hr:12 hr light:dark schedule with light onset of 0600 each day in a temperature- and humidity-controlled room.

Apparatus

The equipment used in this study consisted of four identical BRS-LVE operant conditioning chambers. All chambers were located within individual sound-attenuating cubicles equipped with ventilation fans. Each operant chamber (26.7×30.5×24.1 cm) was equipped with a house light and a dipper mechanism for the delivery of 0.01 ml milk reinforcement (Eagle brand sweetened condensed milk diluted 1:2 v:v with water). An operant lever (4 cm above the grid floor) was located on each side of the food well. Directly above each lever was a cue light to indicate the correct lever. All response and reinforcement parameters were controlled by a PDP8e computer utilizing SCAT software.

Procedure

Prior to soman exposure all rats were trained to lever press for liquid milk reinforcement on a fixed ratio 1 (FR1) schedule using the method of successive approximation. During this initial training phase, a response on either lever resulted in reinforcement. Daily training continued until the rats were able to respond 100 times within a 30-minute period. Following this training the rats were divided into four groups and administered subcutaneously saline (10 rats), 75 μg/kg soman (14 rats), 85 μg/kg soman (21 rats) or 95 μg/kg soman (25 rats). One hour after soman administration all rats were observed for signs of cholinergic toxicity using a scale developed by Penetar (18). This scale rates animals from 0 (no signs) to 5 (severe signs) on six physiological and behavioral parameters; motor signs (fasciculations and seizures), secretory signs, lacrimation, eyeball protrusion, general state, and coordination.

All rats, including the saline control group, were then provided a period in which to recovery from the acute soman toxicity. During this time all animals were weighed daily. Animals showing a weight loss of greater than 25 g from the preinjection body weight were injected subcutaneously twice daily with 10 cc of Ringer's Lactate solution to treat the dehydration due to fluid loss during the acute poisoning stage. Additionally, all animals with greater than 5 g weight loss were provided with a wet mash solution consisting of ground rat chow and water. At the conclusion of the 14-day recovery period or at reattainment of the preinjection body weight, whichever occurred last, animals were reduced to approximately 85% of their postrecovery body weight and operant training was resumed.

Rats were retrained to lever press, if necessary, and then allowed to respond on a FR1 schedule using either lever until they received 100 reinforcements during a 30-minute session. When a rat began to respond adequately under the FR1 schedule (100 responses/session), alternation training began. Initially, rats were simply required to alternate between the two levers on a FR1 schedule with a 2-sec ITI. The correct lever was indicated by a lighted cue light above it. Correct responses produced reinforcement and were followed by all lights in the chamber being turned off for the ITT. This was followed by the illumination of both the house light and the cue light over the opposite lever to indicate beginning of the next trial. Responses on the incorrect or unlighted lever were counted as errors and not rewarded. The ratio schedule and the ITI were gradually increased from the initial requirement to the terminal level of FR20 with an ITI20 as each animal demonstrated proficiency as successively higher levels. Both the FR and the ITI were increased by 2 whenever an animal obtained more than 20 reinforcements within a 10-minute period. When the animals reached FR14 and ITI14, the time allowed to obtain 20 reinforcements was lengthened to 15 minutes. Training sessions were given 40 min per day, 5 days per week until rats attained criterion performance of no more than 25% responses on the incorrect lever for 3 consecutive days or until postexposure training had been conducted for 100 sessions, whichever came first. The 100-day cutoff was included because this was greater than three standard deviations above the mean number of sessions required by the saline control animals to reach criterion.

At the conclusion of behavioral training, all rats were anesthetized with intraperitoneal sodium pentobarbital, and then perfused with a 10% solution of formalin through a 20-gauge catheter inserted into the left ventricle of the heart. An incision was made in the right ventricle to allow excess fixative to escape. The brains were removed, immersed in 10% formalin solution, fixed, processed for paraffin embedding and stained for light microscopy, utilizing hematoxylin and eosin stain. A veterinary pathologist then examined each brain for lesions and rated it either 0 (no visible lesions), 1 (minimal lesions with less than 10% neuronal necrosis), 2 (mild lesions with 10–20% neuronal necrosis), 3 (moderate lesions with 20–40% neuronal necrosis) or 4 (severe lesions with greater than 40% neuronal necrosis observed throughout the brain). Areas examined for lesions included the hippocampus, amygdala, pyriform cortex, septal nucleus, thalamus and cerebral cortex.

RESULTS

Administration of soman produced significant signs of cholinesterase toxicity on four of the six parameters observed. No significant differences were observed between the saline controls and any of the soman groups for lacrimation and eyeball protrusion when the animals were observed one hour after injection. Soman administration produced significant differences between the three soman dose groups

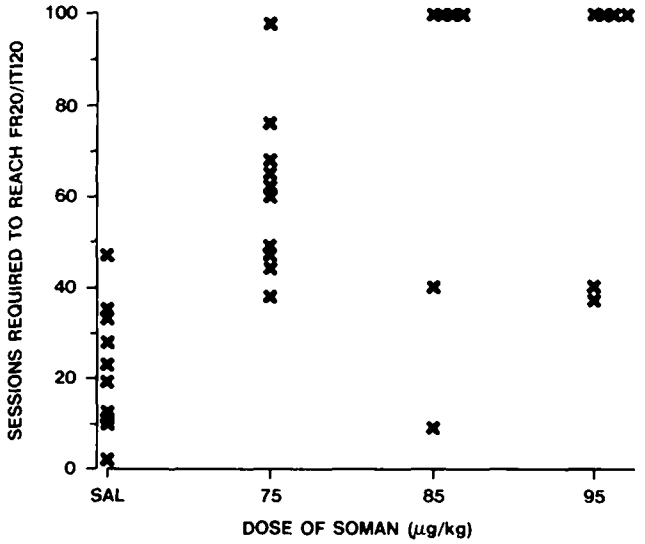

FIG. 1. Sessions required to reach the terminal performance level of FR20/ITI20. Each × represents the number of sessions required by an individual animal to reach FR20/ITI20.

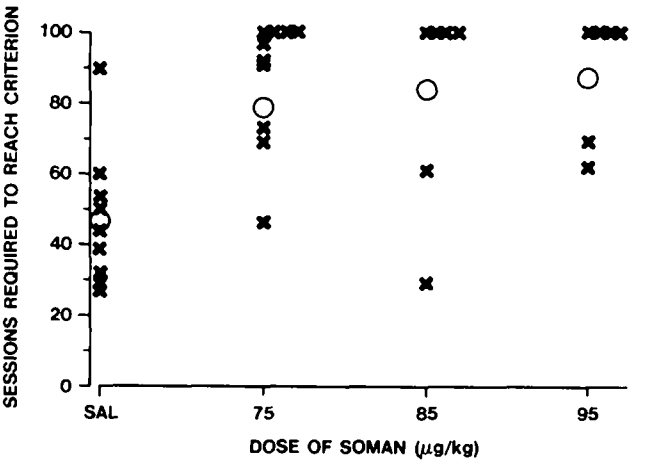

FIG. 2. Sessions required to attain criterion performance levels. Each × represents the number of sessions required to attain criterion performance. The ○ represents the mean number of sessions within the soman dose groups.

and the saline controls on the other four measures; motor sign, secretory signs, general state and coordination. These effects were consistent across both trained and observers (interrater reliability=0.91). A series of Kruskal-Wallace one-way analysis of variance nonparameteric analyses were then performed on just the soman-exposed animals to determine whether the three soman doses would induce a dose-dependent variation in effect. After correcting for ties and controlling for multiple tests, the analysis of the motor symptom scores revealed significant differences between the three doses, $H(2)=10.68$, $p<0.01$. A significant dose difference was also observed on the secretion observations, $F(2)=9.299$, $p<0.01$, and a marginally significant effect was found on the measure of general state, $H(2)=6.14$, $p<0.05$. Although all soman-exposed animals were more affected than the saline controls, no significant dose effects were observed between the three soman doses on the coordination measure. Thus, we observed that administration of soman did produce a significant dose-related increase in a majority of measured signs of acute cholinesterase intoxication. The one-hour cholinesterase intoxication scores appear to be related to the lethality of soman which was determined at one week postexposure. Although only 28% of the animals given 75 μg/kg soman were dead within the first week, 71% and 73% of those animals given 85 or 95 μg/kg soman, respectively, died during the first week after exposure. Although the majority of these animals died within the first 24 hours, a few animals given 85 or 95 μg/kg soman died more than 72 hours after soman administration.

Figure 1 displays the number of days each surviving animal required to reach the terminal level of FR20 and ITI20. As may be seen in this figure, most of the saline controls had little if any trouble reaching this requirement. When data from the soman-exposed animals were examined, a significant dose-dependent effect was seen, $F(3,28)=6.85$, $p<0.01$. Although all but one of the animals receiving 75 μg/kg soman were able to perform the task, they required significantly more sessions to reach the terminal level of performance (Tukey post hoc analysis, $p<0.05$) than did the saline controls. Animals receiving 85 or 95 μg/kg soman performed significantly worse than either the saline controls or the group receiving 75 μg/kg soman (Tukey post hoc analysis, $p<0.05$). Only two animals in each group were able to perform at the terminal level prior to the 100 days of training cutoff. For all analyses, those animals who were unable to perform the task at the terminal level were given scores of 100 days. This allowed inclusion of these animals in the analyses since they were performing the task, even though their performance was not at the terminal level.

Because two-thirds of the animals in the two higher soman dose groups were incapable of performing the alternation task at the terminal level, the results of the analyses of sessions required to reach criterion are not surprising (Fig. 2). The saline control animals required a mean of 46.9 (±5.32 S.E.M.) training sessions to attain criterion performance. This was significantly less than any of the soman-exposed groups, $F(3,28)=7.60$, $p<0.001$. A Tukey post hoc analysis found no significant difference between the three soman-exposed groups in the number of sessions required to attain criterion performance. The animals receiving 75 μg/kg soman had a mean of 79.5 (±5.99 S.E.M.) sessions to criterion, while the animals receiving a 85 or 95 μg/kg soman had mean values of 81.5 (±12.45 S.E.M.) and 87.33 (±8.21 S.E.M.) respectively. These results are somewhat misleading because many of the animals receiving 75 μg/kg soman did attain criterion performance prior to the 100-session cutoff. Conversely the majority of animals receiving either 85 or 95 μg/kg soman were incapable of performing at the terminal level, let alone approaching criterion performance. Even if the study had been extended another 100 days, it is doubtful whether many of these animals would have been capable of criterion performance.

Soman-injected rats displayed neural damage consistent with that previously reported by (8), as may be seen in Figs. 3 and 4. Both the dorsal and ventral hippocampus, thalamic and amygdaloid nuclei and pyriform cortex displayed varia-

FIG. 3. Panel A represents an area of laminar cortical necrosis in the pyriform cortex. Note area of pallor and shrunken, distorted neurons, adjacent to linear area of vacuolar change. Four small foci of mineralization are also present (bar=400 micrometers). Panel B displays a higher magnification of the area of pallor in Panel A. This shows shrunken and necrotic neurons, malacic parenchymal change and a focally extensive area of gliosis (bar=200 micrometers).

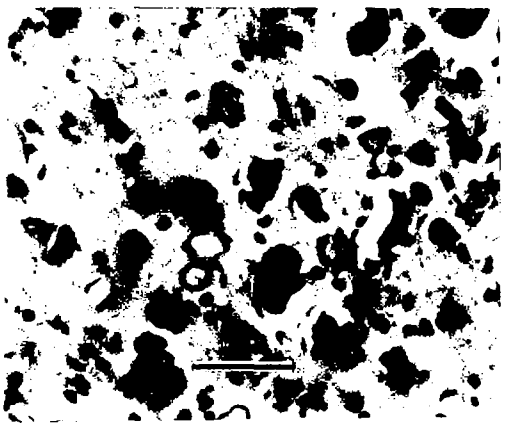

FIG. 4. High magnification of an area of mineralization. Small dark shrunken neurons are also present (bar=25 micrometers).

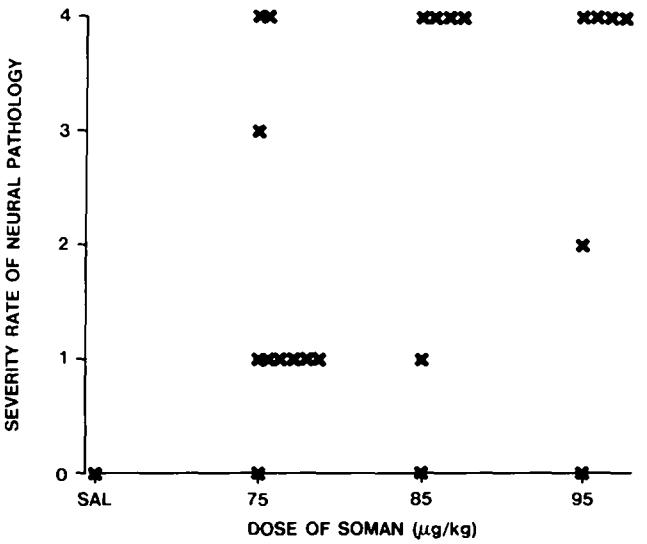

FIG. 5. Overall rated severity of neural damage as a function of soman dose administered.

ble degrees of damage, including neuronal necrosis with or without mineralization, spongiosis, malacia, gliosis and depletion of neurons. In the more severe cases, the lesions included loss of parenchymal brain substances, resulting in cortical atrophy with subsequent dilation of the lateral ventricles. When the overall neural lesions were rated between 0 and 4 as described, the amount and severity of damage was found to be dose-dependent (Fig. 5). Seven of the ten animals receiving 75 μg/kg soman displayed minimal or no pathology. Only 2 animals in each of the 85 and 95 μg/kg soman-exposed groups displayed moderate or less neural lesions. All other animals displayed severe pathology throughout the examined portions of the brain. Significant correlations between the severity of neural injury and both the number of sessions required to reach the terminal performance level of FR20/ITI20 ($r=.80$) and the number of sessions required to attain criterion performance ($r=.67$) were found across all soman doses.

DISCUSSION

In those animals receiving soman, the observed neural lesions appeared to be most severe in the hippocampus and other parts of the limbic system, as has been observed in other studies (6,8). Additionally, the behavioral deficits observed in these animals were strongly reminiscent of the behavioral deficits seen in animals with experimental lesions of the hippocampaus and septal nuclei (4). In addition to failing to learn the task, severely affected animals displayed an extreme hyperreactivity, including a marked aversion to handling. This hyperreactivity did not diminish over time. If anything, the severely affected animals became more hyperreactive.

Administration of various doses of soman produced a dose-related increase in some, but not all signs, of cholinesterase toxicity, and in lethality. However, there was no significant dose-dependent difference in the number of days required to attain criterion performance levels. The lack of a dose-dependent difference may be due, at least in part, to the

cutoff point chosen (100 sessions). If the animals were allowed more training sessions, a significant difference between animals given 75 μg/kg and those given the two higher doses may have been observed.

Further support for the interaction between dose and time to learn the task is found in our observation of a significant correlation between the severity of neural damage and the two measures of alternation learning. Across all doses of soman, only those animals exhibiting no or minimal pathology (0 or 1) performed better than the mean for each group. All animals exhibiting severe lesions were unable to attain the criterion performance levels. That is, two populations of soman-exposed animals were observed: those exhibiting no or minimal neural damage and those with moderate or severe lesions. Animals of each type varied in every soman dose group. However, the proportion of each type varied as a function of administered soman dose. At low doses of soman, such as 75 μg/kg, a high proportion of animals demonstrated mild damage and were capable of learning the task, although at a retarded rate. At higher doses, more animals demonstrated severe pathology and thus required extensive training or were totally incapable of learning the complex task. At both ends of the dose spectrum there were animals capable of learning the task. However, the proportion of these animals dropped as the dose increased. Animals capable of learning the task displayed mild signs of cholinesterase toxicity and later displayed minimal neural lesions. These animals were able both to learn the alternation task at FR20/ITI20 and later to peform this task at criterion performance levels. Those who were unable to learn the task also demonstrated severe neural lesions. Thus, we conclude that although soman does produce a decrement in the ability to learn a cued alternation task, this decrement is related to the severity of acute toxicity symptoms at time of soman administration and the pathology seen at time of necropsy. Neither measure of incapacitation is directly related to the specific dose of soman but rather to the individual reaction to the soman.

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