LETTERS TO THE EDITOR

Effects of Single Exposure to Toluene on Operant Behavior and Brain Toluene Levels in Rats

Key words: Behavior—Brain toluene levels—Exposure—Operant conditioning—Rats—Toluene—Toxicity

Although behavioral effects of short-term exposure to toluene have been studied by the methods of operant conditioning using various reinforcement schedules,¹⁾ the dose-effect patterns of toluene exposure are complicated for several reasons.

The dose, the amount of substance administered, is determined not only by the exposure concentration but also by the exposure duration in the exposure experiment wherein the dose cannot be described by one variable. This seems to be one of the reasons why the dose-effect patterns of toluene exposure were complicated.

On the other hand, the observed effects of the substance are expected to be, in some cases, not the function of the total amount administered, but a reflection of its concentration in the target organ at the time of observation.

From this viewpoint, we studied the effects of single exposure to toluene on the performance on a variable interval 60-sec (VI60") schedule and estimated the brain toluene levels after exposure in rats. This is a preliminary study to find out the behavioral effects of toluene as a function of the toluene concentration in the central nervous system.

Nine-week-old male Sprague-Dawley rats (Charles River Japan, CD) were housed individually, maintained at approximately 85% free feeding weight and trained on a VI60" schedule with appetitive reinforcement (45-mg standard pellets were used as a reinforcer for lever-press response) for 8 weeks (total 22 hr). The test cages (standard type Skinner box) placed in a semisoundproof box were controlled by a minicomputer system. Three rats showing a stable response rate were selected for the exposure experiment.

The apparatus used for toluene exposure was identical to the one illustrated in detail in our previous report.²⁾ Toluene vapor, generated by the saturated vapor pressure method, was diluted twice with clean air and was introduced into the exposure chambers. The volume of each exposure chamber was 60 l and a gas flow rate of 8 l/min was maintained. The exposure concentration in each chamber was monitored by a gas-chromatograph with a flame ionization detector. The exposure concentration of toluene rapidly increased, reaching the intended value within 30 min, and was maintained at that value for the exposure period.

Each rat was exposed to various concentration of toluene (1000, 2000, 3000

Fig. 1. Effects of toluene on VI60'' performance after exposure for 4 hr.

Response rates in every 10-min period for a 120-min test session are plotted as the percentage of the average response rate for a 120-min pre-test session after air exposure in each rat.

ppm) for 4 hr on days 4, 7 and 10 of the experiment. Exposure concentrations, subjects and days of experiment were arranged by Latin square. On day 1, the rats were exposed to air for 4 hr as the pre-test control. On the other days, neither exposure nor training was performed. Immediately after the termination of exposure, rats were placed in the Skinner box and the number of lever-pressings and reinforcements in every 10-min period was recorded for a 120-min test session.

To measure the brain toluene levels after exposure, a total of 12 Sprague-Dawley rats (7 weeks old and equivalent in weight to the animals of the behavioral experiment) were used. They were exposed to toluene for 4 hr at the same concentrations as those in the behavioral experiments (1000, 2000 and 3000 ppm). The rats were decapitated 10 or 60 min after the termination of exposure and the brains were removed. Toluene in the whole brain was extracted, according to the methods used by Nakagaki *et al.*,³⁾ by homogenization in 10 ml of CS₂ containing 0.1 μ l of ethylbenzene as an internal standard, and was measured by a gas-chromatograph with a flame ionization detector. A glass column (2.6 mm $\times$ 1.6 m) packed with UCW982 was used.

The effects of toluene exposure were evaluated by comparing the response rate after exposure with the pre-test performance (average response rate of pre-test session for 120 min was set at 100%). Fig. 1 shows the response rate

Fig. 2. Brain toluene levels 10 and 60 min after 1000, 2000, and 3000 ppm exposure for 4 hr.

Fig. 3. Relationship between the VI60'' performance and the estimated brain toluene levels.

The mean and the standard deviation of response rates in 3 rats from 0 to 20 min and from 50 to 70 min after exposure are plotted against the brain toluene levels at 10 and 60 min after exposure.

of each rat during the 120-min test session after exposure. The total number of responses increased after 1000 and 2000 ppm exposure in all rats. The response after 3000 ppm exposure showed different changes among the subjects. Not only the average response rate for the 120-min session but also the time course of the response rate after exposure were affected by toluene exposure.

The brain toluene levels after exposure are shown in Fig. 2. The brain toluene concentration ($\mu\text{g/g}$ wet tissue), being proportional to the exposure concentration, decreased to one-half within 1 hr. Fig. 3 indicates the relationship between the response rate and the brain toluene level estimated from the results in Figs. 1 and 2. The mean and the standard deviation of response of three rats from 0 to 20 min and those from 50 to 70 min after exposure (Fig. 1) were plotted against the mean toluene levels in the brain measured at the corresponding time (Fig. 2). It is clear from Fig. 3 that toluene produces, at low brain toluene levels, an increase in the response rate which is reversed at high levels, so that an inverted U-shaped curve was obtained between the toluene concentration in the brain and the lever-pressing behavior. This behavioral effect seems to be directly dependent

on the brain toluene level, and not affected by the exposure concentration nor by the time after exposure, at least under the present conditions. These results appear to be compatible with most of the previous reports demonstrating the inverted U-shaped curves between the exposure concentration and the behavioral effect.¹⁾

As compared with the experiments using intraperitoneal or intravenous administration, it is rather difficult to explain the dose-effect relationships in the exposure experiments, especially when the function curve is nonlinear and biphasic, because the dose involves two variables, the concentration and the duration of exposure.

However, the behavioral effects of toluene would be described in a relatively simple manner if the brain toluene level instead of the concentration or the duration of exposure is used as the independent variable (Fig. 3). This study indicates that the effects of toluene on the schedule controlled behavior may be the function of brain toluene levels at the time of observation of the behavior under some conditions similar to those used in this paper. To ascertain this relationship, further studies are needed using a larger number of subjects and different routes of administration.

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