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DEVELOPMENT OF SPONTANEOUS, AGE-RELATED NONCONVULSIVE SEIZURE ELECTROCORTICAL ACTIVITY AND RADIAL-MAZE LEARNING AFTER EXPOSURE TO m-XYLENE IN RATS

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Abstract. It has been hypothesized that exposure to neurotoxins may result in accelerated ageing of the central nervous system (CNS). The present study investigated the effects of a 3-month (6 hr daily, 5 days/week) inhalation exposure of rats to m-xylene, at concentrations of 100 and 1000 ppm, on the spontaneous neocortical spike and wave discharges (SWD) and spatial learning in an eight-arm radial maze. According to the literature, the SWD activity increases and the ability to solve spatial problems worsens as the animal gets older (8). The testing in the maze (one trial daily for five days) was performed two months after the exposure. The SWD activity was assessed on the basis of the number and duration of SWD bursts in one-hour EEG recordings performed before the exposure, on day 28, 56 and 84 of exposure, and then on day 14, 28, 42, and 84 after the exposure. In rats exposed to 1000 ppm m-xylene, unlike in the controls, neither decrease in the number of omission errors nor decrease in response speed was noted in the course of training in the radial maze, which suggested a learning deficit. However, the development of the age-related SWD activity was significantly retarded in these rats when compared with the controls. In rats exposed to 100 ppm m-xylene, the effects on maze behaviour and the SWD activity were similar as in rats exposed to 1000 ppm m-xylene but they were less pronounced. The study has provided an experimental evidence for persistent changes in the CNS functions, resulting from a subchronic exposure to m-xylene. The changes, however, cannot be interpreted simply as an accelerated brain ageing.

INTRODUCTION

There is a growing suspicion that exposure to industrial and environmental neurotoxins may enhance the progression of the age-related degenerative changes in the nervous system. Experimental evidence of such an effect, however, is lacking

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(see 21, 26). If exposure to a neurotoxin accelerates the process of brain ageing, then the age-related changes should proceed faster in the exposed subjects than in the unexposed ones. In the rat, like in other mammals, a memory deficit, pronounced especially in spatial tasks, is an important symptom of advanced age (8). On the EEG level, the number and/or duration of spontaneous SWD bursts in the neocortex has been proposed as a good index of the biological age of the brain (5). The occurrence of the SWD bursts, reminiscent of human absence epilepsy, has been noted in laboratory rats of different strains. They progressively increase in number and become longer as the animal gets older (2, 24). According to the existing evidence, this increase in the SWD activity with age is due to progressive atrophic changes in the CNS cholinergic system (5). In humans, atrophy of this system developing with age is believed to be responsible for presenile and senile dementia (4). In our earlier experiments (12), an accelerated development of the SWD activity was found in rats after a prolonged (3 months) intake of ethanol, a suspected stimulant of brain aging (14). This effect might be regarded as an experimental confirmation of the assumed contribution of neurotoxin exposure to the processes of brain ageing.

Xylene is one of organic solvents commonly used. It is the main compound of many lacquer and paint thinners. Commercial xylene is a mixture of ortho-, meta- and para-isomers among which the meta-form is dominant. Neurotoxic properties of xylene, and other volatile aromatic hydrocarbons, are unquestionable (3). It is also of interest that presenile and senile dementia is more frequently diagnosed in patients with history of past long-term occupational exposure to paints and paint thinners than in those unexposed (13, 16). It makes one to assume that such an exposure may promote the development of age-related CNS dysfunctions. The present experiment was performed in order to find out whether and in what way 3-month inhalation exposure to m-xylene could influence the development of the SWD activity and performance in a radial maze (a spatial task) in the rat.

MATERIALS AND METHODS

Animals

Twenty male rats of the LOD-Wist stock, outbreds, 8 mo. old at the beginning of the experiment, were used. All animals had two chronic, monopolar recording electrodes implanted epidurally over the parietal cortex of both hemispheres, and two bipolar electrodes implanted bilaterally into the dorsal hippocampus. The construction of electrodes and the surgical technique was described in detail in earlier reports (10). In all the rats, the cortical activity was characterized by the occurrence of spontaneous SWD bursts, which was determined on the basis of preliminary EEG recordings. The animals were divided into three groups: two experimental groups exposed to m-xylene vapours at the concentrations of 100 ppm (group X-100), and 1000 ppm (group X-1000), and the control group (group C). The animals were housed in single rat cages under standard laboratory conditions (12/12 hrs L/D cycle with light on at 6.00 AM) with food and water *ad libitum*, except the period of the maze training.

Exposure procedure

M-xylene pure (REACHIM) was used. The rats of group X-100 and X-1000 were exposed to m-xylene vapours in 1.3 m^3 dynamic inhalation exposure chambers for six hours daily (from 8.00 AM to 2.00 PM, five days a week except Saturdays and Sundays and two days during which the EEG recordings were performed), during the period of 12 weeks. The rats of group C were sham-exposed. The details of exposure methodology were described in the previous works from this laboratory (17).

EEG recording and analysis

One-hour EEG recordings were performed between 9.00 AM and 1.00 PM on the day preceding the exposure, on day 28 and 56 of exposure, and on day 14, 28, 56 and 84 after the exposure. For the recording, the rats were transferred to recording compartment and placed into opaque plastic containers (25 × 25 × 35 cm). Next, cables connecting electrodes with inputs of the electroencephalograph, were attached. The recording started 10–15 min later. The EEGs were recorded on an 8-channel electroencephalograph, with time constant set at 0.3 s and high frequency filter set at 35 Hz. The electroencephalograph was in a room adjacent to the recording compartment. The behaviour of the rats during the recording was monitored with the aid of a TV system.

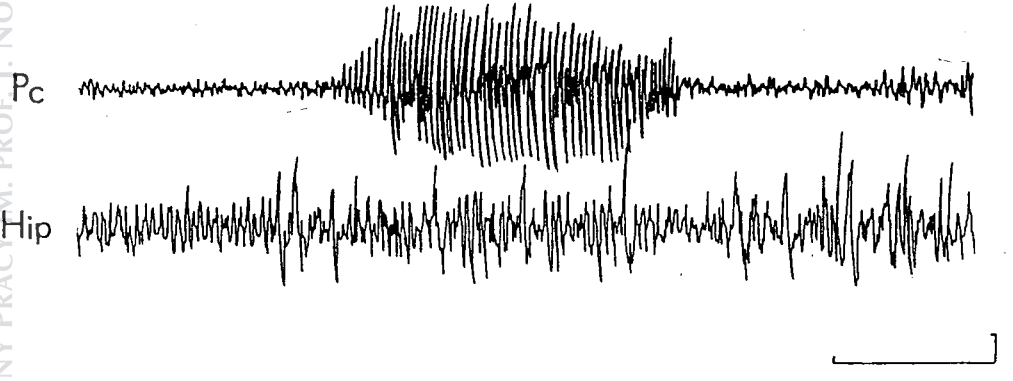

Fig. 1. A section of the rat EEG with the SWD burst (underlined) in the cortical derivation. Denotation of traces: Pc – parietal cortex, monopolar derivation, Hip – dorsal hippocampus, bipolar derivation. Callibration: vertical – 200 uV, horizontal – 3 s.

The following numerical values were obtained on the basis of visual inspection of the electroencephalograms:

a) the number and duration of the SWD episodes (Fig. 1);

b) the percentage distribution of the following states (levels of vigilance):

i) active arousal (AA) – EEG sections characterized by irregular, low amplitude cortical activity and theta rhythm in the hippocampus, not shorter than 10 s;

ii) slow wave sleep (SWS) – sections characterized by an irregular, high amplitude activity both in the cortex and in the hippocampus, not shorter than 10 s;

iii) paradoxical sleep (PS) – sections characterized by irregular, low-amplitude cortical activity and very regular, high amplitude theta rhythm in the hippocampus following directly the SWS episodes;

iv) transitional state (TS) – all the remaining sections which did not meet the AA, SWS, and PS criteria (Fig. 2).

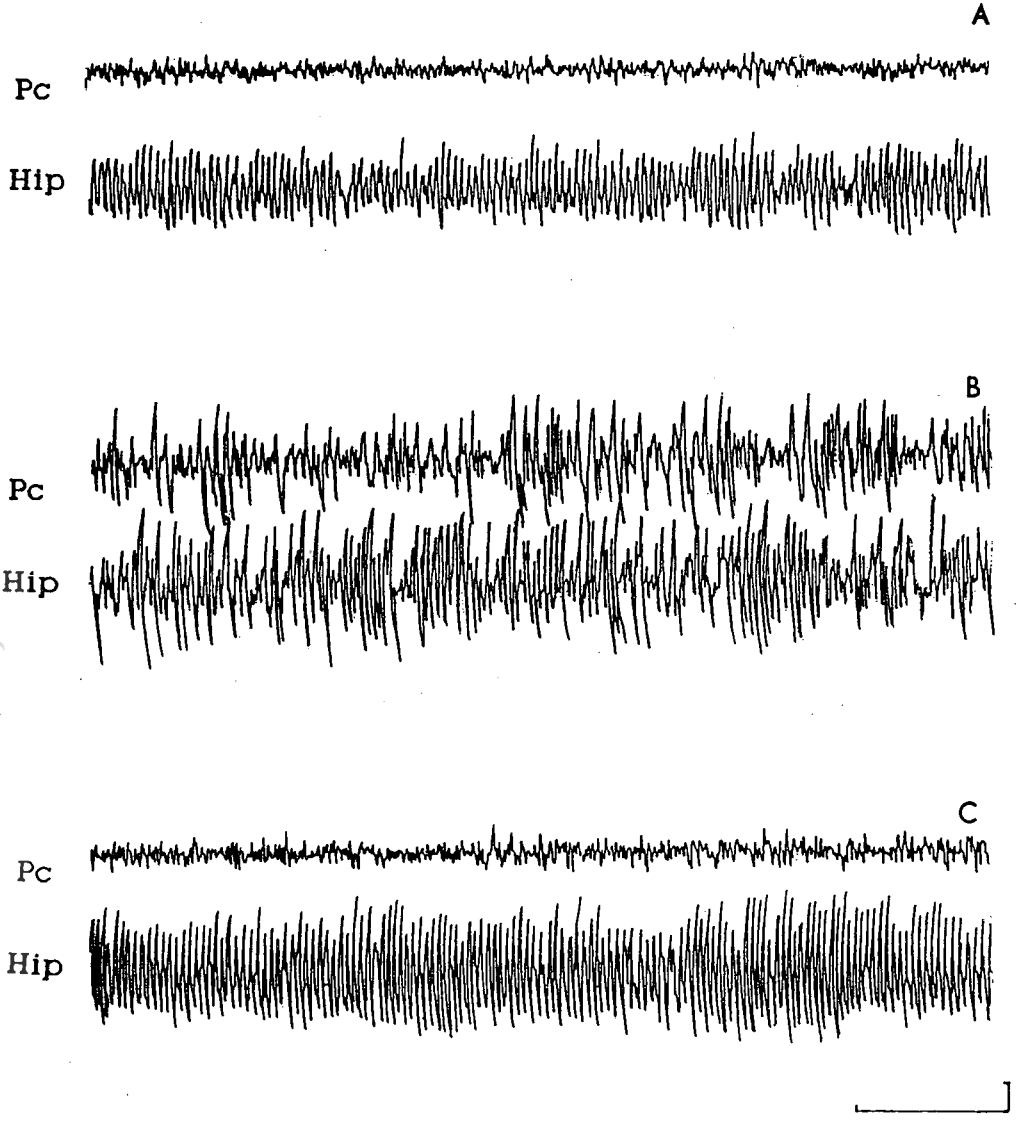

Fig. 2. Selected sections of the rat EEG during the distinguished states. Abbreviations to the right: A, active arousal (AA), B, slow wave sleep (SWS), C, paradoxical sleep (PS). Denotation of traces as on Fig. 1. Callibration: vertical – 10 uV, horizontal – 3 s.

Behavioural testing

The maze used in the present experiment was made of a steel foil, painted white, and consisted of a central, circular platform of 40 cm in diam. from which eight arms radiated at equal angles. The arms were 30.0 cm long, 12.5 cm wide and had 15.5 cm high walls which met at the edge of the central platform. A small 0.2 ml plastic container was fastened on the back wall of each arm.
The maze was located in the centre of a 2 × 4 × 3 m room, 80 cm above the floor level. The walls of the room were painted white. The cupboards, flower pots and paintings hinged on the walls served as spatial cues.

The testing in the radial maze lasted two weeks (from day 70 to day 83 after the exposure) and consisted of two stages. During the first stage (adaptation) the rats were familiarized with the maze. During the second stage (training) the effectivenes in finding water in the maze was investigated.

During the adaptation stage each rat was allowed to explore the maze for 5 min on five consecutive days. The arms contained no water. The total number of entries, the number of arms omitted (omission errors) and the number of reentries (perseveration errors) were noted.

Two days before the beginning of the training stage and for the period of its duration (five days) the rats had access to water in their home cages for 5 min a day only. Before each trial, the containers at the back wall of each arm were filled with water. Each rat received one daily trial on each of five consecutive days. At the beginning of each trial the rat was placed on the central platform of the maze and allowed to freely enter and obtain water at the end of each arm. A trial ended when all eight arms had been entered or 5 min had elapsed. An arm entry was recorded when the rat's four paws were on the floor of the arm. The total number of entries, the number of omission errors, the number of perseveration errors and the number of seconds required to complete the trial were recorded. Immediately after the end of each trial, the rat was transferred to its home cage and given water in an open 25 ml container for 5 min. The amount of water drunk during this time was measured.

Body weight was routinely measured.

Statistics

A one-way nonparametric or a two-way parametric ANOVA was applied for statistical evaluation of the obtained results (22,27). Differences were regarded as significant when $p < 0.05$.

RESULTS

Body weight

Figure 3 shows changes in body weight of the exposed and control rats in the course of the experiment. The two-way ANOVA (groups x successive measurements) revealed no significant effect of the group factor.

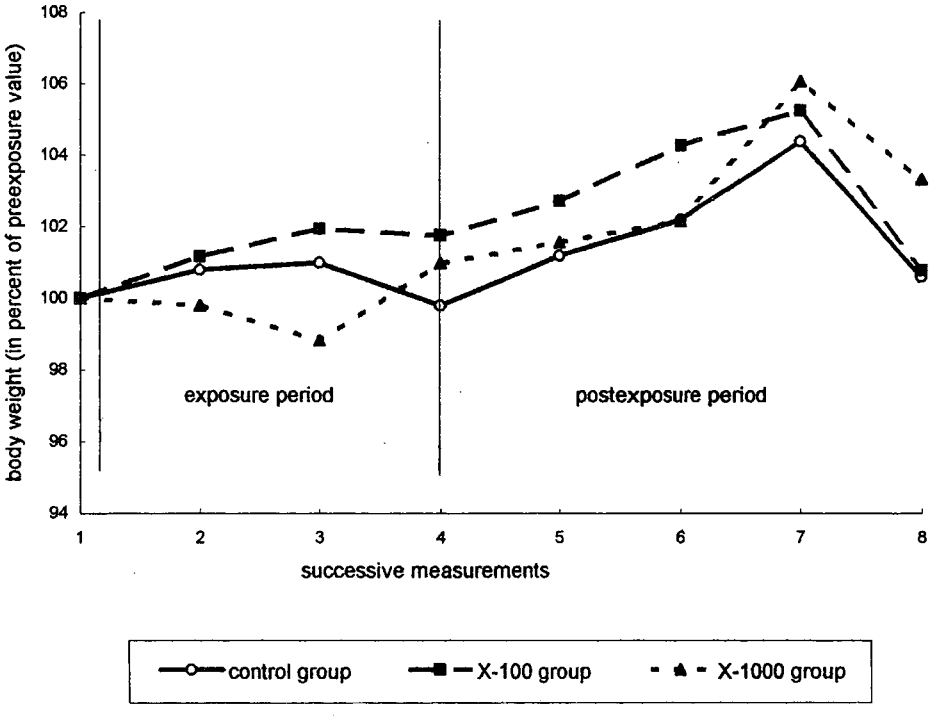

Fig. 3. Changes in body weight of rats exposed to m-xylene vapours at the concentrations of 100 ppm (X-100 group) and 1000 ppm (X-1000 group), and unexposed animals (control group) in the course of the experiment. Abscissa (successive measurements):

1 – a day before the exposure onset;
2 – 30th day of exposure;
3 – 60th day of exposure;
4 – 90th day of exposure;
5 – 14 days after exposure;
6 – 28 days after exposure;
7 – 42 days after exposure;
8 – 84 days after exposure.

Note: A 3–5% drop in body weight at the end of the experiment resulted from limited access to water during the maze training.

Effect of exposure on the development of the SWD activity

From the reports of other authors (6) as well as from our own observations (11) it is known that the SWD episodes occur predominantly during a transitional state, i.e. a state characterized by frequent shifts in electroencephalographic arousal. Therefore, the assessment of the SWD activity was based on the number and duration of the SWD episodes during the transitional state. It was preceded, however, by the analysis of the percentage distribution of all distinguished states in consecutive recording periods.

Generally, the transitional state dominated in consecutive recording periods. The mean percentage contribution of this state during all the recording periods was 70.45 (SD = 16.42) in group C, 74.42 (SD = 16.96) in group X-100, and 77.06 (SD = 14.14) in group X-1000 (Fig. 4).

The mean percentage contribution of the AA state was 15.64 (SD = 11.37) in group C, 14.73 (SD = 11.15) in group X-100, and 13.58 (SD = 11.11) in group X-1000.

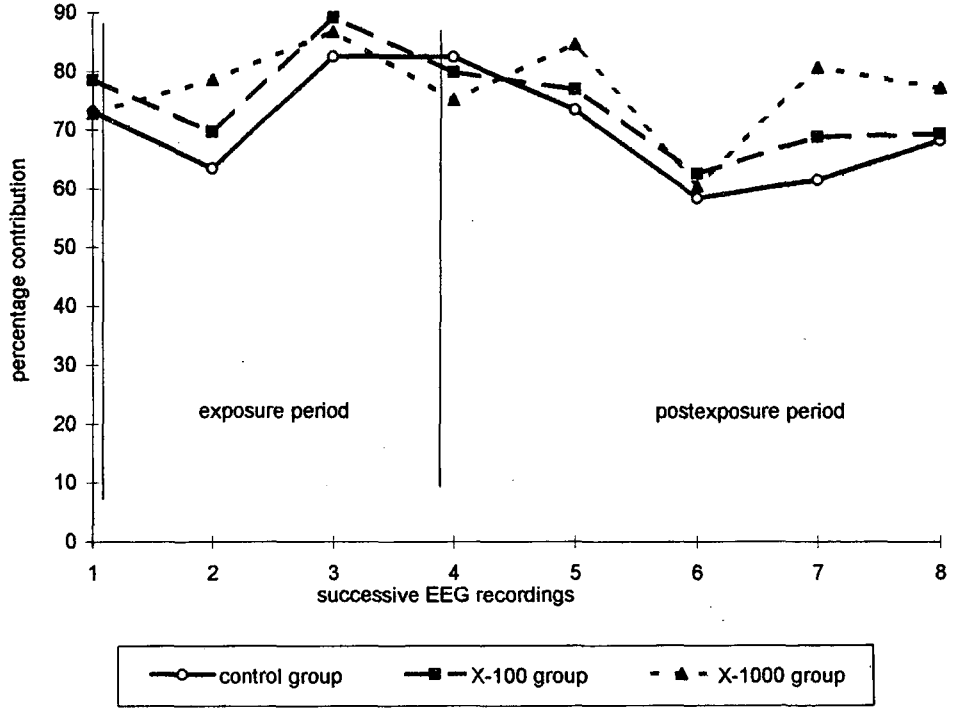

Fig. 4. Changes in the percentage distribution of the transitional state in successive one-hour recording periods in rats exposed to m-xylene vapours at the concentrations of 100 ppm (X-100 group) and 1000 ppm (X-1000 group), and in the unexposed rats (control group). Abscissa (successive EEG recordings) as in Fig. 3.

The mean percentage contribution of the SWS state was 14.19 (SD = 15.07) in group C, 11.06. (SD = 13.98) in group X-100, and 9.60 (SD = 10.64) in group X-1000.

The two-way ANOVA (groups x consecutive recordings) revealed neither significant effect of the group factor nor significant group x successive recordings interaction in the case of any of the above states.

The PS episodes appeared sporadically and the contribution of the PS state never exceeded 0.5% of the total recording time.

There was a large individual variability in the number and the duration of the SWD activity within each group. The mean number of the SWD bursts during the first one-hour recording (before the exposure) was 119.5 (SD = 70.7) in group C, 110.5 (SD = 31.4) in group X-100 and 150.9 (SD = 53.1) in group X-100. The mean total duration of the SWD activity was 749 s (SD = 425.9) in group C, 803 s (SD = = 270.2) in group X-100 and 1035 s (SD = 374) in group X-100. A nonparametric, one way ANOVA revealed no differences between groups with respect to any of the two measures.

For further analysis, direct values concerning the SWD activity in each rat were transformed into percentage, assuming the values from the first (before the exposure) recording day to be 100%. In the case of relative number of SWD episodes the analysis revealed no significant effect of any of the main factors. The groups x

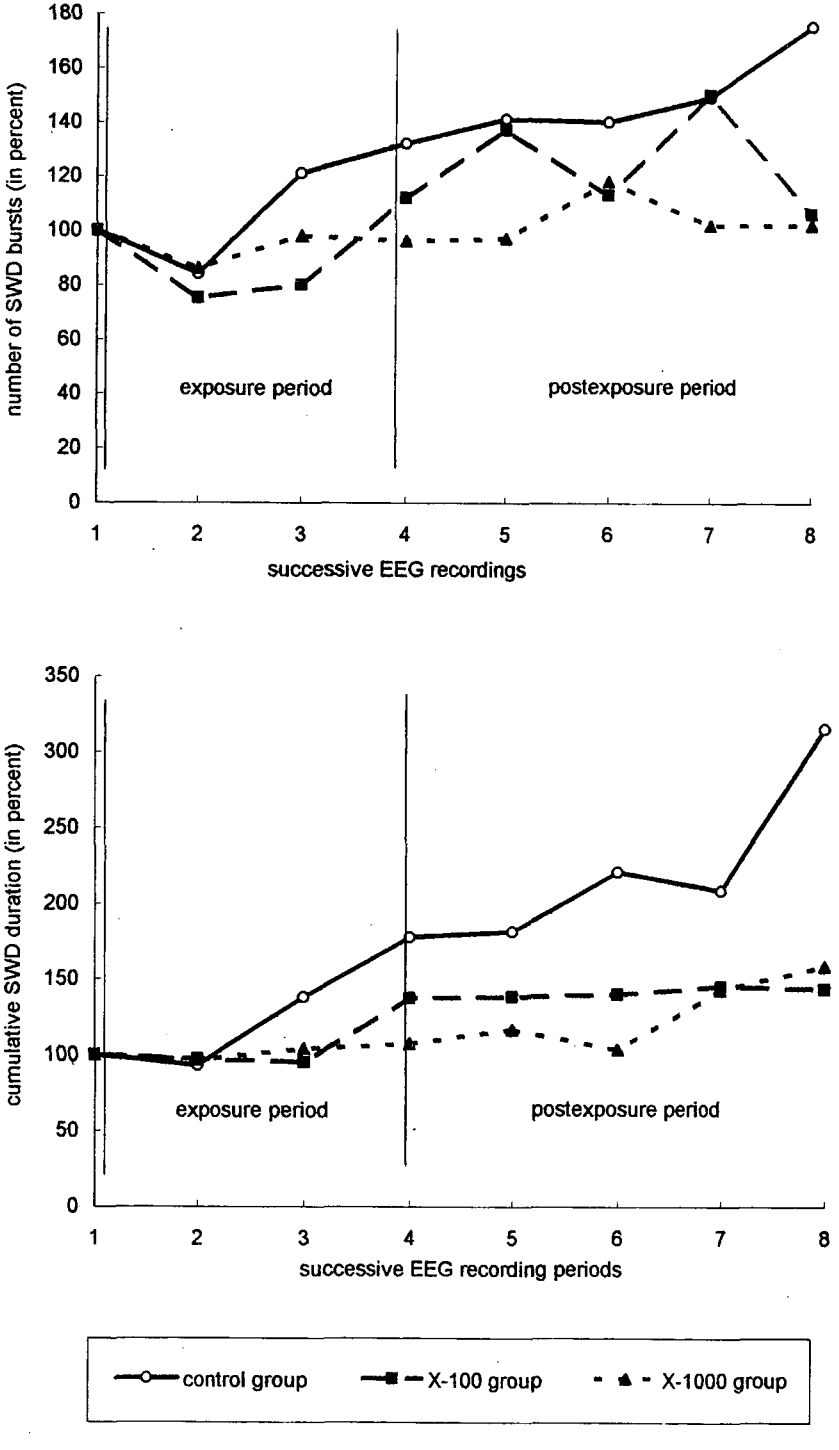

Fig. 5. Changes in the number of SWD bursts (upper diagram) and in the cumulative duration of the SWD activity in successive one-hour recording periods. The remaining description as in Fig. 4.

consecutive recordings interaction, however, was significant ($F(2,17) = 5.90$, $p < 0.02$). Subsequent comparisons between groups revealed significant differences only on day 84 after exposure ($F(2,136) = 8.74$, $p < 0.001$); on that day, the number of SWD episodes was significantly higher in group C than in group X-100 and group X-1000, but the exposed groups did not differ from each other (Fig. 5 upper diagram).

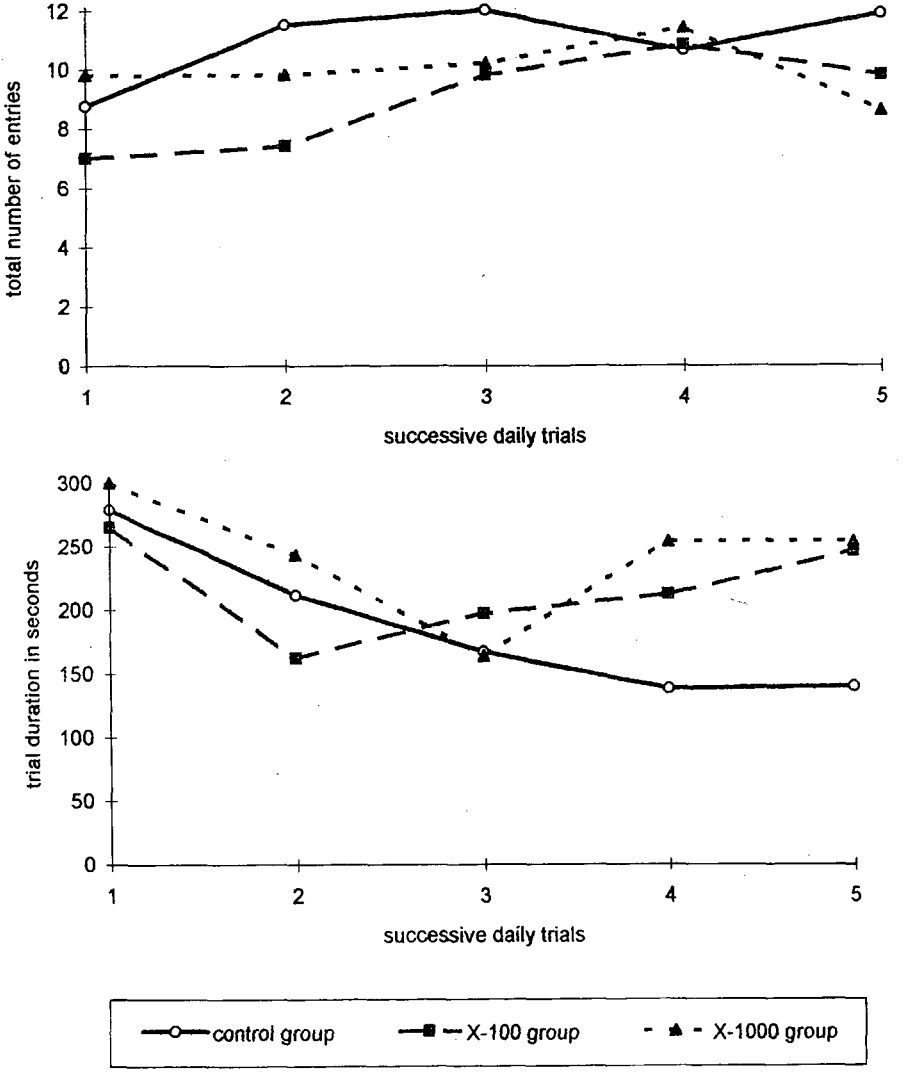

Fig. 6. Changes in the total number of arm entries (upper diagram) and trial duration (lower diagram) in successive daily trials of the maze training in rats exposed to m-xylene at the concentrations of 100 ppm (X-100 group) and 1000 ppm (X-1000 group) and in the unexposed rats (control group).

In the case of the cumulative duration of the SWD activity, the effects of the main factors were insignificant, but the groups x consecutive recordings interaction was significant ($F(2,17)) = 3.73$, $p < 0.05$). Comparisons between groups within con-

secutive recording periods revealed differences only during the 84th day after the exposure ($F(2,136) = 4.20$, $p < 0.02$); in group C the cumulative duration of SWD episodes was significantly ($p < 0.05$) longer than in both the exposed groups. Comparisons between successive recordings within each group revealed differences in group C only; on the last recording day, the cumulative duration of SWD episodes was significantly higher than on the first (before the exposure), on the second (28th day of exposure) and on the third (56th day of exposure) recording day (Fig. 5, lower diagram).

Effect of exposure on radial maze performance

The ANOVA (groups x days) revealed no differences between groups with respect to the total number of entries, number of omission and perseveration errors during the adaptation stage of the radial maze test. The groups x days interaction was also insignificant.

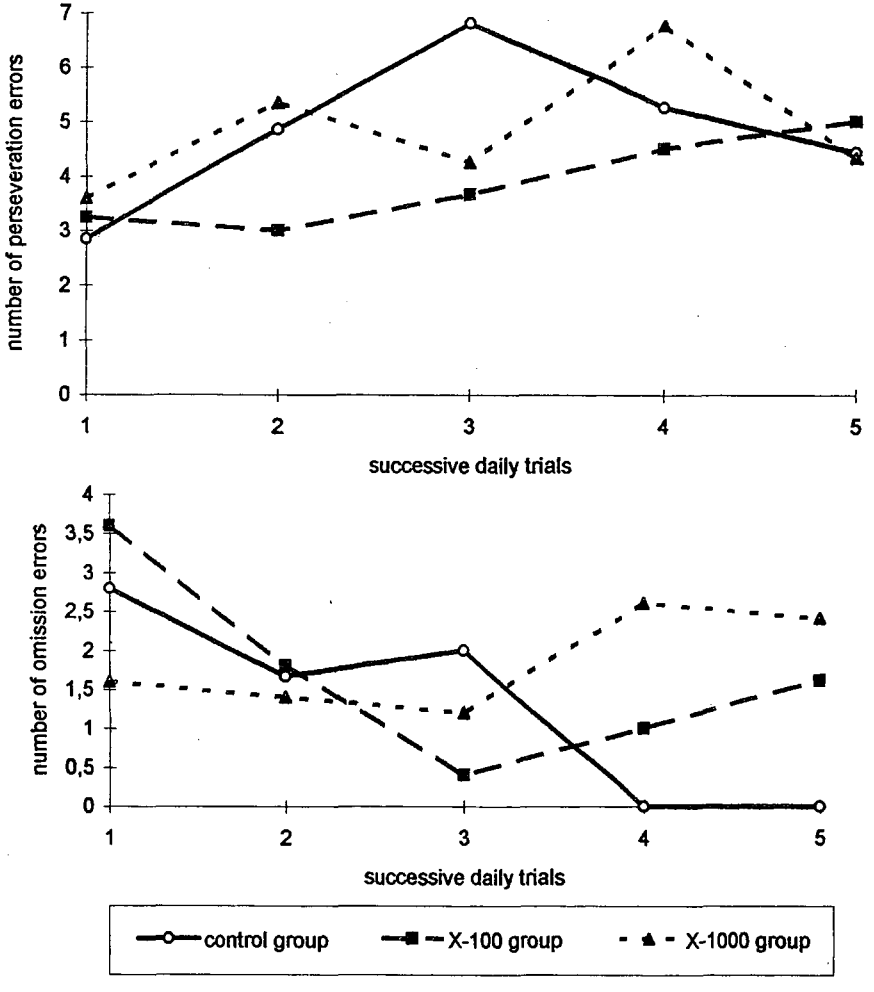

Fig. 7. Changes in the number of perseveration errors (upper diagram) and omission errors (lower diagram) in successive daily trials of the maze training. The remaining description as in Fig. 6.

In the case of the training stage, the analysis revealed no significant effect of the group factor on the trial duration, but the effect of days and the group x days interaction were significant ($F(1,15) = 5.95$, $p < 0.03$ and $F(2,75) = 7.13$, $p < 0.01$, respectively). Between group comparisons within consecutive days revealed significant differences on day 5 ($F(2,75) = 3.20$, $p < 0.05$); on that day, the trial duration in group C was significantly shorter than in both the exposed groups. Comparisons between consecutive days within each group revealed no differences in the case of groups X-100 and X-1000. Differences appeared only in group C ($F(4,60) = 5.52$, $p < 0.001$); on days 3, 4 and 5 the rats needed significantly less time to complete the trial than on day 1 (Fig. 6, lower diagram).

In the case of the total number of entries into the arms neither the effects of the main factors nor the interaction were significant (Fig. 6, upper diagram).

As regards the omission errors, the effect of the group factor was not significant, the effect of days was significant ($F(1,15) = 9.22$, $p < 0.05$) as well as the group x days interaction ($F(2,15) = 8.21$, $p < 0.01$). Successive comparisons between groups within consecutive days revealed no differences, but the comparisons between consecutive days within each group showed differences in group C ($F(4,60) = 3.20$, $p < 0.02$) and in group X-100 ($F(4,60) = 5.71$, $p < 0.001$). In both these groups the highest number of omission errors was observed on day 1 of the training, but it decreased on the following days. The rats of group C made no omission errors on days 4 and 5 (in both cases $p < 0.05$ in comparison with day 1). In rats of group X-100, the number of omission errors was the lowest during day 2 ($p < 0.05$ in comparison with day 1) and day 3 but it increased again on days 4 and 5. In group X-1000, the number of omission errors did not change across days (Fig. 7, lower diagram).

In the case of the perseveration errors, neither the effects of the main factors nor the interaction were significant (Fig. 7 upper diagram).

The analysis of the amount of water drunk within a 5-min period after each trial showed a significant effect of the group factor ($F(2,15) = 6.16$, $p < 0.02$); the rats of group X-1000 drunk significantly more water than the rats of group C, but not the rats of group 100. The effect of days was not significant (Fig. 8).

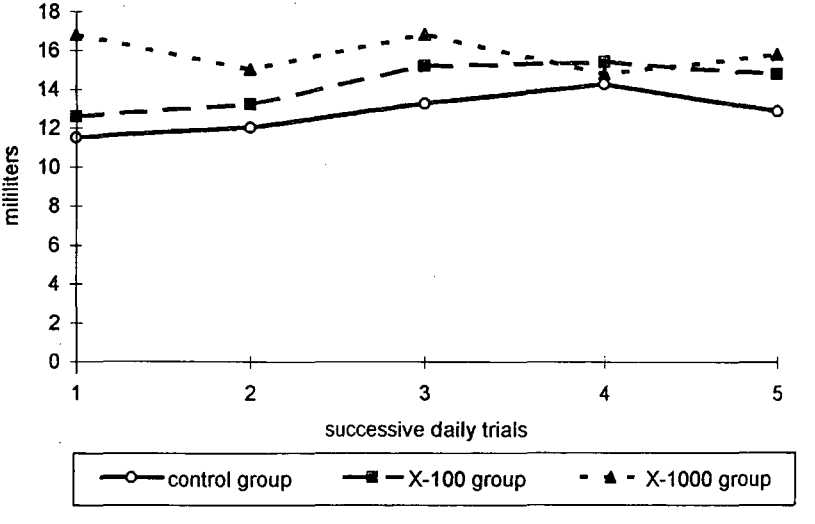

Fig. 8. A diagram showing changes in the amount of water drunk in a 5-min period after successive training trials in the radial maze. The remaining description as in Fig. 6.

DISCUSSION

The results obtained in the present studies showed that a three-month inhalation exposure to m-xylene at the concentrations of 100 or 1000 ppm: i) exerts no overt effect on the health status of the rats, which is suggested by lack of group differences in body weight throughout the experiment; ii) does not seem to influence the level of electroencephalograhic arousal; iii) retards, however, the development of spontaneous cortical SWD activity and impairs the behaviour in the radial maze. It is worth emphasizing that both the latter effects appeared at both exposure levels. Moreover, they were detectable more than two months after exposure, which suggested that they were persistent if not permanent.

A lack of effect of exposure to m-xylene at the applied concentrations on body weight is consistent with observations of other authors (17). The absence of any overt effect on the level of electroencephalographic arousal, were also noted earlier in this laboratory in rats exposed to 1000 ppm m-xylene for 6 months (Korsak, personal information). It contradicts, somehow, the human evidence for EEG changes in workers under long-term exposure to mixtures of organic solvents (19) and in volunteers exposed acutely to m-xylene (23). The reported changes, however, were subtle and spectral analysis of EEGs was required for their detection. The results concerning the SWD activity and maze behaviour are confusing. In our previous experiments, performed on the same strain of rats and under the same testing conditions, the most prominent difference between 24- and 6-month-old rats was a markedly increased SWD activity and a high number of perseveration errors in the maze (Gralewicz et al., in preparation). The retarded development of the SWD activity, and lack of an excessive number of perseveration errors in the exposed rats from the present experiment, suggest by no means an accelerated brain ageing. On the other hand, the remaining aspects of behaviour of the exposed rats in the maze during the training stage were hardly normal. A lack of a gradual shortening of the duration, as well as the persistence of omission errors in rats of the X-1000 group throughout the training, may suggest either a depressed locomotor activity or a learning deficits. In some earlier experiments from this laboratory, a depression of spontaneous locomotion was noted 24 hrs after a 6-month but not after a 3-month inhalation exposure to 1000 ppm m-xylene (17). Moreover, the absence of any differences between the exposed and control animals during the adaptation stage makes suppressed motor activity a rather unlikely explanation of a poorer performance of the exposed rats during the training stage in the maze. It might, perhaps, reflect a dysfunction in long-term memory, attention deficit or a decreased sensitivity to environmental stimuli. The present data do not allow for a more definite conclusion. It is worth mentioning here that somewhat similar deficits in solving a spatial test (water maze) was also observed after a subchronic (4 weeks) exposure to 80 ppm toluene (25), a solvent apparently less neurotoxic than m-xylene (17). An intriguing effect of exposure to 1000 ppm m-xylene is a significantly higher consumption of water during 5 min after each trial. A 5-min period was certainly too short to enable full satiation. Owing to that, the amount of water ingested during this time by the rats tells nothing about the physiological demand of water. For the time being, the most plausible explanation of a higher amount of water drunk by the exposed rats outside the maze seems to be an attention deficit and, therefore, a reduced distractability by external stimuli. It might result in a more effective

drinking. The latter supposition is in line with the proposed explanation of lack of progress in maze performance across trials seen in the exposed rats.

Considering the deficits in maze performance in our exposed rats, as well as reports showing a decreased content of acetylcholine in some brain areas of the xylene exposed rats (15), the prevention of the age-related deterioration of the cholinergic system seems to be an unplausible explanation of the retarded development of the SWD activity in our animals. One may speculate that exposure to m-xylene results in some changes in the CNS which contradict the effect of the age-related progressive degradation of the cholinergic system on the SWD activity. One of them may be an increase in catecholaminergic transmission. It has been found, that exposure to a high concentration of xylene (2000 ppm. 6 hr/day, for 3 days) increases the amount and turnover of catecholamines in some parts of the rat brain (1), and it has been demonstrated that the SWD activity is inhibited when the catecholaminergic transmission is increased (18).

Another possible reason for apparently retarded development of the SWD activity in exposed rats might be a decreased cortical excitability (9), a likely result of xylene-induced reduction in the efficiency of the axonal transport (20). The latter effect has been assumed to be the major cause of long-lasting or permanent functional deficits which may develop in humans and experimental animals exposed to solvents (20). A persistently reduced cortical excitability may also account for the disturbed maze behavior in our xylene exposed rats.

It should be stressed that, as far as changes in the SWD activity are concerned, the effect of inhalation exposure to m-xylene is apparently opposed to that seen after prolonged intake of ethanol (12). It may suggest a diverse rather than common mechanisms of neurotoxic action of these substances (7). Another reason for these discrepancies might be the difference in exposure conditions. The rats receiving 10% ethanol solution as the only fluid accessible 24 hrs a day in their home cages were likely to be more intoxicated than those breathing xylene vapour at the concentration of 1000 ppm for 6 hrs a day.

To sump up, the results of the above experiments reveal that subchronic exposure to m-xylene results in long-lasting disturbances in maze behaviour and influences the development of spontaneous cortical SWD activity. However, the deficits in maze behaviour do not mimic precisely those characteristic for old animals, and changes in the SWD activity are opposite to those developing with age. It suggests that the spatial pattern of biochemical and/or morphological changes in the CNS resulting from m-xylene exposure differs from that which develops in the course of natural ageing.

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