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PERSISTING BEHAVIOURAL AND ELECTROENCEPHALOGRAPHIC EFFECTS OF EXPOSURE TO CHLORPHENVINPHOS, AN ORGANOPHOSPHOROUS PESTICIDE, IN LABORATORY ANIMALS

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Abstract. Organophosphorous compounds (OPs) constitute a large proportion of insecticides used all over the world. Their insecticidal properties and acute toxicity in nontarget species derive from the inhibition of acetylcholinesterase (AChE) which disturbs the cholinergically mediated neurotransmission. OPs do not accumulate in living organisms and the acute signs and symptoms disappear as the AChE activity returns to normal level. Therefore, they are regarded as relatively safe. However, as some literature data suggest, after either acute or prolonged exposure to OPs subtle neurobehavioural impairments may persist long after normalization of AChE activity. The possibility that OPs exposure may induce such long-term effects is nowadays a problem of great concern for the regulatory agencies. Here we present a review of studies from our laboratory aimed at detecting neurobehavioural effects of exposure to chlorphenvinphos, an OP pesticide ((2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate) – CVP)) in laboratory animals.
In Poland, CVP is manufactured (250 tons/year) and used for crop protection. In a series of experiments we have demonstrated that:
a) in rabbits, two i.p. exposures to CVP at the same sublethal dose at three-month interval resulted in a similar inhibition of blood AChE activity but the effect of the second exposure on body temperature and hippocampal EEG was smaller and less consistent than that of the first one. This would indicate that some permanent changes within the CNS may occur even after a single exposure to CVP;
b) in rats, under conditions of repeated i.p. exposure to CVP (one injection/day for ten days) at a symptomatic (3.0 mg/kg) dose inhibiting blood and brain AChE activity by about 80%, the tolerance to CVP, assessed from the spontaneous locomotor behaviour, developed within four to five days. However, single exposure to CVP at a symptomatic (3.0 mg/kg) or subsymptomatic (1.0 mg/kg, less than 50% AChE inhibition) dose, or repeated exposure (one injection/day, for ten days) at subsymptomatic doses (1.0 mg/kg or 0.5 mg/kg) resulted in subtle changes in complex behaviours detectable after AChE activity in blood and in the brain had returned to the normal level. The changes – neophobia in the open field, an increased and more persistent emotional response to a stressful stimulus, and increased EEG arousal response to an external pain signalling stimulus – suggest an increased reactivity of the system or systems responsible for the induction of fear;

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c) direct intrahypothalamic injections of CVP, unlike those of oxotremorine, a direct stimulant of cholinergic muscarinic receptors, did not induce overt changes in the animal (rabbit) behaviour and EEG. This would indicate that the changes in the CNS functions after CVP exposure may be the consequence of increased cholinergic activity due to AChE inhibition rather than to a direct stimulation of cholinergic muscarinic receptors by CVP.

The above findings provide experimental evidence that health effects of exposure to CVP, may persist after recovery of AChE activity in blood and in the brain.

INTRODUCTION

Organophosphrous compounds (OPs) constitute a large proportion of insecticides used all over the world. They belong to the family of anticholinesterases, i.e. compounds able to inhibit the activity of cholinesterases (ChE). The insecticidal properties and the acute toxicity of OP insecticides in nontarget species derive from inhibition of acetylcholinesterase (AChE), i.e. the cholinesterase decomposing the neurotransmitter acetylcholine (ACh). Inhibition of AChE results in an accumulation of ACh in cholinergic synapses, which leads to hyperexcitation of postsynaptic neurons or end organs mediated by the action of muscarinic or nicotinic ACh receptors. This gives rise to the well-known somatic, vegetative, and central signs and symptoms of acute OP intoxication enumerated meticulously by Richardson in a recent paper (39). The majority of signs and symptoms of acute poisoning usually disappear as the level of ChE activity returns to normal.Therefore, organophosphate insecticides were assumed to be relatively safe. It is known, however, that acute exposure to some OPs may cause delayed polyneuropathy (organophosphorous--induced delayed neuropathy — OPIDN), a bilateral degeneration of long axons resulting in sensory and motor deficits (31). Fortunately, owing to the imposed requirements to test all OPs for their neuropathic potential, the neuropathic OPs are being identified and eliminated from the market. However, apart from OPIDN, other delayed or persistent effects of acute or repeated OP exposure were also noted. Early epidemiologic studies (17,35) reported depressive disorders, memory impairment and oculomotor imbalance in workers exposed repeatedly to OP insecticides. EEG abnormalities were found in acutely poisoned workers months after the level of AChE activity returned to normal (13). Savage and co-workers (42), in their comparison of acutely exposed and control workers, found that the exposed group showed significant changes in tests designed to measure abstraction, mood, flexibility of thinking and simple motor skills. Poorer performance in tests to assess memory, attention, visuomotor function, and motor skills after OP poisoning were also reported by Rosenstock et al. (40). The observations cited above suggest that persistent changes in the CNS functional state may result not only from a long-term exposure to OP compounds (35) but also from acute poisoning.

To date there are few animal studies supporting the above possibility. Using the functional observational test battery (FOB) it has been found that adverse effects are detectable several weeks after an acute OP dose (15), and after ChE levels returned to normal (36). On the other hand, Costa and Murphy (9) found no alterations in passive avoidance learning in rats after a repetitive dosing with disulfoton.

Some data suggest that OP exposure may result in a persistent decrease in muscarinic receptor function (46,47) and an elevated response to muscarinic cholinolytics (38).

Numerous OPs, differing in chemical structure, metabolism and biocidal potential, are currently in use. Some of them, apart from being ChE inhibitors, interact with cholinergic receptors directly as their agonists or antagonists (1,2,16). Hence, there is the possibility that long-term effects of exposure to different OPs may differ quantitatively and/or qualitatively although the ChE inhibition resulting from exposure to each of the compounds might be similar. Therefore, animal studies aimed at detection of such effects need to be performed separately for each extensively used OP pesticide.

CHLORPHENVINPHOS: GENERAL INFORMATION

Chlorphenvinphos (2-chloro-1-(2,4-dichlorophenyl)vinyl)diethyl)phosphate) is the active component of several preparations (Sapecron, Enolofos, Birlane, Dermaton, Steladone, Supona). In the United States, all registered uses of CVP were cancelled in 1991. In many European countries, however, chlorphenvinphos (CVP) is used for crop protection. In Poland, CVP is manufactured (250 tons/year) and used mainly for control of the Colorado Beetle. Three CVP preparations are in use: Chlormecide (5.5% CVP), Enolofos (44% CVP) and Metofos (5.5% CVP). About 50 workers are engaged in the process of manufacturing. The population at risk of exposure during application amounts to thousands.

In the external environment CVP is degraded slowly due to its high resistance to hydrolysis. It may result in the accumulation of CVP in soil as well as in plant roots. Within the mammalian organism, however, CVP is metabolized rapidly and the products are not toxic. In the rat, after oral administration of a single dose of radiolabeled CVP, almost 100% of radioactivity is being eliminated from the body within 2–4 days, mainly with urine (12,25,26). CVP is oxidatively dealkylated in the microsomal fraction of the liver. The relative speed of this process varies greatly between species, being 1, 8, 24 and 88 for rat, mouse, rabbit and dog, respectively. That is why CVP is highly toxic to the rat (LD_{50} p.o. = 10.0 mg/kg) and almost nontoxic to the dog (LD_{50} p.o. = 12000 mg/kg).

In humans as well as in experimental animals, signs and symptoms of acute CVP toxicity are similar as in the case of other OP pesticides. In Poland, there were several cases of acute CVP poisoning, some of them fatal (28,37). Interestingly, in humans, serum (unspecific) ChE (sChE) appears to be much more sensitive to CVP than the red blood cell ChE (rbcChE) i.e. AChE (24). In the rat, on the contrary, rbcChE is being inhibited by CVP more efficiently than the sChE (25).

Studies on workers (manufacturers, formulators), exposed to OP compounds including CVP, revealed a high prevalence of the neurovegetative syndrome (trembling of eyelids and fingers, enhanced dermatographism and sweating). Pyramidal or cerebellar symptoms were noted in 34%, distal sensory neuropathy in 31%, equilibrium disturbances in 26.9%, and decreased conduction velocity in peroneal nerve in 24.3% of cases (43,44,45). Changes in EEG in the form of increased beta activity and occurrence of theta waves (29,30,43,44), increased irritability and feeling of anxiety (3) were also noted. No information is available at present about the persistence of these effects after discontinuation of the exposure. Here we present

a summary of our experiments on rats and rabbits aimed at finding out whether acute or repeated exposure to CVP could produce effects detectable after the return of ChE activities to the preexposure level.

EFFECTS OF ACUTE CVP EXPOSURE ON SOME PHYSIOLOGICAL PARAMETERS IN RABBITS

Our first experiment concerning CVP neurotoxicity was performed on rabbits. We intended to find out whether the biochemical, physiological and electrophysiological effects of a single i.p. exposure could be reproduced by a second exposure after a time sufficient for a full recovery from the effects of the first one. The rabbits, bearing chronic intrabrain electrodes for EEG recording from neocortex and the hippocampus, were given intraperitoneally CVP in doses: 22.0, 33.0, 50.0, 75.0, or 112.0 mg/kg (one animal per dose). Cortical and hippocampal EEG, ChE activities in blood, and rectal temperature were measured 30 min before the injection and at selected time intervals after the injection.

Three months later each of the rabbits was given CVP in the same dose as the preceeding one and the effects of both injections on hippocampal EEG, rectal temperature and ChE activities were compared. The first injections resulted in a decrease in sChE and rbcChE activities, a decrease in rectal temperature and an increase in the contribution of theta activity in the hippocampal EEG, and the magnitude of these effects showed almost a linear relationship to the CVP dose. After the second injection the linearity was observed only in the case of the ChE activities. Changes in the rectal temperature were mixed; increase, decrease or no effect were noted, and the hippocampal EEG response, (increased theta activity) was less evident than that evoked by the first injection (Table 1, Figs. 1–3). These results suggested that even a single acute exposure to CVP might result in some changes in the rabbit organism persisting for months after the exposure.

Table 1. Changes in rbcChE (AChE) activity after two i.p. injections of chlorphenvinphos performed at 90 day interval in rabbitas (Adapted from Gralewicz et al. 1989)

CVP dose (mg/kg)		Activity of AChE in percent of the preinjection value (Hours after injection)					
		0.5	1.5	3.0	6.0	24.0	96.0
22.0	a	96.0	75.6	69.0	63.0	74.33	74.0
	b	**85.6**	**77.9**	**42.1**	**44.7**	**64.3**	**86.0**
33.0	a	68.2	87.0	75.3	53.2	38.6	65.1
	b	**52.3**	–	**15.8**	**0.0**	**9.1**	**31.0**
50.0	a	22.7	13.2	7.6	10.2	26.4	46.7
	b	**49.9**	**44.1**	**43.1**	**0.0**	**0.0**	**23.7**
75.0	a	15.8	6.5	2.0	8.0	30.0	58.3
	b	**23.0**	**20.0**	**9.0**	**6.5**	**8.1**	**19.4**
112.0	a	21.2	18.1	9.6	2.2	21.2	43.0
	b	**11.4**	**14.0**	**0.0**	**2.9**	**17.0**	**53.7**

a – first injection, b – second injection.

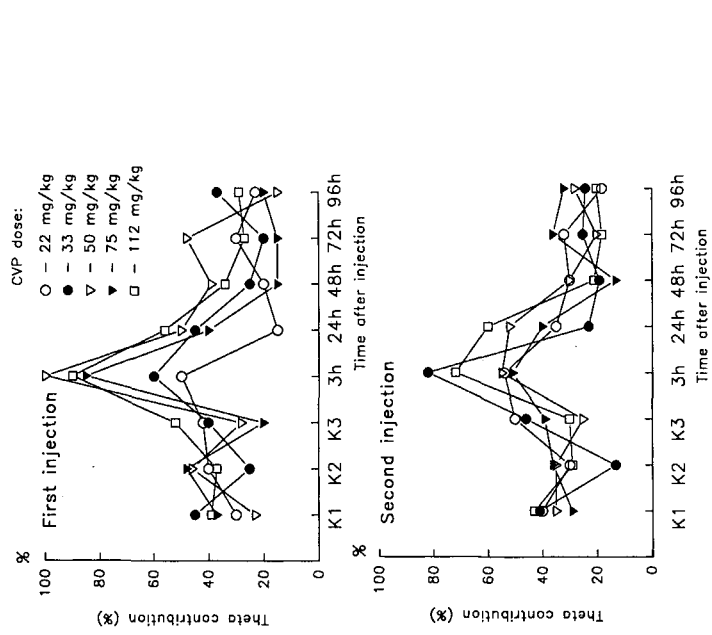


Fig. 2. Changes in the amount of 4–7 Hz theta rhythm in the hippocampal EEG of rabbits after repeated i.p. administration of CVP at different doses. Ordinates: percentage amount of 4–7 Hz theta activity in EEG samples. Abscissa: time of EEG recording (K_1, K_2 and K_3 – control recordings performed 48, 24 and 0.5 hours, respectively, before the injection). Upper panel: effect of the first injection. Lower panel: effect of the second injection performed on day 90 after the first one (Adapted from: Gralewicz et al. 1989).

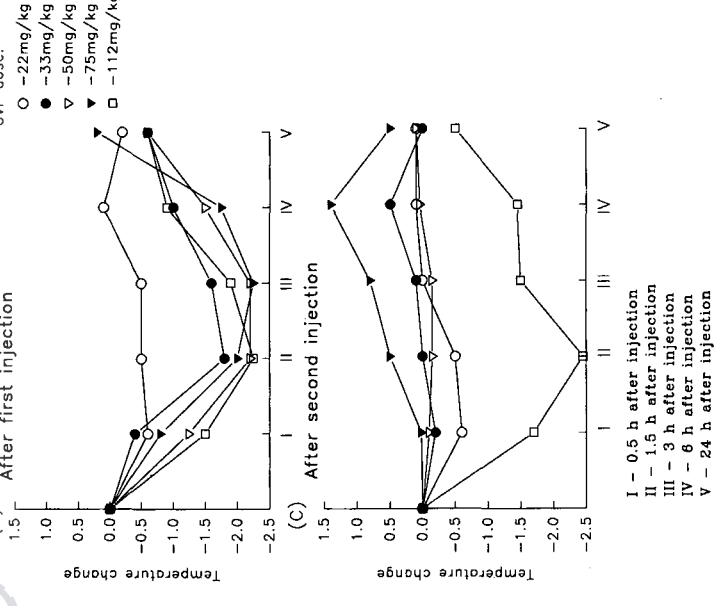


Fig. 1. Changes in rectal temperature in rabbits after repeated i.p. administration of chlorphenvinphos at different doses. Ordinates: temperature difference between the pre- and postinjection values (centigrade scale). Abscissa: time after injection (hours). Upper panel: effect of the first injection. Lower panel: effect of the second injection performed on day 90 after the first one (Adapted from: Gralewicz et al. 1989).

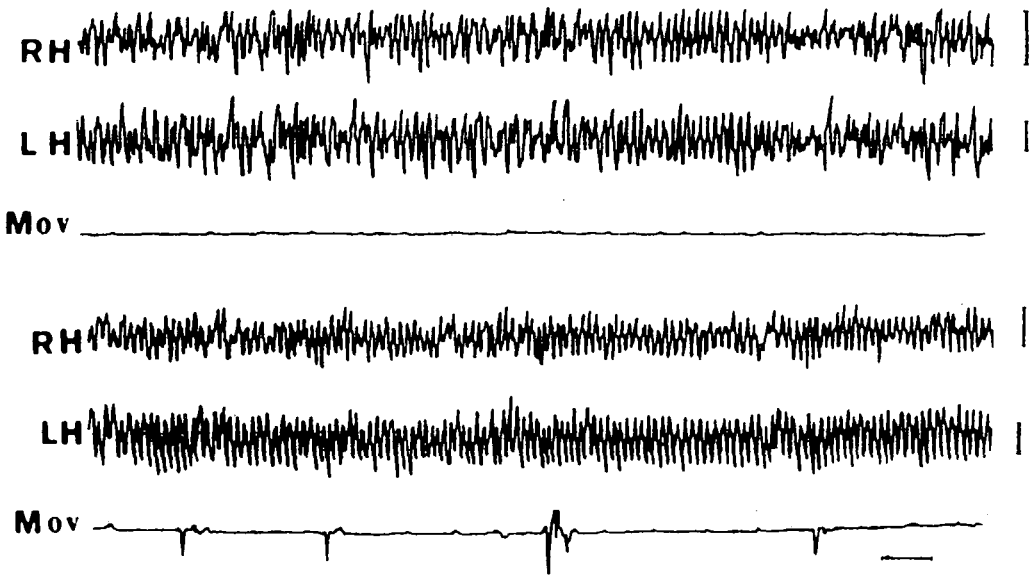


Fig. 3. Sections of EEGs illustrating the effect of an i.p. injection of CVP (50 mg/kg) on the hippocampal activity. A – hippocampal EEG before the injection; B – hippocampal EEG 30 min after the injection. RH – right dorsal hippocampus, LH – left dorsal hippocampus. MOV – activity recorded on the movement channel. Callibrations: 1s, 200 μV.

DEVELOPMENT OF TOLERANCE TO CVP UNDER CONDITIONS OF REPEATED EXPOSURE

It is well established that repeated exposure to ChE inhibitors often results in development of tolerance, i.e. disappearance of the subject sensitivity to the test compound. The mechanism consists in a reduction in density of postsynaptic, muscarinic receptors in the presence of an excess of ACh. This adaptive change develops within few days and is being maintained as long as the decreased ChE activity persists (8,34,41). In one of our experiments we tested the development of tolerance to CVP on rats housed permanently in cages equipped with rotating wheels. The animal could go at will in or outside the wheel through a large opening. Running takes place mainly during hours of dark. The daily distance run within the wheel may amount to several kilometers.

Three groups of rats (n = 5 in each) were given ten daily i.p. injections of olive oil alone (Group O) or CVP diluted in olive oil in a dose of 1.0 mg/kg (Group L) or 3.0 mg/kg (Group H) within the period of two weeks. It is worth noting that 3.0 mg/kg constitutes about one third of the CVP LD_{50} (27) and induces signs and symptoms typical of OP poisoning. Figs. 4 and 5 illustrate changes in the rat locomotor activity within the rotating wheel produced by these injections. As one may see from Fig.5, repeated injections of oil alone (Group O) or CVP in the dose of 1.0 mg/kg (Group L) produced almost no change in this behaviour. In rats receiving CVP in the daily dose of 3.0 mg/kg (Group H) the wheel running was severely reduced on the first three days but on day 5 it returned to the preexposure level.

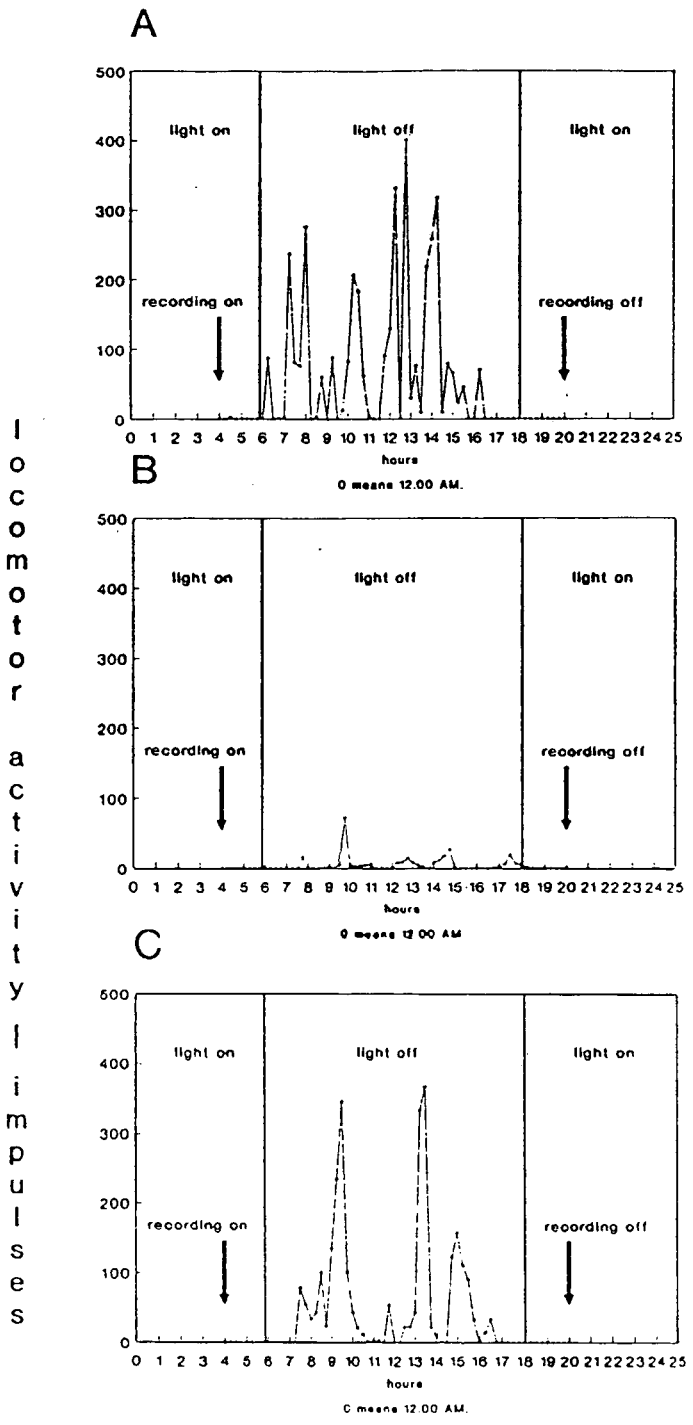


Fig. 4. Three consecutive original records illustrating the daily distribution of the locomotor activity in the rotating wheel of the same rat before the exposure to CVP (A), during day 1 (B) and day 5 (C) of the repeated exposure to CVP at the daily dose of 3.0 mg/kg. (Adapted from: Łuczak and Gralewicz 1992).

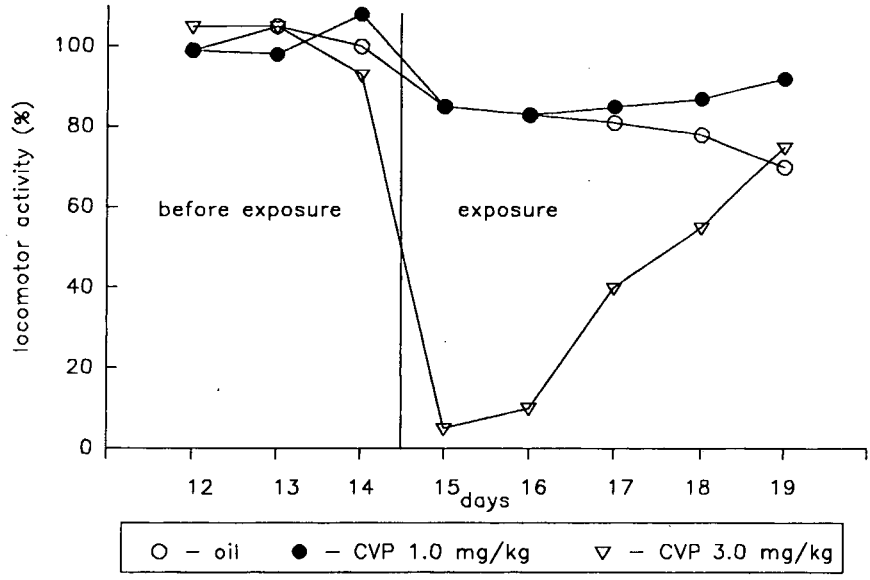
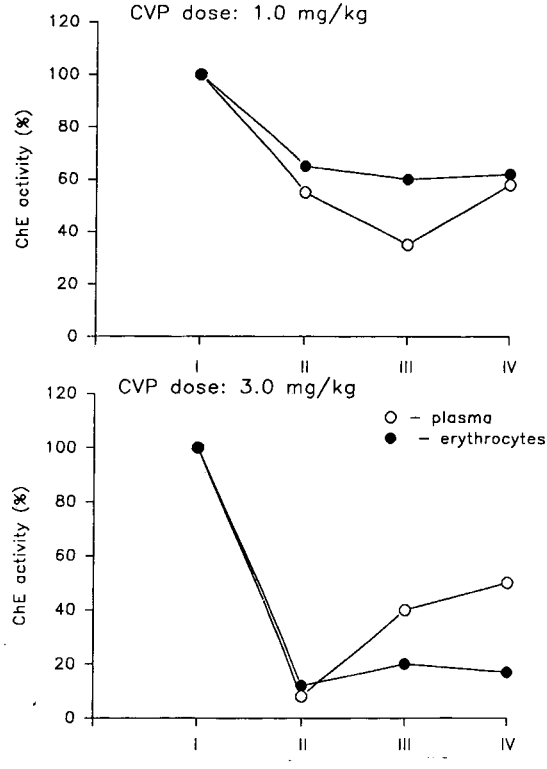


Fig. 5. Diagrams illustrating the effect of repeated i.p. injections of oil or CVP at doses of 1.0 or 3.0 mg/kg on the rat locomotor activity in rotaing wheels. Ordinates: normalized values of locomotor activity (the activity recorded on day 11 = 100%). Abscissa: successive days of the experiment. (Adapted from: Łuczak et al. 1992).



I – control
II – 3 h after first injection
III – 3 h after 5th injection
IV – 3 h after 10th injection

Fig. 6. Diagrams illustrating changes in plasma and rbcChE activity of rats exposed repeatedly (ten injections, one injection/day) to CVP at the doses of 1.0 mg/kg (A) and 3.0 mg/kg (B). (Adapted from: Łuczak et al. 1992).

Determinations of ChE activity, performed in parallel on separate groups of rats, revealed that in the H group, 3h after the fifth injection, the rbcChE and ChE of selected brain regions was inhibited almost to the same extent as 3h after the first one (Fig. 6 a and b). The above data show that in the rat, in conditions of repeated daily exposure to CVP at even high symptomatic doses, tolerance develops quickly; basic motor skills and perceptual abilities normalize within few days, notwithstanding the continuation of exposure and persisting marked depression of blood and brain ChE activities. However, as shown below, already after a single exposure to CVP subtle changes in rat behaviour can be detected even after a complete restitution of the ChE activity.

EFFECTS OF A SINGLE EXPOSURE TO CVP ON RAT'S BEHAVIOUR

In the following experiment three groups of male rats, 4–5 months old, were given a single i.p. injection of pure olive oil (control group) or CVP diluted in oil in the dose of 1.0 mg/kg (group 1.0 mg/kg) or 3.0 mg/kg (group 3.0 mg/kg).

The experiment consisted of two parts; a biochemical part and a behavioural one. In the biochemical part, samples of animals from each group were killed at preselected time intervals after the exposure, and ChE activity was determined in blood and in the brain (51). In the behavioural part the rats (n = 10 in each group) were subjected to selected behavioural tests. The following behaviours and processes were tested:

- spontaneous motor activity (open field test);
- short-term spatial memory (a response to change test in a T-maze);
- the magnitude and duration of the pain-induced emotional (fear) response (a hot-plate test);
- learning of an instrumental response (one-way active avoidance test).

The open-field activity was tested three times a week for two weeks before and two weeks after the exposure. The response to change test was performed on days 2, 4, 9 and 11 after the exposure. The hot-plate test was performed on days 18 and 19 and then on days 52 and 53 after the exposure. The one-way active avoidance test comprised three daily acquisition and three daily extinction sessions performed on days 23–29 after the exposure.

Three h after the single i.p. injection of 1.0 mg/kg CVP, the depression of ChE activity did not exceed 50% in blood and in the brain alike, and 14 days were enough for almost full recovery of the enzyme activity in all tested compartments. In case of i.p. injection of 3.0 mg/kg CVP, the ChE activity measured 3 h after the injection was about 10% of the control value in the case of blood (rbc and sChE activity alike), and slightly above 10% in the case of the brain. The rbcChE and brain ChE returned to preexposure levels within 21 days. Fig. 7 shows temporal distribution of the behavioural testing vs. changes in rbcChE activity after the exposure. In both groups, all tests, except the initial part of the postexposure testing in the open field and in T-maze, were performed when the ChE activity was within the normal range. An effect of exposure was detected only during testing in the open field and on the hot plate. In the open field, in sessions performed on days 8, 10, and 12 after the exposure a new object (a small box) was placed in the center of the arena. It resulted in a significant increase of locomotor and exploratory activity in rats of control groups but not in the rats of the exposed groups (Fig. 8).

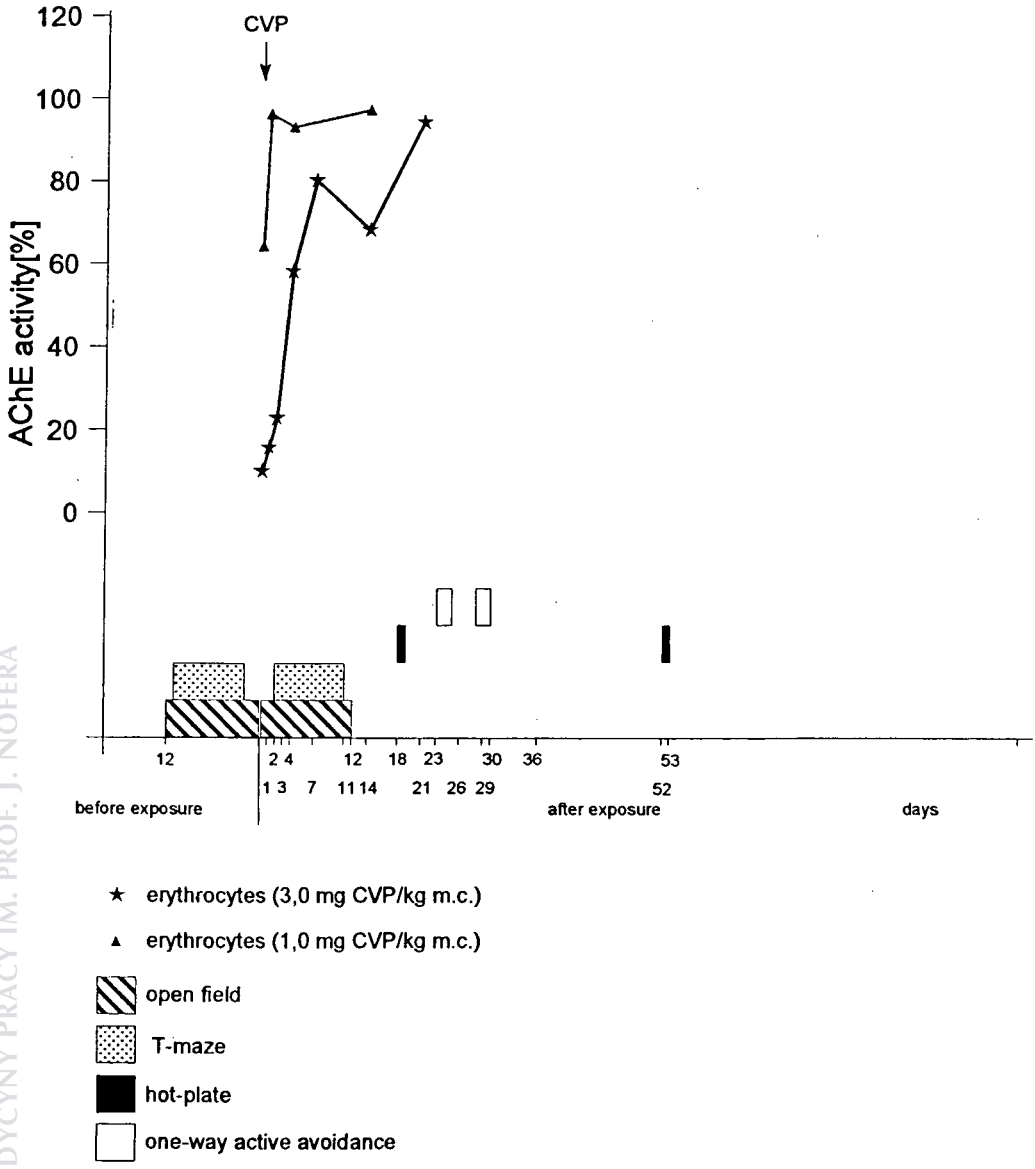


Fig. 7. A diagram illustrating the temporal distribution of behavioural testing vs changes in rbcChE (AChE) activity in rats exposed acutely to CVP at the dose of 1.0 mg/kg or 3.0 mg/kg. (Adapted from: Soćko 1996).

Testing on the hot-plate revealed no differences between groups in the paw-lick latency to heat ($54 \pm 0.5°C$) before a 2-min intermittent footshock. However, in group 3.0 mg/kg in the trial performed immediately after the footshock as well as in that performed 24 h after the footshock, the shock-induced increase in paw-lick latency (an index of the strength of the emotional (fear) response) was significantly higher

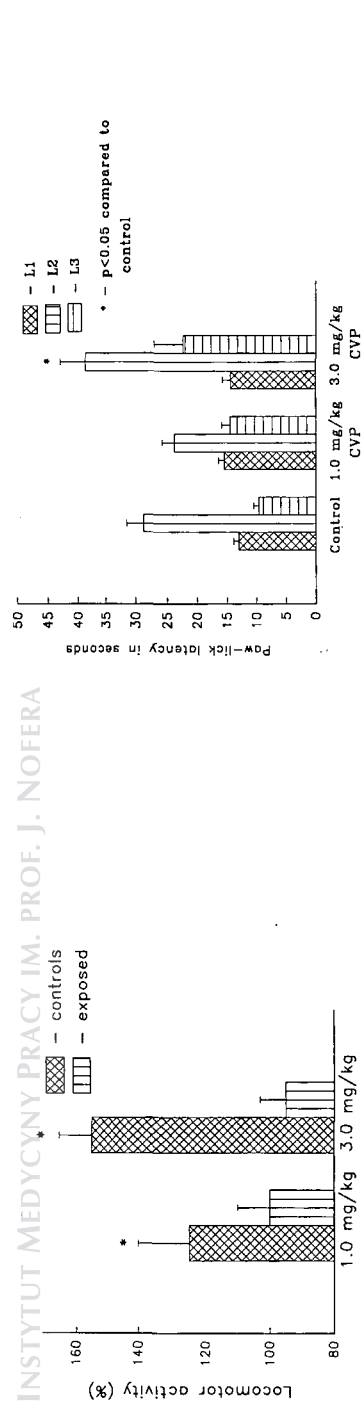


Fig. 8. A diagram illustrating the effect of novelty (the appearance of a new object) on the open-field locomotor (upper graph) and exploratory (lower graph) activity of rats exposed acutely to CVP at the dose of 1.0 mg/kg or 3.0 mg/kg, and their respective control groups. * – $p < 0.05$ in comparison with respective values noted in the same group during testing without a new object (Adapted from: Sočko 1996).

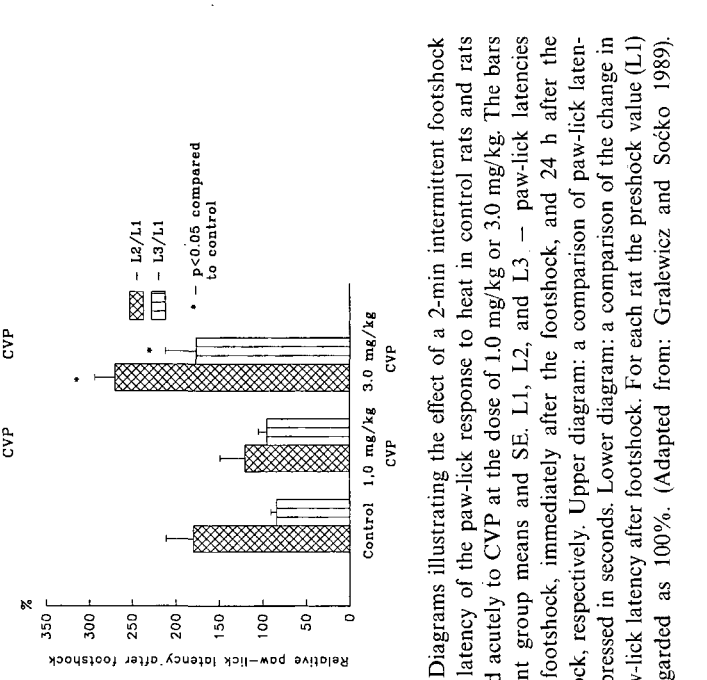


Fig. 9. Diagrams illustrating the effect of a 2-min intermittent footshock on the latency of the paw-lick response to heat in control rats and rats exposed acutely to CVP at the dose of 1.0 mg/kg or 3.0 mg/kg. The bars represent group means and SE. L1, L2, and L3 – paw-lick latencies before footshock, immediately after the footshock, and 24 h after the footshock, respectively. Upper diagram: a comparison of paw-lick latencies expressed in seconds. Lower diagram: a comparison of the change in the paw-lick latency after footshock. For each rat the preshock value (L1) was regarded as 100%. (Adapted from: Gralewicz and Sočko 1989).

compared to that seen in the remaining groups (Fig. 9). It suggests that in this group the effect of shock on behaviour was stronger and more persistent than in the remaining rats.

To sum up, the results of the behavioural tests suggest that after a single i.p. exposure to CVP at symptomatic (3.0 mg/kg) or subsymtomatic (1.0 mg/kg) dose some subtle changes in the rat behaviour may be detected after a time sufficient for complete or almost complete recovery of ChE activity. These changes seem to consist in an elevated fearfulness and an increased persistence of the emotional (fear) response induced by a stressful (pain) experience.

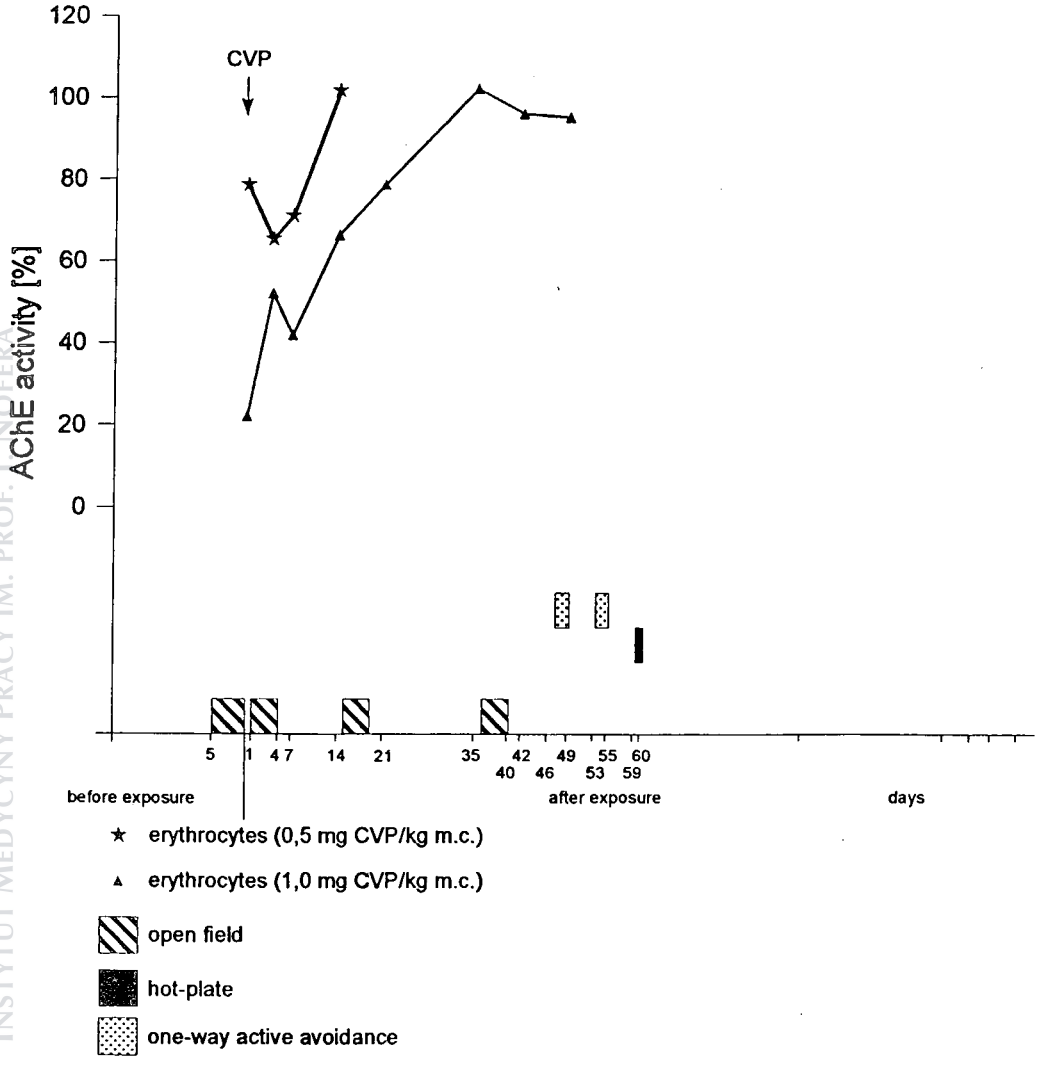


Fig. 10. A diagram illustrating the temporal distribution of behavioural testing vs changes in rbcChE (AChE) activity in rats injected repeatedly (ten times) to CVP at the daily dose of 1.0 mg/kg or 3.0 mg/kg. (Adapted from: Soćko 1996).

EFFECTS OF REPEATED EXPOSURE TO CVP ON THE RAT POSTEXPOSURE BEHAVIOUR

In the next experiment we studied behavioural effects of repeated i.p. exposure to CVP at subsymptomatic doses. As before, three groups of rats were used. Each rat was given ten i.p. injections within a period of two weeks (one injection/day for five days a week). The rats were injected with pure olive oil (control group) or CVP diluted with oil in a dose of 0.5 mg/kg (group 0.5 mg/kg) or 1.0 mg/kg (group 1.0 mg/kg). A part of these animals served for determination of ChE activity at preselected time intervals after the last exposure and another part for behavioural testing.

In the case of the 0.5 mg/kg dose, the ChE activity measured 3 h after the last (tenth) exposure was lowered by no more than 40% and 30% in blood and the brain, respectively, and returned to the preexposure level within 14 days. In the case of the 1.0 mg/kg dose, 3 h after the last exposure the sChE and the rbcChE activities were lowered by about 50% and 78%, respectively. The recovery took about two weeks in the case of sChE, and about five weeks in the case of rbcChE. In the case of the brain ChE, the activities measured 3 h after the last exposure were decreased by 55% (cerebellum) to 65% (frontal pole). The brain ChE activity recovered at a similar rate as the rbcChE.

Fig. 10 shows the sequence of behavioural testing vs. changes in rbcChE activity. The final testing in the open field and all the remaining tests were performed when the level of rbcChE activity was within the normal limits. Only results of the hot-plate test revealed an effect of exposure; in the first postshock trial performed immediately after shocking the paw-lick latencies in group 1.0 mg/kg were significantly longer than in the control group (Fig. 11).

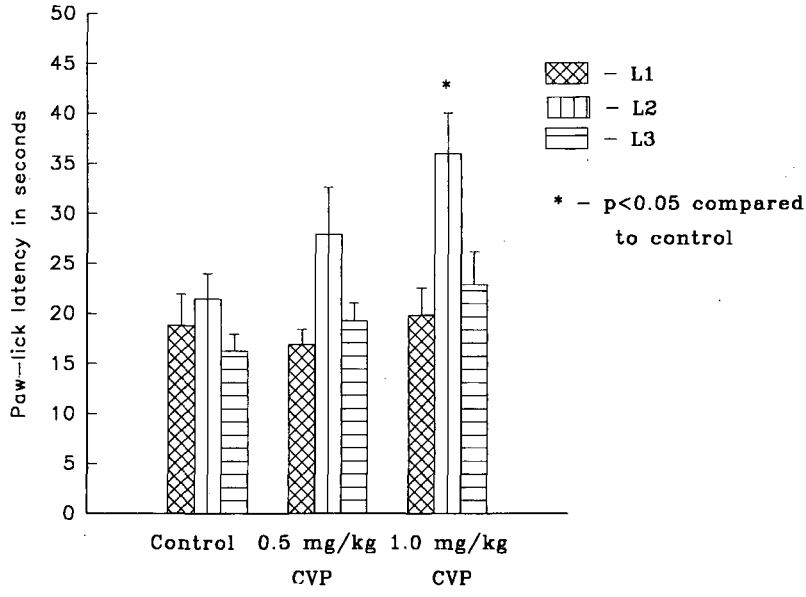


Fig. 11. A diagram illustrating the effect of a 2-min intermittent footshock on the latency of the paw-lick response to heat in control rats and rats exposed repeatedly (ten times) to CVP at the daily dose of 0.5 mg/kg or 1.0 mg/kg. The bars represent group means and SE. L1, L2, and L3 – paw-lick latencies before footshock, immediately after the footshock and 24 h after the footshock, respectively. (Adapted from: Soćko 1996).

In conclusion, the obtained results suggest that a single exposure to CVP at both symptomatic (3.0 mg/kg) and subsymptomatic (1.0 mg/kg) doses might result in subtle changes in animal behaviour which are detectable after normalization of ChE activity in blood and in the brain. These changes include a suppression of the novelty-induced increase in locomotor and exploratory activity in the open-field, and a higher and more persistent footshock-induced increase in the latency of the paw-lick response to heat (hot-plate test). The latter effect is also produced by repeated (ten) exposures to CVP at daily doses of 1.0 mg/kg. The character of the observed behavioural changes suggests a lowered threshold for induction of the emotional fear response in the exposed animals. This conclusion has been supported in experiments on rabbits and rats exposed repeatedly (ten i.p. injections during two weeks) to CVP, in which hippocampal EEG response to an external stimulus was analysed.

PERSISTENT EFFECTS OF REPEATED EXPOSURE TO CVP ON EEG

The hippocampal EEG response to an arousing event consists in appearance of the theta rhythm (Fig. 12). We tested this response on rabbits before and on day 36 after ten i.p. exposures to CVP (one injection/day) at daily doses of 14 mg/kg. Three hours after the last of the ten injections the rbcChE activity was reduced by about 60%. It returned to preexposure level within 35 days. Fig. 13 illustrates the magnitude and the habituation rate of the hippocampal theta response to a series of 30 clicks, before and after the exposure. The clicks were presented at randomly arranged 10–15 s intervals. In the CVP injected rabbits, unlike in rabbits injected with pure oil, the magnitude of the theta response after the exposure was significantly increased but the habituation proceeded at a similar rate as before the exposure. Another test was performed on the same rabbits on days 41, 42 and 43 after the last exposure. In this test the rabbits were presented repeatedly (ten times per session) to a 1000 Hz tone of 10 s duration before and after a conditioning procedure. As a result of this procedure, the tone became a conditioned stimulus signalling an unavoidable footshock and as such it induced signs of fear on the behavioural level accompanied by theta rhythm

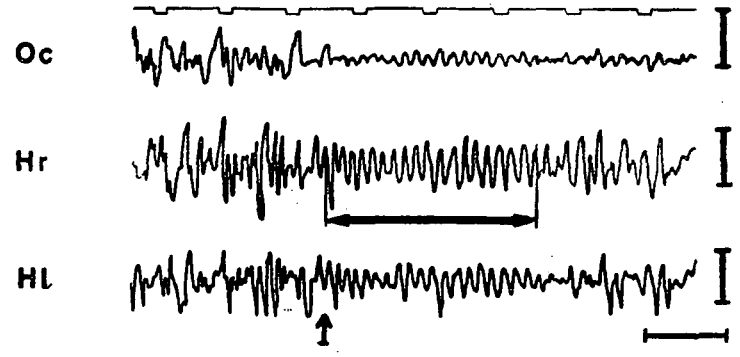


Fig. 12. An EEG section showing the hippocampal arousal response to click. Denotation of traces: Oc – occipital cortex; Hr – right hippocampus, Hl – left hippocampus. The upward directed arrow marks the moment of the click application. The double horizontal arrow marks the beginning and the end of arousal response. Callibration: 200 μV, 1 sec. (Adapted from: Gralewicz et al. 1990).

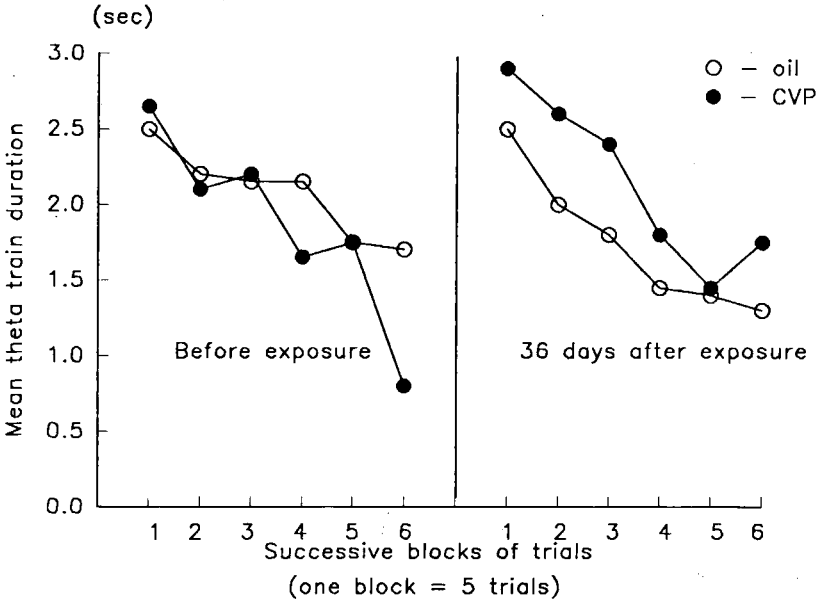


Fig. 13. A comparison of the magnitude and the habituation rate of the hippocampal arousal response to click before and 56 days after repeated (ten times) i.p. exposure to oil or to CVP at the daily dose of 14 mg/kg in rabbits. (Adapted from: Gralewicz et al. 1990).

Table 2. Changes in the contribution of 4–7 Hz theta rhythm in the hippocampal EEG after intrahypothalamic injections of oxotremorine (20 μg) not preceded and preceded by injections of CVP (1360 μg) into the same loci (n = 5) (Adapted from Gralewicz et al. 1994)

		Percentage theta contribution in successive 4 min EEG sections							
		Before injection of oxotremorine				After intrahypothalamic injection of 20 μg oxotremorine			
None	a	44.6	40.2	39.7	39.5	58.0	66.7	64.7	66.4
		(5.5)	(5.1)	(5.5)	(2.1)	(4.0)	(3.6)	(4.7)	(2.9)
	b	–	–	–	–	+	+	±	±
CVP	a	46.1	48.0	34.7	44.6	56.3	63.7	66.5	66.2
1360 μg		(5.4)	(4.0)	(5.2)	(4.0)	(4.0)	(4.1)	(3.6)	(3.6)
	b	–	–	–	–	+	+	±	±

Values in pharentheses – SD; + – behavioural response present, – – behaviournal response absent; ± – behavioural response present after the injection but disappeared before the end of testing.

in the hippocampal EEG. In exposed rabbits, the magnitude of the hippocampal theta response to the tone was significantly higher after the conditioning compared to that before conditioning in the same animals as well as after conditioning in control rabbits (Fig. 14). As revealed by the spectral analysis, mainly the slow (4–7 Hz) cholinergically mediated (4) component of the theta rhythm participated in this increase. A similar effect was observed in rats tested in the same conditions on days 39–41 after a repeated (ten daily i.p. injections) exposure to CVP at 1.0 mg/kg dose (22).

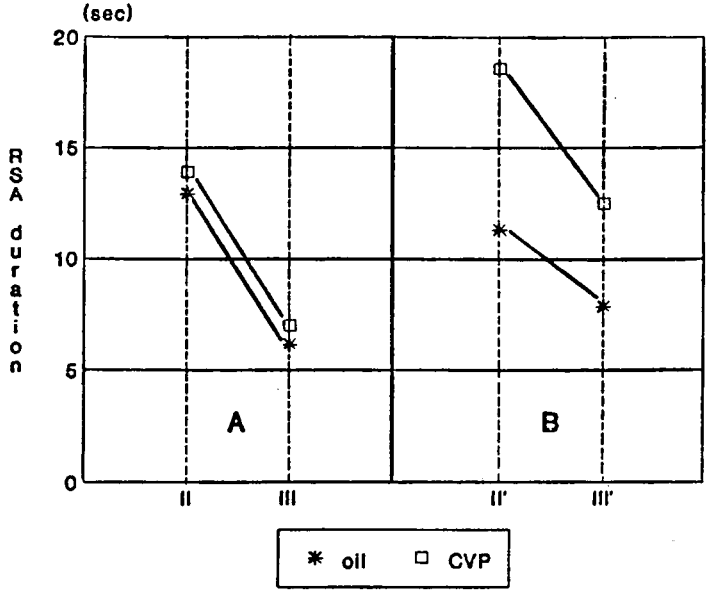


Fig. 14. A comparison of the magnitude of the hippocampal arousal response to a 1000 Hz tone before (A) and after (B) its association with an unavoidable footshock in rabbits. The test was performed on days 41—43 after the repeated (ten times) exposure to oil or to CVP at the daily dose of 14 mg/kg. Ordinates: duration of the theta trains measured from the tone onset. Abscissa: Roman numerals denote successive five trial blocks of a ten-trial session during which the tone was presented. Three sessions were performed each preceeded by a 5-min adaptation period (period I). During the fitst ession (left panel) the tone was presented ten times for 10 sec with 50 sec interval (periods II and III). During the second session (data not shown) an intermittent footshock accompanied the last 5 sec of each tone presentation. During the third session (right panel) the tone was presented in the same way as during the first session (periods II' and III').

EFFECTS OF INTRABRAIN CVP INJECTIONS. IS CVP AN ANTAGONIST OF THE CENTRAL MUSCARINIC RECEPTORS?

An interesting observation from the first experiment (page 4) was the apparent disproportionality between the CVP induced AChE inhibition and the magnitude of the hippocampal EEG response; an increase in the contribution of the theta rhythm in the hippocampal EEG was observed only in rabbits in which the AChE activity after the injection decreased much below 50% of the preinjection value. It was surprising, since in a pilot study on rabbits receiving i.p. injections of eserine (a carbamate anticholinesterase), a continuous theta rhythm in the hippocampus was produced by doses reducing AChE activity by less than 50% (Gralewicz, unpublished data). Similar differences in the magnitude of the hippocampal EEG response to CVP and eserine were observed also in rats (19,50). The theta rhythm dominating the hippocampal EEG after i.p. injections of CVP or eserine (see Fig.3) has a frequency of 4—7 Hz (slow, type 2 theta). According to the existing evidence, its presence reflects the activity of the central cholinergic (muscarinic) system (4). Therefore, the apparently lower ability of CVP, compared to that of eserine, to induce the theta response at a similar level of AChE inhibition, has led us to the conclusion that CVP may interact with central cholinergic receptors as their antagonist.

The validity of the above conclusion was checked in an experiment in which the behavioural and electroencephalographic responses to CVP or eserine, injected directly into the rabbit anterior hypothalamic area, were studied (23). An intrahypothalamic injection of cholinergic (muscarinic) agonists, like carbachol (5 – 10 μg) or oxotremorine (15 μg), induces, within few minutes, an attentive posture with occasional feet thumping (a threat behaviour) and a continuous theta rhythm in the hippocampus. The same response was observed after injections of eserine (200 μg) even if the injection was made 2h after a pretreatment of the hypothalamus with hemicholinium (HC3). HC3 prevents the synthesis of ACh which leads to a depletion of ACh stores and a subsequent blockade of cholinergic transmission. Without ACh, inhibition of AChE could not be sufficient to stimulate postsynaptic cholinergic receptors. Therefore, the result suggests that eserine, apart from being an AChE inhibitor, is also a potent agonist of cholinergic muscarinic receptors. Intrahypothalamic injections of CVP, even at very high doses (1340 μg) produced no effect at all, and injections of oxotremorine, a muscarinic agonist, into the same locus 15 min after CVP injection induced a continuous theta rhythm and feet thumping. This suggests that CVP is neither agonist nor antagonist of the muscarinic cholinergic receptors. Therefore, effects of CVP on the behaviour could not be attributed to a direct action of this compound on cholinergic muscarinic receptors.

CONCLUDING REMARKS

The results of our experiments have demonstrated that in the rat as well as in the rabbit, exposure to CVP results in alterations in the CNS functional state which may persist after normalization of ChE activity in blood and in the brain. The alterations manifest themselves on the behavioural as well as on the EEG level. The changes in behaviour (open-field neophobia, a higher and more persistent emotional response to a painful stimulus) and in EEG (increased hippocampal 4 – 7 Hz theta response to a pain-signalling CS) suggest an increased reactivity of the system (or systems) responsible for induction of fear. It is worth noting that increased anxiety and a feeling of being endangered were common subjective symptoms reported by greenhouse workers exposed to OP pesticides, including CVP (3,44). Results of numerous studies leave no doubt that among the brain systems controlling the induction of negative emotional states the cholinergic system plays the main part (6,7,11). A direct electrical or chemical stimulation of cholinergic elements in the midbrain (5), hypothalamus (10), or hippocampus (33) induces, on the behavioural level, a set of somatic and vegetative symptoms referred to as "emotional defensive response" (5), and, on the level of EEG, a continuous theta rhythm in the hippocampus (10, 33). To date, there are no available data showing reliably that other neurotransmitter systems, apart from the cholinergic one, are directly influenced by CVP. The results of intrahypothalamic injections suggest that the effects of CVP on the cholinergic system are due to the inhibition of ChE and not to a direct interaction of this compound with cholinergic muscarinic receptors. Thus, in the case of CVP, the long-term depression of ChE activity may be the main cause of the increased reactivity of the brain cholinergic system which may last for a longer time than that necessary for a full ChE recovery.

The magnitude and duration of ChE inhibition sufficient to produce long-term alterations of the CNS functional state is an important question which requires clarification. As appears from our studies on rats, some changes in behaviour (e.g. neophobia in the open field) are detectable two to three weeks after a single exposure. This exposure (1.0 mg/kg) induced no overt acute symptoms and resulted in about 45% inhibition of rbcChE activity which returned to a normal level within four days. It may suggest that even low-level exposures to irreversible ChE inhibitors may lead to long-lasting neurobehavioural consequences.

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