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On the Development of Behavioral Tolerance to Organophosphates IV: EEG and Visual Evoked Responses

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WOLTHUIS, O. L., I. H. C. H. M. PHILIPPENS AND R. A. P. VANWERSCH. *On the development of behavioral tolerance to organophosphates IV: EEG and visual evoked responses.* PHARMACOL BIOCHEM BEHAV **39**(4) 851–858, 1991.—Several earlier studies showed that, in contrast with DFP, repeated injections with soman did not lead to behavioral tolerance in rats. The reason for the difference between the effects of these two organophosphate cholinesterase inhibitors was not clear and a neurophysiological approach was undertaken. Four experiments (A, B, C and D) were carried out, each consisting of three groups of rats, SC injected with saline, DFP (600 μg/kg) or soman (60 μg/kg) respectively. In Experiment B and D the rats were trained to criterion in a two-way shuttlebox. Thereafter, the animals of Experiment B were fitted with suitable electrodes and two days later their EEGs and visual evoked responses (VERs) were recorded, 1 and 24 h after a single dose of the above-mentioned compounds. In Experiment D the trained animals were subsequently injected 3 times per week for 4 weeks with the same doses and their performance was tested 5 days per week, 1 and 24 h after injection. After those 4 weeks, when the DFP-treated animals had developed behavioral tolerance, electrodes were fitted and EEGs and VERs were recorded after two days, again 1 and 24 h after injection, as in Experiment B. The difference with Experiments A and C was that these animals were not trained. Otherwise, treatment schedules and recording procedures of Experiment A were identical to those of Experiments B and of Experiment C to those of Experiment D. In all cases the EEGs and VERs were recorded from animals slowly walking in a rotating hollow transparent wheel. The results show a similar pattern in all four experiments. The shapes of the averaged VERs remained essentially the same in all experiments; differences in latencies and amplitudes, which were observed 1 h after the injections of the cholinesterase inhibitors and had disappeared 23 h later, were interpreted as being quantitative in nature. On the other hand, the EEGs showed qualitative differences between the effects of the two organophosphates in all experiments, notably a clearcut extra peak in the low frequency range (2–2.5 Hz) of the EEG power spectrum 1 h after injection of soman, which had disappeared 23 hours later. A slight increase in beta-activity following soman was noted, but was not consistently present in all experiments. The EEG power spectra 24 h after the injection of both organophosphates only showed a high degree of synchronization in the 8 Hz range, compared with their respective saline-injected controls. The differences found could not be ascribed to changes in body temperature, pupil diameter or sensitivity to footshock. It is suggested that this extra peak detectable 1 hour after soman is due to a second, reversible effect of this compound, unrelated to its cholinesterase-inhibiting effect. This low frequency peak may be linked to the persistently recurring behavioral decrements found 1 h after the soman injections and may camouflage the detection or prevent the development of behavioral tolerance to the cholinesterase-inhibiting effects of soman.

Soman DFP EEG VER Organophosphates Tolerance

IN previous studies in our laboratory (15, 21, 26), shuttlebox performance of rats was tested 1 and 24 hours after repeated injections with the irreversible organophosphorous cholinesterase inhibitors DFP or soman. The injections, with doses that caused no overt symptoms, were given three times per week. The aim of the studies was to investigate the mechanism whereby tolerance to organophosphates develops in the hope that knowledge of these mechanisms would provide clues for an alternative therapy against soman, e.g., by mimicking pharmacologically a state of "instant" tolerance. Such a therapy would, in theory, be independent of the type of cholinesterase inhibitor.

In these studies the performance decrements found one hour after the first DFP injections gradually disappeared in the course of approximately three weeks. This phenomenon is called behavioral tolerance and has been reported by several authors for many cholinesterase inhibitors (4, 6, 19). Surprisingly, the performance decrements found one hour after the injections of soman persisted during the six weeks that the experiments lasted. When tested 24 hours after the injections, however, performance in both the DFP- or soman-treated animals was close to that of saline-injected animals. In the course of the biochemical, behavioral and electrophysiological experiments that followed this initial finding, possible explanations for these different behavioral effects of these two inhibitors did not emerge. This left us with the unsatisfactory conclusion that apparently behavioral tolerance does not develop after repeated injections of soman.

Registrations of spontaneous or evoked electrical activity of the brain, together with behavioral methods, have long been known to be useful techniques to detect and to differentiate between effects of several compounds (12) and have in recent years been successfully used in our laboratory (26). Therefore, it was decided to apply these techniques to animals chronically injected

with saline, DFP or soman, using the same procedures as before. The results of four successive experiments show that soman and DFP have quantitively different effects on the visual evoked responses (VERs). On the EEG, however, the effects of these two inhibitors do not only differ quantitatively, but also qualitatively: one hour after the injection of soman an extra low frequency peak is detectable in the EEG power spectrum which is absent one hour after the injection of DFP or saline. Measured 24 hours later, most of the organophosphate-induced changes have disappeared, only a high degree of synchronization at approximately 8 Hz remains. The results indicate that soman, in addition to cholinesterase inhibition, causes a second (reversible) effect that might be responsible for the performance decrements that persistently recur 1 hour after the repeated injections of soman.

METHOD

Animals

Male Small Wistar (Wag/Rij) rats, with a starting body weight of 150–170 g, were used. The animals were bred in the laboratory under SPF conditions, i.e., hysterectomy derived, bacteriologically controlled and kept under sterile conditions. All animals were experimentally naive. During the whole experimental period and during testing the animals were kept in thermostatically controlled environment (23 ± 1°C).

Procedures

Four experiments were carried out:

After a single injection:

Experiment A: EEG and VER measurements in untrained animals.

Experiment B: EEG and VER measurements in trained animals.

After repeated injections:

Experiment C: EEG and VER measurements in untrained animals.

Experiment D: EEG and VER measurements in trained animals.

In each experiment three groups were present, injected subcutaneously with saline, DFP or soman respectively. In all experiments the dose of DFP was 600 μg/kg and the dose of soman was 60 μg/kg. Group size in all cases was 6–8 animals.

In Experiment B the animals were trained for active avoidance in a two-way shuttlebox as described before (21,26). Briefly, the animals received 20 trials per day at time intervals of 1 min (±20% random) and were trained to avoid footshock (250 μA, constant current principle) by moving into the other compartment within 10 s after a light stimulus was presented. After the animals had reached criterion, which was 80% or more correct avoidance responses (CARs), preparations to obtain the neurophysiological parameters were started. The rats were anaesthetized with hexobarbital-Na and a small hole was drilled in the skull, 6.0 mm caudal and 3.6 mm lateral of bregma, i.e., over the caudal part of cortical area 17, where in our rats the largest visual evoked potentials are found. The holes ended at the dura mater; a silver electrode of 0.4 mm diameter was fitted into the hole and fixed with dental cement. A silver reference electrode (connected to earth) was placed on the outside of the skull between the eyes and was also fixed in place (tip free) with dental cement. Recordings of the EEGs and VERs were carried out approximately 48 hours later and took place 1 and 24 hours after the injections.

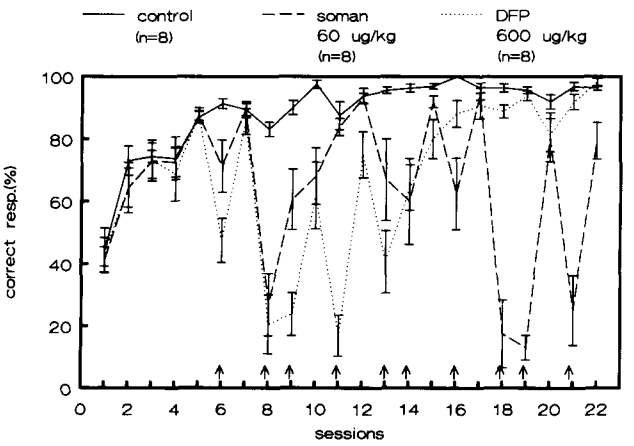

FIG. 1. Shuttlebox performance of rats repeatedly injected subcutaneously (see arrows at bottom) with saline (—), 600 μg/kg DFP (....) or 60 μg/kg soman (- - -). The animals were injected three times per week and tested 5 days per week, except during weekends between sessions 8 and 9, 13 and 14, 18 and 19. It can be seen that, in contrast with DFP, tolerance does not develop after repeated injections of soman. The effects on the EEGs are shown in Fig. 3 (Experiment D).

As in earlier experiments (25), for these EEG and VER measurements the rats were placed in a vertical motor-driven hollow wheel of Plexiglas, with a diameter of 50 cm and a tread of 14 cm. Circumference speed of the wheel was 10 cm/s. On one side of the wheel, opposite the axial connections to the electromotor, there was a central hole in the Plexiglas through which the rats could be put in and taken out of the wheel and through which the thin wires ran that connected the electrodes to the amplifiers. Total recording time was 10 min, i.e., 5 min for EEG recordings and 5 min to record the VERs. These recordings took place in a test cubicle (2 × 2 × 2.5 m), sound-isolated and dark. Each animal was allowed a 5-min adaptation period in the running wheel before recordings started.

For the EEGs, filters with cutoff frequencies at 0.3 and 27 Hz (3 dB) were used, frequencies outside this range were filtered out. Sample frequency was 50 Hz. In essence, the technique was the same as used before (25), with the difference that from each 5-min recording period per animal 5 epochs of 10 s (instead of 20 s) were randomly selected for a Fast Fourrier Transformation (FFT). FFT to obtain a power spectrum (total power) was carried out for each 10-s period, the results of the 5 epochs were averaged per animal and subsequently for a group of similarly treated animals. A calculation of the mean frequency was not performed, since in the case of soman 2 peaks appeared in the power spectrum and a calculated mean frequency would not provide relevant information.

The VERs were recorded during the 5-min period that followed. Frequencies outside 0.3–100 Hz were filtered out and the EEG sequela during 500 ms following each of the 100 flashes were first stored and averaged per animal (by a HP Vectra RS/16 computer) and then per group of similarly treated animals. Flashes were generated at a frequency of 0.5/s (±20% random) by a xenon photoflash, placed at a distance of 1 m and aimed at the deepest point of the wheel. Each flash lasted 1 ms and the

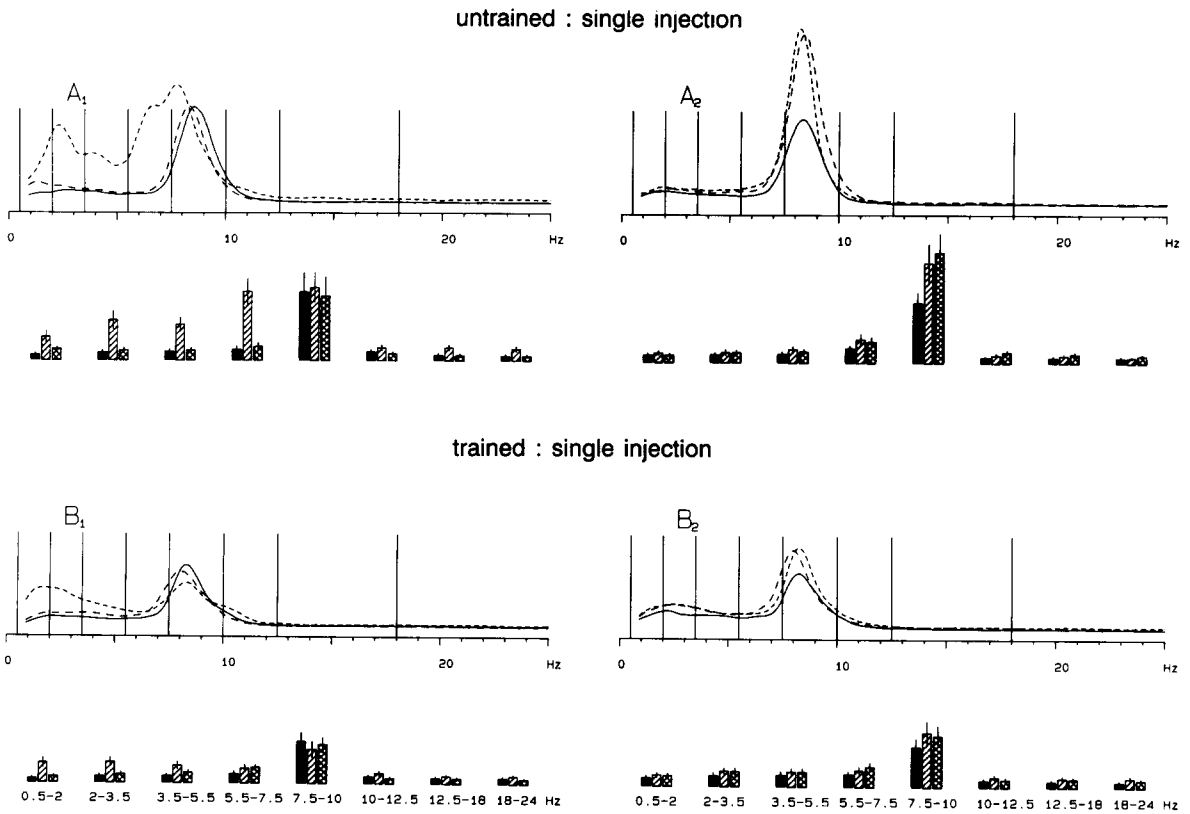

FIG. 2. The averaged power spectra of the EEGs of untrained (A1, A2) or trained (B1, B2) rats, 1 hour (A1 and B1) or 24 hours (A2 and B2) after a single SC injection. The curves represent the continuous spectrum and is subdivided by vertical lines to delineate the frequency classes. The bars (±S.E.M.) show the results for each frequency class. Excursions in the vertical direction represent the power (V^2) in arbitrary units. In both Experiments A and B three treatment groups were present, each group of 6–8 animals, which were injected with saline (— and black bar), 60 μg/kg soman (- - - and hatched bar), or 600 μg/kg DFP (— — — or double-hatched bar). During recording the animals were walking in a motor-driven hollow wheel (circumference speed 10 cm/s). Particularly noteworthy is the increase in low frequency 1 h after soman treatment (A1 and B1) and the synchronization in the EEGs of the OP-treated animals 24 h after injection.

intensity at the deepest point of the wheel was 40 luxsec. No ambient lighting was provided, the cubicle was dark.

For the results in Table 4 the averaged VERs of each individual animal were traced by hand, i.e., by joystick and cursor on the computerscreen, and the amplitudes and latencies of each VER component were determined. This was done to avoid artefacts as a result of computer averaging: e.g., an artificial lowering of the amplitude and broadening of the N1 peak which may occur during computer averaging when the latencies of these peaks differ between animals.

In Experiment D the animals were injected 3× per week (Monday, Wednesday and Friday) during 4 weeks with saline, DFP or soman in the above-mentioned doses, after they had reached criterion. Shuttlebox performance was tested 5 days per week, 1 and 24 hours after the compounds were injected (except on Saturdays). At the end of this period the animals were anaesthetized, electrodes were fitted and EEG and VER recordings were obtained as described for Experiment 1.

In Experiment A the EEGs and VERs were obtained from animals that had not been trained. EEGs and VERs were obtained as described for Experiment B, i.e., again 1 and 24 hours after a single injection of saline, DFP or soman.

In Experiment C the animals were repeatedly injected with the same doses and according to the same schedule as mentioned for Experiment D. However, the animals were not trained. EEGs and VERs were obtained, as in Experiment D, after 4 weeks.

Rectal Temperature

These organophosphates are known to cause a decrease in body temperature (24). Hence, core temperatures were measured in repeatedly injected animals, 1.5 and 24.5 h following the injections at session 14 of Experiment D, as well as 1.5 h after the last injection.

Pupil Diameter

Since the organophosphates cause a pupillary constriction, which may affect the VERs, the pupillary diameters were measured 1.5 and 24.5 h following session 14 of Experiment D, as well as 1.5 h after the last injection. The measurements were carried out under a microscope with an ocular micrometer, at a standard distance and under photocell-controlled standardized lighting conditions.

Footshock Sensitivity

Footshock sensitivity was assessed by measuring the flinch reaction with an "up and down method" (9,22). The animals

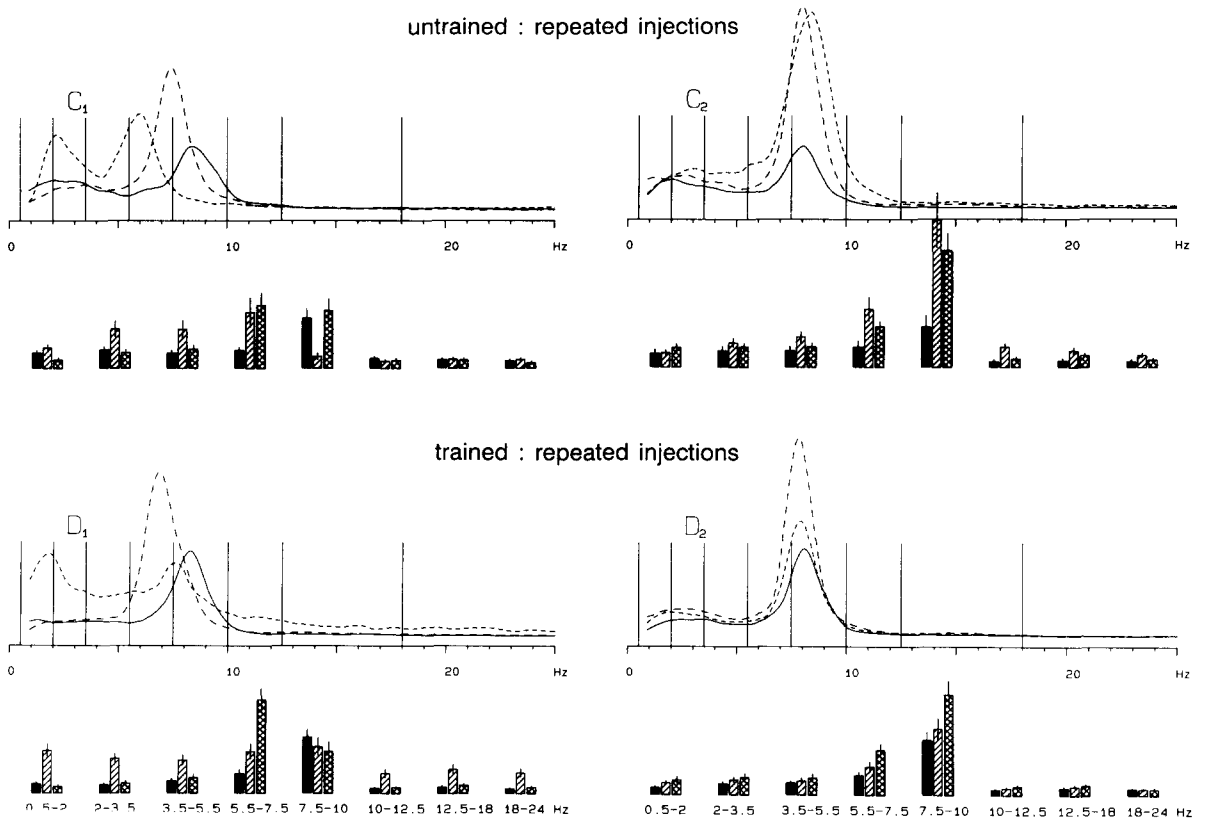

FIG. 3. The averaged power spectra of the EEGs of untrained (C1, C2) and trained (D1, D2) rats repeatedly injected SC with saline, soman (60 μg/kg), or DFP (600 μg/kg) measured 1 h (C1, D1) or 24 h (C2, D2) after injection. Layout and coding of the treatment groups of both Experiments C and D is the same as in Fig. 2. Performance of the animals of Experiment D is shown in Fig. 1.

were situated in a Plexiglas cage standing on a grid floor. When the animals had all four feet on the grid floor, the grid floor was briefly electrified and the presence or absence of a reaction of the animal was noted; a positive flinch reaction consisted of a quick withdrawal of the forepaw from the grid floor. In a sequential fashion the current (constant current principle) was increased in steps of 10 μA when the animals did not react and was decreased in steps of 10 μA when they did. If the absence of a flinch reaction at a set current level was followed by the presence of a flinch at 10 μA higher, or vice versa, this was called a reversal. The threshold for each animal was determined from 4 positive and 4 negative reversals. The flinch threshold was assessed 1 and 24 h after the first and the tenth injection in a separate experiment in which untrained animals were treated with the repeated injection schedule that was used for group C.

Chemicals

DFP (diisopropylfluorophosphate) and soman (pinacolyl methylphosphonofluoridate) were synthesized by Dr. H. P. Benschop from the Prins Maurits Laboratory TNO. Both compounds were at least 99% pure. Solutions were freshly prepared before injection.

Statistics

The multiple *t*-test of Welch (16), including Bonferoni's correction, was used for all comparisons. When the term significant is used this means $p<0.05$, two-tailed.

RESULTS

The effects of repeated injections on performance (Experiment D) are shown in Fig. 1. As before (21,26), behavioral tolerance following repeated administration of soman was not found, in contrast with the behavioral tolerance found after DFP.

The four experiments were performed at intervals of several weeks or even several months; seasonal changes might be responsible for the variability between the results of the different experiments. However, within each experiment the results are directly comparable.

The results of the EEG analyses are shown in the Figs. 2 and 3. In essence, the results of all four treatment groups exhibit the same trend: 1 h following soman the EEG power spectra show the presence of an extra low frequency peak at approximately 2 Hz. This peak was absent in the DFP- and saline-treated animals, the differences with the results obtained with soman were highly significant. One hour after injection, both organophosphates also induced a shift to lower frequencies of the main power peak, when compared with the EEG power spectra from saline-treated animals.

In the EEG spectra obtained 24 h after the injection of soman the low frequency peak had disappeared; at that point in time the power spectra of both organophosphate-treated groups only showed a high degree of synchronization at a frequency of approximately 8.5 Hz, i.e., close to the main peak frequency of the saline-treated animals.

The results of the averaged VERs are shown in Fig. 4 and in Table 1. In Fig. 4 it can be seen that the averaged VERs mea-

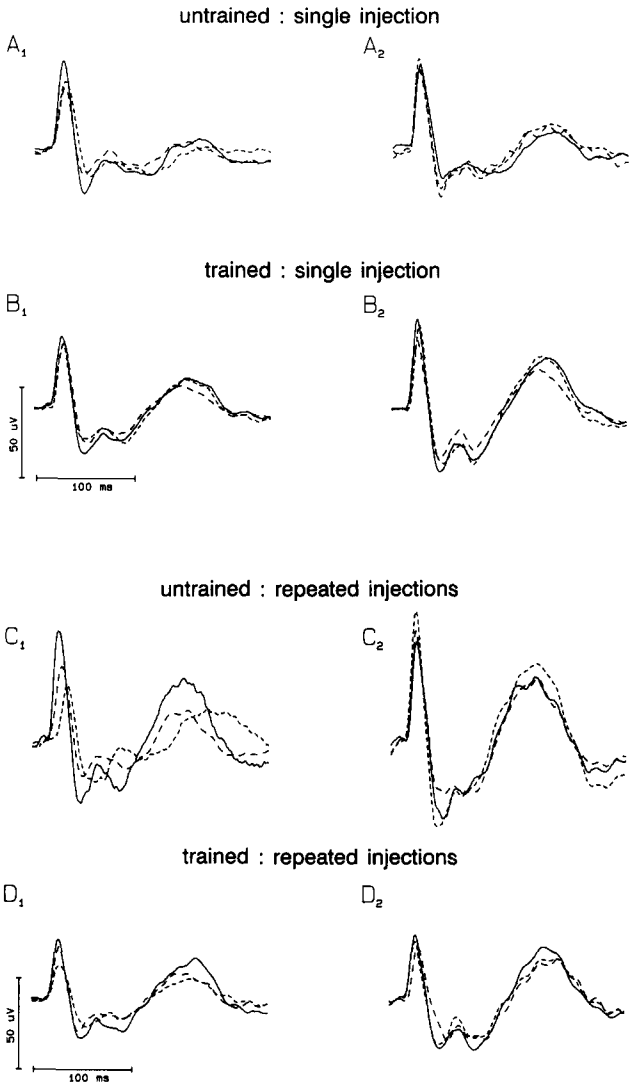

FIG. 4. The averaged visual evoked responses (VERs) of all animals of the four Experiments A, B, C and D. Each experiment consisted of three treatment groups (n=6–8 animals per group). The VERs were first averaged per 100 flashes (0.5/s ± 20% random) per animal and subsequently per group of similarly treated animals. Negative peaks are plotted upwards, positive peaks downwards. The measurements were performed immediately following the EEG registrations shown in Figs. 2 and 3. For treatment and treatment coding of the curves see legend of Fig. 2. Note that the shapes of the VERs are hardly changed. In general, 1 h after the injections (left vertical column) the latencies of the various peaks (e.g., N1, P2, N3) of the OP-treated animals seem slightly increased and the amplitudes somewhat decreased. Compare with Table 1. These effects have disappeared 24 h after the injections (right vertical column).

sured 1 h after the single or repeated injections with organophosphates show a small decrease in amplitude of all VER components and a slight increase in latency of the early VER components. In general these effects are clearest in the soman-treated animals and can best be seen in the N1, the P2 and the N3 components of the averaged VERs. Hence, only the values of the amplitudes and latencies of those components are shown in Table 1. For the sake of clarity we omitted the calculated values of the other VER components measured 1 h after injection, as well as all values obtained 24 h after injection, when most changes had disappeared.

Body temperature (Table 2) and pupil diameter (Table 3), measured 1.5 and 24.5 h after the injections at session 14 of Experiment D, were not altered significantly. This was also the case when these parameters were measured 1.5 h after the last injection (not shown).

In a separate experiment with untrained animals and repeated injections as in group C, flinch thresholds were measured 1 h and 24 h following the first (not shown) and the tenth injection with the same doses: these thresholds were not significantly changed in any of the determinations (Table 4).

DISCUSSION

The effects of the repeated injections with saline, DFP (600 μg/kg) and soman (60 μg/kg) on performance in Experiment D (Fig. 1) demonstrate once again that in contrast with the effects of DFP, development of tolerance to soman was not observed (Fig. 1).

Since it is reported that DFP (7) and soman (18) have antinociceptive effects, there was a theoretical possibility that this effect of soman in our rats would be much stronger than those of DFP. Thus an occasional avoidance-failure of those animals that had received soman an hour before would go unpunished since the animals would not feel the footshock. Once perceived, they might persevere in their behavior, which might cause the observed drop in performance. As the sole explanation this possibility seemed theoretical since, in the absence of footshock, extinction of acquired shuttlebox performance with the present technique was found to be extremely slow: approximately 20–30% in two weeks (unpublished). Nevertheless, it was decided to measure footshock sensitivity 1 and 24 h after a single and ten repeated organophosphate (OP) injections. A change in footshock sensitivity was not found, neither after a single nor after ten (see Table 4) injections.

The results of some earlier unpublished preliminary experiments confirmed the notion that EEGs and VERs recorded from animals that performed a simple motor task, i.e., slowly walking (10 cm/s) in a rotating hollow wheel, would show a lower variability compared with recordings from freely moving animals exhibiting all sorts of behaviors, such as sniffing, rearing, grooming, etc. In subsequent experiments (25) this technique was used succesfully and has also been applied in the present experiments.

The differences in effects of DFP and soman on the averaged visual evoked responses (VERs) seemed quantitative rather than qualitative and may be due to subtle differences in cholinesterase inhibition patterns between the two inhibitors. In this respect it was found earlier that soman at low doses causes effects that have to be ascribed to predominant cholinesterase inhibition in the CNS, whereas after DFP predominant inhibition occurs peripherally [for a discussion see (25)]. At first sight these findings are in disagreement with more recent results of tolerance experiments (21) showing that these inhibitors did not induce large differences in cholinesterase inhibition in homogenates of different brain areas. However, determination of cholinesterase activity in homogenized biological material containing a large number of neurons is not an optimal method to detect subtle differences in inhibition patterns and may have only limited value, particularly shortly after the OP injection.

Another explanation for the small reductions of the VER amplitudes might be that soman at just sublethal doses causes brain damage (8, 13, 17) and in the present experiments repeated

TABLE 1

THE CALCULATED AMPLITUDES AND LATENCIES OF THE N1, P2 AND N3 PEAKS OF THE AVERAGED VERs 1 h AFTER (REPEATED) SC INJECTION(S) OF SALINE, DFP (600 μg/kg) OR SOMAN (60 μg/kg) IN THE EXPERIMENTS A, B, C AND D

Treatment	VER Components	Amplitude	Latency	Amplitude	Latency
		Untrained 1 h After Single Injection (A1)		Trained 1 h After Single Injection (B1)	
Saline	N1	87.9 ± 13.1	38.0 ± 0.4	77.1 ± 10.7	35.0 ± 0.8
	P2	−56.3 ± 10.2	59.6 ± 1.5	−50.6 ± 8.1	57.7 ± 0.9
	N3	12.7 ± 4.5	166.4 ± 6.4	31.7 ± 6.2	165.7 ± 1.4
Soman	N1	78.4 ± 11.7	40.7 ± 1.6	64.3 ± 14.7	36.0 ± 0.4
	P2	−41.1 ± 9.7	63.3 ± 1.9	−39.0 ± 8.8	60.9 ± 1.8
	N3	6.6 ± 3.0	185.3 ± 11.4	30.0 ± 7.8	159.7 ± 2.6
DFP	N1	59.9 ± 12.2	38.9 ± 0.8	67.6 ± 5.5	36.6 ± 0.6
	P2	−35.3 ± 7.7	58.1 ± 1.4	−40.9 ± 3.9	60.1 ± 1.0
	N3	7.4 ± 6.8	157.1 ± 5.8	26.1 ± 3.2	153.3 ± 3.5
		Untrained 1 h After Repeated Injections (C1)		Trained 1 h After Repeated Injections (D1)	
Saline	N1	106.1 ± 9.1	33.9 ± 0.6	67.9 ± 8.0	35.6 ± 1.6
	P2	−79.1 ± 7.6	58.3 ± 1.4	−49.1 ± 8.1	58.6 ± 1.6
	N3	57.3 ± 7.0	162.0 ± 1.8	48.9 ± 3.1	170.7 ± 3.1
Soman	N1	56.9 ± 10.2	42.1 ± 0.9	43.1 ± 11.8	37.6 ± 2.2
	P2	−58.2 ± 6.2	69.1 ± 3.1	−46.1 ± 9.7	57.5 ± 3.0
	N3	41.9 ± 6.0	179.5 ± 7.2	30.1 ± 7.8	153.5 ± 12.5
DFP	N1	72.5 ± 7.8	37.3 ± 0.8	58.4 ± 7.8	36.6 ± 0.9
	P2	−45.3 ± 3.6	59.5 ± 1.2	−37.9 ± 3.7	54.7 ± 1.0
	N3	28.3 ± 3.4	160.8 ± 3.2	31.7 ± 5.1	161.6 ± 4.7

For reasons given in the text the values were obtained by tracing the averaged VER of each animal with a joystick on the computer screen and by subsequently averaging the obtained values per treatment group. Compare with the left vertical column of Fig. 4.

The differences had almost all disappeared 23 h later (not shown).

doses of 60 μg/kg of soman, i.e., each approximately 0.5 LD_{50}, are administered. However, it is known that gross lesions and necrosis occur when convulsions or seizures are predominant; these phenomena are absent in the present animals. Moreover, in earlier experiments (26), light microscopical damage was not found in various brain areas of similarly treated animals. Although very unlikely it might be that very light edematous changes, so far only electromicroscopically seen to emerge 2 h after soman (8) and which are most likely reversible within 24 h, occur earlier and are responsible for the effects seen 1 h after injection. Whether such effects can also be caused by DFP is unknown.

A third possibility that might explain the decrease of VER amplitudes seen 1 h after injection of the OPs is the following. During the 48-h interval between injections de novo synthesis of cholinesterase occurs (21). Upon injection of the organophosphates this newly formed enzyme is reinhibited. It is plausible that the consequences of these repeated reinhibitions will be perceived by the animal and will cause temporary arousal. Arousal may not only suppress the VER after-discharges (10), but may also reduce the amplitude of the early VER components (23). If this explanation is correct it might also explain why these amplitude reductions are best seen after repeated injections (see C1 and D1 in Fig. 4 and in Table 1).

With respect to the EEGs, the most interesting finding is that one hour after soman there is a large significant increase in low frequency activity around 2–2.5 Hz in all four experiments (see Figs. 2 and 3). These low frequency peaks are not seen in any of the groups treated with DFP and are apparently reversible, since 23 h later they have disappeared. Twenty-four hours after the injections only a high degree of EEG synchronization was observed in all organophosphate-treated animals around 8–8.5 Hz, i.e., the dominant frequency in the power spectrum of the

TABLE 2

THE ABSENCE OF A SIGNIFICANT EFFECT OF THESE LOW (REPEATED) DOSES OF ORGANOPHOSPHATES ON BODY TEMPERATURE

After the 6th SC Injection With:	Body Temperature 1.5 h After Administration (°C)	Body Temperature 24.5 h After Administration (°C)
Saline (1 ml/kg)	38.5 ± 0.4	38.2 ± 0.2
Soman (60 μg/kg)	38.4 ± 0.3	38.4 ± 0.2
DFP (600 μg/kg)	38.1 ± 0.3	37.9 ± 0.3

The measurements took place in the animals of Experiment D, immediately after their performance was tested on session 14, i.e., 1.5 h after the 6th injection, as well as 24.5 h following this injection, i.e., immediately after session 15. A similar absence of effects was found 1.5 h after the last injection in Experiment D (not shown).

TABLE 3

THE ABSENCE OF A SIGNIFICANT EFFECT OF THESE LOW (REPEATED) DOSES OF ORGANOPHOSPHATES ON PUPIL DIAMETER

After the 6th SC Injection With:	Pupil Diameter 1.5 h After Administration (mm)	Pupil Diameter 24.5 h After Administration (mm)
Saline (1 ml/kg)	0.85 ± 0.04	0.86 ± 0.06
Soman (60 μg/kg)	0.77 ± 0.07	0.80 ± 0.05
DFP (600 μg/kg)	0.77 ± 0.06	0.81 ± 0.05

These measurements took place in the animals of Experiment D, immediately following the body temperature measurements shown in Table 2.

TABLE 4

MEASUREMENTS OF SENSITIVITY TO FOOTSHOCK BY DETERMINATION OF THE FLINCH THRESHOLDS WITH AN UP AND DOWN METHOD (SEE TEXT)

After the 10 SC Injection With:	Flinch Threshold 1 h After Administration (μA)	Flinch Threshold 24 h After Administration (μA)
Saline (1 ml/kg)	77.2 ± 4.9	82.5 ± 3.3
Soman (60 μg/kg)	84.2 ± 3.7	80.7 ± 5.2
DFP (600 μg/kg)	82.0 ± 6.8	67.3 ± 15.3

These thresholds were determined in a separate experiment (n = 6 per treatment group), 1 and 24 h after the 10th SC injection. The injection schedules with saline, DFP (600 μg/kg) or soman (60 μg/kg) were the same as in the animals of Experiment C. A separate experiment with nontrained animals was chosen, because exposure to nonescapable footshock may interfere with subsequent performance tests.

saline-treated walking rats.

In some experiments (see A1, B1 and D1, Figs. 2 and 3) there is also an increase in β-activity 1 h after soman, but this is not consistent and has mostly disappeared 23 h later.

The significance of this increase in delta activity in awake animals one hour after soman treatment is obscure. In the awake state, delta activity is thought to represent an aspecific effect that has been observed after cobalt cortical implants and after large doses of alcohol [see (12)]. It has also been suggested (11,20) that this slow wave activity may be associated with behavioral impairment, which would explain the performance decrements observed one hour after soman in the present experiments. However, the evidence is scanty and the literature on this topic is inconclusive.

In a retrospective study of sarin-exposed humans and in a prospective study of sarin-exposed rhesus monkeys, Burchfiel and Duffy (5) found a persisting increase in EEG β-activity. Even if the species differences are disregarded it is difficult to compare their data with the results of the present experiments. Sarin in low doses is predominantly a peripheral inhibitor and cholinesterase inhibition in vivo by sarin appears rather reversible, in contrast with cholinesterase inhibition by DFP or soman (14). However, these authors did observe an increase of delta and a slowing of theta by visual inspection of the EEGs, which was also observed by others but was not found upon spectral analysis. It may be that these effects are more pronounced in the rat. Whatever the case may be, these results do not offer an explanation for the present differences between the effects of DFP and soman.

On the neuronal level, the differences between DFP and soman cannot be readily explained on the basis of differences in cholinesterase inhibition, because no significant differences were found earlier in the degree of enzyme inhibition in various areas of the CNS 1 h after DFP or soman (21). It is possible, however, that these differences may be linked to a second effect of soman, not related to its cholinesterase-inhibiting effect. This is the reversible effect of soman on nicotinic and muscarinic acetylcholine receptors (3) or on the receptor-ionic channel complex (1,2), which have been reported by the groups of Eldefrawi and those of Albuquerque. Unfortunately, a direct comparison between DFP and soman under the same experimental conditions has not been carried out and to explain the present findings on the basis of these receptor-linked effects of soman remains speculative. But if true, we may have to alter a previous statement "that apparently behavioral tolerance does not develop after repeated injections of soman" (21). It may be that after repeated doses of soman, as for a large series of other cholinesterase inhibitors (4, 6, 19), behavioral tolerance does develop but is camouflaged by this secondary effect of soman which keeps on causing performace decrements during the first hours after the injection of this compound. On the other hand, this secondary effect of soman may block the development of tolerance.

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