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RETARDATION OF THE AGE-RELATED INCREASE IN SPONTANEOUS CORTICAL SPIKE-WAVE DISCHARGES (SWD) IN RATS AFTER A 28-DAY INHALATION EXPOSURE TO AN INDUSTRIAL SOLVENT, PSEUDOCUMENE (1,2,4-TRIMETHYLBENZENE)

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Abstract. It has been hypothesized that exposure to neurotoxins may hasten the process of brain ageing. Volatile hydrocarbons are in common use as solvents and their neurotoxic properties are acknowledged. In the rat, the age-related neurodegenerative changes in the brain develop together with an increased occurrence of bursts of spontaneous spike-wave discharges (SWD) in the neocortex. Therefore, the number and/or duration of SWD bursts may serve as an index allowing to distinguish between young and old brains (7). Measuring the SWD activity after exposure may thus reveal the effect of the studied neurotoxicant on brain ageing. Pseudocumene (1,2,4-trimethylbenzene, TMB), is a component of industrial solvent mixtures. The present study investigated the effect of a 4-week (6h/day, 5 days/week) inhalation exposure to TMB at concentrations of 0, 25, 100 or 250 ppm on the occurrence of SWD bursts. EEG recordings were performed before and one day, 30 days and 4 months after exposure. In rats exposed to TMB at 0 and 25 ppm, the level of the SWD activity increased progressively after exposure. In rats exposed to TMB at 100 or 250 ppm, the SWD activity did not increase during the post-exposure period or even dropped below the pre-exposure level. This result indicates that the persistent changes in the rat central nervous system (CNS), following the exposure to TMB, differ in some respects from those which develop in the course of normal ageing.

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INTRODUCTION

It has been assumed that even a low-level exposure to neurotoxins may render the neural cells susceptible to premature senescence and death (31,38). If it is true, the symptoms of CNS senility should appear earlier in the exposed subjects than it could be expected from the subject's chronological age. In laboratory rats of many inbred and outbred strains, spontaneous bursts of rhythmic 7–11 Hz spike-and-wave discharges (SWDs) are present in

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neocortical EEGs during a state of awake immobility (5,7,9,27). The bursts increase in number and/or duration as the animal grows older and according to some authors (5, 7), these measures may serve as a diagnostic marker to distinguish between young and old brains. The increased occurrence of SWD bursts may be accompanied by poorer performance of memory tasks (5). It made some authors assume that the increase in SWD activity with age reflects the progress of age-related neurodegenerative changes in the CNS which, in humans, lead to senile dementia (7,13). Measuring the SWD activity may thus appear a useful tool in detecting the effects of exposure to neurotoxicants on the development of such changes (14).

Volatile aromatic and aliphatic hydrocarbons are in common use as paint and lacquer thinners, fuels, solvents and degreasers. Occupational exposure to these compounds may result in long-lasting neurobehavioural disturbances (11,12,17,20,21,26,30,32). It is worth noting that in persons with the history of a long-term exposure to paint thinners the prevalence of presenile and senile dementia is three times as high as in the non-exposed population (28). It supports the assumption, that exposure to neurotoxic substances may accelerate the age-related neurodegenerative processes in the CNS and suggests that industrial solvents have the potential to induce such effects. In an earlier experiment we checked this supposition by measuring the effect of a 3-month inhalation exposure (6 hours daily, 5 days/week), to m-xylene (1,3-dimethylbenzene), an important component of many industrial solvent mixtures, on the development of SWD activity in rats. Unexpectedly, the results revealed retarded rather than accelerated development of SWD activity in rats exposed to 100 or 1000 ppm m-xylene within a three-month observation period after exposure (15). Thus, at least in the case of m-xylene, the exposure seemed to ameliorate rather than accentuate the effects of age on the development of SWD activity. However, particular components of solvent mixtures may differ with respect to neurotoxic potency which makes it possible that exposure to other compounds will influence the development of SWD activity in a different way than exposure to m-xylene does.

The purpose of the present work was to find out whether and in what way the exposure to pseudocumene (1,2,4-trimethylbenzene-TMB) could influence the development of SWD activity in rats. Like m-xylene, TMB is one of the main components of commercial solvent mixtures and a measurable component of high-octane fuels (25,39,40). Literature data from animal studies concerning TMB toxicity are scarce (8) and those concerning long-term neurotoxic effects of exposure to this compound are lacking. Recently, it has been shown in our laboratory that a four-week inhalation exposure of rats to TMB at concentration of 100 or 250 ppm results in a transient decrease in sensitivity to pain and disturbances in rota-rod performance (24) as well as in a serious impairment of passive avoidance learning (16). The latter effect was revealed over a month after the exposure to 100 and 250 ppm TMB, which suggested that it was long-lasting, if not permanent. As mentioned above, a deficit in passive avoidance, likewise the increased SWD activity, is characteristic of old rats (5). It would thus be interesting to examine whether exposure to TMB under the same exposure conditions would also result in a faster development of SWD activity.

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MATERIALS AND METHODS

Animals

Male Wistar rats (of our Institute's breed), 5 mo. old at the beginning of the experiment, were used. They were housed at a standard laboratory conditions (temperature – 22°C, light cycle – 12 h light/12 h darkness with light on at 6:00 AM, humidity – 60%). The rats were caged one per cage, and had free access to food. Each rat was implanted with chronic intrabrain electrodes. Based on the preliminary EEG recording the animals were divided into four groups: the sham exposed group (TMB0 group n = 9), and three groups exposed to TMB at the following concentrations: 25 ppm (TMB25 group, n = 10), 100 ppm (TMB100 group, n = 9) and 250 ppm (TMB250 group, n = 10). The group means and SDs of the SWD burst number and the total SWD duration per hour were similar in each group.

Electrode implantation

The implantation of electrodes into the brain was performed using a stereotaxic instrument, under general (pentobarbital sodium, 50 mg/kg i.p.), and local (1.0 ml of 2% polocainum, s.c. into the wound area) anaesthesia. The plane between Bregma and Lambda was levelled to horizontal. Two bipolar electrodes, each made of two twisted strands of stainless steel teflon-coated wire (diam: 200u) were implanted bilaterally into the fronto-parietal cortex (2.0 mm anterior to Bregma and 2.0 mm lateral to the midsaggital suture) in such a way that the uninsulated (0.5 mm) tip of one strand penetrated the brain about 1.0 mm in depth and the second touched the cortical surface. Two other electrodes of a similar construction were implanted bilaterally into the dorsal hippocampus (3.5 mm posterior to Bregma, 2.0 mm lateral to the midline and 4.5 mm ventral to the skull surface). A stainless-steel jeweler screw fixed in the skull bone over the cerebellum served as ground during recording. The outer ends of the electrodes were soldered to a miniature female connector and the whole assembly was fixed to the skull with dental cement. After the surgery the animals were allowed to recover for a 14-day period.

Exposure

The rats of TMB25, TMB100 and TMB250 groups were exposed to TMB vapours (pseudocumene, 825442, FLUKA) 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, for four weeks. The rats of the TMB0 group were sham-exposed (the air flowing through the chamber contained no solvent). The details of exposure methodology were described in former papers from this laboratory (23).

EEG recording and analysis

One-hour EEG recordings were performed four times: on the day before exposure (recording 1), on the first day after the discontinuation of exposure (recording 2), one month and four months after exposure (recording 3 and 4, respectively). All recordings were performed between 9:00 AM and 1:00 PM. The equipment and the recording procedure were described in detail in an earlier work (15). For each rat, EEG transients from one cortical and one hippocampal derivation were analysed. As mentioned in the introduction, the occurrence of SWD bursts is limited to the

state of awake immobility. They do not appear when the animal is aroused or in a state of deep sleep or paradoxical sleep (10,14). Therefore, during the analysis the states preferable and unpreferable for the occurrence of SWD bursts were distinguished basing on the known relationship between the animal behaviour and the morphology of cortical and hippocampal EEG (14,15).

The following numerical values were obtained for each one hour EEG section:

a) the number and the total duration of SWD bursts (Fig 1);

b) the percent contribution of the following states:

i) high arousal (HA), ii) slow-wave sleep (SWS), iii) transitional state (TRANS).

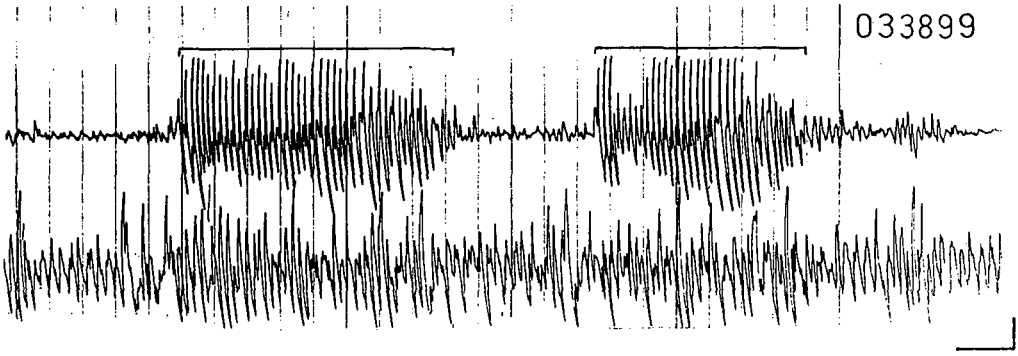

Fig. 1. A fragment of rat electroencephalogram. Upper trace: frontoparietal ECoG with SWD episodes (overlined). Lower trace: hippocampal EEG. Callibrations: vertical 200 uV, horizontal 1.0 s.

EEG sections characterized by a continuous hippocampal theta rhythm (4–9Hz) and cortical desynchronization were classified as HA if they lasted for at least 10 s. The 10-s limit was implied by our previous studies (14). At the behavioural level the HA state corresponds to long bouts of exploratory activity and to the state of paradoxical sleep. The sections of irregular activity of large amplitude in the hippocampus and in the cortex, at least 10 s long, were classified as SWS. The part of the recording which remained after subtracting the cumulated duration of HA and SWS, was classified as the TRANS state. It was characterized by changes in the hippocampal and cortical EEG morphology occurring at intervals shorter than 10 s and by the presence of SWD bursts. Behaviourally, the TRANS state corresponded to awake but relaxed immobility with occasional postural adjustments.

Statistics

The differences were estimated with variance analysis. Multiple comparisons were performed with the Tukey test (41).

RESULTS

Body weight

In the case of body weight the analysis (two-way ANOVA, groups × measurements) revealed no significant effect of the group factor. The groups × measurements interaction was also insignificant (data not shown).

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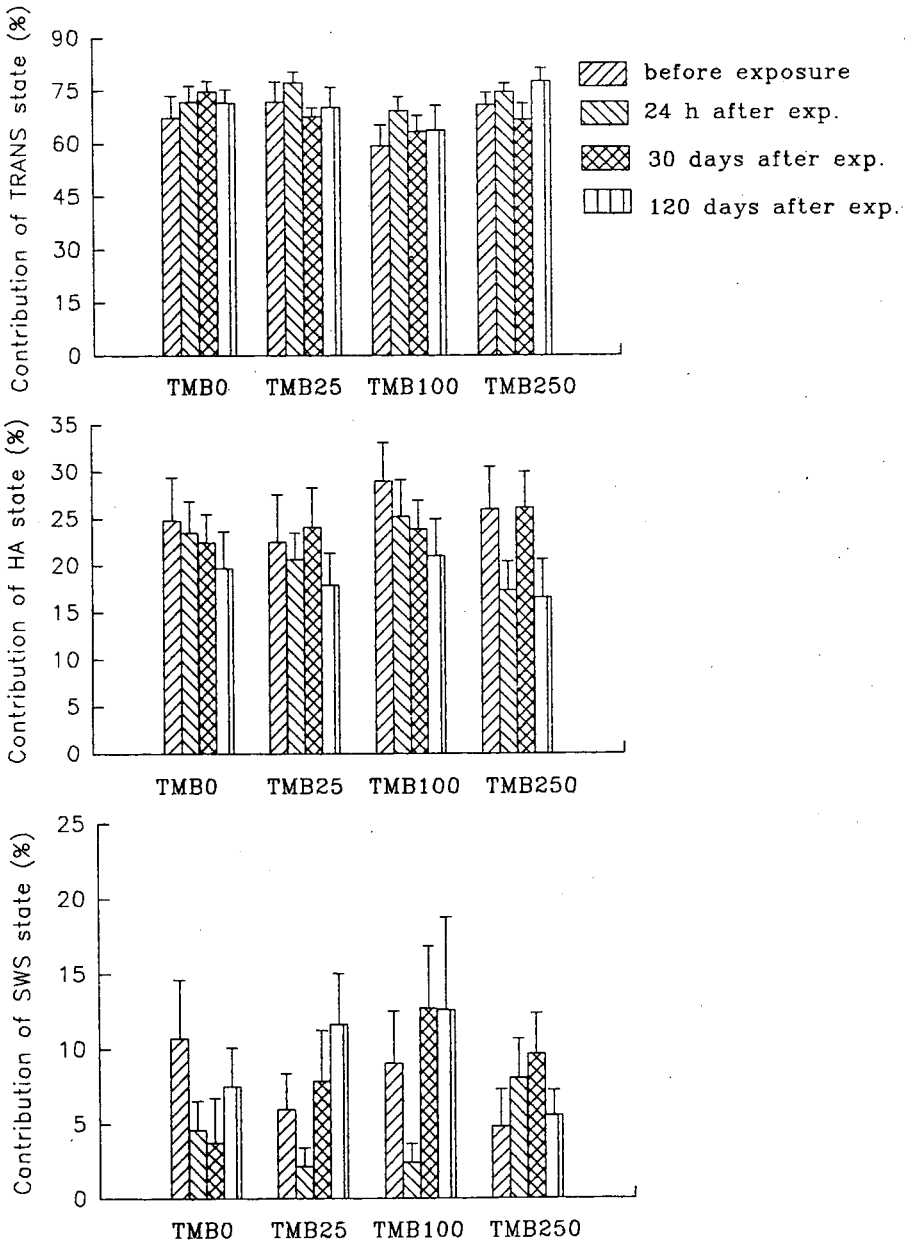

Fig. 2. Diagrams showing the effect of a four-week inhalation exposure to TMB on the contribution of TRANS (upper diagram, HA (middle diagram), and SWS (lower diagram) states in the rat EEG during successive one-hour recording periods. The bars represent group means and SE. Denotation of groups: TMB0 — a sham exposed group; TMB25, TMB100 and TMB250 — groups exposed to TMB at concentration of 25, 100 and 250 ppm, respectively.

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EEG activity

Visual inspection of one-hour EEG sections revealed that in all the groups and during all the recording periods, TRANS was the dominant state (about 70%). The contribution of the HA state was slightly above 20%, and that of SWS — about 10%. The ANOVA revealed that in the case of each state the effects of both main factors as well as the groups × recordings interaction were not significant (Fig. 2). In the case of the SWD bursts occurrence during the TRANS state, the groups × recording

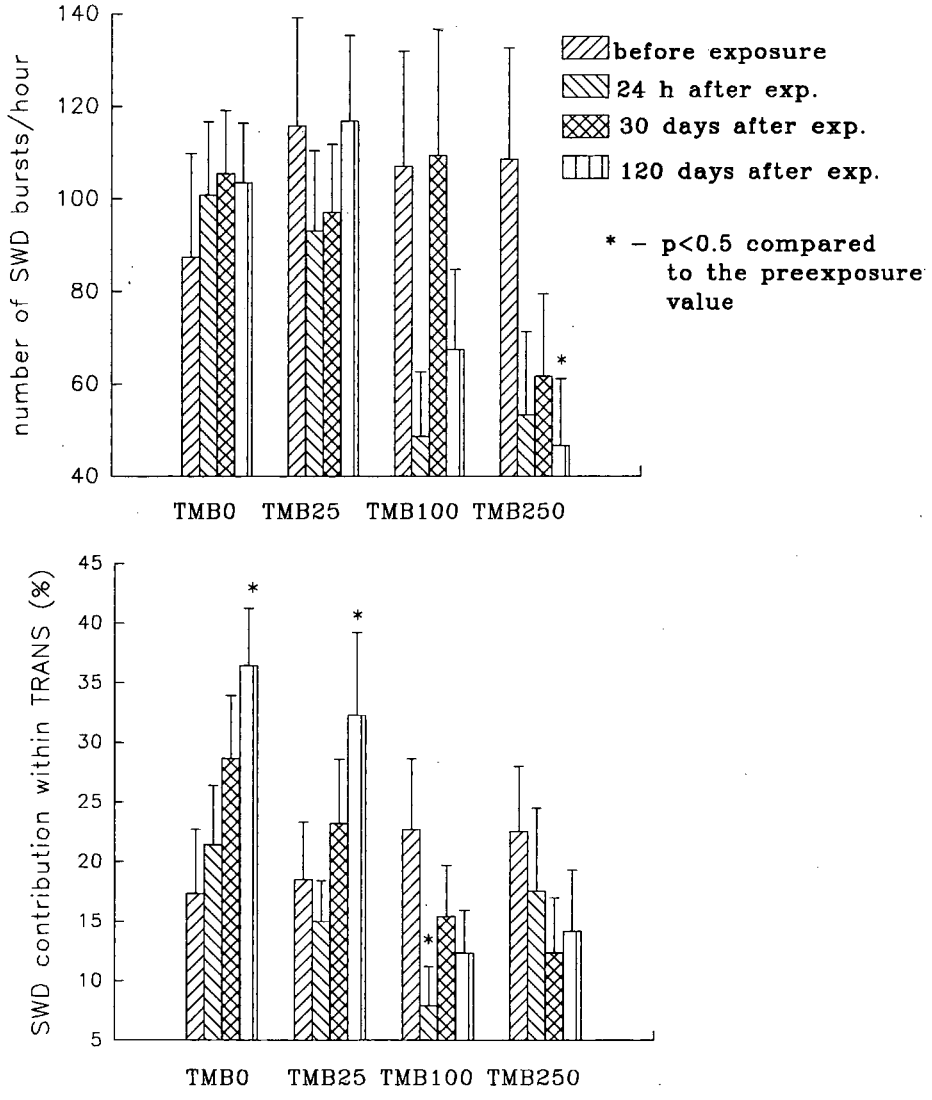

Fig. 3. Diagram showing the effect of a four-week inhalation exposure to TMS on the SWD burst occurrence (upper diagram) and on the percent contribution of SWD activity within TRANS state (lower diagram) during successive one-hour recording periods. The bars represent group means and SE. Denotation of groups as in Fig. 2. * — $p < 0.05$ in comparison to the preexposure value in the same group.

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interaction was found to be significant ($F(3,34) = 13.34$, $p < 0.0001$). Generally, after the exposure the occurrence of the SWD bursts increased in TMB0 and decreased in TMB100 and TMB250 groups. The differences between successive recordings, however, were significant only in TMB100 group ($F(3,102) = 3.19$, $p < 0.05$) where SWD episodes in recording 4 were significantly less frequent than in recording 1 and 3 (Fig. 3, upper diagram).

The analysis of the percent contribution of SWD activity within the TRANS state revealed no significant effects of both main factors but the groups × recording interaction was significant ($F(3,34) = 34.73$, $p < 0.0001$). Subsequent analysis (one-way ANOVA) revealed significant differences between the groups in recording 4 ($F(3,136) = 5.62$, $p < 0.002$); in the group TMB100 and TMB250 the percent contribution of SWD activity during the TRANS state was significantly lower than in the TMB0 group. The comparison of recordings within each group revealed significant differences in the TMB0 ($F(3,102) = 6.93$, $p < 0.001$), TMB25 ($F(3,102) = 6.10$, $p < 0.001$), and TMB100 ($F(3,102) = 3.80$, $p < 0.02$) groups. In the TMB0 and TMB25 groups, the amount of SWD activity increased gradually and in both the groups, it was significantly higher, in recording 4 than in recordings 1 and 2. In groups TMB100 and TMB250, in all the recordings performed after exposure the amount of SWD activity during TRANS state was decreased as compared to that before the exposure but the decrease appeared significant only in TMB100, and only in recording 2 (Fig. 3, lower diagram).

DISCUSSION

The absence of differences in rat body weight in the course of the experiment as well as the similarity in the distribution of the distinguished states, HA, SWS and TRANS, within successive one-hour recording periods indicate no overt effect of the exposure to TMB on the rat health status and on the global level of arousal. However, after exposure to TMB at concentration of 100 or 250 ppm, the age-related progressive increase in the amount of SWD activity during the TRANS state was retarded which suggests a persistent change in the rat CNS functional state. It would thus appear that the effect of inhalation exposure to TMB on the developmant of SWD activity is qualitatively similar to that of m-xylene (15).

The age-related increase in SWD activity, observed also in our rats of the TMB0 and TMB25 groups, has been regarded as a result of a progressive morphological and functional deterioration of the basal forebrain cholinergic system (5,7,13), a major source of unspecific activating influence on the thalamus and the cortex. The retarded of the increase in SWD activity in rats exposed to 100 or 250 ppm TNB might thus be explicable if such an exposure could prevent the rat from the age-related deterioration of the basal forebrain cholinergic system or induce changes compensating somehow for the age-related decline of the cholinergic influence on the corticothalamic structures. The former possibility seems to be unlikely. After a four-week exposure to 100 or 250 ppm TMB the rats show disturbances in passive avoidance learning and the character of these disturbances suggests an impaired movement inhibition (16). Such deficits appear when the cholinergic activity is decreased rather than increased (29). Therefore, the possible effects on other systems should be considered. Unfortunately, the information concerning structural or biochemical changes which may develop in CNS as a result of TMB exposure, is

lacking. The data concerning other methylated benzenes (toluene and xylenes) suggest that exposure may affect utilization and turnover of biogenic amins in the rat CNS (2,3,4,18,22). The dopaminergic system appears to be particularly vulnerable (35,36). On the other hand, it has been shown that an experimental increase in noradrenergic (a-noradrenergic) or dopaminergic neurotransmission inhibits SWD activity (33,37). Moreover, a direct stimulation of the brain dopaminergic system disturbs passive avoidance learning (6). 1984). Providing that TMB influences the brain in a way similar to that for other methylated benzenes, it may be tentatively assumed that the reduction in the SWD activity (present study), as well as the disruption of passive avoidance learning (15) after the exposure may be due to a facilitation of monoaminergic transmission in the rat CNS. Such a change, which could possibly represent a persistent adaptation to the CNS-depressing effect of TMB, might ameliorate the effect of the age-related gradual decline in cholinergic activity on SWD generation. Notwithstanding the validity of the above assumption, the obtained results reveal that:

i) a four-week inhalation exposure to TMB at relatively low concentration (the MAC value adopted for TMB = 20 ppm (1)), may produce persistent functional changes in the rat CNS;

ii) these changes, however, differ in nature – at least on the level of ECoG – from those developing spontaneously with age;

iii) the similarity of the effects of exposure to TMB and m-xylene on the development of SWD activity suggests a common mechanism of action. However, considering the differences in the exposure duration (TMB – 4 weeks, m-xylene – 12 weeks) and the concentration at which an overt effect was observed (TMB – 100 ppm, m-xylene – 1000 ppm), TMB appears to be a more potent neurotoxicant than m-xylene. This supposition will be investigated in future experiments.

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