Psychopharmacology 48, 153–158 (1976)

Psycho-
pharmacology

Acquisition and Loss of Behaviorally Augmented Tolerance to Ethanol in the Rat

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Abstract. The phenomenon of behavioral augmentation of tolerance (BAT) to ethanol (EtOH) in the rat was replicated in studies using the moving belt test of intoxication. Rats performing the test daily under the influence of EtOH (2.2 or 2.5 g/kg i.p.) developed tolerance more rapidly than those receiving the same dose *after* each daily session on the belt. However, both groups reached the same maximum level of tolerance. Acceleration of tolerance by BAT was proportional to the frequency of performance under the influence of EtOH when total exposure to EtOH was held constant. The degree of tolerance produced by BAT could not be increased by daily gavage with a large dose (6 g/kg) of EtOH. After termination of EtOH administration, tolerance produced by BAT was lost at the same rate, whether or not daily alcohol-free sessions on the belt test were given. These findings are consistent with the hypothesis that BAT and conventionally produced tolerance differ only in rate.

Key words: Ethanol – Tolerance – Rat – Behavioral augmentation – Rate.

Tolernace to ethanol in the rat has been demonstrated in terms of change in the effect of ethanol upon the performance of a task in a two-turn circular maze (Chen, 1968). Tolerance is acquired more rapidly when the animals are obliged to perfrom the task daily under the influence of ethanol than when they receive the same dose of ethanol *after* completion of the task each day (Chen, 1968; LeBlanc et al., 1973). The effect of this performance requirement has been designated "behavioral augmentation of tolerance" (Kalant et al., 1971). This phenomenon has been interpreted in different ways. Chen (1968, 1972) considers it a form of learning, distinct from "physiological" tolerance to ethanol. However, a replication and modification of Chen's study (LeBlanc et al., 1973) indicated that the distinction was not clear. Animals receiving ethanol *after* the daily task performance did reach the same level of tolerance as the others; both groups showed signs of physical dependence together with the tolerance, and neither group became more tolerant when given large daily doses of ethanol by stomach tube. In our view, the available evidence did not demonstrate a clear difference between learned tolerance and physiological adaptive change in the central nervous system, which tolerance is ordinarily thought to reflect.

The present communication reports the results of studies which define further some characteristics of behaviorally augmented tolerance, preparatory to a more detailed comparison between it and physiological tolerance to ethanol. These studies were designed to answer the following questions. Is behavioral augmentation task-specific, i.e., is it seen only with the circular maze or is it demonstrable in other types of performance tests? Is it an all-or-none phenomenon, or can it be graded in intensity according to the schedules of training? Is it reversible, and if so, on what time scale compared to the loss of conventionally defined tolerance to ethanol? If it is reversible, is the rate of reversal affected by differences in activity of the animals during the post-alcohol period?

METHODS

The experimental subjects were male Wistar rats purchased from Woodlyn Farms, Guelph, Ontario, at an initial body weight of 80–100 g. They were housed singly and fed standard laboratory chow and water. They were trained on the moving-belt test (Gibbins et al., 1968) during their period of growth, and were fully trained by the time they had reached a weight of approximately 300 g. Their weight was then held at 300 ± 15 g by appropriate restriction of the daily ration of chow. The food was provided at 4 p.m. each day,

Table 1
Schedule of injections

Group	Test days		Treatment days	
	before run	after run	before run	after run
1 Controls	ethanol	saline	saline	saline
2 Physiological	ethanol	saline	saline	ethanol
3 Behavioral	ethanol	saline	ethanol	saline

and was consumed completely before the start of training or test sessions next day.

In the moving-belt test, animals are obliged to remain on a motor-driven belt which moves continuously over a shock-grid. If the animal puts one or more paws on the grid, it receives a shock and activates a cumulative timer. The effect of ethanol, pentobarbital and other drugs is seen as a monotonic dose-dependent increase in time off belt. Slight modifications of the apparatus, for convenience of training and maintenance, had no significant effect on the dose-response or blood level-response curves (LeBlanc et al., 1969). Details of these changes are available from the Addiction Research Foundation.

For intraperitoneal (i.p.) injection, ethanol was prepared as an 8% (w/v) solution in physiological saline. For oral administration by gavage, it was made up as a 25% (w/v) solution in tap water. The actual procedures were different for each experiment, and therefore are described separately under the results. Since different groups of rats, obtained at various times of the year, differ somewhat in sensitivity to ethanol, the dose to be used during tests under ethanol was adjusted to give comparable degrees of initial effect in all experiments. For this reason the test dose was 2.5 g/kg in Experiment 1, and 2.2 g/kg in the other experiments.

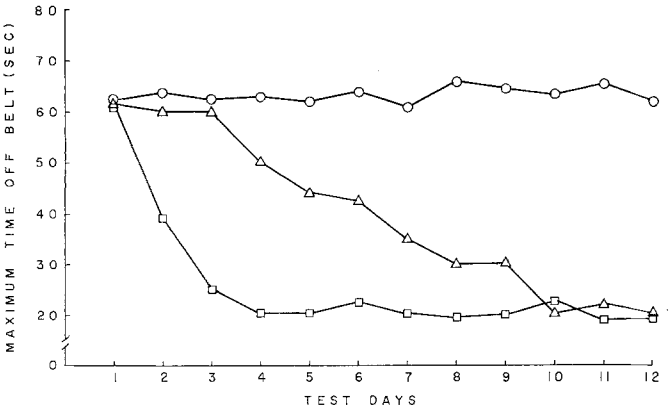

Fig. 1. Error scores on successive tests of performance on the moving-belt apparatus under the influence of ethanol (2.5 g/kg i.p.), by rats on different treatment schedules described in Table 1: ○, controls; △, physiological group: □, behavioral group. Successive numbered test days are separated by three treatment days

RESULTS

Experiment 1. Twelve rats were trained to a stable level of performance on the moving-belt test, with less than 1% error on any given 2-min test run. They were then ranked in order of the maximum impairment produced by an i.p. injection of ethanol (2.5 g/kg). Each animal was given 6 2-min trials, beginning 2.5 min after the injection of ethanol, and separated by 2.5-min rest intervals. The impairment score was the maximum error (time off belt) in any of the six trials. Within successive groups of three in the ranking list, the animals were then randomly assigned to 3 separate groups, thus generating 3 groups matched with respect to their ranges of alcohol sensitivity. The 3 groups were designated controls, behavioral group, and physiological group, as defined by Chen (1968) on the basis of their respective subsequent treatments.

During the experimental period each animal was tested daily on the moving belt apparatus, and received one i.p. injection before the test and another immediately after. The compositions of the injected solutions for each group are indicated in Table 1. The moving belt test was repeated under ethanol (2.5 kg) every fourth day.

The results (Fig. 1) were consistent with those obtained in the earlier study using the circular maze test (LeBlanc et al., 1973). Tolerance, as shown by a decrease in error score under the constant dose of alcohol, became evident first in the behavioral group, and developed maximally by test day 4. The physiological group developed tolerance more slowly, but did reach the same maximum by test day 10–12. Analysis of variance confirmed the strong effects of treatments ($F = 48.90$; $df = 2, 9$; $P < 0.001$) and of days ($F = 36.08$; $df = 11, 99$; $P < 0.001$) in the behavioral and physiological groups. For these two groups the interaction of treatment by days was highly significant during the period of days 2–9 inclusive ($F = 11.05$; $df = 8, 99$; $P < 0.001$), confirming the difference in rate of attainment of the final plateau. The controls failed to change significantly over time.

A replication of the experiment with twice as many animals, but limited to 6 test days, yielded virtually the same result. The data will be presented elsewhere as part of a larger study.

Experiment 2. In the preceding experiment, as well as in the earlier study involving the circular maze task (LeBlanc et al., 1973), the behavioral groups received daily training under the influence of ethanol, since the procedure was identical on test days and training

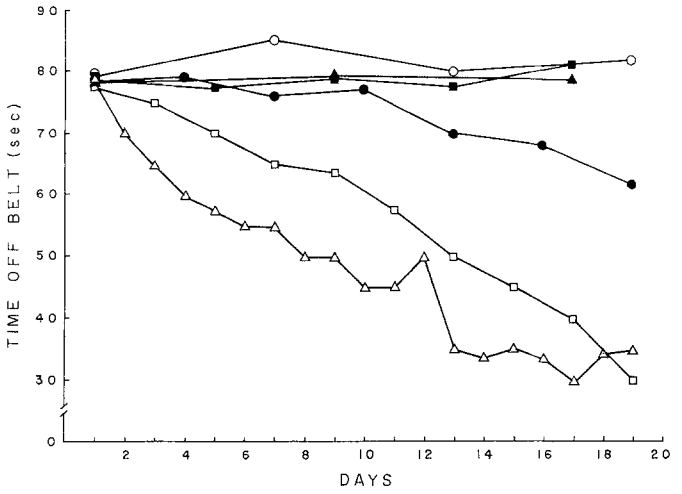

Fig. 2. Effect on ethanol on time off belt (moving-belt test) as a function of frequency of testing under ethanol. Each group underwent a session on the moving-belt apparatus every day; on days for which no score is shown, no ethanol was given. On the test days shown, ethanol (2.2 g/kg) was given before the moving-belt session. Frequency of testing under ethanol: Group 1 (▲) every 8 days, Group 2 (○) every 6 days, Group 3 (■) every 4 days, Group 4 (●) every 3 days, Group 5 (□) every 2 days and Group 6 (△) daily

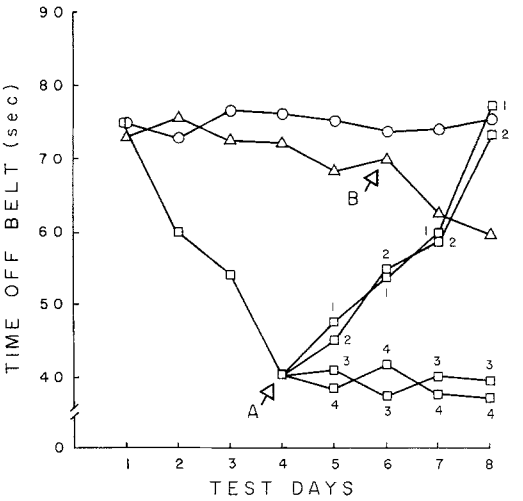

Fig. 3. Acquisition and loss of tolerance to ethanol on the moving-belt test under various chronic treatments: ○, saline controls; △, "physiological" group; □, "behavioral" group. Arrow A indicates change of treatment for the behavioral group. Group 1 was allowed to withdraw from ethanol in their home cages. Group 2 was given moving-belt trials without ethanol during the withdrawal period. Group 3 was switched to daily gavage with ethanol (6 g/kg). Arrow B indicates the start of gavage of the "physiological" group. Consecutively numbered test days were separated by three treatment days

days. In order to determine whether the effect of behavioral augmentation of tolerance was quantal or continuously variable, different groups of animals were exposed to different intensities of exposure to training under alcohol.

Forty-eight animals, trained to the stated criterion on the moving belt test, were randomly assigned to six groups matched on the basis of peak impairment on an initial test after an i.p. dose of 2.2 g/kg of ethanol, as explained for the slightly higher dose (2.5 g/kg) in Experiment 1. All groups were then given daily runs on the moving belt apparatus, receiving i.p. injections of ethanol in a dose of 2.2 g/kg before the test with differing frequencies for the various groups. The groups were differentiated by the fact that testing under the influence of ethanol was conducted every day or every second, third, fourth, sixth and eighth day respectively.

As shown in Figure 2, tolerance developed most rapidly in the group tested daily under ethanol, and progressively less rapidly in those tested every second and every third day. The remaining groups did not develop tolerance at all. These visual impressions are borne out by the statistical analyses. Analysis of variance of the results for each group separately showed no significant decrease in impairment in Groups 1, 2 and 3 on successive tests. However, there were significant reductions for Group 4 ($F = 51.02$; $df = 6, 42$; $P < 0.001$), Group 5 ($F = 511.5$; $df = 9, 63$; $P < 0.001$) and Group 6 ($F = 920.9$; $df = 18, 126$; $P < 0.001$). Comparisons among the latter three groups showed that the slope for Group 4 was significantly less than those for Groups 5 and 6 ($t = 11.69$ and 11.75 respectively, $P < 0.001$ in both cases). The slopes for Groups 5 and 6 did not differ significantly from each other, but this is attributable to a "bottoming-out" effect in Group 6, indicated by a highly significant ($P < 0.001$) non-linear component in the change over time. This corresponds graphically to the fact that Group 6 reached a maximum level of tolerance by day 13 of the test period, while Group 5 reached the same level by day 17 to 19.

Experiment 3. This experiment was designed to yield further information concerning the relation between behavioral and pharmacological tolerance, by examining the maintenance or loss of behaviorally augmented tolerance under various conditions. Forty-eight trained rats were divided into 3 groups of 8, 8 and 32 respectively, matched on the basis of the peak impairment on the moving-belt test produced by an initial test dose of ethanol (2.2 g/kg intraperitoneally). The groups were then subjected to chronic treatment and testing schedules identical with those described under Experiment 1, the 32-animal group being in this case the behavioral group.

Over the first 4 test days, the results (Fig. 3) replicated those of Experiments 1 and 2. Analysis of

variance showed no significant linear change of test score over time in the control group. The behavioral group showed rapid development of tolerance as reflected by a highly significant decline over time ($F = 703$; $df = 1, 93$; $P < 0.001$), and by test day 4 had reached a level corresponding to the maximal tolerance shown in the previous experiment. At this point it was divided into four equal sub-groups, matched on the basis of their levels of impairment on the fourth test day. All sub-groups continued to be tested every fourth day under the ethanol-saline injection sequence, but differed with respect to the treatment on intervening days:

1. Sub-group 1 was given no further treatment, being simply left in the home cages.

2. Sub-group 2 was given daily runs on the moving-belt test, receiving saline both before and after each run on the non-test days.

3. Sub-group 3 received the saline-alcohol sequence on non-test days.

4. Sub-group 4 was transferred to daily intubation with ethanol (6 g/kg) in lieu of the previous treatment. It received runs on the moving-belt apparatus only on test days, and on those days it received the usual 2.2 g/kg test injection before the run, saline after, and the balance of its 6 g/kg dose by intubation subsequently.

Both withdrawal sub-groups (i.e. 1 and 2, which did not receive alcohol between test days) showed a progressive loss of tolerance over exactly the same time course (Group 1: $F = 73.36$; $df = 1, 28$; $P < 0.001$; Group 2: $F = 321.5$; $df = 1, 28$; $P < 0.001$). Both groups had returned to their initial levels of alcohol sensitivity by test day 8 (Fig. 3). In contrast, the two sub-groups which had been transferred to the "physiological" and gavage schedules continued to show the same level of tolerance as they had at the point of change-over. This is comparable to the results of Phase II in the maze study (LeBlanc et al., 1973).

The original physiological group gradually developed a small degree of tolerance over the first 6 test days, when compared with the controls. The linear change over this time was significant ($F = 11.64$; $df = 1, 35$; $P < 0.01$). At this point, the physiological group was transferred to a schedule of daily gavage identical to that used for the fourth sub-group of the original behavioral group. Their scores also changed linearly over the next two test days ($F = 9.27$; $df = 1, 14$; $P < 0.01$), and the slope became significantly steeper after the change of treatment (first and second slopes were -0.9 and -5.1 s/day respectively, $P < 0.05$). This served to verify that the animals in this group were in fact capable of developing a greater degree of tolerance than they had previously shown, and also that the dosage of ethanol employed by gavage was able to produce this. These findings are compared to those in Phase III of the maze study (LeBlanc et al., 1973).

DISCUSSION

The present studies indicate that the phenomenon of behavioral augmentation of tolerance is not specific to the maze test employed in earlier work (LeBlanc et al., 1973). It applies equally well (Fig. 1) to the moving-belt test which is based on an aversive rather than an appetitive performance, involves different skills, and is affected by a higher dose range of alcohol. With this test there was also a slower development of tolerance in the physiological group than had been found with the maze test, despite a higher daily dose of alcohol. This finding does not prove, but is consistent with, the hypothesis advanced previously that the functional deficit produced by ethanol, rather than the actual concentration of ethanol itself, is the stimulus to the development of tolerance (Kalant et al., 1971). Otherwise, one would expect that a higher dosage level should produce a greater rate of tolerance development, independently of the complexity of the task or its temporal relation to the administration of alcohol. This hypothesis is also consistent with the observation (Fig. 2) that behavioral augmentation of tolerance is a graded phenomenon, occurring more rapidly when the density of training under alcohol is greater.

Tolerance acquired under a behavioral augmentation schedule was lost rapidly (Fig. 3) when training under alcohol was stopped, regardless of whether non-alcohol runs were carried out on intervening days between alcohol test sessions. Moreover, the rate of loss of tolerance in the two groups was closely similar to that previously reported for a group which had acquired tolerance by the conventional "physiological" method (LeBlanc et al., 1969).

In confirmation of the findings in the previous study with the maze test (LeBlanc et al., 1973), once behaviorally augmented tolerance had reached its maximum levels it could not be increased by gavage with a much larger alcohol dose. At the same time, once behaviorally augmented tolerance had reached maximum levels it was effectively maintained by a technique (physiological schedule) which was clearly less effective in *producing* tolerance initially.

It may be argued on theoretical grounds that the "physiological" groups in Experiments 1 and 3 were not really fundamentally different from the "behavioral" groups, since they received repeated test sessions under ethanol at 4-day intervals. Thus, they might be considered as low-intensity behavioral augmentation groups. The empirical argument against this interpretation is based on the finding (Fig. 2) that the groups

receiving tests under ethanol every fourth, sixth and eighth day did not develop tolerance during the time limit of the experiment. This suggests that, with the present test procedure, very low frequency of testing under ethanol does not constitute behavioral augmentation.

These findings indicate that behavioral augmentation of alcohol tolerance is independent of a particular task or testing schedule. This is wholly consistent with the results of our earlier study on generalization of behavioral augmentation of tolerance (LeBlanc et al., 1975). None of the characteristics so far studied permits any distinction between the final state achieved by behaviorally augmented tolerance and that produced by more conventional pharmacological means. The results, therefore, do not provide any confirmation of the existence of two separate types of tolerance, even though they cannot disprove the possibility.

Acknowledgments. The authors are grateful to L. Currin, C. E. Fahlgren, and A. Wilson for their technical assistance.

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Received December 23, 1975; Final Version April 13, 1976