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BEHAVIORAL SENSITIVITY TO AMPHETAMINE AFTER REPEATED EXPOSURE TO AN ORGANOPHOSPHOROUS PESTICIDE IN THE RAT. EFFECT OF COEXPOSURE TO RESTRAINT

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Abstract. In the work environment, chemical stressors coexists frequently with physical or psychological stressors. The purpose of the present experiment was to find out whether the effects of a repeated exposure to chlorphenvinphos (CVP), an organophosphorus pesticide, could be modified by a concurrent exposure to restraint, a psychological stressor. The experiment was performed on male Wistar rats. CVP was administered ten times (one injection/day) at doses of 0.5 or 1.0 mg/kg i.p. (1/20 and 1/10 of LD50, respectively) within a period of two weeks. A half of the rats from each group were immobilized in restraint chambers for 120 min/day starting 10–15 min after CVP injection. In each rat, the effect of 0.5 mg/kg of amphetamine (AMPH) and 0.75 mg/kg of scopolamine (SCOP) on motor activity in an open-field was tested three weeks or six weeks (in rats exposed to 0.5 mg/kg or 1.0 mg/kg doses of CVP, respectively) after the last exposure day. No clear cut effect on the behavioral responsiveness to AMPH or SCOP were noted in rats subjected to repeated restraint, repeated 0.5 mg/kg doses of CVP, or combination of these two stressors. In rats exposed to CVP at the 1.0 mg/kg doses, the behavioral response to AMPH was augmented and this effect was not apparently altered in rats coexposed to restraint The above result indicates that the repeated exposure to CVP may lead to functional alterations within the central nervous system and that coexposure to restraint neither facilitates nor prevents these alterations from development.

Key words:
Organophosphorous pesticide, Restraint, Behavioral sensitization, Amphetamine, Rat

INTRODUCTION

The nervous system is the primary target for many chemicals, some of which are commonly used in industry, agriculture and household. Its functional state is also influenced by various physical or psychological stimuli. The character, intensity and occurrence of non-chemical stimuli in a given environment (e.g. work place, home) may vary considerably depending on the technological processes, local conditions and interpersonal relations.

Many published reports indicate that various stimuli, chemical, physical or psychological, applied once or repeatedly, may trigger a process leading to a long-lasting change in the organism response to the initiating stimulus upon its reexposure, but also to other stimuli. This process has been termed as time-dependent sensitization (TDS), as it proceeds after the initiating stimulus termination [1–3]. The existing evidence suggests that a shift in functional balance between the neurotransmitter systems occurs in TDS and that the dopaminergic and glutamatergic systems play a key role in the TDS development [4,5]. From the present study point of view, the most intriguing fact is that the changes can proceed in opposite directions;

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the responsiveness to a test stimulus may either increase or decrease after the exposure. According to Antelman et al. [2,3] the direction of the change is not dependent on the specific properties of the initiating stimulus, e.g. its modality or chemical structure and pharmacological properties, but simply on its potential to induce the stress response, i.e. activate the hypothalamo-pituitary-adrenal axis. In their experiments, the exposure to week stressors led to an increase, and the exposure to strong stressors to a decrease in the behavioral response to a test stimulus applied weeks later.

In the work environment one may be exposed to a variety of stressful stimuli. Stress response may be induced by chemicals (not necessarily neurotoxic), which evoke unpleasant sensory sensations, physical stimuli (noise), overcrowding, the knowledge of being in a contact with something dangerous, and many other factors. Stress responses induced by each of the stressors may differ considerably in magnitude. Hence, as the Antelman et al. data suggest [2], the long-term consequences of exposure to one stressor may be opposite to those produced by another stressor. The question is what kind of long-term functional changes would develop after the organism was subjected to the action of several stressors. As far as the consequences of a chemical exposure are concerned, two situations need consideration. First, when a physical or psychological stressor accompanies the exposure to a chemical stressor (a common situation in many occupational settings), and second, when exposure to a chemical stressor occurs days or weeks after an exposure to a psychical or physical stressor. The present experiment concerns the first situation.

Based on the observations made by Antelman et al [2], one may expect that the long-term behavioral changes, developing after a low- and high-level exposures to a chemical stressor, will be opposite in character. Results apparently consistent with such expectation were obtained in our previous experiments in which rats were exposed repeatedly to chlorphenvinphos (CVP), an organophosphorous pesticide at low (0.5 mg/kg i.p.) or moderate (1.0 mg/kg i.p.) daily doses and tested for their behavioral responsiveness to amphetamine (AMPH) or scopolamine (SCOP). The adopted exposure-test interval, three weeks or six weeks in case of low and moderate dosing, respectively, sufficed for restitution of acetylcholinesterase activity in blood and the brain [6–8]. It has appeared that the daily dosing with 0.5 mg/kg of CVP made the rats hypersensitive, whereas dosing with 1.0 mg/kg CVP made them hyposensitive to the locomotion stimulating effect of AMPH or SCOP. The rise in the plasma corticosterone level after a CVP exposure [9] proves that exposure to this pesticide induces a stress response. After the 0.5 mg/kg dose, this response is probably weaker than after the 1.0 mg/kg one, which might account for the opposite effects of the lower and higher doses on the behavioral sensitivity to AMPH and SCOP. It is conceivable that the presence of a physical or psychological stressor during the CVP exposure would result in a stronger stress response than that produced by CVP alone. One may expect than that in animals subjected to an additional stressor during exposure to CVP at low (0.5 mg/kg) or moderate (1.0 mg/kg) doses the postexposure changes in the behavioral responsiveness to AMPH or SCOP would be reversed or augmented, respectively, compared to those developed after exposure to CVP alone. Such a result would indicate that non-chemical stressors concurrent with exposure to a neurotoxic chemical could determine not only the quantity but also the quality of at least some effects of the exposure. The purpose of the present experiment was to check the above assumption.

MATERIALS AND METHODS

Animals

The experiment was performed on 72 male Wistar rats (IMP:EPIF), outbreeds. The rats were 3-4 months old, with body weight of 310–380 g at the experiment onset. For two weeks before the start of the experiment and during the experiment they were housed in single rat cages at 22°C ± 0.5°C, with a light/dark cycle of 12/12 h (light on at 6:00). Standard rat food pellets (Murigran) and tap water were accessible *ad libitum*. Body weight was measured routinely once a week and before each injection.

Chemicals

CVP (2-chloro-1-(2,4-dichlorphenyl)vinyl diethyl phosphate), CAS REG. No [470-90-6], technical grade, was obtained as a gift from the manufacturer (ORGANIKA-AZOT, Jaworzno, Poland). AMPH (d-amphetamine sulphate) and SCOP (scopolamine hydrobromide – SCOP) were purchased from SIGMA. For injections, CVP was diluted in peanut oil. AMPH and SCOP salts were dissolved in bidistilled water. All chemicals used were administered intraperitoneally at 1.0 ml/kg volume.

Apparatus

The rat motor activity was assessed with the use of a computerized 4-unit set of activity chambers (PROFEX Ltd, Białystok, Poland). The set was located in a room, 6 • 2 • 3 m, neighbouring the animal rooms. It was illuminated with a row of four white luminescence bulbs located at the ceiling. The ambient temperature and humidity inside the testing room were the same as in the animal rooms. Each activity chamber consisted of clear acrylic open field box (63 • 63 • 40 cm) with 2 tiers of infrared motion sensors spaced 2.5 cm apart. The first and second tier of sensors were 4.0 cm and 15.0 cm from the cage floor. Each cage was equipped with a calculating system which transformed the beam interruptions into the location of the animal within the cage, 5 times per second. Raw data were stored in the cage memory. After the end of a test session the cage memory content was downloaded to a computer memory and subjected to further analysis with the aid of a computer program.

Testing the rat motor activity

Except two 1h habituation sessions, all remaining sessions consisted of two parts, preinjection and postinjection, each lasting 50 min. After completion of the preinjection part, the rat was transferred to its home cage for 8–10 min. During this time the activity chambers were thoroughly cleaned. Then the injection was made and the rat was put into the activity chamber for the next 50 min measurement.

The effect of the injected drug on the rat motor behavior was assessed by comparing the distance (DIS) covered by the rat during the postinjection and preinjection measurements. The DIS value was a sum of all shifts of the point representing the rat's body. Location of this point within the cage was calculated on the basis of interruptions of the beams emitted by the lower tier. The configuration of the beam interruptions changes not only during locomotion but also during nonambulatory movements (face and body washing, exploratory head movements etc.). Therefore, the DIS value may be regarded here as a global index of motor activity.

Immobilization

The rats were immobilized in cylindrical plastic chambers (restrainers), 250 mm long and 65 mm in the inner dia. The rear end of the restrainer was opened. A hole, 20 mm in dia., was cut in the middle of the front rounded end. The rear end could be locked by a cylindrical plug. The rat was placed in the restrainer in such a way that its muzzle and tail protruded outside through the holes in the front end and in the plug. Immobilized rats were left in their home cages for the fixed period of time and then freed.

Experimental procedure

At the start of the experiment the rats were habituated to the activity cages. The habituation consisted of two 1 h sessions performed two days apart. After habituation the rats were divided into eight groups: O1, OSt1, MP and MPSt, and O2, OSt2, DP and DPSt (Table 1). Care was taken to make the groups as similar as possible with respect to the mean body weight. Then the rats were treated with the selected "stressors" once a day, five days a week for two weeks. Rats of the O1, O2, OSt1 and OSt2 groups were given pure oil, and rats of the remaining groups were given CVP at doses of 0.5 mg/kg (groups MP and MPSt) or 1.0 mg/kg, (groups DP and DPSt). Ten to fifteen min after the injection the rats of the OSt1, OSt2, MPSt and DPSt groups were placed in the restrainers for 120 min. Rats of the remaining groups were left undisturbed in their home cages till the next injection. Three weeks (groups O1, OSt1, MP, and MPSt) or six weeks (groups O2, OSt2, DP, and DPSt) after the last treatment day, the rats were tested for their response to AMPH and

Table 1. Experimental groups and the treatment procedure

Group	Treatment (ten times, once a day during two weeks)	Number of days between the last treatment day and test session 1 (with AMPH)
O1 (n = 8)	Oil	21
OSt1 (n = 8)	Oil + 120 min restraint	21
MP (n = 10)	0.5 mg/kg of CVP	21
MPSt (n = 10)	0.5 mg/kg of CVP + 120 min restraint	21
O2 (n = 8)	Oil	42
Ost (n = 8)	Oil + 120 min restraint	42
DP (n = 10)	1.0 mg/kg of CVP	42
DPSt (n = 10)	1.0 mg/kg of CVP + 120 min restraint	42

SCOP challenges*. This part of the experiment consisted of three sessions, denoted as sessions 1, 2 and 3, with two-day between-session interval. After a 50 min preinjection testing, the rats were injected with 0.5 mg/kg dose of AMPH in session 1, with physiological saline (SAL) in session 2, and with 0.75 mg/kg dose of SCOP in session 3. The doses of AMPH and SCOP were established in a pilot experiment.

Statistical analysis

Statistical comparisons were performed with the use of a two-way ANOVA (groups x sessions) for repeated measures. Tukey test was used for multiple comparisons [10]. In case of the body weight data, the analysis was performed on relative values assuming the body weight on the first day of exposure as 100%. In case of motor activity measurements, direct values as well as relative values ((postinjection DIS/preinjection DIS) • 100) were subjected to the analysis.

RESULTS

The effect of exposure(s) on body weight

Two days before the start of the exposure the mean body weight varied from 355.2 g (±7.5 g) in group O (groups O1 and O2 combined), to 362.3 (±11.2) in group DP. Significant differences between groups appeared during the exposure. The values of six measurements were taken into account: three from the period of exposure (exposure days 3, 7, and 10) and three from the postexposure period (postexposure days 7, 14 and 21). The results are presented in Fig. 1. The effects of both main factors, as well as the interaction were significant (groups: $F(5,66) = 48.15$, $p < 0.0001$; measurements: $F(1,66) = 521.84$, $p < 0.0001$; interaction: $F(5,66 = 279.82$, $p < 0.0001$). Generally, group O differed significantly from all remaining groups, groups MP and DP did not differ from each other but both differed significantly from the immobilized groups, ie. from the MPSt, DPSt and OSt (OSt1 and OSt2 combined) groups. The immobilized groups did not differ from each other. In group O, body weight increased steadily during the experiment duration. In groups MP and DP, the increase was apparently inhibited at the beginning of the exposure and then it started to rise again at a similar rate as in group O. At no time point, the MP and DP groups differed between themselves but both differed from the immobilized groups. In all immobilized groups, body weight decreased significantly at the beginning of exposure

* The interval between the last exposure day and the day of the first pharmacological challenge was established on the basis of the results of earlier experiments [8]. They showed that after ten daily i.p injections of CVP at a dose of 0.5 mg/kg or 1.0 mg/kg, the acetylcholinesterase activity in blood and in the brain returns to normal level within 14 and 35 days, respectively.

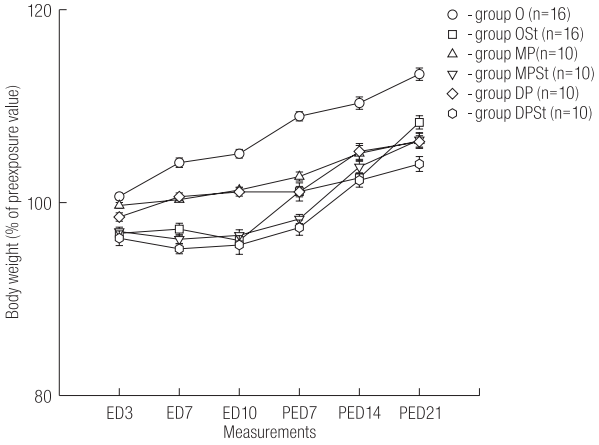

Fig 1. The comparison of changes in body weight of rats during, and three weeks after the repeated (ten times, once per day) exposure to following stresors: oil i.p.1.0 ml/kg (group O – control), CVP at daily i.p. doses of 0.5 mg/kg (group MP), or 1.0 mg/kg (group DP), to 120 min restraint (group OSt) or 120 min restraint + CVP at daily doses of 0.5 mg/kg (group MPSt) or 1.0 mg/kg (group DPSt). ED – exposure day, PED – postexposure day. Body weight on the first day of exposure was regarded as the reference (100%).

which was most evident in the DPSt group. The above results may be summarized as follows:

- the exposure to CVP or immobilization, each exerted negative influence on body weight;
- the effects of CVP at doses of 0.5 mg/kg and 1.0 mg/kg were similar quantitatively;
- the negative effect of repeated restraint was stronger than the effect of dosing with CVP.

The effect of exposure(s) on the behavioral response to amphetamine or scopolamine

Owing to the different time interval between exposure and the pharmacological challenge test, the data obtained on groups tested three weeks and those tested six weeks after the last treatment day were analysed separately. In both parts the groups were compared with respect to:

- the direct DIS values in the last habituation session (first 50 min) and preinjection parts of sessions 1, 2 and 3;
- the direct DIS values in the preinjection and postinjection parts of sessions 1, 2 and 3;
- the relative DIS values in sessions 1, 2 and 3.

A) A three-week treatment-test interval (Low CVP doses and restraint: groups O1, OSt1, MP and MPSt)

The comparisons of DIS values in session 0 and preinjection parts of sessions 1, 2 and 3

The analysis showed a significant effect of the session factor ($F(1,32) = 14.98$, $p < 0.001$) and significant interaction ($F(3,32) = 26.45$, $p < 0.0001$). The subsequent comparisons between groups in successive sessions revealed no significant differences. Comparison between sessions showed significant differences in group MP ($F(3,96) = 11.67$, $p < 0.0001$) and in group MPSt ($F(3,96) = 10.83$, $p < 0.0001$). In both these groups, the preinjection DIS value in session 2 was significantly higher than that in sessions 0 and 1, and the preinjection value in session 3 was significantly higher than that in session 0 (Fig. 2A).

The comparison of direct preinjection and postinjection DIS values in sessions 1, 2 and 3

The analysis showed a significant effect of the measurement factor ($F(1,32) = 23.13$, $p < 0.0001$) and significant groups x measurements interaction ($F(3,32 = 14.70$, $p < 0.0001$). Subsequent comparisons revealed no differences between groups in successive measurements but in each group the differences between successive measurements were significant. In session 1, all groups showed significantly higher postinjection DIS values (i.e. after the AMPH injection) than the preinjection ones. In session 2, the significant difference between the postinjection and preinjection DIS values was found only in group MP: the preinjection value being significantly higher than the postinjection one, and in session 3, the postinjection value was higher than the preinjection one only in group OSt1 (Fig. 3A).

The comparison of relative postinjection DIS values in sessions 1, 2 and 3

The analysis showed a significant effect of the session factor ($F(1,32) = 21.56$, $p < 0.0001$) and significant groups x sessions interaction ($F(3,32) = 8.57$, $p < 0.0005$). In none of the sessions the differences between groups were found. The comparison between sessions revealed differences in group O1 ($F(2,64) = 6.41$, $p < 0.005$), group MP (($F(2,64) = 9.72$, $p < 0.0005$) and group MPSt ($F(2,64) = 5.91$, $p < 0.005$). In these groups, the relative postinjection DIS values in session 1 (after AMPH) were significantly higher than those in session 2. In none of the

groups the results of sessions 2 (after SAL) and 3 (after SCOP) differed significantly (Fig. 4A).

The above comparisons indicate:

- no overt alterations in the rat spontaneous activity and in the magnitude of the behavioral response to AMPH or SCOP three weeks after repeated daily restraint;
- no alteration of the behavioral responsiveness to AMPH or SCOP after repeated daily i.p. administration of CVP at the 0.5mg/kg doses. It results, however, in a facilitated conditioning of the AMPH response to the experimental context.

B) A six-week treatment-test interval (Moderate CVP doses and restraint: groups O2, OSt2 DP and DPSt)

The comparisons of DIS values in session 0 and preinjection parts of sessions 1, 2 and 3

The analysis showed a significant effect of the session factor ($F(1,32) = 6.63$, $p < 0.02$) and significant sessions x groups interaction ($F(3,32) = 28.00$, $p < 0.0001$). In the last habituation session the groups did not differ between themselves. After exposure, the significant differences were found in session 1 only ($F(3,128) = 5.17$, $p < 0.005$); in this session the DPSt group was significantly more active as compared to groups OSt2 and DP, but not to group O2 (Fig. 2B).

The comparisons between sessions revealed significant differences in group DPSt ($F(3,96) = 5.09$, $p < 0.005$). In this group, the activity level in session 1 was insignificantly higher than in session 0 and significantly higher than in sessions 2 and 3.

The comparison of direct preinjection and postinjection DIS values in sessions 1, 2 and 3

The analysis revealed significant effect of measurements ($F(1,32) = 57.59$, $p < 0.0001$) and significant interaction ($F(3,32) = 16.86$, $p < 0.0001$). The differences between groups were found in the preinjection ($F(3,192) = 3.14$, $p < 0.05$), and postinjection ($F(3,192) = 4.19$, $p < 0.01$) measurements only from session 1. In the preinjection measurement, the activity level of group DPSt appeared to be significantly higher but only when compared to group DP. In the postinjection measurements, groups DP and DPSt were apparently more active than groups O2 and OSt2, but the difference reached the statistical significance only when groups OSt2 and DP were compared (Fig. 3B).

The comparisons between successive measurements revealed significant differences in all groups. In session 1 (with AMPH), all groups showed the postinjection DIS value significantly higher than the preinjection one. In session 2 (with SAL), in none of the groups the postinjection and preinjection values differed significantly. In session 3 (with SCOP), the postinjection values were significantly higher than the preinjection ones in group OSt2 DP and DPSt, but not in group O2.

The comparison of the relative postinjection DIS values in sessions 1, 2 and 3

The effect of the session factor ($F(1,32) = 52.09$, $p < 0.0001$) and the interaction ($F(3,32) = 24.32$, $p < 0.0001$) was significant. The subsequent comparisons between

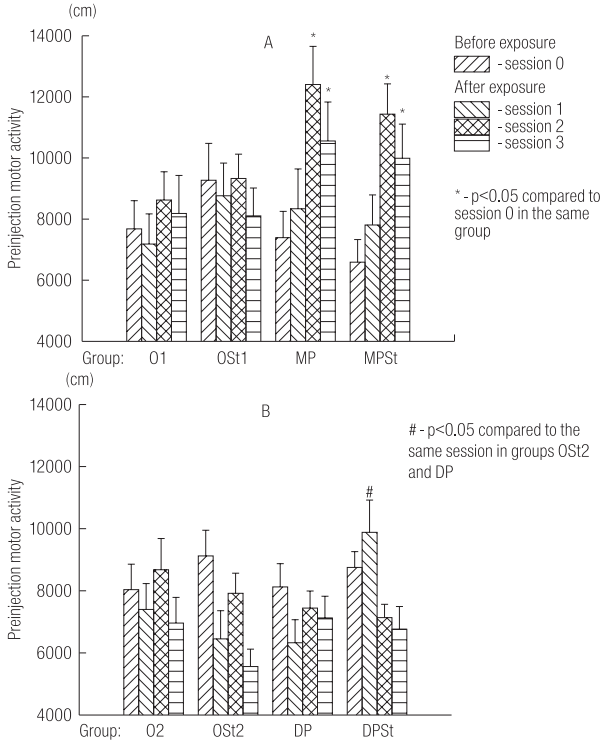

Fig. 2. The comparison of groups with respect to the direct preinjection DIS values (distances) in session 0 (before the exposure to stressors) and sessions 1, 2 and 3 (after the exposure). The bars represent means and SEM of the total distance (in cm) covered by the rat during a 50 min measurement preceding the injection.The rats were injected with 0.5 mg/kg of AMPH in session 1, with physiological saline in session 2, and with 0.75 mg/kg of SCOP in session 3. Denotations of groups as in Fig. 1. A – the results obtained in groups tested on days 21-25 after the last day of exposure; B – the results obtained in groups tested on days 42-46 after the last exposure.

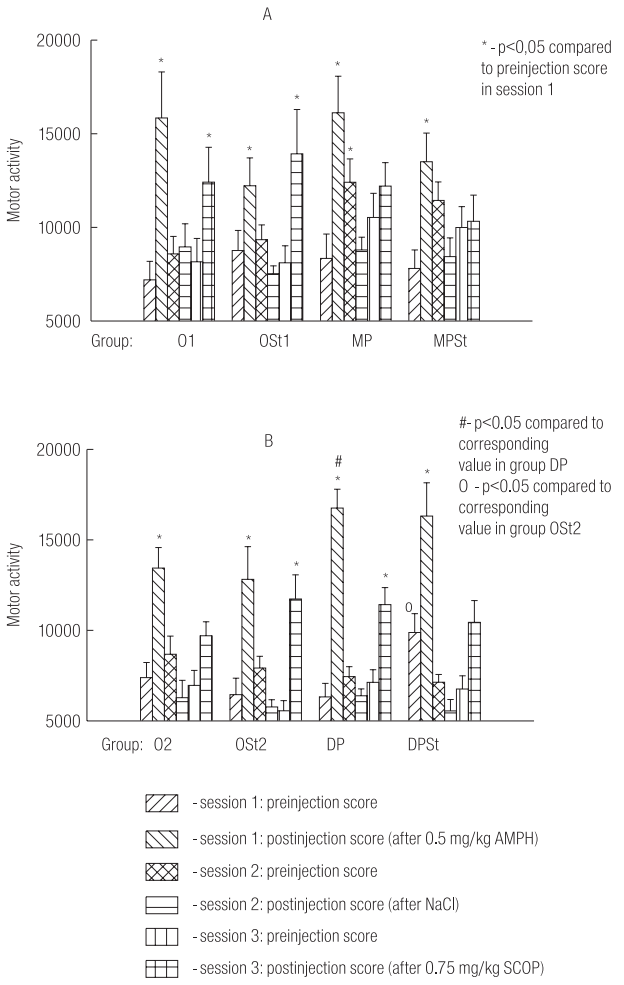

Fig. 3. The comparison of groups with respect to the direct preinjection and postinjection DIS values in sessions 1, 2 and 3. The bars represent means and SEM of the total distance (in cm) covered by the rat during a 50-min preinjection and a 50-min postinjection measurements. Remaining description as in Fig. 2.

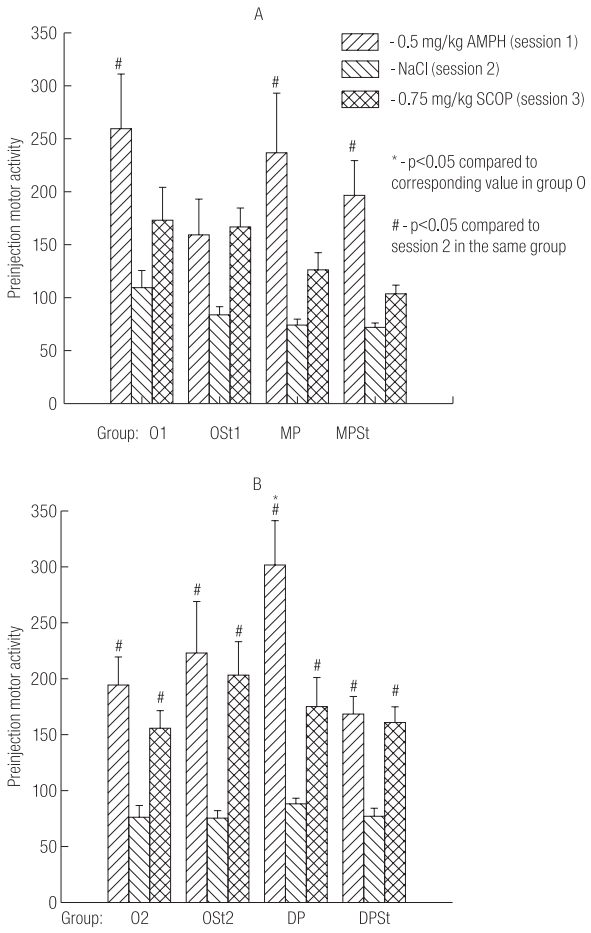

Fig. 4. The comparison of groups with respect to the relative DIS values ((postinjection DIS/preinjection DIS) • 100) in sessions 1, 2 and 3. Remaining description as in Fig. 2.

groups revealed differences in session 1 only ($F(3,96) = 6.61$, $p < 0.001$); the increase in activity was significantly more pronounced in group DP than in groups O2 and DPST. The comparison between sessions revealed differences in all groups. In all groups, the relative DIS values were significantly higher in sessions 1 and 3 as compared to session 2 (Fig 4B).

The results of the above comparisons indicate:

- no overt alterations in the rat spontaneous activity and in the magnitude of the behavioral response to AMPH or SCOP six weeks after repeated daily restraint;
- an increased behavioral responsiveness to AMPH but not SCOP after repeated daily i.p administration of CVP at doses of 1.0 mg/kg;
- no change in responsiveness to AMPH after applying the restraint during the CVP exposure. Restraint results, however, in a transient increase in spontaneous activity.

DISCUSSION

The obtained results confirm the negative effect of restraint on body weight [11]. The effect of exposure to CVP alone is less obvious. In groups MP and DP there was a retardation of the body weight increase, but this effect was apparently unrelated to the dose. An inspection of the data from our previous experiments shows that the dose-effect relationship was evident when the pesticide injection was followed by testing for the acute behavioral effects [6], but not when the rats were returned to their home cages immediately after the injection [12]. It allows

to suspect that some procedural factors (i.e. what the animal is required to do after the pesticide administration) may influence the effect of CVP on body weight.

The results of the pharmacological challenges are not fully consistent with our expectancies. First, based on the data from our earlier experiments [6,13,14], we expected an increased behavioral reactivity to AMPH and SCOP in group MP and an opposite effect in group DP. Second, assuming that the combined stressor, restraint + CVP, is more stressful than each of these stressors alone, we expected that after the exposure the locomotion stimulating effect of AMPH and SCOP would be diminished not only in group DPSt but also in group MPSt, i.e. that the differences between the MPSt and DPSt groups would be only quantitative (a stronger reduction in group DPSt). Third, based on numerous literature data [e.g. 15–18], we expected an augmented reactivity to AMPH in the OSt groups. None of these expectancies have been fulfilled in the present experiment. A facilitated conditioning of the acute AMPH response (increased locomotion) to the test environment seems to be the only effect of the low-level CVP exposure (doses of 0.5 mg/kg), and the effect of the moderate-level exposure (doses of 1.0 mg/kg) consists in increased rather than decreased responsiveness to the locomotion-stimulating effect of AMPH. It would be very interesting to find out whether the absence of the conditioned increase in activity in groups DP and DPSt was due to the CVP doses (too high?) or to the time between the last CVP injection and the AMPH challenge (too long?). A repeated restraint alone did not apparently produce any behavioral consequences. The only effect which might, tentatively, be ascribed to the combination of this stressor with the CVP exposure was the increased spontaneous activity on the first postexposure test session.

Several factors may be responsible for the inconsistency between the results obtained in groups MP and DP and those obtained in similarly treated groups in the previous experiment. The fact that the effect of exposure to CVP at the 1.0 mg/kg doses resembled that noted earlier after exposure to CVP at the 0.5 mg/kg doses, might suggest that in the present experiment the efficacy of the pesticide was diminished. A recent spectrophotometric analysis has shown that the inconsistencies cannot be accounted for the differences in chemical composition of CVP preparations. There remain, however, the differences in the experimental procedures and possible differences in the material used.

In the previous experiment, unlike in the present one, motor activity of the rat was assessed on the basis of a direct visual inspection which means that the sensory stimuli generated by the experimenter's presence in the test environment could influence the rat's behavior. We do not know at present whether and to what degree these procedure differences could be responsible for the inconsistency between the previous and present data. A preliminary test suggests a suppressive effect of the experimenter's presence in the test room on the rat's behavior in the activity cages.

The differences in the rat populations might also contribute to the differences in the results obtained in the previous and the present experiments. In the previous study we used Wistar rats, outbreeds, from our breeding colony (IMP/dak). Like many other laboratory rat populations, the population of IMP/dak is characterized by a large (up to 80%) proportion of subjects with an inherited propensity for spontaneous absence-like seizures [19]. The rats used in the present experiment came from a „non-epileptic" line obtained by a successive crossbreeding of the IMP/dak subjects with no spontaneous seizures. It cannot be excluded that the rats of this line and those from the general IMP/dak population differ also in other traits and that these differences contributed to the differences in the data obtained in the previous and present experiments.

The genetic endowment could also contribute to the absence of an overt effect of restraint on the behavioral reactivity to AMPH and SCOP in the OSt groups. In other words, it is possible that rats of the IMP:EPIF line are resistant to some effects of stress. Experiments on mice showed that the effects of restraint on the behavioral reactivity to pharmacological challenges [20, 21], the function of the dopaminergic structures [22,23] and the magnitude of the stress response [24] are all strain-related. Another factor preventing the effect of restraint on the responsive-

ness to AMPH in the present experiment may be the fact that the adopted procedure enabled relatively fast adaptation [25,26].

In spite of the inconsistencies between the expected and the obtained results, the data from the present experiment show once again that the exposure to CVP may result in neurobehavioral alterations detectable after time sufficient for restitution of AChE activity. It is evidenced by the significant increase in activity in the preinjection parts of sessions 2 and 3 in groups MP and MPSt, and by the significantly augmented behavioral reactivity to AMPH in the DP group. They also show that the introduction of an additional, non-chemical stressor during the exposure to CVP results in little or no alteration in the effect of exposure.

According to numerous literature data, exposure to variety of chemical stressors can lead to a long lasting increase in behavioral reactivity to AMPH, and this increase is due to the exposure-induced activation of the hypothalamo-pituitary-adrenal axis and the raised level of plasma glucocorticoid [2,3,16,24,27–29]. It has been shown that exposure to CVP results in enhanced plasma corticosteroids [9], which might account for the increased sensitivity to AMPH in groups DP and DPSt. However, restraint also induces the increase in the corticosteroid level [16]. The comparison of the effect of CVP and restraint on body weight during the exposure (present experiment) indicates that of the two stressors the restraint was the stronger one. In view of the above, the absence of overt consequences of the restraint, applied alone or in combination with CVP, on the response to AMPH and SCOP is hard to accept. It is worth noting, however, that according to some recently published reports, restraint may exert a protective rather than potentiating influence against the effect of a chemical stressor [30]. It has also been shown that in conditions of repeated restraint, the restraint-induced increases in the mRNA of the corticotropin-releasing factor (CRF) diminish and the responsiveness to new stressors declines [27,28]. These observations may account for, at least in part, the absence of the effect in group OSt, although they are of little help in explaining the results of the MPSt and DPSt groups.

According to the data obtained in previous studies [6] the repeated exposure to CVP at a dose of 0.5 mg/kg or 1.0 mg/kg results, respectively, in an increased or decreased responsiveness to SCOP. In the earlier experiment the postexposure responsiveness to AMPH and SCOP was tested on separate groups of animals, whereas in the present one each group was tested with both drugs and the testing with SCOP followed the testing with AMPH. It cannot be excluded that the AMPH injection changed the rat responsiveness to SCOP and that the AMPH-induced change overshadowed the change resulting from the earlier CVP exposure.

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