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BRIEF COMMUNICATION

Forced Turning Induced by Toluene[1]

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ISHIKAWA, T. T. AND H. SCHMIDT, JR. *Forced turining induced by toluene.* PHARMAC. BIOCHEM. BEHAV. 1(5) 593–595, 1973. – Repeated daily toluene inhalation produces circling in rats. This effect may be specific to toluene since xylene fails to elicit turning. The turning follows toluene inhalation and is not associated with histological lesions of the brain. Forced circling can be reestablished more rapidly 15 days after last toluene inhalation than 21 or 34 days thereafter. These latter conditions require about as many exposures to toluene as were required to institute turning originally.

Toluene Forced turning

TOLUENE, a common petrochemical glue solvent, has been demonstrated to have neurotoxic effects [3]. Bieliauskas and Huprich in a behavioral study of toluene inhalation observed forced circus movements [4]. These latter, not being a primary focus of the Bieliauskas and Huprich study, were not extensively examined. It is the intent of the present paper to describe the occurrence of forced turning and investigate residual effects of toluene inhalation upon subsequent redevelopment of toluene induced forced turning.

METHOD

Animals

Thirty male albino rats of approximately 90 days of age were used. These animals were of the Sprague-Dawley strain and had not been used in other experiments

Apparatus

The apparatus used consisted of 2 30 × 30 × 60 cm Plexiglas boxes. Installed within each of these boxes was a 22 × 22 × 25 cm Skinner box. These latter only served to confine the rat within a well defined space rather than as a source of data.

Air was pumped into the larger box by means of a piston pump. the air bubbled through toluene in a 125 ml bottle. Initially, approximately 50 ml toluene was placed in the bottle. Tubes connected the toluene bottles with the boxes. The box exhausted through other tubes to outside the laboratory thus maintaining the interior of the box near atmospheric pressure.

Procedure

The rats were placed in the inhalation boxes for half an hour per day on a daily basis. During this period, the rats inhaled toluene vapor. The vapor consisted of 4–5 ml toluene in 40–50 liter of air. After the passage of the inhalation period, the rat was placed either on the floor or in a square box, 110 cm on a side to ascertain whether the rat turned. If the rat did not turn within 20 min after removal from the inhalation chamber, it was removed to its home cage.

When forced turning was observed, the animal was given 7 additional consecutive daily exposures to toluene as described above. Subsequent to this the toluene was withdrawn. The period of withdrawal was 15 days for Group 1, 21 days for Group 2, and 34 days for Group 3. The rats remained in their home cages during withdrawal. After the

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withdrawal period the rats received toluene as described above until the recurrence of turning was observed.

RESULTS

Toluene induced forced turning develops only gradually. The mean number of exposures to the onset of turning during initial acquisition was 7.6 for the 30 animals in this experiment. The mean values for the groups were 6.9, 7.4, and 8.5 exposures with a standard deviation of 1.14 exposures. An F test between these 3 groups was not significant, suggesting that they were random samples from the same population with respect to toluene exposures required for the development of forced turning.

Prior to the onset of forced turning it was possible to observe several distinct effects of toluene. First of all, toluene inhalation produced hypnosis in all rats. Hypnosis was manifested by a loss of righting reflex. This effect appeared only rather slowly on the first exposure but more rapidly subsequently. Second, scratching was observed in the animals. This scratching occurred on only one side in a given animal. It always involved a hind leg and the scratching was directed at the level of the lower costal margin. Third, initially the rats entered the inhalation chamber readily or at least with no well directed struggling. After 2 or 3 exposures the rats struggled vigorously. Fourth, prior to the onset of turning by an exposure or 2 some, though not all, animals developed a pattern of what appeared to be partial turning alternating rapidly between clockwise and counter-clockwise movements. It was subsequent to all of this that forced turning developed.

When forced turning had developed one or both of 2 patterns occurred. All animals showed forced turning when they had righted after hypnosis. Some animals turned during the induction of hypnosis with toluene. The initial direction of turning (the first direction turned on a given day) was consistent. A significant correlation was between initial direction turned during original development of forced turning and recurrent development after withdrawal ($r = 0.48$, $p<0.01$). On the other hand, if proportion of clockwise turns i.e., ratio of clockwise turns to total turns, was considered the mean proportion was 54%, not significantly different from 50% clockwise turning. More telling is that the distribution of proportion of clockwise turns did not significantly differ from a binomial distribution. The mean proportion of clockwise turns did not arise from the two humps of a bimodal distribution. On the average, rats tended to turn equally in both direction. Odd–even reliability was obtained with respect to the relative proportion of clockwise turns. This was obtained by comparing the proportion of clockwise turns on 3 consecutive odd days after the criterion was reached as compared with the proportion of clockwise turns on 3 consecutive even days. The Pearson product moment correlation was 0.68 a significant value ($p<0.001$). Thus the direction of turning on the part of an individual rat is on the average consistent and not random.

The mean number of trials to redevelop the turning response, 15, 21, and 34 days after withdrawal was 1.5, 6.0, and 5.2 exposures, respectively. Only the value for 15 days of withdrawal was significantly less than the number of exposures required for the development of initial turning ($t = 5.24$, $df = 9$, $p<0.001$). The number of exposures to reestablish forced turning is significantly less than the mean number of exposures required after 15 days of withdrawal when compared with the average of comparable values after 21 and 34 days ($F = 24.26$, $df = 1/26$, $p<0.001$). There was no significant difference between the number of exposures required 21 and 34 days after withdrawal.

DISCUSSION

The results reported indicate that forced turning occurs as a function of prolonged toluene exposure. This occurs after a number of toluene induced effects. There is some residual effect of toluene inhalation.

One control which needs some discussion here is whether prolonged daily retention in the box may not suffice to induce turning. Two sets of observations would appear to preclude such a conclusion. First, groups of rats were placed in the boxes without toluene administration, all of the other conditions being the same. Turning did not develop. Second, a subsequent group of 4 rats was treated with toluene until turning had taken place on 3 sucessive days following toluene inhalation. On the next day, comparable quantities of xylene were pumped into the chamber. No turning ensued. One day later turning was elicited in all 4 animals when toluene was again administered. In addition to controlling for time in box, this observation indicates that toluene has some specificity for producing forced turning. Although it cannot be inferred from this that xylene would be ineffectual in producing turning under all circumstances. An interesting observation in the same vein yet to be undertaken would entail the use of benzene in conjunction with toluene and alone.

The data demonstrate that the forced turning is a reversible effect. A prolonged residual effect of toluene inhalation which can be evoked as forced turning when subsequent toluene inhalation has been administered was also demonstrated. This effect does not appear a consequence of brain damage since none was found during histological examination. Cross sections of brain were made by paraffin embedded material at 20 μ and staining with cresyl violet. Thus the effect would appear to depend upon the pharmacological activity of toluene rather than some destructive possibility. The residual effect would appear to depend upon retention at and slow release of toluene from unknown sites.

The obvious problem, of course, is why does turning occur at all? Forced turning has been demonstrated in many instances and species all of which entail clear lateralization whether chemical or structural [1, 2, 5–12]. In such instances the direction of turning is predominantly in one direction or the other. This latter is in contrast to the present finding wherein the distribution of frequency of turns approximates a normal distribution with a mean frequency of 50% clockwise turns. this latter would seem to imply a rather rapid shift of dominant lateralization within some individuals evidenced by equal frequencies in the direction of turning with other animals turning predominantly in one direction, clockwise or counterclockwise.

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