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Long-Term Behavioral Changes in Rats Following Organophosphonate Exposure[1,2]

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RAFFAELE, K , D HUGHEY, G WENK, D OLTON, H MODROW AND J McDONOUGH *Long-term behavioral changes in rats following organophosphonate exposure* PHARMACOL BIOCHEM BEHAV 27(3) 407–412, 1987 —The organophosphorus compound soman irreversibly inhibits cholinesterase in both the central and peripheral nervous systems High doses of this compound produce seizures and death in animals Surviving animals exhibit neural lesions and behavioral abnormalities The behavioral effects of a single exposure to soman were evaluated in rats injected with 50 μg/kg or 85 μg/kg soman or with saline Each rat was tested for either activity in an open field or performance in a 14 choice point multiple T-maze All rats were then tested for reactivity to tactile stimuli Some rats exposed to soman showed increased activity in the open field, learning deficits in the Stone maze, and increased reactivity to tactile stimuli, while others showed behavior similar to that of controls An increase in reactivity was correlated with increased open field activity and with poor performance in the Stone maze Rats which had received soman and were abnormal in behavioral tests were more likely to have abnormal brain pathology than rats which had received soman and were normal in behavioral tests

Animal behavior Soman Learning Activity Reactivity

EXPOSURE to organophosphorus compounds that irreversibly inhibit acetylcholinesterase may produce a variety of behavioral changes After accidental exposure, humans have demonstrated impaired memory as well as reduced vigilance and concentration, they may become depressed, anxious and irritable [6,11] Rats given soman (1,2,2-trimethylpropyl-methylphosphonofluoridate), a potent and irreversible cholinesterase inhibitor, may suffer significant deficits in the acquisition of operant behaviors, such as are generated on a differential reinforcement of low rates of responding (DRL) schedule [7], lever press active avoidance task [2] or two lever operant alternation task [9]

The present study was designed to improve our understanding of the long-term behavioral effects produced by a single exposure to soman Rats were exposed to saline, 50 μg/kg soman or 85 μg/kg soman and tested on three behavioral tasks Because soman may produce lesions in the hippocampus and septum as well as in other limbic regions of the brain [5,10], the tasks chosen were sensitive to disruption by other, experimentally-induced types of lesions in the septo-hippocampal system and, thus, were likely to detect behavioral changes produced by soman

METHOD

Subjects

Eighty male Sprague-Dawley rats weighing 400 to 500 g at the start of behavioral testing were used in this study Throughout testing, the rats were maintained at 85% of free feeding weight, with the addition of 5 g of body weight per week to allow for normal growth Prior to and during the study, all rats were individually housed in stainless steel rack cages with ad lib access to water

Apparatus

Open field activity Open field activity was measured in a wooden box 77×95 cm with walls 38 cm high The walls were unpainted whereas the floor was painted white and divided into 16 areas, each 18×23 cm These areas were marked by black lines

Stone maze A straight runway and a small maze were utilized to shape rats to run through a maze to obtain food reinforcement The straight runway measured 180×10 cm and was made of clear Plexiglas The small maze was made

[1]The experiments reported here were conducted according to The Guide for the Care and Use of Laboratory Animals (1978) 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 assertions 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 of the Department of Defense

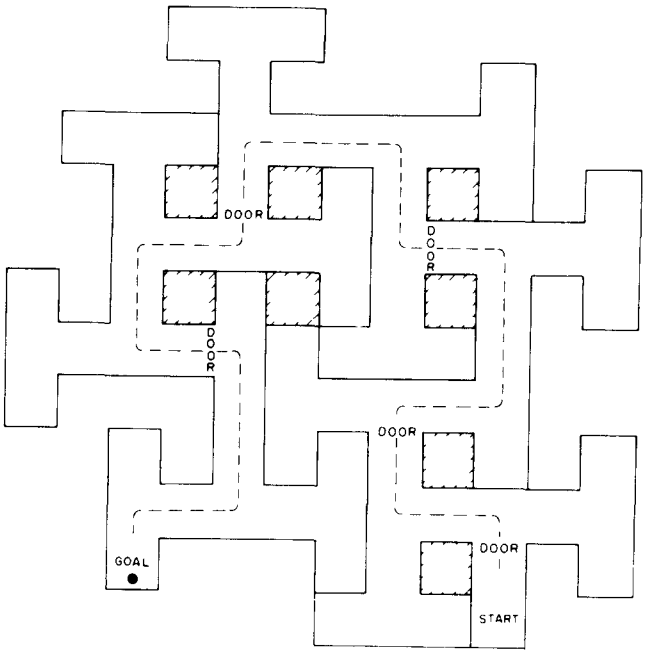

FIG 1 Diagram of the floor plan of the Stone maze

of Plexiglas sheets fastened together and placed in the activity apparatus to create a variety of paths through the box

The Stone maze [4] consisted of a multiple T-maze containing 14 choice points, with sliding panels (doors) located at four points within the maze (Fig 1) The individual alleys measured 12 cm wide The maze was located in an alcove with overhead lighting A fan in the corner was used to remove the alcohol vapors after cleaning

Procedure

Soman was obtained frozen from the stock solution at USAMRICD and diluted in chilled saline to a concentration of 85 μg/ml All rats were individually weighed to the nearest 0 1 g and randomly divided into three groups Each rat was injected SC with saline (1 0 cc/kg) or with an appropriate volume of soman to produce a dose of either 50 or 85 μg/kg Twenty rats were injected with saline, 25 with 50 μg/kg soman, and 35 with 85 μg/kg soman Surviving animals were handled and weighed daily for the next three weeks Animals demonstrating a weight loss were given a wet mash diet every day until recovery of pre-injection weight Those animals losing more than 25 g were given daily SC injections of 12 ml Ringers lactate solution until recovery Three animals injected with 50 μg/kg soman (12% lethality) and fourteen injected with 85 μg/kg soman (40% lethality) died within the first two weeks after injection No animals died after this period Surviving animals were placed on the food deprivation schedule three weeks after soman exposure One week later, at the start of the testing procedure, rats from each of the three groups were randomly divided Ten rats from each exposure group were utilized in the locomotor activity test Ten of the remaining rats from each group were trained in the Stone maze The remaining two rats injected with 50 μg/kg and one rat injected with 85 μg/kg were not utilized Following completion of the appropriate test procedure, each rat was tested for reactivity to tactile stimuli

Open Field Activity

Test procedure Each rat was placed in the center of the apparatus for a 10 minute test session on two consecutive days Activity was recorded as the total number of areas entered within each test session A rat was considered to have entered a square if the two front paws were within that area All testing was done with the experimenter blind to the rat's experimental group

Stone Maze

Test procedure Each rat was trained for five days to go down the straight runway for chocolate milk Milk was initially held in front of the rat and gradually moved farther down the alley At the end of this phase, all rats ran the entire length of the alley to obtain the milk reinforcement

After training in the alley, each rat was trained in the small maze for five days During the first two days, the rats were placed in the maze in pairs and allowed to explore for 10 minutes, during which time chocolate milk was continuously available in one corner For the next three days rats were individually placed in the maze and removed after drinking chocolate milk in the opposite corner

The rats were then trained in the Stone maze On the first day of training, the rat was placed in the goal box and allowed to consume 1 0 ml chocolate milk The rat was then transferred to the start box and allowed to go through the maze for a second reinforcement (1 0 ml chocolate milk) Each of the four doors was closed after the rat had passed through it, thus preventing the rat from going back through the maze Time was measured, using a stopwatch, from the opening of the start door until the animal reached the goal box After drinking the milk, the rat was removed from the apparatus and the floor was cleaned using a 30% ethanol solution

While the rat was in the maze, the experimenter recorded its movements on a diagram of the maze If the rat did not reach the goal after 10 minutes in the maze, it was moved to the goal, allowed to drink the reinforcement, and removed from the maze Each rat was tested one trial per day, six days per week, until achieving a criterion of less than ten minutes to complete the maze on 40 trials After three weeks, any rat not consistently reaching the goal in less than ten minutes was given two trials per day with at least one hour between trials Any rat that did not consistently reach the goal in less than ten minutes after two weeks of this training was withdrawn from the study

Performance on the Stone maze was evaluated as follows An error was defined as placement of both forepaws more than 5 cm into an incorrect alley, one that was not the shortest path to the goal For each trial in which the rat finished the maze within the 10 minute time limit, two types of errors were measured A *choice point error* was defined as the first error a rat made at a given choice point on the correct path Therefore errors of this type could range from 0 to 14 (the total number of choice points) *Total errors* included not only choice point errors but also all other types of errors, such as repeated errors and all entries into either end of an incorrect arm The mean number of each type of error was determined for each rat for blocks of five trials for the 40 complete trials

Reactivity

Each rat's reactivity to four types of stimuli was meas-

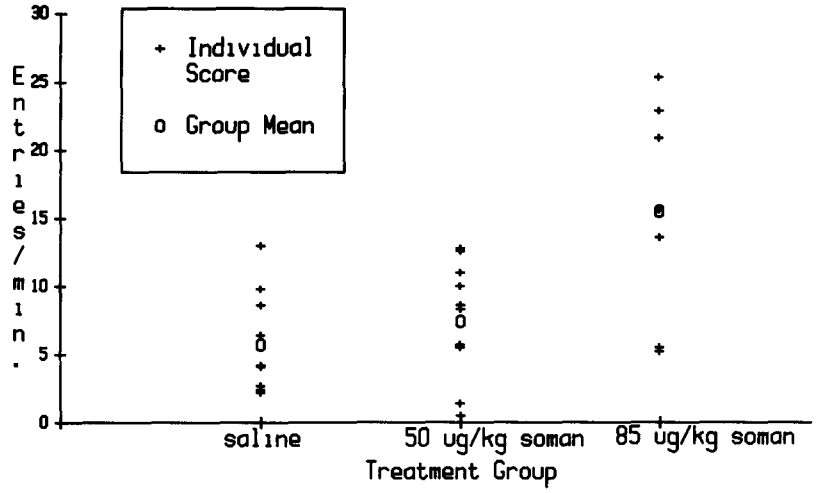

FIG 2 Mean scores and distribution of individual scores for each exposure group in open field activity

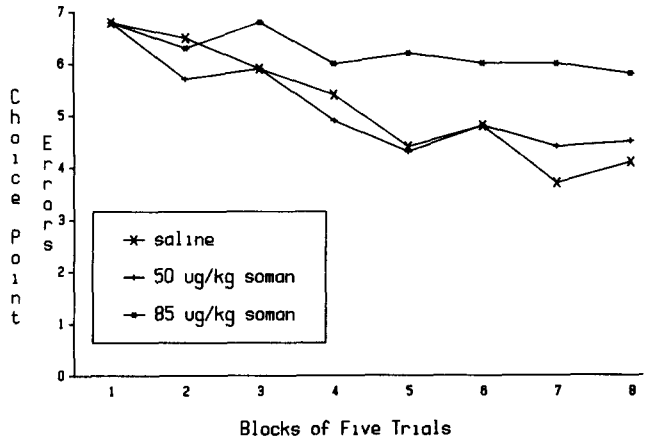

FIG 3 Mean number of choice point errors in the Stone maze for each exposure group, in blocks of 5 trials

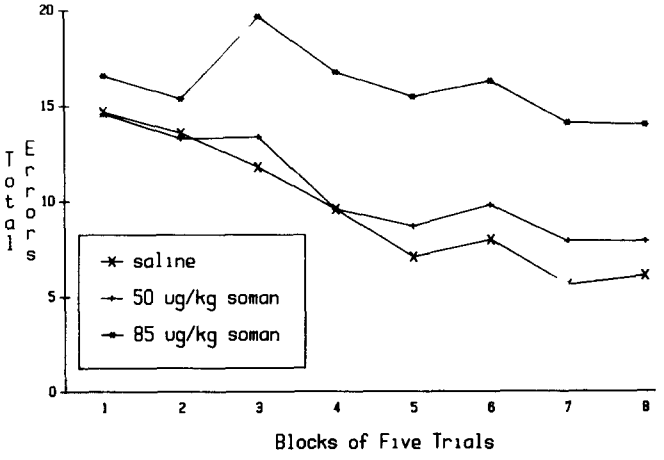

FIG 4 Mean number of total errors in the Stone maze for each exposure group, in blocks of 5 trials

ured [1] With the experimenter "blind" to the animal's experimental group, the home cage was removed from the colony room and placed on a flat surface A wire mesh cylinder 30 cm in diameter and 90 cm high was placed around the cage to prevent escape and to allow the rat to jump without injuring itself The four types of stimuli used were a puff of air to the back, a touch on the back with a stick, a touch on the nose with a stick and an attempt to pick up the rat with a gloved hand The magnitude of reaction to these stimuli was rated on a scale of 0 (least intense) to 3 (most intense) Individual scores for all stimuli were added to obtain a total reactivity score for each rat (minimum score=0, maximum score=12) Because rats had received differing amounts of handling during prior tests, the scores were analyzed separately for rats tested in the open field and for those trained on the Stone maze

Histology

After the completion of behavioral testing, rats were anesthetized with sodium pentobarbital (Harvey Laboratories, Inc , Philadelphia, PA 19140) and perfused intracardially with 0 9% saline and then with 10% formalin-saline The brain was removed and fixed in a 30% sucrose/10% formalin-saline solution The brain was frozen and cut into 20 μm sections, starting at the level of the septum and continuing posteriorly through the hippocampus Every tenth section was mounted and stained with cresyl violet and luxol fast blue The sections were examined microscopically for pathology in four regions likely to be affected by soman (hippocampus, amygdala, thalamus, piriform cortex) and also for ventricular dilation In a procedure which enables comparison of pathology among rats [7], each region was rated from 0 to 3 0=no damage (normal tissue), 3=severe damage (large amount of cell loss, spongy tissue, some gliosis, some necrotic cells) Ventricular enlargement was also rated from 0 to 3 0=no enlargement, 3=severe enlargement Ratings for all brain regions were added to obtain a pathology score for each rat (minimum score=0, maximum score=15)

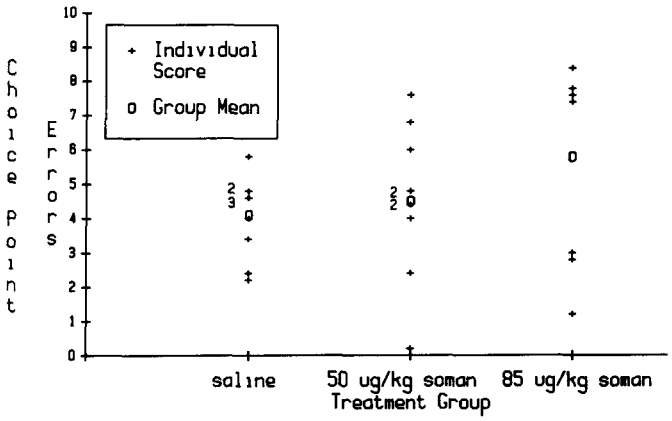

FIG 5 Distribution of individual choice point error scores for each exposure group, on the last block of trials (35–40) in the Stone maze

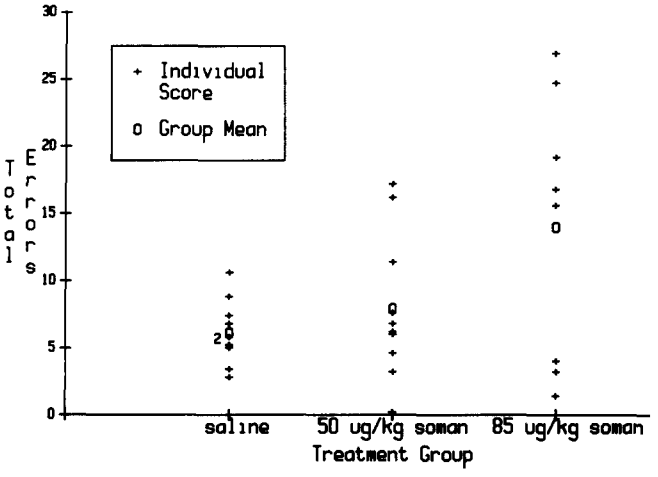

FIG 6 Distribution of individual total error scores for each exposure group, on the last block of trials (35–40) in the Stone maze

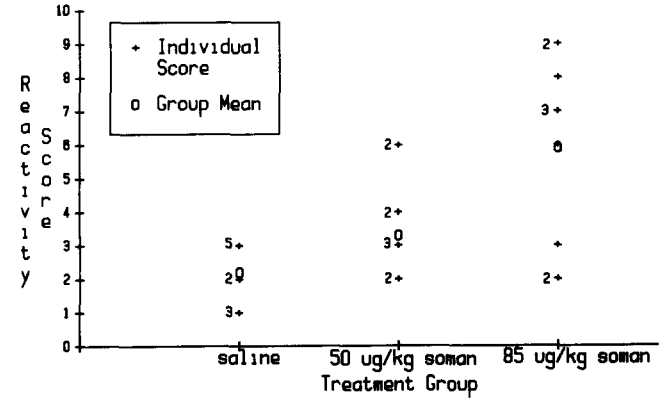

FIG 7 Mean scores and distribution of individual reactivity scores for each exposure group (rats tested following open field activity)

RESULTS

Open Field Activity

Control rats slowly increased their level of exploration in the open field, first into adjacent areas and then along the adjacent walls Rats in the low dose group began the session in a similar manner, but began increased exploration more rapidly In contrast, in the high dose group five of the ten rats immediately began to move rapidly around the apparatus, sometimes running across the center but more often running rapidly around the walls, making complete circuits of the apparatus Three of the rats in the high dose group had convulsions during the testing and failed to move from the area in which they were placed The two remaining high dose group rats behaved similarly to control rats (Fig 2)

Statistical analyses failed to demonstrate a significant difference in activity levels between the three groups when all rats were included in the comparison, $F(2,27)=1\ 18$ However, when the rats in the high dose group that had seizures during testing were excluded from the comparison, the rats in the high dose groups were more active than were the rats in both the control (Scheffe's Test, $df=2,24$, $p<0\ 05$), and in the low dose groups (Scheffe's Test, $df=2,24$, $p<0\ 05$) In addition, the behavior of rats receiving the high dose of soman varied more than the behavior of those receiving either saline, $F(7,9)=5\ 45$, $p<0\ 05$, or the low dose, $F(7,9)=5\ 22$, $p<0\ 05$

Stone Maze

Initially all rats made errors in the Stone maze At the start of training, control rats made errors by entering one or both arms of a blind T, or by returning towards the start and entering or re-entering dead ends which they had already passed This type of error became less frequent as training progressed By the end of training, control rats had significantly decreased the incidence of both choice point errors, $F(7,3)=59\ 6$, $p<0\ 005$, and total errors, $F(7,3)=39\ 7$, $p<0\ 01$ Additionally, the control rats rarely entered more than one arm of a dead-end T

Two rats receiving 85 μg/kg soman were unable to consistently reach the goal in less than 10 minutes The behavior of five of the remaining eight rats was markedly dissimilar from that of the control rats After making an incorrect choice, these rats would remain within the arms of the T maze, alternating between the arms As many as 15 errors would be made before these animals would go back down the stem and return to the correct path This type of behavior, *perseveration,* would create a large increase in total errors (but count as only one choice point error) In addition, rats in the high dose group would, on occasion, enter sequentially all dead ends within a section of the maze and then retrace their path many times prior to proceeding to the next section of the maze This behavior would increase both types of errors Performance of rats in the high dose group was extremely variable However as a group, there was no tendency for the high dose group to demonstrate a reduction over time in either choice point errors, $F(7,1)=15\ 29$, $p=0\ 20$, or total errors, $F(7,1)=4\ 54$, $p=0\ 35$

Rats receiving the low dose of soman also demonstrated great variability in maze performance Two rats in this group showed the same type of repetitive behavior displayed by rats in the high dose group, whereas seven behaved similarly

to the control rats Overall, rats receiving the low dose of soman showed an improved performance over time, as measured by reductions in both types of errors [choice point errors, $F(7,3)=18\ 91$, $p<0\ 05$, total errors, $F(7,3)=12\ 13$, $p<0\ 05$] (Figs 3 and 4)

Although control rats decreased their errors over training and the high dose soman exposure group did not show a consistent decrease in errors, these differences were not significant for any specific block of trials [choice point errors, $F(14,38)=1\ 90$, $p<0\ 01$, total errors, $F(14,38)=1\ 15$, $p=0\ 35$] The lack of significant differences was due to the great variability of the rats receiving either the low or the high dose of soman (Figs 5 and 6) Variability of the total error score for both the low and the high dose soman groups was significantly greater than that for the saline-treated control group [high dose vs saline group $F(7,9)=9\ 58$, $p<0\ 05$, low dose vs saline group $F(9,9)=3\ 79$, $p<0\ 05$], and variability of the number of choice point errors was significantly greater for the high dose rats than for the saline controls, $F(7,3)=3\ 97$, $p<0\ 05$

Reactivity

Normal rats oriented to the stimuli but did not display aggression or struggle when picked up Twelve of the eighteen rats tested in the high dose group and four of the twenty rats tested in the low dose group demonstrated exaggerated reactivity to the test stimuli These animals typically jumped several cm in the air, attacked the stick, and reacted aggressively when the gloved hand attempted to pick them up This heightened reactivity continued throughout the entire period of testing, in spite of the daily handling used during some of the testing procedures (Fig 7)

The statistical analysis of the dose-response relationship of reactivity revealed that soman dose was related to an increase in reactivity for the animals in the activity test, $F(2,27)=9\ 89$, $p<0\ 001$, as well as in Stone maze procedure, $F(2,25)=8\ 86$, $p<0\ 001$ Rats receiving the high dose of soman were significantly more reactive that those receiving either the low dose or saline (Tukey post hoc analysis, activity test group, $p<0\ 05$, Stone maze test group, $p<0\ 05$) The significant increase in reactivity was highly correlated to the increase in open field activity shown by the high dose rats in the earlier test ($r=80$) Reactivity was also significantly correlated to performance on the Stone maze for rats in the high dose group ($r=80$)

Histology

Rats in the high dose group had pathology scores ranging from 0 to 15, with a mean score of $9\ 25\pm2\ 62$ The scores were clearly divided into two non-overlapping groups rats with pathology scores ranging from 0–2 had normal scores in reactivity (0–3) and in the Stone maze (mean choice point errors in the last block of trials ranged from 1 2–3 0), rats with pathology scores ranging from 12–15 had high scores in reactivity (4–9) and in the Stone maze (mean choice point errors in the trial block ranged from 7 4–8 4)

Rats in the low dose group had pathology scores ranging from 0 to 12, with a mean score of $4\ 5\pm1\ 67$ The association between pathology and behavioral abnormalities was not as clear-cut for the low dose group, but there was a trend toward higher pathology scores in rats with abnormal behavior scores Pathology scores of rats with high Stone maze error scores (mean choice point error score of 6 0–7 6 in the last block of trials) ranged from 4 to 12 Pathology scores of rats with low Stone maze error scores (mean choice point error scores of 0 2–4 8 in the last block of trials) ranged from 0 to 5

DISCUSSION

A single exposure to soman caused increased activity in the open field, impaired performance on the Stone maze and increased reactivity to various tactile stimuli These behavioral changes were highly correlated across all tasks That is, rats showing increased reactivity also had higher levels of activity in the open field Other rats showing increased reactivity demonstrated poorer performance in the Stone maze This correlation was especially pronounced in rats receiving 85 μg/kg soman

The behavioral changes measured by these tests remained apparent throughout the testing procedure, which was not completed until nearly four months after injection of soman Rats with poor performance at the beginning of testing remained deficient throughout the sequence with no evidence of recovery of normal functioning Even after extensive handling while being tested in the Stone maze several high dose rats continued to react aggressively to tactile stimuli Many soman-injected rats demonstrated exaggerated reactions to normal handling procedures often trying to jump out of their cages when the cage was being removed from the rack and attempting to escape or bite the experimenter when being picked up

Variability of performance among the soman-exposed rats was significantly greater than among the control rats The behavior of some soman-exposed rats in both the low dose and in the high dose groups was indistinguishable from that of the saline-injected controls on the tasks used However, other rats in both soman-exposed groups were clearly abnormal and hyperreactive The proportion of affected rats was dose-dependent More surviving rats in the high dose group (13/18) than in the low dose group (7/20) scored outside the normal range on at least one of the three tasks Individual differences in behavior were associated with differences in brain pathology Those rats with substantial pathology had abnormal scores in reactivity and the Stone maze, while those with minimal pathology had normal scores This result agrees with that found previously [7] soman-exposed rats were tested for DRL acquisition

Some rats in both soman-exposed groups failed to learn the Stone maze The repetitive behavior these animals demonstrated, both in the dead-end T's and in the segments of the maze, was continued throughout the entire testing procedure Animals with experimentally produced lesions of the fimbria-fornix and aged rats also have difficulty learning this maze and show increased activity in the open field task ([3], Shapiro, personal communication) Therefore, the behavioral changes seen in soman-exposed animals may be attributable to the soman-induced lesions in the hippocampus, which were evident in the pathology of rats which demonstrated behavioral changes, but not in that of rats which behaved like controls (cf [9])

A single subLD$_{50}$ exposure to organophosphorus compounds may produce neural lesions in exposed animals [8] The severity of the lesions may be a function of dose [9] These studies demonstrate concommitant behavioral changes, persisting long after the termination of soman exposure, which also vary as a function of soman dose Although animals given electrolytic neural lesions in the same areas affected by soman may show recovery of function after a period of time, animals receiving soman do not show any recovery The reasons for the apparent total lack of recovery indicate the necessity of future research

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