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Effects of Subchronic Administration of Soman on Acquisition of Avoidance-Escape Behavior by Laboratory Rats

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GELLER, I., R. J. HARTMANN, JR. AND E. M. GAUSE. *Effects of subchronic administration of soman on acquisition of avoidance-escape behavior by laboratory rats.* PHARMACOL BIOCHEM BEHAV **23**(2) 225–230, 1985.—Holtzman male Sprague-Dawley rats were given four injections of saline or soman at 31 μg/kg or 46 μg/kg. The injections were given every 3 days during a 2-week period. Tail vein blood samples, drawn 24 hr before the first injection and 24 hr after the last injection, were analyzed for acetylcholinesterase (AChE) inhibition. For the low and high soman groups, whole blood AChE was inhibited 20 and 24%, respectively, while plasma AChE was inhibited 17 and 25%, respectively. Parallel saline injections produced a small inhibition of whole blood AChE and an increase in activity (negative inhibition) of plasma AChE. During the second week of soman administration rats began training on a discriminated shock avoidance task. The seven animals administered 46 μg/kg soman did not learn the lever-pressing avoidance response during a period of 120 days. Five of eight saline rats and four of seven 31 μg/kg soman rats learned the avoidance response. There was no significant difference between learners relative to rate of learning of the avoidance task. All of the rats learned to escape the shocks. These data indicate that subchronic soman inhibits learning of new behaviors by laboratory rats.

Discriminated-avoidance Escape Acquisition Soman Rats Blood AChe Plasma AChE

ONGOING research in our laboratories has been concerned with the effects of subchronic administration of soman on performance of operant behavior by laboratory rats. For animals trained to perform these operant tasks, administration of soman at 5, 10, 20 or 40 (8–70% LD-50) μg/kg once every 3 days during a 30-day period yielded unsystematic data which generally reflected a reduction in operant rates during the injection periods with a return to baseline control levels after cessation of soman for those animals that survived the injections. There have been several other reports indicating persistent behavioral changes in survivors of near-lethal doses of soman ([5]; McDonough, Smith and Smith, personal communication). The latter investigators reported that after a single near-lethal dose of soman, rats were unable to learn to perform on a DRL schedule as well as control animals. The effect persisted up to 70 days post drug when the experiment was discontinued. These studies used acquisition or learning of a task rather than performance as the dependent variable. The purpose of the present investigation was to determine if rats could learn a discriminated avoidance escape task after receiving several administrations of soman.

METHOD

The subjects were 40 Holtzman male Sprague-Dawley rats, 90–120 days old at the start of the experiment. They were housed in standard rat cages and maintained on ad lib food and water in a laboratory with a 12:12 light/dark cycle. The animals were trained on the avoidance-escape procedure in a Skinner box which contained a lever for the rat to depress, a speaker for the delivery of auditory stimuli and a grid floor through which shocks could be delivered to the animals' feet. The method for training rats on the procedure has been described previously [1, 2, 3]. Briefly, it was as follows: Fifteen sec after the start of the experimental session a 5-sec auditory stimulus (tone) was activated. The tone was terminated contiguously with a 2-sec shock to the animals' feet. The rats could avoid the shock by responding in the presence of the tone. Such responses (termed avoidance responses) shut off the tone and delayed the onset of the next auditory stimulus for a new 15-sec period; in this way the rat could postpone the next shock by 20 sec. Responses made in the presence of the shock (termed escape responses) served to terminate the shock and delay the onset of the next auditory stimulus for a new 15-sec period. Avoidance efficiency was calculated as percentage of responses to tones, and escape efficiency as percentage of responses to shocks. The training sessions were of 1-hr duration and were conducted on Monday through Friday of each week.

Soman (obtained from Chemical Research and Development Center, Aberdeen Proving Ground, MD) was diluted to 50 μg/ml. It was kept at −80 degrees C until ready for use. The selection of doses for the study was based on a preliminary acute LD-50 study with age-matched rats (LD-50=62.0 μg/kg).

The doses of soman selected for a subchronic dosing

TABLE 1

EFFECT OF SUBCHRONIC SOMAN UPON BLOOD AChE LEVELS OF DISCRIMINATED AVOIDANCE RATS

			AChE Activity			
			Whole Blood		Plasma	
Group	(N)	Time	μmol/hr/ml	% Inhib.	μmol/hr/ml	% Inhib.
Saline		Pre-	45.9 (±1.7)	0	17.4 (±1.8)	0
Control	(8)	Post-	43.7 (±2.9)	4.8	21.0 (±2.1)	−20.7
31 μg/kg		Pre-	42.5 (±1.3)	0	15.7 (±0.9)	0
(0.5 LD-50)	(7)	Post-	33.9 (±2.6)	20.2	13.0 (±1.2)	17.2
46 μg/kg		Pre-	41.8 (±1.9)	0	19.5 (±1.9)	0
(0.75 LD-50)	(7)	Post-	31.8 (±1.4)	23.9	14.6 (±1.6)	25.1

Soman or saline as indicated were administered 4 times over 10 days; Blood samples taken 24 hr before first dose (Pre-) and 24 hr after fourth dose (Post-).
Values shown are group means ± S.E.M.

protocol for rats to be employed in the discriminated avoidance acquisition experiment were 0.5×LD-50 (31 μg/kg) and 0.75×LD-50 (46 μg/kg) to be administered every third day for a total of four doses. Rats were assigned to one of three groups of ten rats each; one group received saline 1 ml/kg, one 31 μg/kg soman (0.5×LD-50) and the third received soman at 46 μg/kg (0.75×LD-50). An additional ten animals were added to the 46 μg/kg group to assure enough survivors for the conduct of the avoidance experiment. Prior to initiation of training on the avoidance behavior, groups were dosed with either soman or saline twice—Day 1 and Day 3. Avoidance training was initiated on Day 6, and animals were injected with soman/saline immediately after the behavioral session on Day 6 and Day 9. Blood samples were obtained from all animals on Day 0, 24 hr before the first soman injection and on Day 10, 24 hr after the fourth soman injection.

RESULTS

A number of rats did not survive the soman treatments and two saline rats were dropped from the study at its inception, one because of illness and one because it was lying on its back, thereby insulating itself from the shocks and not pressing the lever. The final number of rats used in the study were eight controls and seven in each soman group. Of the 14 surviving soman rats, only three of the 46 μg/kg group showed any effects to soman injections. These symptoms included mouth movements in two rats and hypoactivity in a third. The symptoms were evident only during post-drug hr 1.

The dosing protocol employed for the avoidance animals produced a lowering of blood AChE activity; the effects are shown in Table 1 for each treatment group. While the whole blood AChE level of the saline control group declined approximately 5% over the 10-day interval of soman administration, the lower soman dose group (0.5×LD-50) exhibited a 20% inhibition and the higher dose group (0.75×LD-50) exhibited a 24% inhibition 24 hr after the fourth soman injection. Plasma AChE activity was stimulated 21% by the saline injections, but was inhibited to the extents of 17 and 25%, respectively, by the soman injections.

Figures 1, 2 and 3 contain avoidance and escape data obtained for each individual rat for each treatment condition. The ordinate indicates percent avoidance efficiency (x—x) plus S.E.M.s and percent escape efficiency (●— —●) minus S.E.M.s. The efficiency scores were calculated as percentage of responses to stimuli/total stimuli (avoidance) or percentage of responses to shock/total shocks (escapes).

Figure 1 shows the effects of subchronic saline injections on acquisition of the avoidance and escape behavior. Escape responding efficiency reached 100% for all saline rats. Five of the eight rats learned to avoid with efficiency scores of better than 50%; avoidance efficiency reached 95% for Rats A-1 and A-19 and ranged from 50 to 80% for the other three rats that learned the avoidance response. Although Rats A-16, A-22 and A-28 did make avoidance responses, their efficiency remained below 40%.

Figure 2 shows the effects of subchronic soman at 31 μg/kg (0.5×LD-50) on acquisition of the avoidance-escape behavior. Escape responding efficiency reached 100% for all rats. By the ninth week of training, escape responses for Rat A-17 began to diminish, reaching zero level by week 16. Direct observation of the rat revealed it to be lying on its back and avoiding shocks by insulating itself with its fur. Four of the 31 μg/kg rats acquired the lever-pressing avoidance response. Rats A-5 and A-8 stabilized at better than 60% efficiency while avoidance efficiency for Rats A-11 and A-26 ranged from 25 to 75%. Two of the rats in this group made no avoidance responses during the period of 16 weeks.

Figure 3 shows the effects of subchronic soman at 46 μg/kg (0.75×LD-50) on acquisition of avoidance-escape behavior. None of the 46 μg/kg soman animals acquired the avoidance response during a 16-week training period. Escape responding efficiency reached 100% for five rats in this group. Periodic observation of Rats A-33 and A-36 showed them to be standing and taking the shocks, during the early part of the study, and consistently lying on their backs throughout the sessions beginning the 11th week of the study. It appeared as though Rat A-3 might learn to avoid but this rat reached a maximum efficiency of 35% during week 4 and then became progressively worse, reaching a low of 12% during week 16. Analysis of variance with repeated measures

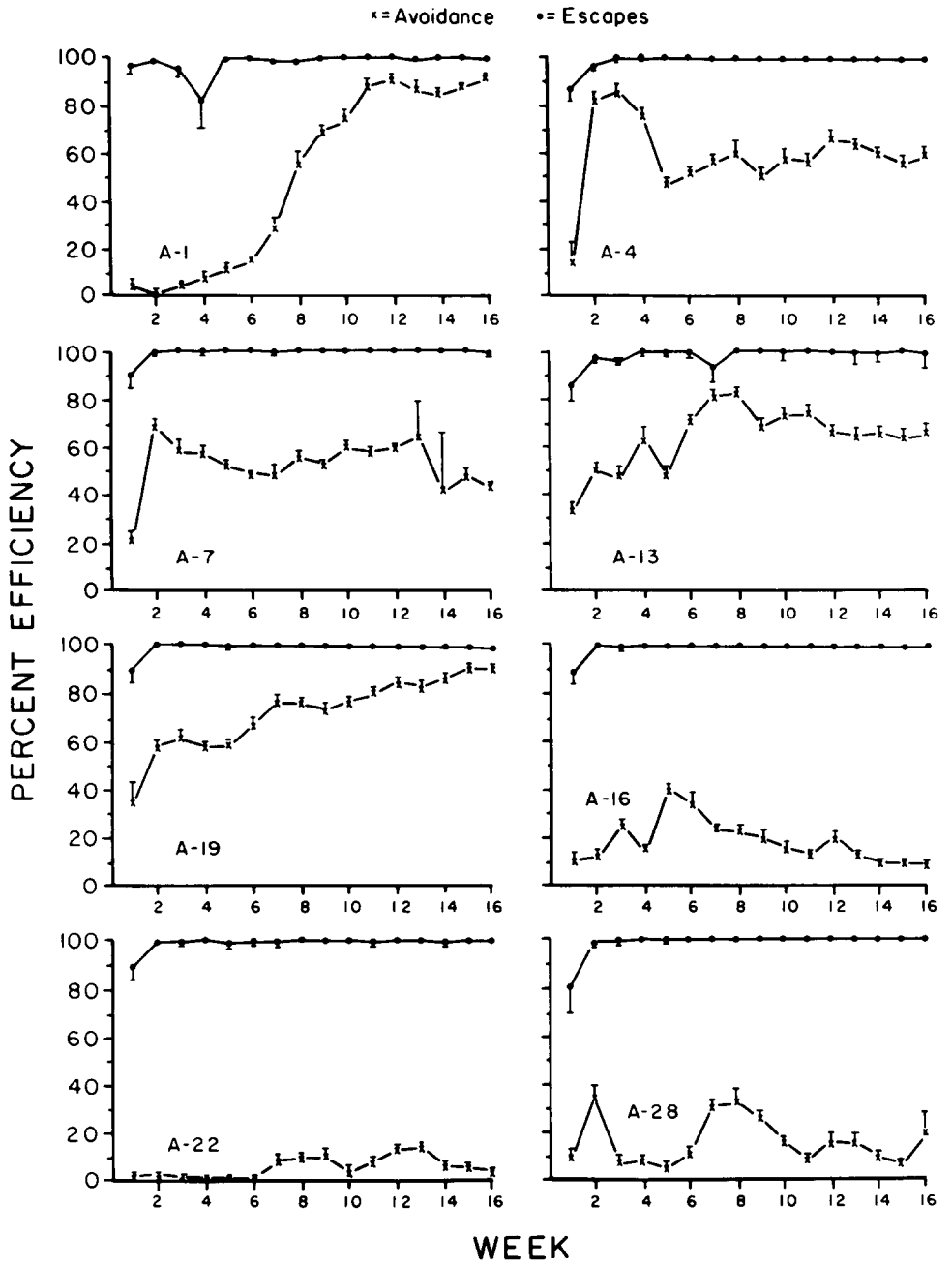

FIG. 1. Effects of subchronic saline administration on acquisition of a lever-pressing avoidance and escape response by laboratory rats. Avoidance efficiency=responses during stimuli/total stimuli × 100 and escape efficiency=responses during shocks/total shocks × 100.

[9], revealed significant dose effects (see Table 2A). Acquisition data of the saline or 31 μg/kg soman rats were significantly different from the data of the 46 μg/kg soman rats. The data of the saline rats didn't differ significantly from those of the 31 μg/kg soman animals. There was a significant dose × week interaction indicating that avoidance behavior improved over time for the saline and 31 μg/kg groups but not for the 46 μg/kg group.

Analysis of variance with repeated measures for the learners of the saline and 31 μg/kg groups (see Table 2B) revealed no significant dose effects in avoidance acquisition but there was a significant dose × week interaction indicating an improvement in avoidance behavior over time for both groups.

DISCUSSION

The findings of this experiment show that none of seven rats treated on four occasions with 46 μg/kg soman (0.75×LD-50) were able to learn a discriminated avoidance task during a training period of 16 weeks. This was true

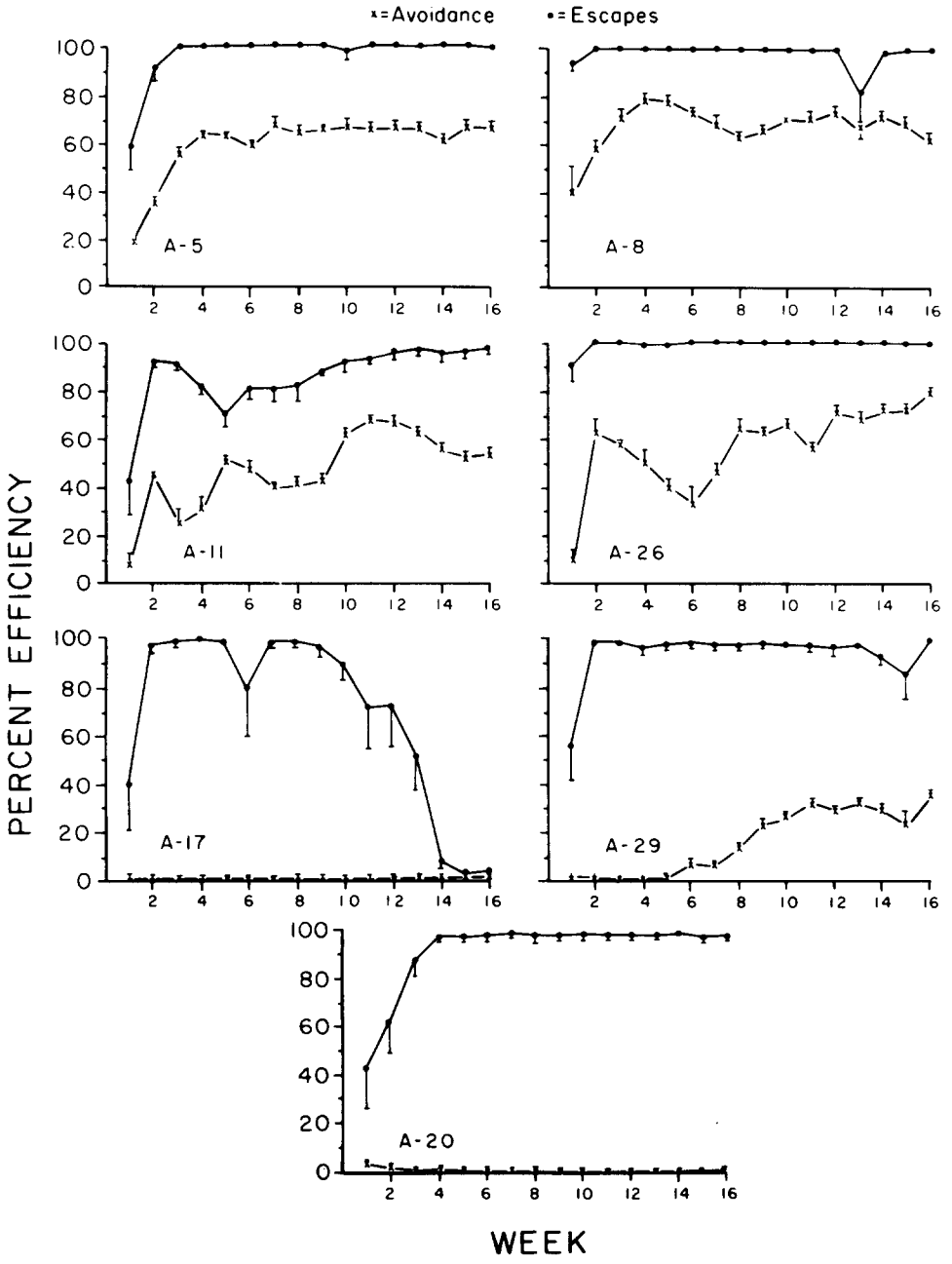

FIG. 2. Effects of subchronic administration of 31 μg/kg soman on acquisition of a lever-pressing avoidance and escape response by laboratory rats. Avoidance efficiency=responses during stimuli/total stimuli × 100 and escape efficiency=responses during shocks/total shocks × 100.

despite the fact that overt neurological symptoms following soman injection were minimal and none of these animals convulsed. However, this does not rule out the possibility of the occurrence of petit mal convulsions or silent brain seizures which may have occurred even though they were not observed. In any case, overt symptomatology may not be a necessary condition for behavioral effects to occur.

The possibility that the consistent failure of the 46 μg/kg soman group to avoid the shock could reflect a soman-induced motor deficit can be ruled out because five of seven animals in this group escaped the shocks as well as the saline control and 31 μg/kg soman groups (Figs. 1, 2, 3).

In the present study, soman inhibited the learning of the discriminated avoidance task by untrained rats during a 16-week period, while it was observed previously (Geller *et al.*, unpublished observations) that soman produced a temporary decrement in performance by rats of the avoidance task, an effect that disappeared with repeated soman administration. These observations are in agreement with those of Wolthuis and Vanwersch [10] who demonstrated that soman and other

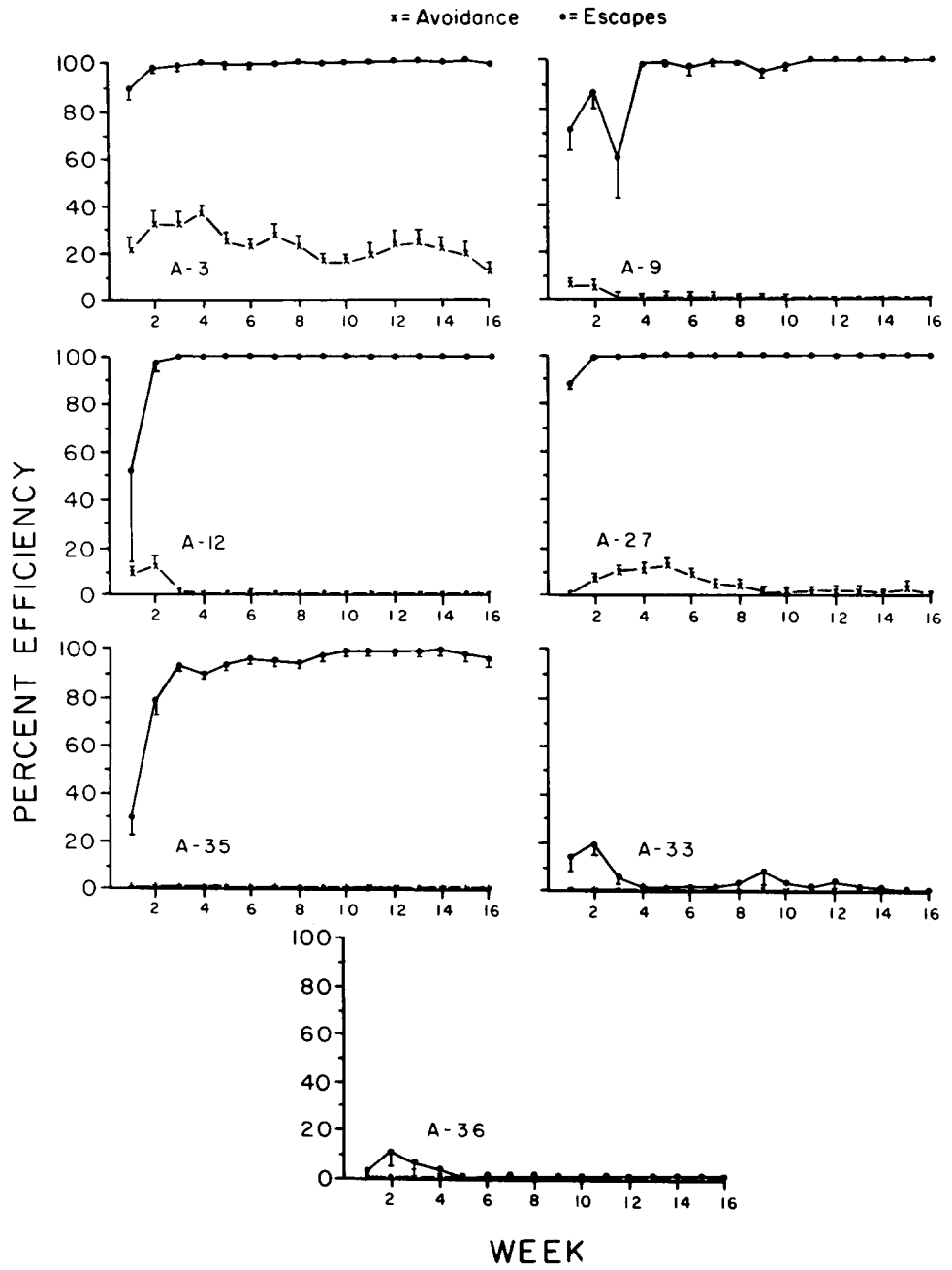

FIG. 3. Effects of subchronic administration of 46 μg/kg soman on acquisition of a lever-pressing avoidance and escape response by laboratory rats. Avoidance efficiency=responses during stimuli/total stimuli × 100 and escape efficiency=responses during shocks/total shocks × 100.

cholinesterase inhibitors, at doses that did not produce overt symptomatology, interfered with the learning by rats of 2-way shuttlebox avoidance, while retention tests of a passive avoidance response were less sensitive to the effects of soman.

The inability of soman treated rats to learn a discriminated avoidance response may be explained by the observations of a number of other investigators who have described the occurrence of soman-induced neuropathology and brain lesions in laboratory animals [4, 6, 7, 8].

Petras [7] studied brains of soman injected rats and uninjected controls macroscopically after giving soman in doses ranging from 79.4 to 114.8 μg/kg. He rated animals for soman-induced neurological signs. Brain damage was found in four of four animals that had seizures and in three of four that showed limb tremors. Widespread axon degeneration in forebrain and midbrain was traceable to hindbrain and spinal cord. The pattern of degeneration was not similar to fetal anoxia or delayed neurotoxicity caused by tri-ortho-cresylphosphate. Animals didn't have to experience convul-

TABLE 2(a)

A REPEATED MEASURES ANOVA COMPARISON OF SALINE AND SOMAN RATS RELATIVE TO THE RATE OF ACQUISITION OF AVOIDANCE BEHAVIOR*

Treatment		Saline	Soman (31 μg/kg)	Soman (46 μg/kg)	
n=		8	7	7	
Mean		42.01	35.86	4.78	
S.D.		25.27	28.57	8.58	
Source	*df*	Mean square	F		*p*
Dose	2	46208.81	5.59		0.012
Week	15	633.33	5.01		0.000
W × D	30	250.06	1.98		0.002

*In the repeated measures analysis of variance, the same 22 individuals are compared at all 16 weekly periods (See [9]).

TABLE 2(b)

A REPEATED MEASURES ANOVA COMPARISON OF SALINE AND 31 μg/kg SOMAN RATS RELATIVE TO RATE OF ACQUISITION OF AVOIDANCE BEHAVIOR IN LEARNERS*

Treatment		Saline	Soman 31 μg/kg)		
n=		5	4		
Mean		59.43	57.86		
S.D.		9.23	8.26		
Source	*df*	Mean square	F		*p*
Dose	1	86.77	0.07		0.800
Week	15	1462.04	6.64		0.000
W × D	15	70.16	0.32		0.992

*In the repeated measures analysis of variance, the same 9 individuals are compared at all 16 weekly periods (See [9]).

sions for the damage to occur. He also reported soman-induced brain lesions in cats and monkeys.

McLeod *et al.* [6] gave rats 106, 133 or 165 μg/kg soman. Survivors were killed and brain sections examined by light microscopy. A consistent pattern of neuronal degeneration was observed in brains of only those animals that had experienced convulsions after soman. Lesions appeared to be like those reported for hypoxia or status epilecticus.

Lemercier *et al.* [4] poisoned rats with one lethal or sublethal subcutaneous injection or by several less strong, weekly doses of soman. Those surviving animals that experienced respiratory failure or repeated and prolonged convulsions exhibited neuronal changes similar to those of hypoxic encephalopathy. Lesioned gray structures were generally poor in AChE.

Other investigators have demonstrated soman-induced behavioral changes with concomitant CNS damage. Mays *et al.* [5] tested rats on a passive avoidance task after giving them soman. All of her controls learned the avoidance task while 69% of the soman rats did not. Five of nine animals that avoided had neuropathology while 18 of 19 animals that did not avoid had neuropathology. McDonough *et al.* (personal communication) found that of nine soman treated rats tested on the DRL procedure that were not as proficient as controls in learning the DRL task, five showed evidence of CNS damage. Consideration of the above strongly suggests that our behavioral observations may be due to soman-induced brain lesions. Histological evaluation is planned to investigate this possibility.

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