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Learning Factor in Rapid Tolerance to Ethanol-Induced Motor Impairment

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BITRÁN, M. AND H. KALANT. *Learning factor in rapid tolerance to ethanol-induced motor impairment.* PHARMACOL BIOCHEM BEHAV **39**(4) 917–922, 1991.—Exposure of male Wistar rats to a single moderate dose (1.7 g/kg, IP) of ethanol (EtOH), followed by intensive intoxicated practice on the moving belt apparatus (a total of 12 min during the first hour after EtOH injection), results in functional tolerance to the motor-impairing effects of a second dose given either 8 or 24 h later. In the absence of intoxicated practice, or after a considerably reduced opportunity for it (a total of 4 min during the first hour after EtOH injection), the same dose of EtOH fails to produce tolerance. Thus, not only the opportunity to practice, but also its extent and possibly its quality are important determinants in the rapid development of intersessional tolerance. In contrast to its rapidity of development, no significant loss of this tolerance is evident three weeks after the tolerance acquisition sessions.

Ethanol Motor impairment Rapid tolerance Rat Intoxicated practice

EXPERIMENTAL observations made during the last two decades have considerably altered the traditional view of tolerance as an adaptation to the drug exposure per se, requiring long, repeated exposures to the drug. In particular, extensive study of the effects of environmental, behavioral and temporal factors on the expression of tolerance has contributed greatly to a contemporary view of tolerance as a complex multifactorial adaptive response.

Functional tolerance, understood as an acquired decrease in central nervous system sensitivity to the effects of a drug as a result of previous exposure (15), has been shown to be subject to control by environmental stimuli. In fact, tolerance itself can become a conditional response as a result of pairing between the initial unconditional acute homeostatic response to the drug effect and identifying environmental cues that regularly precede administration of the drug (5,21).

In addition, it is now well established that individuals with identical pharmacological histories may display dramatically different levels of drug tolerance. Studies of tolerance to amphetamines (1), barbiturates (35) and ethanol (EtOH) (2, 3, 13, 24, 26, 32, 37) have consistently shown that administration of the drug *just before* behavioral training sessions affords greater development of tolerance than equal drug exposure *after* identical training sessions. This process has been referred to elsewhere as behaviorally augmented tolerance (26).

The fact that drug tolerance has proven to be amenable to analysis in the context of both associative and instrumental learning suggests that this adaptive reaction should have a built-in learning factor that can express itself to different degrees according to the specific experimental conditions employed (14).

Studies designed to assess the rate of development of chronic tolerance to EtOH indicate that this rate is highly dependent upon the test measure examined (19, 20, 33). However, intersessional tolerance to various effects of EtOH has been demonstrated 24 h after a single IP injection of EtOH. This phenomenon, designated as *rapid tolerance*, has been reported for EtOH-induced hypothermia in mice (4) and rats (21), for EtOH-induced ataxia in mice (dowel-balance task) (8) and rats (tilt-plane test) (21), and for loss of righting reflex in rats (21). Unfortunately, the term *rapid tolerance* is not always used in this specific sense. It has been used by some authors in reference to intrasessional tolerance, following either a single dose (9) or multiple doses (6,17) within the one session. These are really instances of *acute tolerance*; the importance of this distinction is dealt with in the Discussion section below.

In this paper we report a rapid model for the development of behaviorally augmented tolerance to EtOH-induced motor impairment in the rat. In this system, exposure of rats to a single moderate dose of EtOH, followed by intensive intoxicated practice, conferred functional tolerance to the effects of an identical dose given 8 or 24 h later. The same dose of EtOH, followed by minimal or no intoxicated practice, failed to produce rapid tolerance.

METHOD

Subjects

Male Wistar rats weighing about 150 g when purchased (Charles River, Montréal, Canada) were individually housed in

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an environmentally controlled room at 21–23°C and 40% relative humidity, with lighting on from 0700 to 1900 h. Water and standard Purina rat chow were available ad lib. When the rats reached 300 g b.wt., they were held at this weight by appropriate restriction of the daily chow ration.

Moving Belt Test

Training period. In this test, the rats were trained to walk on a motor-driven metal mesh belt that moved continuously over a shock grid (27). When the rat put one or more paws off the belt, it received a mild foot-shock and a cumulative timer was activated to record the total time-off-belt during a 2-min trial. The rats were trained to a criterion of 99% correct performance (i.e., not more than 1.2 s off belt during any 2-min trial). Training sessions began within the first week after arrival of the animals in the vivarium.

Test sessions. Unless otherwise stated, the motor impairment was measured for each rat in six 2-min trials starting at 7, 17, 27, 37, 47 and 57 min after an IP injection of EtOH (1.7 g/kg as a 17% w/v solution in saline). The impairment score for each rat in each test session is the maximum time off belt in any trial within that session; in almost all cases, this occurs on either the first or second trial.

Upon completion of the last trial in each session, 50 μl of blood was taken from the cut tip of the tail for gas chromatographic determination of blood ethanol concentration (BAC) (23). The experimenter conducting the trials was kept blind with respect to the treatments that the rats had received.

Experiment 1

Thirty-six rats trained to criterion on the moving belt test (MBT) were randomly assigned to one of three groups: Control, Before, and After (n = 12 each). On both Test 1 (t = 0 h) and Test 2 (t = 8 h), Control rats received an IP injection of saline (1 ml/100 g b.wt.) just before the MBT and a second saline injection immediately after completion of it. On Test 1, Before rats were injected with EtOH (1.7 g/kg), given the MBT, and then injected with saline after completion of the last trial. The same procedure was carried out on Test 2. Animals of the After group received saline before the MBT on Tests 1 and 2, and EtOH (1.7 g/kg, IP) after each test. On Test 3 (24 h) and Test 4 (3 weeks later), all rats were given EtOH (1.7 g/kg) before the MBT and saline after it.

Experiment 2

Twenty-four rats trained to criterion were randomly assigned to a Control or an Experimental group (n = 12 each). On Test 1 (t = 0 h) the Control rats received a saline injection before the MBT, and a second saline injection after it. The Experimental group were injected with EtOH (1.7 g/kg) before the MBT and saline after it. On Test 2 (24 h later), both Control and Experimental groups were injected with EtOH before the MBT, and saline after the last trial. Three weeks later (Test 3), both groups were again tested after receiving the same dose of EtOH IP.

Experiment 3

Forty-eight rats trained to criterion on the MBT were randomly assigned to two subgroups (n = 24 each) named Moderate (M) or Intensive (I) practice, according to the number of trials they would receive on the MBT during Test 1. Each group was further subdivided into Control and Alcohol subgroups. On Test 1, Control-M rats received a saline injection before the MBT, which consisted of only two 2-min trials starting at 7 and 17 min after the injection. A second saline injection was given at 59 min after the first injection. Alcohol-M rats received EtOH (1.7 g/kg IP) before the MBT, which also consisted of two 2-min trials, and a saline injection 59 min after the EtOH. For the Control-I and Alcohol-I subgroups, the treatments were as described above for the corresponding M subgroups, except that the MBT consisted of six 2-min trials staring at 7, 17, 27, 37, 47 and 57 min after the initial injection.

On Test 2 (t = 24 h) all rats in both M and I groups were injected with EtOH, tested for six 2-min trials as above, and then injected with saline.

Statistical Analyses

In each experiment, several rats failed to show any appreciable alcohol effect on performance in the MBT, and were found to have much lower BACs than the other rats of the corresponding groups. This was taken as evidence of unsatisfactory injections, the EtOH probably having been injected into the intestine or intramuscularly, rather than IP. Therefore, the results for any rat that failed to show at least 30 s maximum impairment score were excluded from the analysis.

Statistical comparisons were carried out by general linear model ANOVAs, using the NCSS program for IBM-PC, followed by post hoc comparisons of specific groups, when appropriate.

RESULTS

Experiment 1

Administration of a single EtOH injection followed by intoxicated practice on the MBT (a total of 12 min in the first hour after injection) resulted in a significant decrease of the motor impairment produced by identical EtOH injections given 8 h and 24 h later (Fig. 1). Overall ANOVA of the maximum impairment scores showed a significant effect of Tests, $F(2,23) = 3.66$, $p < 0.04$. Post hoc comparisons by Duncan's multiple range test showed that the mean score on Test 1 (74.7 ± 6.0 s) was significantly greater than that on Test 2 (54.2 ± 6.0, $p < 0.05$) or Test 3 (55.4 ± 6.3, $p < 0.05$). However, the time-courses of motor impairment on Tests 2 and 3 were almost identical (Fig. 1) and the maximum impairment scores did not differ significantly. BACs at the end of Test 1 did not differ significantly from those obtained after completion of Tests 2 and 3 [210 ± 5, 217 ± 4 and 217 ± 6 mg/dl respectively; $F(2,24) = 0.97$, $p > 0.60$], suggesting that the observed decrease in impairment was the result of functional tolerance rather than a change in EtOH disposition. Moreover, the largest differences in impairment were evident at 7 and 17 min after the injection of EtOH, too early for the manifestation of a difference in EtOH metabolism.

Intergroup comparison of the time-course of motor impairment on Test 3 is shown in Fig. 2. Overall ANOVA of the maximum impairment scores showed a highly significant effect of Groups, $F(2,26) = 7.30$, $p < 0.003$. Post hoc comparisons by Scheffé's test showed that the Before group score (55.4 ± 6.1 s) was significantly less than that of the After group (77.1 ± 5.5, $p < 0.05$) and the Control group (85.8 ± 5.2, $p < 0.01$), but the latter two groups did not differ from each other. All three groups presented similar BACs after completion of the test [221 ± 2, 223 ± 4 and 217 ± 6 mg/dl for Control, After and Before groups respectively; $F(2,21) = 0.49$, $p > 0.62$].

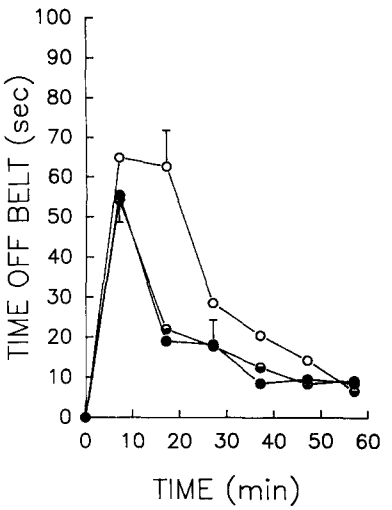

FIG. 1. Time-course of ethanol-induced motor impairment in Experiment 1: change in performance of Before group on Test 1 (○ t=0 h), Test 2 (◒ t=8h) and Test 3 (● t=24 h). On each test day, all rats were injected with ethanol (1.7 g/kg, IP) and tested on the moving belt apparatus at 7, 17, 27, 37, 47, and 57 min after the injection. Vertical bars represent largest S.E.M. for any trial within each group.

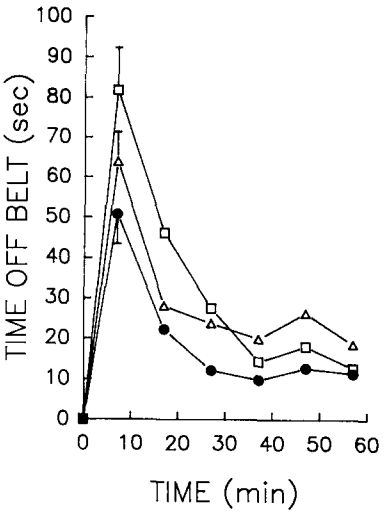

FIG. 3. Time-course of ethanol-induced motor impairment (Experiment 1): intergroup comparison of performance on Test 4 (t=3 weeks). □: Control group; △: After group; ●: Before group. All rats were tested after an IP injection of ethanol (1.7 g/kg).

Tolerance in the Before group, though somewhat reduced, persisted for up to 3 weeks (Fig. 3). Overall ANOVA of the maximum impairment scores showed a significant effect of Groups, $F(2,24)=3.54$, $p<0.05$. Post hoc comparisons showed that the mean value for the Before group (50.7 ± 7.3 s) was significantly less than that of the Controls (81.8 ± 9.1, $p<0.02$ by Fisher's LSD test). The mean value for the After group (63.8 ± 8.1) was not significantly different from that of either of the other two groups. No significant intergroup differences were observed in BACs after completion of Test 3 [241 ± 4, 235 ± 7 and 238 ± 4 mg/dl for Control, After and Before groups respectively; $F(2,24)=1.55$, $p>0.23$].

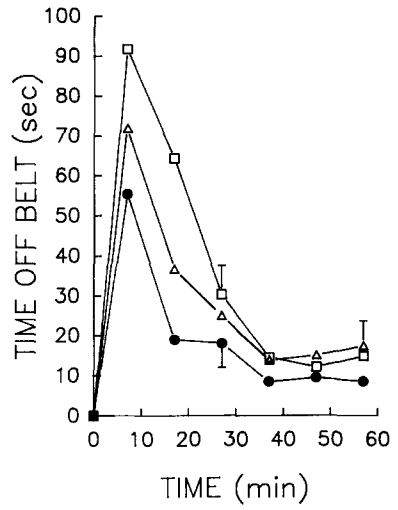

FIG. 2. Time-course of ethanol-induced motor impairment (Experiment 1): intergroup comparison of performance on Test 3 (t=24 h). □: Control group; △: After group; ●: Before group. On Test 3, all rats received ethanol (1.7 g/kg, IP) before being tested on the moving belt apparatus as described in Fig. 1 legend.

Experiment 2

Administration of a single dose of EtOH resulted in the development of tolerance to the motor impairing effect of a second identical dose given 24 h later, without the need for an intervening dose at 8 h. This tolerance persisted for at least 3 weeks (Fig. 4). Overall ANOVA of the maximum impairment scores on the three tests showed a highly significant effect of Tests, $F(2,35)=6.41$, $p<0.0043$. Post hoc comparisons by Fisher's LSD test showed that the mean score on the initial test (80.1 ± 7.3 s) was significantly greater than those on the tests at 24 h (44.5 ± 7.0; $p<0.01$) and 3 weeks (56.6 ± 7.9; $p<0.04$). However, the latter two did not differ significantly from each other ($p>0.05$). The 3-week impairment scores of the Experimental group, however, were significantly lower than those of the Controls tested under EtOH at 3 weeks (not shown).

The change in motor impairment in the Experimental group between Day 1 and Day 2 (Fig. 4) was not the result of altered drug disposition, as BACs at the end of testing each day did not differ significantly (220 ± 3 and 213 ± 3 mg/dl for Days 1 and 2 respectively; $t=1.6$, $p>0.10$). In addition, the difference in impairment between Tests 1 and 2 cannot be attributed to nonspecific variability in baseline response, because no significant differences were observed between the impairment scores after the first EtOH exposure of the Experimental group on Day 1 and the Control group on Day 2 (data not shown).

Experiment 3

The time-course of EtOH-induced motor impairment on Test 2, 24 h after Test 1, is shown in Fig. 5. An overall ANOVA of maximum impairment scores in all four treatment groups showed a significant effect of Groups, $F(3,41)=4.44$, $p<0.0086$. Post hoc comparisons by means of Fisher's LSD test at $\alpha=0.05$ showed that the maximum impairment score for the Alcohol-I group (82.7 ± 0.5 s) was significantly lower than those for Con-

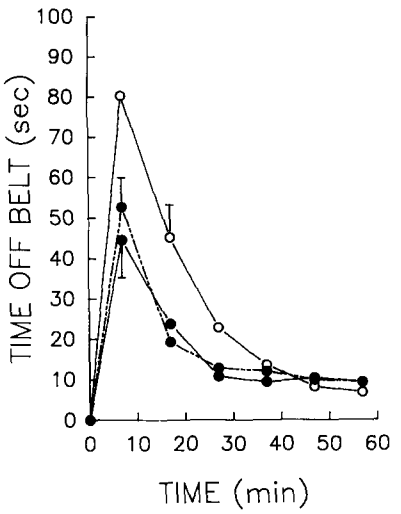

FIG. 4. Time-course of ethanol-induced motor impairment (Experiment 2): sequential change in performance of Experimental group on Test 1 (○; t=0 h), Test 2 (–●–; t=24 h) and Test 3 (- -●- -; t=3 weeks). All tests were carried out after IP injection of 1.7 g/kg ethanol.

trol-M and Control-I groups (100.2±5.2 and 106.9±4.6 s respectively), but the Alcohol-M group (96.2±5.0 s) did not differ significantly from any of the other three groups.

In order to maximize the chances of showing some degree of tolerance in the Alcohol-M group, a repeated measures ANOVA was carried out with the results of the first four trials in all four groups on Day 2. There were highly significant main effects of Groups, $F(3,41)=8.89$, $p<0.0001$, and Trials, $F(3,123)=202.29$, $p<0.0000$, and a significant interaction of Groups × Trials, $F(9,123)=2.56$, $p<0.01$. These results confirmed that the degree of impairment was not only lower in the Alcohol-I group than in the others, but that it also decreased more rapidly over repeated trials. Moreover, post hoc comparisons by Fisher's

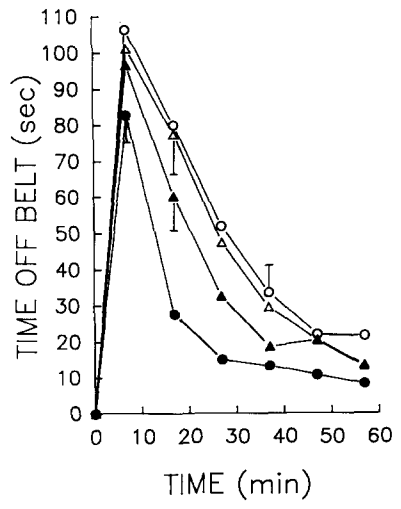

FIG. 5. Time-course of ethanol-induced motor impairment (Experiment 3). Intergroup comparison of performance on Test 2 (t=24 h). △: Control-Moderate practice (4 min practice on Test 1); ▲: Alcohol-Moderate practice; ○: Control-Intensive practice (12 min practice on Test 1); ●: Alcohol-Intensive practice. On Test 2, rats received ethanol (1.7 g/kg IP) followed by six trials on the moving belt test.

LSD test, at $\alpha=0.05$, now showed that the Alcohol-M group differed significantly from the Alcohol-I group but not from the Control-M group, whereas the Alcohol-I group differed from all the other groups. The Control-M and Control-I groups did not differ when tested under EtOH on Day 2.

BACs in the four groups on Day 2 were very closely similar: Control-M 211±5, Alcohol-M 212±4, Control-I 214±3 and Alcohol-I 218±4 mg/dl respectively. One-way ANOVA confirmed that these were not significantly different, $F(3,36)=0.18$, $p>0.90$.

DISCUSSION

The results of this study confirm that tolerance to EtOH-induced motor impairment can be demonstrated in rats with only two exposures to EtOH, provided that the first drug exposure is followed by intensive intoxicated practice of the response used to measure the effect of EtOH.

It has been known for some time that intersessional tolerance to EtOH-induced hypothermia or ataxia can be elicited in mice, in a similar paradigm consisting of two administrations of EtOH 24 h apart (4, 8, 30). The present demonstration that the inclusion of a behavioral augmentation component can result in equally rapid development of tolerance to EtOH-induced motor impairment in the rat emphasizes the importance of practice as an initial determinant in rapid tolerance. The fact that moderate practice was not sufficient to make the subjects tolerant indicates, as noted by others (7,26), that the extent of practice is a crucial factor. Since the Moderate-practice rats were tested only at 7 and 17 min after the first EtOH injection, at which time they were still markedly intoxicated, it is tempting to speculate that the development of tolerance requires not simply the opportunity for intoxicated practice, but conceivably also the opportunity for practice over a sufficiently long time to experience recovery from the impairing effects of the drug, as in the case of the Intensive-practice group. That this practice effect is indeed related to learning and memory is suggested by the fact that, in Experiments 1 and 2, the Control and After groups which received two tests under the influence of EtOH, but separated by a 3-week interval, did not develop tolerance.

The experimental system used in this study can be considered to give rise to intercessional tolerance as a result of a "chronic" treatment consisting of "n" EtOH exposures, in which n=2 in the present case. Nevertheless, it may have implications for the phenomenon of acute (intrasessional) tolerance and its relation to chronic tolerance. A considerable body of evidence indicates that tolerance to EtOH can develop within an hour or less (acute tolerance), during a single EtOH exposure (25, 30, 36). An even greater body of data substantiates the development of tolerance over a much more protracted time course [see (15)]. It is not yet possible to state with certainty the relationship, if any, between these types of tolerance, mainly because they have been studied over, and defined by, vastly differing time courses. However, a similar effect of intoxicated practice on acute, rapid, and chronic tolerance would be at least suggestive of a common mechanism.

Results presented here, showing that "chronic" tolerance can be demonstrated with only two exposures to the drug effects, are consistent with the idea that "acute" tolerance is the innate unconditional adaptive response to the experienced drug effects, and the "chronic" tolerance (of which the "rapid" model is the shortest possible instance) involves either retardation of the decay of this innate response or enhancement of the rate of its reappearance on subsequent exposures, by either learning (including conditioning) or more extensive pharmacological "practice." In keeping with the latter, the use of higher treatment doses can result in the development of tolerance to the ataxic and other

effects of EtOH in paradigms which do not include the opportunity for intoxicated practice (8, 10, 18).

The foregoing hypothesis is consistent with the recent finding (16) that rapid tolerance and chronic tolerance show striking similarities with respect to their pattern of relative magnitude in different rat strains, and their asymmetry of cross-tolerance between EtOH and pentobarbital. The present experimental system is well suited for this type of comparative study. In the present work, intersessional tolerance was demonstrated as early as 8 h after the first EtOH exposure. Conceivably, the use of smaller doses (to prevent build-up of EtOH levels) would permit shortening of the interval between the two EtOH injections so as to determine the minimum interval required to produce this type of tolerance. Lengthening of the inter-injection interval, on the other hand, will reveal the critical period beyond which the experience derived from the first injection can no longer influence the response to the second. Earlier work (26) suggests that this critical limit may be of the order of 3–4 days, but it will be necessary to define it with greater precision in order to derive clues as to the underlying neuronal process that sets the limit.

From our results, it is clear that when spaced either 8 or 24 h apart, the two EtOH injections elicited comparable degrees of tolerance. Moreover, the extent of tolerance produced after 8 h seemed maximal, as no further increase was produced by a third injection given 16 h later. It is conceivable that the tolerance found at 8 h after the initial exposure was really an artefact explicable in terms of circadian variation in sensitivity to EtOH. However, this argument seems untenable in the light of the finding by Gallaher et al. that the same degree of tolerance was produced by the same initial dose in different animals, even if their first exposures occurred at different times of the day (8).

In sharp contrast to the rapidity of development, no significant loss of tolerance was evident 3 weeks after the tolerance acquisition sessions. Though further experiments are clearly needed to determine the actual rate of loss of tolerance in this paradigm, it is increasingly clear that the persistence of tolerance can be influenced by behavioral or environmental factors, and this may explain why reports of its duration vary widely.

In this context, it is interesting to note that, in analogy to memory processes and chronic EtOH tolerance (28), the development and/or expression of rapid tolerance (same time frame as in the present work) is prevented by the protein synthesis inhibitor anisomycin (34). This finding suggests that de novo protein synthesis is essential in all three processes. The finding that memory for learned avoidance or discrimination tasks, 24 h after a single training session, is facilitated by immediate posttraining administration of epinephrine, naloxone, or muscarinic antagonists (11, 12, 29, 31) raises interesting possibilities for further exploration of the cellular mechanism(s) of tolerance.

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