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Neurobehavioral Effects of Repeated Sublethal Soman in Primates

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GAUSE, E. M., R. J. HARTMANN, B. Z. LEAL AND I. GELLER. *Neurobehavioral effects of repeated sublethal soman in primates.* PHARMACOL BIOCHEM BEHAV **23**(6) 1003–1012, 1985.—Juvenile male baboons were trained to perform a match-to-sample discrimination task; effects of repeated sublethal exposure to the organophosphate nerve gas, soman, upon task performance were then explored. Both acute and subchronic exposure schedules were employed, and soman potency was verified by assay of soman-induced inhibition of acetylcholinesterase activity in whole blood, plasma, and erythrocytes. A characteristic profile of behavioral effects encompassing immediate, persistent, and delayed effects was observed. Immediate dose-related effects of soman included: increases in mean session response time, increases in errors, and decreases in extra responses. Seizures were also observed at the highest dose of soman employed (5 μg/kg). The increase in mean session response time was due to intermittent lapses in responding to stimuli (attentional deficits). Both the attentional deficits and intermittent generalized seizures were also persistent effects, with both occurring randomly after acute exposure to 5 μg/kg soman. Preliminary evidence suggests that occurrence of attentional deficits was associated with the occurrence of generalized and/or focal seizures; and that these effects may reflect irreversible lesions which become more threatening to the animal with increasing time. An additional, delayed effect was a sudden marked increase in the incidence of extra inconsequential responses which occurred several weeks after cessation of soman exposures.

Soman Discrimination behavior Baboon Attentional deficits Blood acetylcholinesterase Seizures

ORGANOPHOSPHATE compounds capable of inhibiting acetylcholinesterase activities include various compounds employed as insecticides as well as the extremely potent, irreversible inhibitors classified as nerve gases. Exposure to toxic doses of centrally-acting organophosphate compounds causes an accumulation of endogenous acetylcholine at both muscarinic and nicotinic sites within the brain, with severe biological consequences. One of the OP nerve gases is soman, O-1,2,2-trimethylpropylmethylphosphonofluoridate. Following exposure to toxic concentrations of soman, immediate symptoms include salivation, muscle tremors, prolonged generalized convulsions, and respiratory failure [17]. The lethal mechanism is considered to be failure of the respiratory system [13].

Extensive and irreparable CNS damage attributable to soman exposure has been described. Widespread axon degeneration was seen in the forebrain and midbrain regions of rats; it was concluded that overt seizures and neurological signs need not be present for damage to occur [22]. On the molecular level, acute, near-lethal doses of soman resulted in marked increases in glucose utilization in rat brain [17,23]. Repeated exposure to sublethal doses of soman produced decreased ligand binding to muscarinic receptors in rat brain [5]. The time course of recovery for the muscarinic receptors was compared to the recovery of brain cholinesterase activity; recovery of the enzyme activity slightly preceded the recovery of receptor ligand binding, suggesting that the two systems are associated [5,18].

From the types of brain damage produced by soman, it has been hypothesized that humans surviving soman exposure might have impairments of skilled movements, memory, cognition, autonomic regulation and psychiatric disorders [21,22].

Humans exposed to less toxic organophosphate compounds have shown persistent changes in mental functions. Elevated levels of anxiety were reported in men exposed chronically to OP pesticides, but not in those exposed acutely [15]. Metcalf and Holmes [20] found that workers exposed occupationally to OP compounds exhibited disturbed memory and difficulty in maintaining alertness and appropriate focussing of attention. Electroencephalographic measurements indicated that 9/12 workers complaining of lethargy, drowsiness, sleep problems and narcoleptic-like symptoms exhibited narcoleptic sleep records [20]. Duffy *et al.* [6] conducted EEG measurements upon industrial workers exposed accidentally to sarin, another OP nerve gas, and found statistically significant changes in brain electrical activity persisting more than a year after exposure. Differences included increased beta activity, increased delta and theta slowing, decreased alpha activity, and increased amounts of rapid eye movement during sleep.

Acute exposures of humans to an OP nerve agent described as EA-1701 produced psychological effects characterized by difficulty in sustaining attention and a slowing of intellectual and motor processes. Subjective effects of these same exposures included feelings of being generally slowed down, agitated, tense, and confused—conditions described as a state of altered awareness [3].

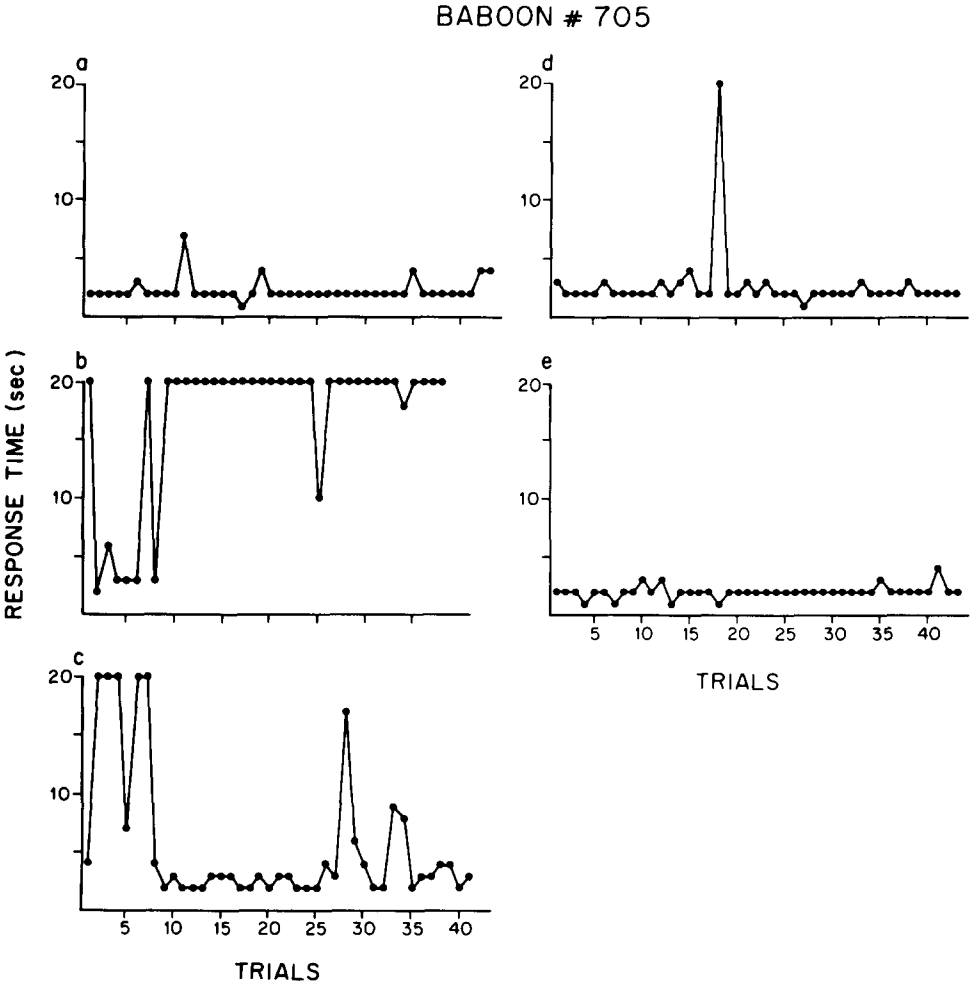

FIG. 1. Effects of 5 μg/kg soman upon trial-by-trial response times of Baboon 705 performing the MTS Discrimination Task. (a) day before soman treatment; (b) session initiated within 20 min after soman injection (whole blood AChE inhibited 63%; no overt neurological symptoms); (c) 24 hr after soman; (d) 48 hr after soman; (e) 120 hr after soman.

An investigation of neurobehavioral effects of soman exposure upon primate performance of a complex task which requires the integration of CNS functions and measures cognitive ability, alterness, memory and focussing of attention is in progress by our laboratory. Data presented in this paper describe effects of soman upon performance of a match-to-sample discrimination task by baboons; these results represent an extension of findings reported previously [9], as well as results of more recent experiments.

METHOD

Experimental Animals

Six male juvenile baboons *(Papio cynocephalus)* obtained from the Southwest Foundation for Biomedical Research Breeding Colony were employed. During the experiments reported herein, the ages and weights of these animals varied from 23–40 months and 6–10 kg.

Primate Discrimination Task

The match-to-sample (MTS) discrimination task employed, the training of baboons, and the collection and analysis of data resulting from the performance of this task have been described previously [9].

Blood Acetylcholinesterase Assay

Acetylcholinesterase (AChE) activities of whole blood, plasma and washed erythrocytes were determined by a coupled spectrophotometric assay based on the method of Ellman [8]. Acetylthiocholine was employed as substrate, as it provides a sensitive assay suitable for the comparison of multiple blood samples [4, 24, 26]. Blood was drawn into heparinized syringes, chilled, and assayed without delay. Percent hematocrit was measured; blood was then diluted and an aliquot reserved for RBC count and whole blood AChE. The remainder of the sample was centrifuged at 1000 g to separate diluted plasma from RBC pellet; plasma was subjected to a high-speed spin (3000 g) to remove small cells and debris and diluted further before assay for AChE; the RBC pellet was washed 1 time by resuspension in chilled saline and resuspended in saline for assay. All fractions were assayed at a 1:600 dilution ratio (in duplicate) and RBC

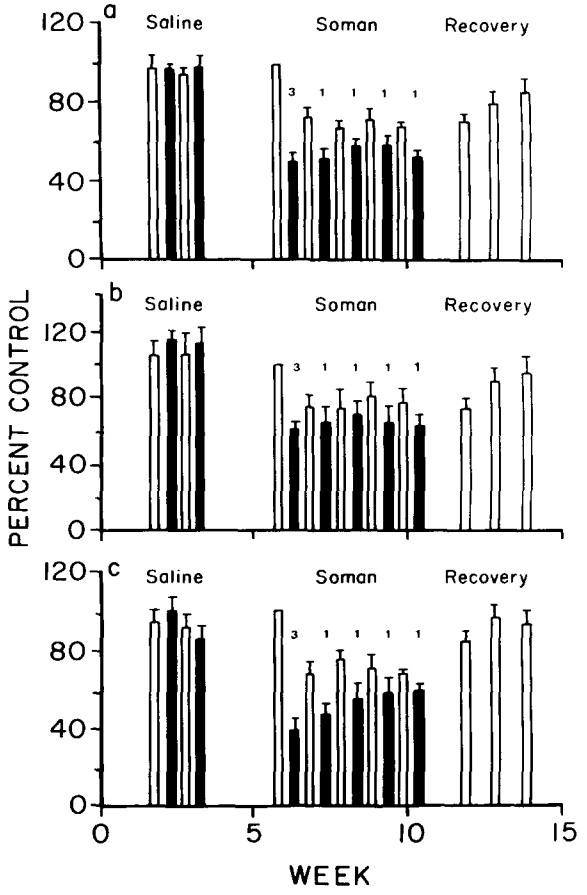

FIG. 2. Effects of subchronic soman exposure protocol upon baboon blood AChE activities. Blood samples were obtained immediately before the soman or saline injections preceding behavioral sessions, and within 20 min after completion of the 2-hr sessions. During recovery periods, blood was sampled after completion of the behavioral sessions at weekly intervals. Values shown are group means and S.E.M. for whole blood (a), plasma (c) and erythrocytes (b) expressed as percent control. Control (100%) level taken as value immediately preceding the initial 3 μg/kg dose of soman for each individual animal.

counts were performed on the dilutions assayed. The assay mixture contained 2.0 ml Tris buffer, 0.05 M, pH 7.4; 150 μl substrate, 30 μl dithiobisnitrobenzoic acid, and 1 ml diluted sample. Initial velocity was measured over the first 0.5 min after addition of sample.

Soman Exposures

Soman was obtained from USAMRICD in dilute solution and kept frozen (Revco, −80) until used. Appropriate dilutions in saline were prepared and baboons were administered intramuscular (gastrocnemius muscle) injections of soman as indicated. Soman was injected 10 min before initiation of the behavioral session. Blood samples were obtained immediately before each soman injection and within 20 min after completion of the 2-hr behavioral session, or approximately 150 min after soman.

Statistical Analysis

Grouped data were compared statistically by means of *t* and F (Repeated Measures ANOVA) tests. Single cages (individual baboon) experimental designs were compared by a one factor (Experimental Phase) randomized ANOVA program allowing for unequal sample sizes. Calculations were performed on an Apple IIe microcomputer with software by Human Systems Dynamics (Stats Plus and ANOVA II).

TABLE 1

PRE-SOMAN LEVELS OF BABOON BLOOD ACETYLCOHLINESTERASE ACTIVITIES

Whole Blood (μmol/hr/ml)	Plasma (μmol/hr/ml)	Erythrocytes (μmol/hr/cell × 10^6)
69.6 (13.4)	45.0 (9.9)	6.03 (0.96)

Mean and S.D. values of 3 determinations for each of 6 baboons prior to first subchronic soman exposure.

RESULTS

The effects of acute soman exposures upon MTS discrimination performance, and the persistence of these effects have been described in an earlier publication [9]. Additional analyses of some data obtained in these acute exposures are presented herein; however, the bulk of the information presented here is concerned with the effects of subsequent subchronic soman exposure upon baboon discrimination task performance and the persistence of these effects.

Acute Soman Exposures

Particular care was taken throughout all of these experiments not to administer a lethal dose of soman. The LD50 value for this species, sex, age, and route of administration has not been reported previously nor determined in this study; however, estimated values for other nonhuman primates indicate that the LD50 for this animal model is probably in the range of 6.5–7.5 μg/kg [16]. Accordingly, the highest dose administered to any baboon during the acute exposure experiments was 5 μg/kg; and subsequent soman injections were always withheld until blood AChE activity of each individual animal returned to at least 80% of the last pre-soman baseline level.

As reported previously, there were no effects of acute soman exposure upon discrimination task performance except at the highest dose levels employed (4 and 5 μg/kg). Within 1–24 hr of administration of 4–5 μg/kg soman, increases of mean session response times, decreases in extra inconsequential responses, decreases in responsiveness to visual stimuli, and increases in errors were observed. These effects generally lasted only 1–2 days. An additional delayed effect, a marked increase in extra responses, was consistently observed to occur 2–4 weeks post-soman and generally persisted much longer than the immediate effects; in one animal the elevated level of extra responses did not return to pre-soman baseline levels for 10–16 weeks [9].

The reported increase in mean session response time was a prominent effect of 4–5 μg/kg acute soman [9] and this increase generally persisted for one or more days after soman. Detailed analyses of the computerized recordings of these behavioral sessions revealed that this increase in mean session response time reflected a pattern of responses to stimuli in which one or more trials were missed entirely while the

TABLE 2

VARIATION IN BABOON BLOOD HEMATOLOGICAL VALUES DURING FINAL 3 WEEKS OF SUBCHRONIC SOMAN EXPOSURE AND SUBSEQUENT 3 WEEKS OF RECOVERY

Parameter	Sample	1 μg/kg	1 μg/kg	1 μg/kg	RW 1	RW 2	RW 3	Reference*
Erythrocytes	Pre-	5.07 (0.56)	5.31 (0.37)	5.31 (0.30)	5.09 (0.36)	4.96 (0.34)	5.15 (0.26)	4.97 (0.28)
($\times 10^6$/mm^3)	Post-	5.27 (0.23)	5.39 (0.37)	5.14 (0.22)	—	—	5.10 (0.36)	
MCV	Pre-	82.3 (7.5)	76.4 (3.4)	75.0 (2.3)	77.0 (2.0)	76.6 (3.4)	78.8 (2.0)	80 (5)
(μ^3)	Post-	77.3 (1.2)	75.4 (1.7)	75.8 (3.1)	—	—	77.8 (3.3)	
MCHC	Pre-	32.3 (0.6)	32.8 (1.3)	33.2 (0.8)	32.4 (1.1)	32.8 (0.8)	33.8 (1.5)	32 (1)
(pg)	Post-	32.3 (1.2)	32.6 (1.1)	32.8 (1.9)	—	—	34.2 (0.8)	
Hemoglobin	Pre-	12.8 (0.3)	13.2 (0.9)	13.2 (0.6)	12.7 (0.9)	12.6 (0.4)	13.4 (0.7)	12.6 (0.9)
(g/dl)	Post-	13.0 (0.5)	13.2 (0.9)	12.8 (0.2)	—	—	13.3 (0.9)	
Reticulocytes	Pre-	2.9 (1.5)	1.3 (0.5)	1.4 (0.5)	1.6 (0.6)	1.2 (0.6)	1.0 (0.6)	1.2 (0.4)
(%)	Post-	2.5 (0.5)	1.6 (0.4)	1.5 (0.5)	—	—	0.9 (0.8)	
Leukocytes	Pre-	7.57 (1.5)	7.60 (1.15)	7.10 (2.47)	8.44 (2.77)	8.20 (2.41)	7.34 (2.75)	7.6 (2.7)
($\times 10^3$/mm^3)	Post-	9.43 (1.76)	9.42 (2.51)	10.96 (4.54)	—	—	9.00 (3.18)	
Neutrophils	Pre-	33.7 (2.5)	38.6 (20.6)	34.0 (9.3)	30.4 (10.4)	25.2 (7.5)	32.4 (7.5)	44 (13)
(%)	Post-	40.7 (11.5)	42.2 (11.4)	44.6 (20.5)	—	—	31.2 (11.0)	
Lymphocytes	Pre-	62.7 (2.9)	58.4 (19.1)	60.4 (11.1)	65.2 (9.7)	70.4 (5.8)	65.0 (7.0)	53 (13)
(%)	Post-	57.3 (11.0)	53.4 (9.6)	51.4 (19.0)	—	—	66.0 (10.1)	

Values are means and S.D.; N=5 baboons.

*Hack, C. A. and C. A. Gleiser, *Lab Anim Sci* **32**: 502–505, 1982.

trials to which the animals did respond were generally performed as accurately, and sometimes as quickly as normal. Operationally, missed responses are defined as the failure to respond within 20 sec to either side stimuli, or within 30 sec to center stimuli.

Examples of this pattern of disrupted responding are shown in Fig. 1(a)–(e), which illustrates the effects of 5 μg/kg soman upon the individual trial response times during the 2-hr behavioral session, and the return to pre-soman responding patterns over a 6-day period for one baboon. Prior to receiving the highest dose of soman (5 μg/kg), baboon 705 exhibited a response time of approximately 2 sec to most of the 43 trials in the session (Fig. 1 (a)); the mean session response time was only slightly greater than 2 sec, or 2.23 sec, and for only one trial was the response time greater than 5 sec (6.8 sec). The trial-by-trial record for the session which was initated within 20 min of receiving 5 μg/kg soman on the following day is shown in Fig. 1(b). After missing the initial trial of this session, 705 responded quickly and correctly to the next 5 trials (approximately 35 min time span); the next trial was missed, followed by a normal correct response; however, after this, 705 let the next 36 trials go by without responding (a lapse of approximately 48 min) before making a correct but slow response; 8 more trials were missed (approximately 24 min) before the animal made another correct but very slow response. This animal did not exhibit any neurological signs such as convulsions, tremors, fasciculations, or salivation upon administration of soman. By the following day (24 hr post-soman), baboon 705 appeared to exhibit a significant behavioral recovery (Fig. 1(c)); the first response was normal, the next 3 trials were not attempted, followed by a correct, but slow response and 2 more missed trials before regaining his normal rate of correct responding after 20 min. The normal pre-soman baseline pattern of responding was maintained for about 1 hr before the animal began to exhibit a distinct increase in variability of rate of responding. By the second post-soman day, shown in Fig. 1(d), 705 appeared to have essentially regained his baseline level of responding quickly and rapidly except for one lapse; and after a weekend period of rest, he appeared to have fully regained his baseline rate of responding (Fig. 1(e)). This animal received no additional soman for the succeeding 5 months; however, he intermittently exhibited similar lapses in responding in which 1–5 trials at a time were missed on 16 different widely-spaced behavioral test days.

Two other baboons exhibited neurological symptoms shortly after receiving the highest dose (5 μg/kg) of soman. Baboon 735 experienced a generalized convulsion within minutes after the soman injection; he was immediately administered atropine sulfate and appeared to recover. He did not, however, respond to any of the trials during the 2-hr session immediately following the seizure. His response pattern was normal on the following day, but on the second day after soman, he missed 6 trials in a row (approximately 18 min lapse in responding). Since this time, both lapses in responding and documented seizures have become increasingly frequent for this animal in the absence of soman exposure. These generalized seizures/convulsions generally persist for 30–60 min.

The other baboon which exhibited overt symptoms when administered 5 μg/kg soman was baboon 573. This animal did not immediately appear to be affected by the soman injection; however, by the end of the 2-hr session he was in a crouched position, holding his midsection as if in discomfort. During this session he had a total of 25 min of lapses in responding. In the months after this exposure, this animal began exhibiting rather long lapses in responding with increasing frequency. During many of these lapses he ap-

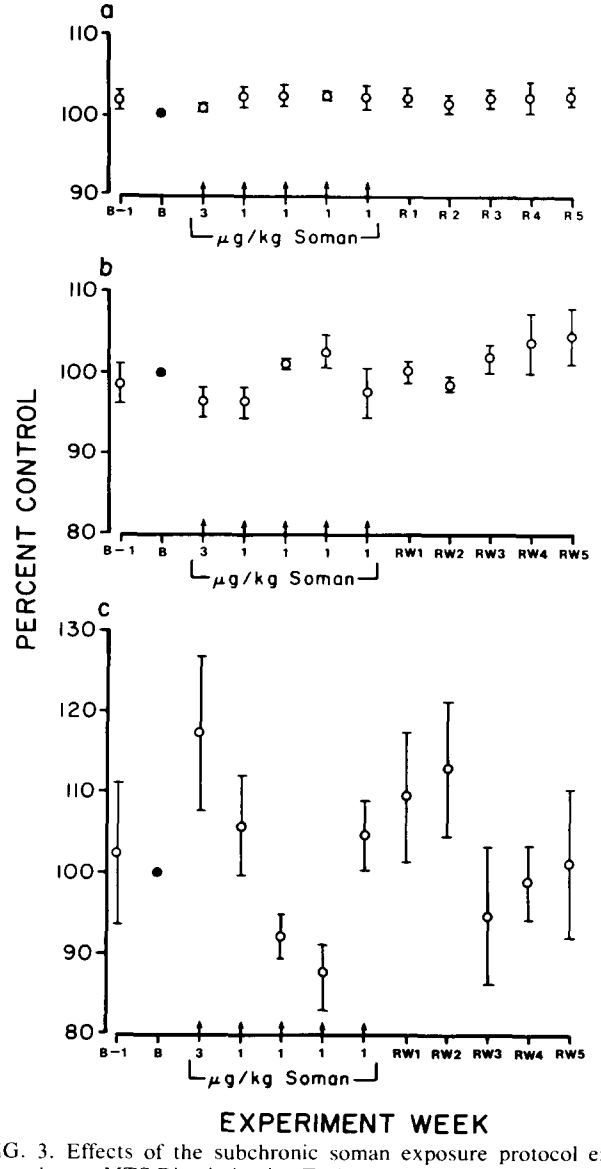

FIG. 3. Effects of the subchronic soman exposure protocol employed upon MTS Discrimination Task parameters. The mean of the 5 daily sessions during the week for each animal was compared to that animal's mean for the baseline week and expressed as percent control; these values were then averaged across subjects and the mean and S.E.M. values plotted as a function of experimental week. Baseline week (100% Control) was the week immediately preceding the initial subchronic (3 μg/kg) dose of soman. Statistical significance was examined by means of a Repeated Measures ANOVA comparing Experimental Week with Baseline Week. (a) Percent correct responses ($F=1.042$; $p=0.428$); (b) Percentage of trials worked ($F=1.234$; $p=0.293$); (c) Mean session response time ($F=1.584$; $p=0.137$).

peared to be comatose; however, at other times this animal gave no indication of being drowsy during such lapses. Other animals exhibiting similar lapses occasionally appeared to be dozing or lethargic.

None of the animals exhibited significant lapses in responding until after they had received the 4 or 5 μg/kg dose of soman. After receiving this dose, 4 of the 6 baboons began exhibiting these abnormal patterns of responding intermittently; 1 of the 2 remaining animals did not exhibit this type of response pattern until much later (after the first subchronic exposure), when he exhibited lapses in responding for 3 days in a row and died overnight after the third day. In this case, death occurred 5 days after the animal received the initial subchronic exposure of 3 μg/kg. The veterinarian's diagnosis of death was "bloat;" this death therefore cannot be definitely attributed to soman treatment. The 6th baboon has continued to maintain his normal baseline pattern of responding without any lapses to date. The frequency of recurrence of these gaps in responding is different for individual animals; however, for all animals, the frequency appears to be increasing with time.

Subchronic Soman Exposures

After completion of the acute soman exposures, the baboons were maintained on the behavioral performance schedule, but were not administered soman or subjected to any invasive technique for 4 months. Prior to initiation of subchronic soman experiments, animals were administered saline 3 times at weekly intervals. During the subchronic exposure experiments, doses of soman were chosen below the levels tolerated acutely by all animals; blood AChE was also monitored closely. Soman at 3 μg/kg was administered to all animals and exactly 1 week later 1 μg/kg soman was given; the 1 μg/kg dose was then repeated at weekly intervals 3 more times. In other words, the subchronic exposure protocol included 5 weekly injections of soman: 3 μg/kg initially, followed by 1 μg/kg on each of 4 subsequent weeks.

The effects of the subchronic soman exposure protocol employed here upon blood AChE activities are illustrated in Fig. 2. Blood samples were obtained before and after each injection of soman or saline, or at weekly intervals during the recovery period. Figure 2 is a bar graph of the group mean and S.E.M. values for whole blood, plasma, and erythrocyte AChE activities for each week of treatment or recovery. Data are expressed as percent control, with control (100%) level taken as the value immediately preceding the initial 3 μg/kg dose of soman. The actual values of control AChE activities measured for these animals are shown in Table 1. The subchronic exposure protocol produced and maintained some degree of inhibition of both plasma and erythrocyte AChE activities throughout the 5 weeks of exposure; this can be seen from the weekly pre-soman levels (Fig. 2(a)–(c), open bars). It can also be seen that after the initial 3 μg/kg dose of soman, each subsequent 1 μg/kg dose produced some reduction in AChE activities (Fig. 2(a)–(c), shaded bars), but these doses were not sufficient to maintain the inhibition resulting from the 3 μg/kg dose. After the last 1 μg/kg soman dose, both RBC and plasma activities appeared to be returning to control range within 3–4 weeks; blood sampling was therefore discontinued.

In addition to AChE activities, values of hematocrits and RBC counts were monitored throughout the subchronic exposure phase. Measurements of a range of other hematological parameters were also obtained during the latter part of this exposure phase. Over the entire subchronic exposure phase, there was little or no change in either hematocrit values or RBC numbers accompanying the changes in AChE activities (data not shown).

For the last 3 1 μg/kg soman exposures, blood samples taken before the pre-session soman injection and after completion of the behavioral session were submitted to an in-house Clinical Chemistry Laboratory for measurement of the values shown in Table 2. These values are compared to

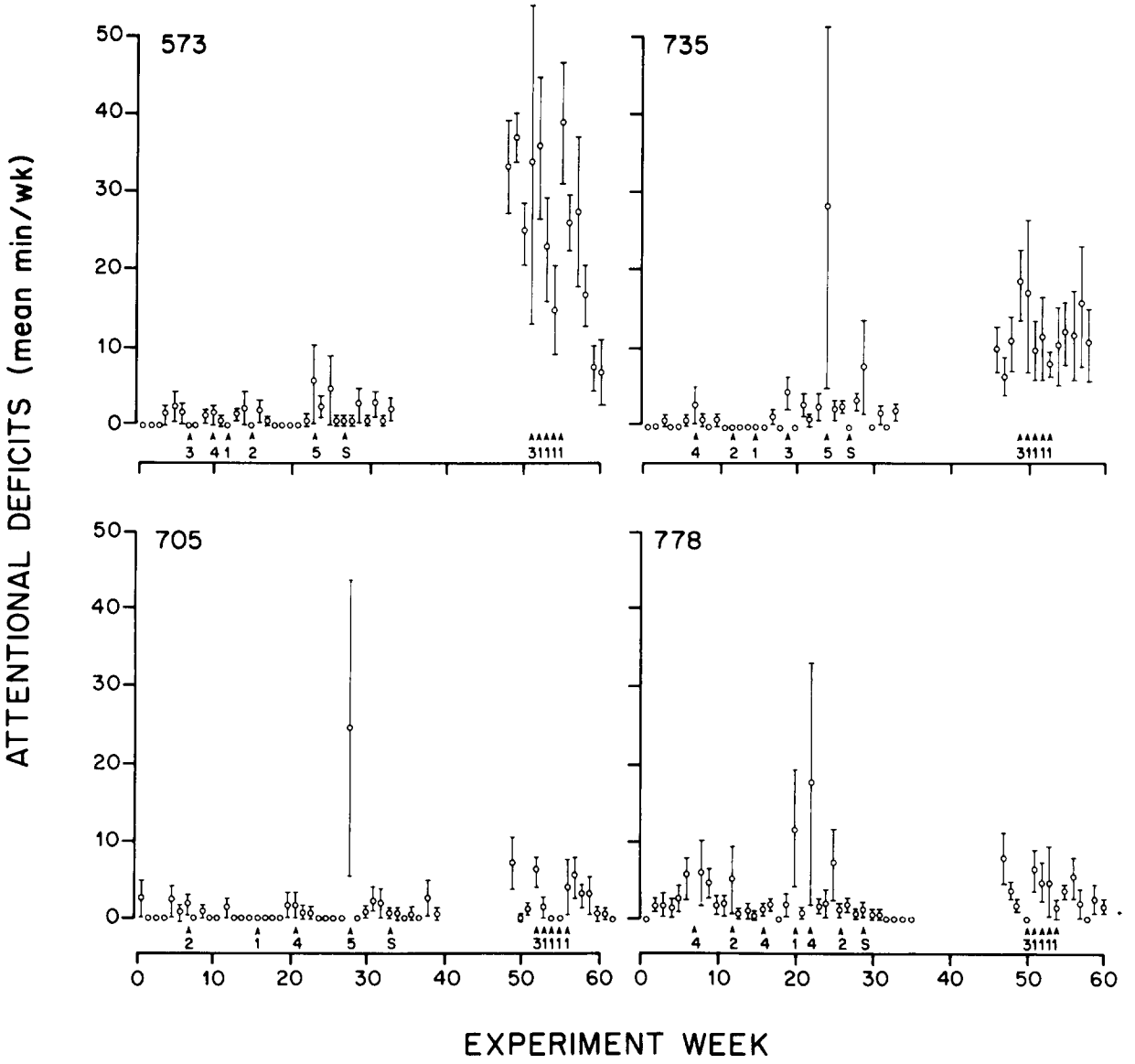

FIG. 4. Profiles of the occurrence of attentional deficits to the MTS Discrimination Task for 4 individual baboons as a function of exposure to soman. Lapses in attention to the task are plotted as weekly means and S.E.M. of lapse duration in minutes. Soman treatments are shown as arrows and numbers corresponding to the dose in micrograms/kg above the horizontal axis; "S" denotes saline treatment. The initial 30–40 weeks comprise the acute exposure experiment which includes 6 weeks pre-exposure baseline, 4–6 acute exposures as indicated, and 6-weeks of no treatment baseline recovery. The subchronic exposure experiment includes weeks 45–62 and consists of 3 weeks pre-exposure baseline, 5 weeks of 1 exposure/week as indicated, and 6 weeks of no treatment baseline recovery. Baboons 573 and 735 exhibited overt neurological symptoms when administered the 5 microgram/kg dose of soman. Another baboon exhibited no attentional deficits throughout the acute exposure experiment or subchronic baseline period; however, after the initial subchronic soman exposure he exhibited deficits for 3 days in a row and died overnight in his home cage. A sixth baboon has not exhibited any attentional deficits to date.

reference values for juvenile male baboons from the same colony published previously by this same laboratory [11]. For all parameters, experimental animal means and S.D. values for all time points were within control range (Table 2), indicating that major changes in blood cell populations were not induced by the subchronic soman experiment.

Effects of this subchronic soman exposure protocol upon operant behavioral parameters expressed as group (N=5) weekly means are shown in Fig. 3 (a–c). For these grouped comparisons, the week immediately preceding the initial subchronic (3 μg/kg) dose of soman was used as the baseline, or 100% control, level of performance; values shown represent weekly means and S.E.M. of all animals. The accuracy of performance of the MTS discrimination task was not affected by this repeated soman exposure; all animals maintained the pre-soman 90–100% level of correct responding (Fig. 3a). The percentage of trials worked was decreased (non-significantly) by the initial higher (3 μg/kg) dose; however, this parameter returned to baseline range for the remainder of the soman exposure period (Fig. 3b). Although

TABLE 3

OCCURRENCE OF ATTENTIONAL LAPSES (MIN/WEEK) IN 4 BABOONS EXPOSED TO SOMAN

Baboon	Experimental Phase	Statistics Mean (S.D.)	ANOVA Comparison	F	p
573	A	0.86 (1.00)			
	B	3.12 (2.02)	A × B	5.70	0.0426
	A′	31.57 (6.28)	A × A′	157.22	<0.001
	B′	28.44 (9.75)	A × B′	48.49	<0.001
	A″	16.60 (9.86)	A × A″	15.44	0.003
735	A	0.20 (0.31)			
	B	7.98 (13.2)	A × B	2.22	0.1725
	A′	8.79 (2.19)	A × A′	102.72	<0.001
	B′	12.54 (4.48)	A × B′	46.33	<0.001
	A″	11.70 (2.15)	A × A″	171.03	<0.001
705	A	0.90 (1.14)			
	B	6.87 (11.8)	A × B	1.62	0.2358
	A′	2.67 (3.71)	A × A′	1.28	0.2954
	B′	2.26 (2.65)	A × B′	1.31	0.2825
	A″	2.36 (2.09)	A × A″	2.16	0.1732
778	A	2.14 (1.81)			
	B	7.02 (7.26)	A × B	2.62	0.1420
	A′	4.41 (3.05)	A × A′	2.06	0.1930
	B′	3.34 (2.60)	A × B′	0.81	—
	A″	2.72 (1.96)	A × A″	0.26	—

Experimental design: ABA′ A′B′A″.

A=6 weeks prior to acute soman; B=week of highest acute soman dose plus 3 subsequent weeks; A′=3 weeks prior to initiation of subchronic soman experiment; B′=5 weeks of subchronic soman experiment; A″=5 weeks post subchronic soman exposures.

the accuracy of responding and the performance efficiency were not affected by this subchronic soman exposure protocol, there were more apparent (non-significant) effects upon mean session response time (Fig. 3c). The initial 3 μg/kg dose of soman produced an increase of approximately 150% in mean response time, and subsequent 1 μg/kg doses produced increases in variability.

As described above, the measure of mean session response time reflects the occurrence of lapses in responding, or deficits in attention to the discrimination task. Depending upon the dose of soman and the individual animal, these subchronic exposure-associated attention deficits occurred on the day of soman administration and/or 1–2 days following soman. These attention deficits occurred throughout the subchronic exposure phase and appeared to increase in frequency with time for most of the animals. However, one baboon (3835) has not exhibited these attention lapses to date. The individual profiles of attentional deficits for 4 of the 6 baboons throughout both pre-exposure, acute exposure, acute recovery, subchronic exposure, and subchronic recovery periods is shown in Fig. 4. These lapses in attention to the MTS task are plotted as weekly means (and S.E.M.) of lapse duration in minutes.

As can be seen from Fig. 4, the occurrence of attentional lapses is essentially an all-or-nothing response which occurs at different times and with differing frequencies of recurrence for different animals. For this subchronic experiment, the relationship between soman dose and the extent of attention deficits during the week appears complex, perhaps reflecting the fact that these are not naive animals. In 3 of the 5 baboons, the initial 3 μg/kg dose produced increases in attentional deficits, while a decrease was seen in one animal and no change in the other (Fig. 4). The succeeding 1 μg/kg doses produced both increases and decreases over baseline levels.

If soman exposure produces central lesions, then post-soman behavioral baselines would be expected to differ from pre-soman baselines, and the experimental design employed in this study could be considered as: ABA′—A′B′A″, in which A is the original pre-soman baseline; B is the acute soman exposure phase; A′ is the post-acute soman recovery baseline and the pre-subchronic soman baseline; B′ is the subchronic soman exposure phase; and A″ is the post-subchronic soman exposure recovery baseline phase. The 4 baboons exhibiting attentional deficits can be treated as 4 single cases of this experimental design [2,14]. The occurrence of attentional lapses (min/week) during each phase for these 4 baboons was subjected to an analysis of variance and the results are summarized in Table 3. Baboons 573 and 735, the 2 animals which have exhibited soman-induced seizures and convulsions, demonstrate marked increases in the extent of attentional deficits per week and significant differences in variability between experimental phases (Table 3). The other 2 baboons shown in Table 3 (705 and 778) also exhibited soman-associated increases (non significant) in attention deficits; 778 alone appeared to be approaching his original pre-soman baseline after the subchronic exposure phase (A″, Table 3).

For baboon 735, the onset of lapses in operant responding appears to be associated with the occurrence of generalized seizures; and the altered pattern of responding persists for several days after a seizure. The relationship between seizures and attentional deficits is illustrated in Fig. 5. This animal was discovered to be recovering from a seizure early one morning in his home cage; no treatment was administered and his regular behavioral session was initiated approximately 45 min later. This seizure occurred 8 days after the last subchronic soman exposure of 1 μg/kg (i.e., during Recovery Week 1). A blood sample obtained the day before this seizure yielded RBC and plasma AChE assays of 81% and 90% of initial (Subchronic Exposure Baseline) levels, respectively. Figure 5(a) is the individual trial response time pattern of the day before this seizure; however, it should be noted that an earlier seizure was recorded 2 days before this session and residual effects of this prior seizure are evidenced in a greater variability in response time than was observed initially prior to soman exposures. Figure 5(b) is the response time pattern of the session which was initiated within 45 min after the generalized seizure on the next day; 735 did not respond to the first 5 trials (15 min lapse), he then began to respond and maintained fairly good responding until the end of the first hr; he again missed a string of 3 trials before beginning to respond slowly on several trials. Difficulty in responding normally was also observed on the following day (Fig. 5(c)).

Baboon 573, the animal which exhibited abdominal cramps 2–3 hr after the 5 μg/kg soman dose and subsequently had a history of frequent attentional lapses, was observed to experience a generalized epileptic-type seizure 19 weeks after the last subchronic soman exposure. Diazepam (5 mg IV) and Prednisone (2 mg IM) were administered and

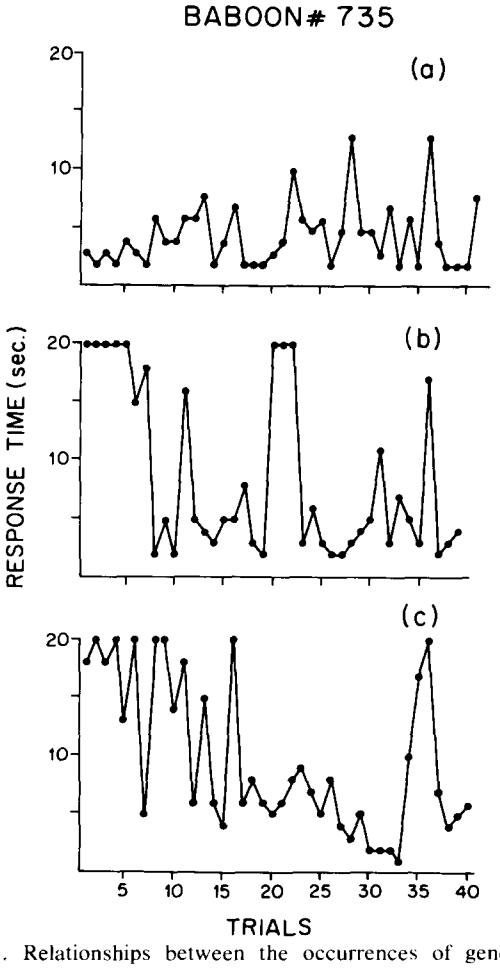

FIG. 5. Relationships between the occurrences of generalized epileptic-type seizures and of attentional deficits to performance of the MTS Discrimination Task (shown as within session trial-by-trial response time patterns). During recovery from subchronic soman exposures, Baboon 735 was experiencing intermittent seizures to which the unusual variability in baseline performance seen in (a) is attributed; a seizure was documented 2 days prior to the MTS session shown in (a); blood was taken after this session and plasma and RBC AChE levels were 90% and 81% of initial baseline level (prior to first subchronic soman treatment). The session shown in (b) was initiated within 45 min after occurrence of a generalized seizure on the morning of the following day. The response time pattern shown in (c) was observed on the next day and presumably reflects residual effects of this seizure as no other seizures were documented in the 24-hr interval between (b) and (c).

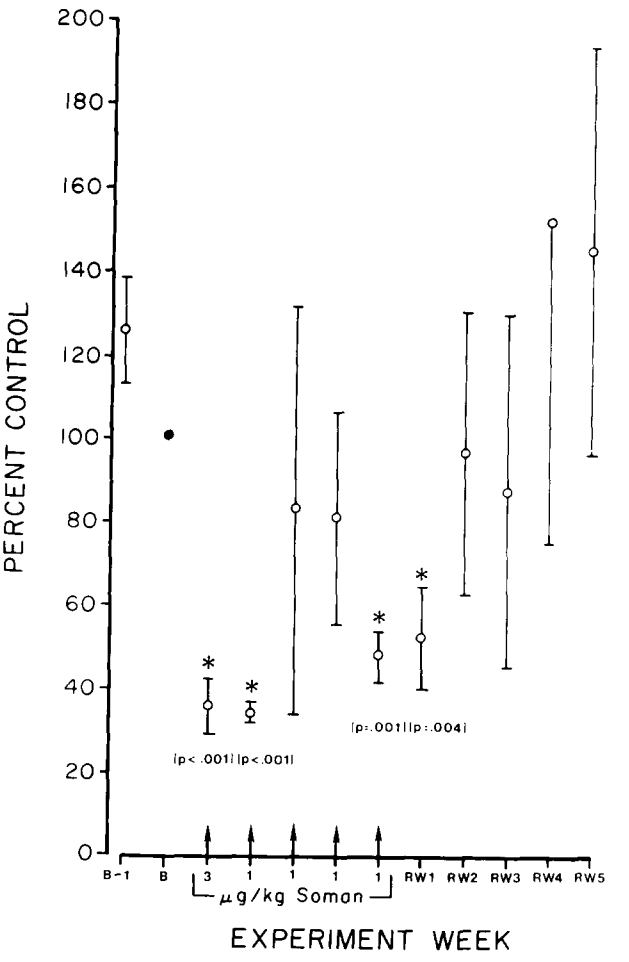

FIG. 6. Effects of subchronic soman exposure upon the incidence of extra inconsequential responses during performance of the baboon MTS Discrimination Task. The mean of 5 daily sessions during a week for each animal was expressed as percent of control week (week prior to initial subchronic soman dose of 3 micrograms/kg); the percent control values were then averaged across animals and these means and S.E.M. plotted. Experimental week is indicated as: week before baseline week (B-1), baseline (B), dose of soman administered during week (3, 1, 1, 1, or 1 microgram/kg), or week of recovery (RW1, RW2, RW3, RW4, or RW5). Statistical significance was examined by a t-test for significance for each experimental week compared to baseline week, and by a Between-Within ANOVA comparing Phase (Pre-soman, Soman, and Recovery) and Experimental Week (Phase: $F=8.742$, $p=0.042$; Week: $F=1.393$, $p=0.280$; Phase × Week: $F=0.603$).

exactly 2 weeks later this animal experienced another similar seizure.

For all animals, a delayed effect upon the incidence of extra inconsequential responses occurred several seeks after the completion of the subchronic soman exposures, similar to the increase reported previously for the acute soman exposures [9]. This marked increase in extra responses occurred during different weeks of the recovery period for different animals and these individual differences in timing are reflected in the increased variability of the group means (and S.E.M.) seen in Fig. 6. It can also be seen that the first 2 subchronic soman exposures depressed extra responses below the baseline level (Fig. 6), an effect which is also consistent with effects of the earlier acute exposures.

DISCUSSION

Repeated, sublethal soman administration appears to produce a characteristic profile of effects upon baboon discrimination behavior performance. This profile encompasses immediate, persistent, and delayed effects.

This characteristic pattern of effects upon discrimination performance has now been observed in 2 different, widely-separated series of experiments. It should be pointed out, however, that both experiments involved the same 6 baboons, and that validation of these findings will require additional studies of more naive animals. It is known that exposure to sublethal levels of soman can produce tolerance to subsequent soman [25], and it is possible that the initial acute

exposures could have produced some degree of neurobehavioral tolerance in our baboons.

Consistent features of the profile include immediate dose-related effects of: (a) increases in mean session response time, reflecting the occurrence of lapses in responding to stimuli; (b) increases in errors; and (c) decreases in the incidence of extra inconsequential responses. An important dose-related immediate effect is the occurrence of seizures after the highest dose employed in these experiments (5 μg/kg). This dose level would appear to be the threshold for seizure induction in the juvenile male baboon. One animal (735) exhibited generalized convulsions and loss of consciousness within 10 min of receiving this dose of soman. Another baboon (573) exhibited abdominal cramps (abdominal epilepsy?) within 2–3 hr after this dose. Both of these symptoms are characteristic of organophosphate nerve agents [3]. Four of the 6 baboons did not exhibit any signs of seizures or other neurological signs at the highest soman dose level, although 2 of these animals did exhibit immediate and recurring attentional deficits. One baboon (3835), however, has exhibited no sign of seizure or attentional deficit to date. This apparent decreased sensitivity of this animal appears to reflect individual differences in susceptibility and not any experimental artefact in dosing with soman as a typical dose-related inhibition of blood AChE with 5 μg/kg producing 77% inhibition was measured.

The lapses in responding, or deficits in attention to performance of the task, and the intermittent generalized seizures are also persistent, as well as immediate, effects. After soman exposure, both these attentional deficits and seizures recur on an intermittent, random basis for periods of weeks to months in the absence of additional soman exposure. In addition, a delayed effect is the sudden, dramatic increase in the incidence of extra responses which occurs weeks after termination of soman exposures and may persist for a number of weeks.

The persistent and delayed effects of sublethal soman—i.e., the residual, intermittent attentional deficits, the recurring generalized seizures, and the marked transient increases in extra responding—are of particular interest. The possible implications of the increases in extra responding have been discussed previously [9].

The soman-associated seizures observed for 2 of the 5 baboons in the present study appear to differ in significant ways from either the spontaneous seizures observed in other *Papio cynocephalus* from the SFBR breeding colony, or the seizures induced by intermittent light stimulation in *P. papio*. One of the 2 baboons (735) convulsed immediately after receiving the highest dose of soman administered (5 μg/kg) and was treated with atropine sulfate; additional seizures occurred in the absence of further soman treatment. For this animal, seizures were generally full-blown tonic/clonic convulsions lasting 30–45 min. The other baboon (573) exhibited abdominal cramps within 2–3 hr after the same dose of soman, and much later exhibited several seizures which were not as severe as those seen in 735, but also lasted 30–40 min. For both of these animals, generalized seizures appear to be occurring more frequently with increasing time. Spontaneous seizures, which are observed in a small percentage of the baboons from the same breeding colony as these 2 baboons, appear to be quite different from the generalized seizures associated with soman exposure. The spontaneous seizures are generally of 20–30 sec duration, occur principally in animals which are born blind, and disappear by the time animals reach 2–3 years of age (R. J. Haines, personal communication). Seizures induced by intermittent light stimulation (ILS) in *P. papio* have been described as paroxysmal responses which generally cease abruptly upon termination of stimulation, except for a small percentage of animals which may exhibit tonic/clonic grand mal seizures in response to stimulation [12]. *P. cynocephalus*, however, have been reported to be much less susceptible to photic seizures than *P. papio* [1]. The recurring soman-associated seizures would appear to be similar to the "exceptionally long seizures with sustained motor activity" induced by bicuculline administration [19].

The persistent attentional lapses described here represent atypical behavior for baboons performing the MTS task in the absence of drug treatment. The MTS discrimination task with baboons has been used in our laboratories for many years and this type of persistent, recurring failure to respond to one or more trials or blocks of trials during a session has not been observed previously. The immediate effects of soman administration upon trial-by-trial responses and response times resemble in some ways the immediate effects of CNS-depressive doses of phenobarbital; however, the phenobarbital effects do not recur after clearance of the drug within a day, whereas the soman-associated effects recur rather frequently on a random basis for at least 1 yr after soman, suggesting the involvement of CNS lesions. In fact, the apparent link between generalized seizures and the onset of the MTS attentional deficits in 2 baboons point to the possibility that the attentional deficits could be manifestations of focal epileptic seizures.

The persistent attentional deficits observed here could represent an operant behavioral analog of the mental confusion defined as "lapses in attention" for humans exposed occupationally to organophosphorous insecticides [7]; or as narcoleptic-like symptoms, or as difficulty in maintaining appropriate focussing of attention [20]. Durham *et al.* [7] concluded that: (1) lapses of attention, due either to "blanking out," an "inappropriate sense of well-being," or other causes, are relatively common in persons with extensive exposure to OPs; and (2) these lapses are recognized only when they are sufficiently prolonged and/or occur during critical situations (such as the operation of aircraft). It is possible that the daily performance of an operant task requiring integrative CNS functions, such as the MTS discrimination task, could provide an uniquely appropriate means of detecting the occurrence of such attention lapses.

It is rather surprising that repeated sublethal soman exposures sufficient to reach a seizure threshold and to cause persistent recurring focal and generalized seizures did not produce strong or persistent effects upon discrimination performance accuracy. There were also no effects upon other performance parameters such as mean response time and percent trials worked except for the intermittent attentional lapses. The lack of strong effect of soman upon discrimination performance contrasts with the pronounced effect of prior repeated exposure to soman upon ability to learn an operant avoidance task [10].

Results of these studies indicate that sublethal soman exposure may produce CNS lesions which are not seriously incapacitating at the time of exposure, but which may worsen and not only impair task performance, but become life-threatening at some later time. The persistence of neurobehavioral effects in the baboon observed here appears to be consistent with the persistent changes in brain electrical activity related to sarin exposure reported by Duffy *et al.* [6].

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