

ASSESSMENT OF LONG-TERM EFFECTS OF EXPOSURE TO TOLUENE BASED ON THE ANALYSIS OF SELECTED BEHAVIORAL RESPONSES WITH PARTICULAR REFERENCE TO THE ABILITY TO TRIGGER BEHAVIORAL HYPERSENSITIVITY IN RATS

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Abstract. Toluene is a major component of numerous commercial organic solvent formulations. It is often listed among the chemicals capable of producing the organic solvent syndrome and a neurobehavioral hypersensitivity condition. The hypersensitivity condition (continued long-term intensification of some behavioral reactions in response to pharmacological or environmental stressors) is usually associated with the increased tonus of the functional dopaminergic system. The aim of our current research was to determine whether, under conditions of inhalation exposure, toluene can produce long-term behavioral changes or modify the intensity of the behavioral response to apomorphine, a dopaminergic receptor agonist. In our experiment, male rats were exposed to 25, 100 and 250 ppm toluene for 4 weeks (5 days/week, 6h/day). The following behaviors were tested: finding water in a radial maze; open field motor activity; acquiring the conditional response of passive avoidance; sensitivity to a thermal pain stimulus (hot plate test) and changes in this sensitivity caused by stress; and acquiring the conditional response of two-directional active avoidance. The behavioral response to apomorphine, i.e. the increased spontaneous locomotor activity, was assessed on day 10 after the termination of the exposure in the rotary drum test.

In the behavioral experiment, significant differences between groups were recorded only for the hot plate test; in the 100 and 250 ppm rats, electric-shock-related anxiety response was stronger than in the control group. In the experiment using pharmacological provocation, the behavioral response to apomorphine in the rats exposed to 100 ppm or 250 ppm toluene was significantly lower. Our results indicate that low concentrations of toluene may produce long-term behavioral changes in rats. However, these changes seem to be linked with reduced rather than increased functional tonus of the dopaminergic system.

Key words:

Toluene, Behavior, Hypersensitivity, Rats

INTRODUCTION

Occupational exposure to organic solvent vapors may cause long-term functional disturbances in the central nervous system (CNS) known as the solvent syndrome. Impaired learning ability and memory, and changes in the sphere of emotions are the main characteristics of the syndrome [1–3]. Solvents are also quoted among

agents capable of inducing multiple chemical sensitivity (MCS) manifested by multiple system ailment syndrome in response to intake of chemicals at doses tolerable by the majority of the population [4–6]. MCS includes mainly CNS symptoms, such as impaired concentration, attention and memory, depression, sleepiness, anxiety and irritability. According to some authors [7,8], MCS

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results from a process known as time-dependent sensitization (TDS). TDS is triggered by an occasional chemical stressor: it continues to develop gradually during several days or even weeks after cessation of the exposure to the chemical stressor and persists for months. In the experimental animals, TDS is manifested by behavioral hypersensitivity (usually detectable by altered motor activity) to chemical (e.g. psychostimulants such as amphetamine or cocaine) or psychophysical (pain, noise, immobility) stressors. There is much experimental evidence that the TDS behavioral hypersensitivity results from an increased functional tonus of the dopaminergic system [9].

Toluene is a major component of many solvent mixtures used for the paint and varnish production. It is listed among the chemicals responsible for the development of the solvent syndrome [1,2]. Some results show that the behavioral effects of toluene exposure resemble those recorded for amphetamine [10]. Amphetamine, an indirect dopaminergic agonist, is a model inductor of TDS and behavioral hypersensitivity condition [9].

The aim of our experiment was to find out whether inhalation exposure to toluene may lead to long-term behavioral changes in the rat and to test changes (if any) in rat behavioral reactivity to apomorphine (a dopaminergic agonist) resulting from toluene exposure. The results of the experiment were expected to support or repeal the conjecture that the behavioral effects of the exposure represented behavioral sensitization associated with exposure-related hyperactivity of the dopaminergic system. The available literature data show that low-dose toluene exposure may produce long-term effects in the functional condition of the dopaminergic system, but the results fail to provide a clear-cut indication either of the mechanism responsible for the changes or for their direction [11].

MATERIALS AND METHODS

Chemicals

Toluene (methylbenzene) from FLUKA and apomorphine from SIGMA were used.

Animals

Male IMP:WIST outbred 4-month (at the experiment onset) rats were used in our experiment. The animals were kept in single breeding cages under standard conditions (12h/12h light on/dark cycle, 22°C-24°C temperature, 50–60% relative humidity). Water and standard food (Murigran pellets) were accessible to the animals *ad libitum*. Body mass was determined once a week. Behavior and general condition of the animals were observed each day between 9:00. and 15:00.

Inhalation exposure

The rats were exposed to toluene in 0.25m³ toxicological chambers with forced air flow. Air flow in the chamber, monitored continuously throughout the experiment, was 15 to 16 air changes per hour. Desired solvent vapor concentrations were obtained by passing the air through a scrubber filled with the test solvent.

The animals were exposed to the chemicals during 4 weeks (5 days/week, 6h/day). The rats were placed in the toxicological chambers in single 25 • 20 • 20 cm wire cages (1 cm dia. mesh). During the exposure, neither food nor water were accessible to the animals.

Procedure and apparatus

The results of the following tests (performed in the order as specified below) were used as the basis for assessing the behavioral effects of toluene exposure:

- radial maze (assessment of short-term memory) – 14–19 days after the exposure;
- rotary drum (assessment of motor activity) – 25 days after the exposure;
- active avoidance (assessment of long-term memory) – 35–45 days after the exposure;
- hot plate (assessment of sensitivity to pain and intensity of pain-related emotional response) – 50 and 51 days after the exposure;
- active avoidance (assessment of the ability to learn) – 53 days after the exposure.

Body mass of the animals was controlled once a week throughout the experiment.

A detailed description of the apparatus used in the experiment (except for that used to test spontaneous activity) may be found elsewhere. [12].

Radial maze

The radial maze test comprised two phases: the assessment of the adaptation before and after the exposure and the main test after the exposure. During the adaptation phase, each rat was allowed to explore the radial maze for 3 min/day during five consecutive days. Radial maze arms contained water. Each day, the following were determined: the number of re-entries to the arms already visited (perseveration errors), the number of omitted arms (omission errors), and the time required for eight selections (duration of test).

During two days preceding the main test, water was accessible to the animals in the breeding cages only for 5 min/day. Before each daily test, the containers placed at each of the arm ends were filled with water. Each test started with placing the rat on the central platform and ended after eight selections of radial maze arms or after 3 min had elapsed. Time required by the rat to make eight selections and the number of perseveration and omission errors were determined.

Rotating wheel

The apparatus used to test motor activity of the animals comprised 8 rotary drums mounted in the breeding cages. Each drum was 70 cm dia. and 10 cm wide. Rotation of the drum by 90 degrees triggered an electric pulse from a contactron excited by each of four magnets placed on the drum periphery; the pulse was then fed to a pulse counter. The motor activity was assessed from counter reading.

Passive avoidance

The test consisted of six trials denoted 1, 2, 3, 4, 5 and 6. Trials 1, 2, 3 and 4 were performed at one-day intervals. Trial 5 was performed after three, and trial 6 after seven days since trial 3. In trials 1 and 2, the rat was placed on the platform and the time after which the animal had stepped down on the floor was recorded. After it had stepped down on the floor, the rat was allowed to explore

the room for 1 min and then transferred to its breeding cage. In trial 3, immediately after the step-down, the rat received a series of electric footshocks (100 ms pulse width, 2.0 mA, 1.0 Hz) for 10 s, and was then immediately transferred to its breeding cage. In trials 4, 5 and 6, the procedure was similar to that in trials 1 and 2, except for the case that the rat had not stepped down on the floor within 180 s and was removed from the platform and placed in the breeding cage.

Time for which the animal had stayed on the platform in the trials after the shock was used as a measure of the long-term memory.

Hot plate

The hot plate test comprised the determination of the latency of the unconditional paw lick response after the rat had been placed on the hot plate. Immediately after it had licked its hind paw, the animal was removed from the hot plate to end the trial. The test consisted of three trials. Immediately after the first trial, the rat was transferred to a 80 • 30 • 30 cm cage, where it received for 2 min a series of 2.0 mA, 100 ms 0.5 Hz electric footshocks from the metal bars of the floor. Trial 2 was performed after 2 or 3 s since the termination of the electric shock series, while trial 3 was performed 24 h afterwards. If the animal had not reacted as expected within 60 s, it was transferred to its breeding cage. The paw lick latencies determined in the consecutive trials were denoted L1, L2 and L3, respectively.

Active avoidance

The rats were trained to shift between two parts of the cage to avoid electric footshock (unconditional stimulus – US). The presentation of the footshock (square-wave 100 ms 2 mA 1.0 Hz electric pulses) was signaled by 500 Hz tone pulsating at 3.0 Hz (conditional stimulus – CS). The trial commenced with the presentation of CS in the part of the cage occupied at the moment by the rat. If, within 5 s since CS start, the rat did not shift to the other part of the cage, the metal floor bars of the cage part occupied by the rat were connected to the electric shock generator. CS and US ceased immediately after the rat had passed to the opposite side of the cage, and the trial was finished. Time



interval between the consecutive trials was 20 to 40 s (mean – 30 s).

Acquiring the active avoidance the response was assessed from the results of one 30-trial session (53 days after the exposure). The number of shocks avoided during consecutive 5-trial blocks served as the basis of the assessment.

Statistical analysis

Non-parametric one-way Kruskal-Wallis ANOVA and the Scheffe test [13] were used to analyze rotary drum and active avoidance test results. Data from the remaining tests were processed by two-way ANOVA for the repeatable measurements. The differences were considered significant when p value was < 0.05 .

Toluene ability to produce the behavioral hypersensitivity condition was assessed from motor activity of the rats placed in rotary drums. The motor activity was assayed after 10 days since the exposure cessation, before and after subcutaneous administration of apomorphine at 1 mg/kg. The duration of each determination was 60 min. The exposure effect was assessed by comparing the pre- and post-injection results with the results of a 60-min determination performed on the day preceding the first day of the toluene exposure.

The differences were assayed by two-way ANOVA for the repeatable results (group measurement). The differences were assumed to be significant when p value was < 0.05 .

RESULTS

Body mass

For the whole duration of the experiment, body mass of the rats in all groups increased. The groups did not differ in the values of the body mass gain.

Radial maze

The results obtained before (adaptation) and after the exposure (main test) were analyzed. For the adaptation, significant differences between groups in the number of the perseveration or omission errors were not recorded. Our comparison of main test results, i.e. trial duration, the number of omission and perseveration errors, and the

total number of entries, did not reveal statistically significant differences between the groups.

Rotary drum

Our statistical analysis did not reveal any significant differences between the groups.

Passive avoidance

The analysis (two-way ANOVA, groups $\times$ trials) showed a significant effect of the trial factor: $F(5,14) = 54.5$, $p < 0.001$. In three last trials (after the application of the electric shock) the latency of staying on the platform was significantly longer than in the trials before the application of the shock. The interaction was significant: $F(15,14) = 2.1$, $p < 0.05$. The between-group comparisons within the individual trials revealed significant differences in the trial performed 48 h after the application of the electric shock. The latency of staying on the platform was shorter for the 25 ppm rats than for the control group. The comparisons between trials within each group revealed longer time of staying on the platform in the trials after the shock as compared to those before its application (Fig. 1).

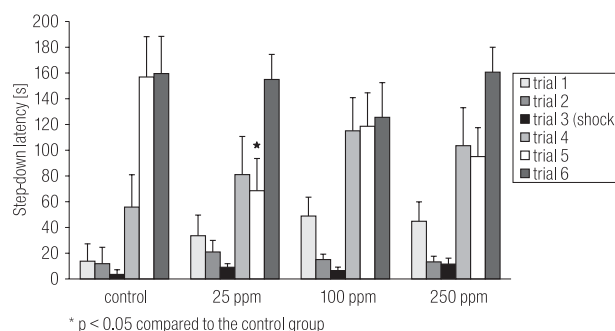


Fig. 1. Step-down passive avoidance learning. Trials 1–3 were performed at 24 h intervals. The step-down response was punished by a 10 s-footshock in trial 3. Trials 4, 5, 6 were performed 24 h, 3 days, and 7 days after trial 3, respectively.

Hot plate

The statistical analysis of the paw-lick latency (two-way ANOVA, groups $\times$ trials) revealed a significant effect of the trial factor: $F(2,44) = 29.2$, $p < 0.001$; the latencies immediately and 24 h after the electric shock were significantly longer than those preceding the shock. The interaction was significant: $F(6,44) = 5.2$, $p < 0.001$. The com-

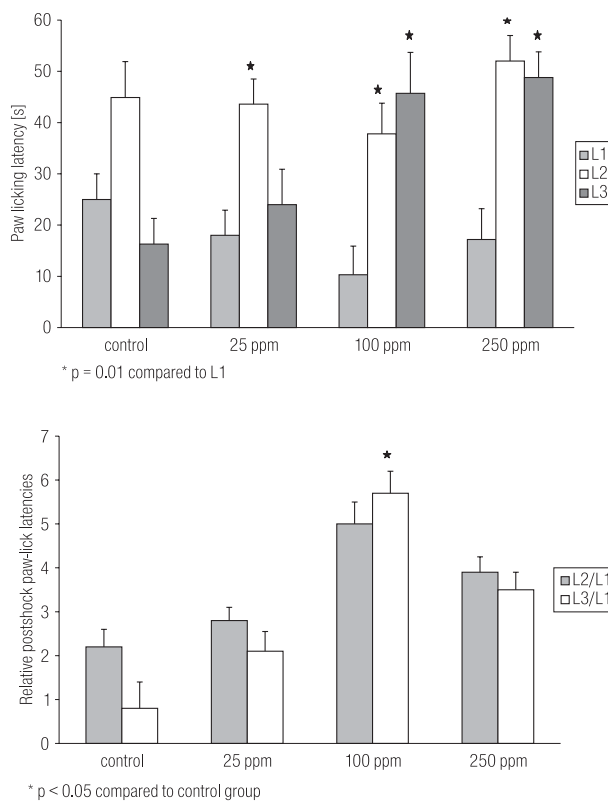


Fig. 2. Hot-plate behavior. Upper diagram: a comparison of the latency of the paw-lick response to a thermal stimulus. L1 – paw-lick latency in trial 1 performed before a footshock, L2 – paw-lick latency in trial 2 performed several s after a footshock, L3 – paw-lick latency in trial 3 performed 24 h after the footshock. Lower diagram: A comparison of the change in the paw-lick latency noted immediately (L2/L1) and 24 h (L3/L1) after footshock.

parisons between the groups revealed: in the control group – longer paw-lick latency immediately than 24 h after the shock; in the 25 ppm group – longer paw-lick latency immediately after than before the shock; in the 100 ppm and 250 ppm groups – longer paw-lick latency immediately and 24 h after than before the shock (Fig. 2). The groups did not differ in the value of the L2/L1 proportion. However, they did differ in the value of the L3/L1 proportion (non-parametric test: $\chi^2 = 8.7$, $df = 3$, $p < 0.05$). For the 100 ppm group, the value of the proportion was higher than in controls.

Active avoidance

To assess the speed of learning, each training session was divided into six five-trial blocks. The level of performing the conditional response (the number of electric shock

avoidances) in the consecutive blocks was analyzed by two-way ANOVA, groups x blocks.

The analysis revealed a significant effect of the block factor; the performance was highest in the last block. The effect of the group factor was insignificant.

Assesment of toluene to initiate behavioral hypersensitivity

Our analysis revealed a significant effect of the measurement factor: $F(1,20) = 4.93$, $p < 0.05$. Statistically significant increase in motor activity after the administration of apomorphine was recorded for the control and the 25 ppm groups (Fig. 3).

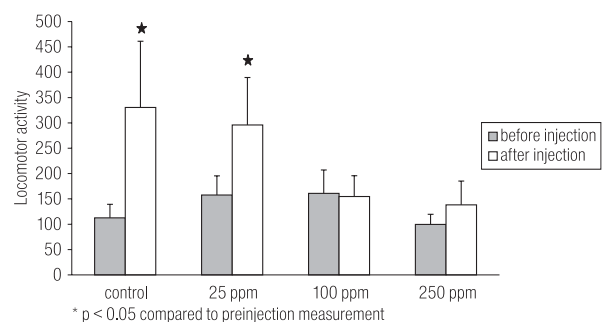


Fig. 3. The comparison of locomotor activity (rotary drum test) before and after apomorphine injection.

DISCUSSION

The results of our experiment show that a four-week inhalation exposure of the experimental rats to toluene (6 h/day, 5 days/week) at 25, 100 and 250 ppm did not significantly affect the health condition of the animals, as evidenced by similar body mass gain in all groups. The exposure did not produce significant changes in the rat behavior in the majority of the experimental situations. Statistically significant differences were noted only in the hot-plate test; for the 100 ppm and 250 ppm rats, the effect of a 2-min electric shock (longer paw-lick latency) was more persistent than in the control and 25 ppm groups. No difference between groups in the results of the active avoidance test and in the L1 and L2 values recorded in the hot-plate test means that it would not be reasonable to assume that the higher L3 values were due to reduced sensitivity to pain. The longer latency of the paw-

lick response in our experimental situation was associated with the animals' persistent attempts to get off the hot plate, a behavior that was competitive to the protective paw-lick response. The differences detected between the groups may indicate that, in the 100 and 250 ppm groups, the neuronal systems responsible for the active forms of the protective behaviors dominated over the systems controlling the unconditional protective responses. This effect could not be due to the presence of toluene or its metabolites in the nervous tissue, as observed seven weeks after the exposure. It is generally recognized that the bulk of absorbed toluene is eliminated from the organism within ten to twenty hours after exposure cessation, and its removal from the nervous system is particularly rapid. [14]. The time interval between exposure cessation and the execution of the individual tests was therefore long enough to remove completely the solvent and its metabolites. Thus, it seems reasonable to suppose that the observed changes were due to toluene interacting with the nervous tissue in the past.

The stronger behavioral response in a dangerous situation may indicate that toluene exposure induces TDS and behavioral hypersensitivity to stressogenic factors. Such condition is attributable to the hyperactivity of the dopaminergic system. Thus, it is reasonable to expect that the response to dopaminergic agonists in toluene-exposed rats is stronger than in those non-exposed.

The effects of this experiment suggest that the behavioral reactivity to apomorphine in the toluene-exposed rats was reduced. Besides, the effect seemed to be directly proportional to the solvent concentration; it was observed only in the 100 ppm and 250 ppm groups. The weaker behavioral response to apomorphine in the exposed rats points to a reduced functional tonus of the dopaminergic system. This change is contrary to that induced by psychostimulants, such as amphetamine or cocaine [15,16]. Thus, it is clear that, in spite of some similarity between behavioral effects of the acute exposure to amphetamine and toluene [10,17], the functional changes in the dopaminergic system caused by each of the two chemicals are reverse.

The results of the experiment deny the conjecture that the behavioral effects of toluene exposure noted in the first

experiment reflected the neurobehavioral hypersensitivity linked to hypersensitivity of the dopaminergic system. On the other hand, the evidently lower behavioral sensitivity to apomorphine in the exposed rats makes us speculate whether the change could underlie the behavioral effects, i.e. higher L3 values in the hot-plate test. If so, the administration of non-specific blockers of this system (e.g. haloperidol) should produce behavioral effects similar to those observed for toluene exposure. Further research is required to verify this conjecture and to determine the type of biochemical changes caused in the dopaminergic system by exposure to toluene and other benzene derivatives.

The available data indicate that a four-week exposure to toluene at a low (80 ppm) concentration does not affect spontaneous motor activity of the experimental rats, but it makes them sensitive to apomorphine [11,18]. The published reports also show that exposure to higher concentrations of toluene does not produce a similar effect [19,20]. In our present experiment we used toluene concentrations identical as those used in our earlier research on benzene derivatives (25, 100 and 250 ppm), expecting that the effect of exposure to 100 ppm might be similar to that observed for the 80 ppm exposure. It is quite likely that our results were due to a drop of dopaminergic receptor density in the CNS (in *nucleus accumbens*) or reduced affinity of those receptors to the agonist, or to a combination of those effects. Suitable biochemical research is required to elucidate this question.

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