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# Contingent Drug Tolerance: Differential Tolerance to the Anticonvulsant, Hypothermic, and Ataxic Effects of Ethanol

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KIM, C. K., J. P. J. PINEL, S. DALAL, T. E. KIPPIN, L. E. KALYNCHUK AND G. J. PAYNE. *Contingent drug tolerance: Differential tolerance to the anticonvulsant, hypothermic, and ataxic effects of ethanol.* PHARMACOL BIOCHEM BEHAV **52**(3) 531-539, 1995.—The kindled-convulsion model of epilepsy was used to study contingent tolerance to ethanol's (1.5 g/kg; IP) anticonvulsant, hypothermic, and ataxic effects in adult male rats. In the present experiments, three groups of amygdala-kindled rats received a series of bidaily (one every 48 h) convulsive stimulations: one group received ethanol 1 h before each stimulation; one group received ethanol 1 h after each stimulation; and another group served as the saline control. Tolerance to ethanol's anticonvulsant effect (Experiments 1 and 2) was greatest in those rats that received ethanol before each convulsive stimulation; whereas, tolerance to ethanol's hypothermic (Experiments 1 and 2) and ataxic (Experiments 2) effects developed in both groups that received ethanol. These results were predicted on the basis of the drug-effect theory of drug tolerance: the theory that functional drug tolerance is an adaptation to the disruptive effects of drugs on concurrent patterns of neural activity, not to drug exposure per se.

Ethanol Alcohol Tolerance Contingent tolerance Behavioral tolerance Seizure Convulsion Kindling Anticonvulsant Hypothermia Ataxia Amygdala

ACCORDING to the drug-effect theory of drug tolerance [see (22,24)], functional tolerance is an adaptation to the disruptive effects of drugs on concurrent patterns of neural activity, rather than to drug exposure per se. One prediction of this drug-effect theory is that the development of functional tolerance to a particular drug effect will be contingent on the expression of that drug effect during periods of drug exposure.

The strongest support for the drug-effect theory has come from experiments that have used the before-and-after design (13). In such experiments, there are two key groups: the subjects in one group receive the drug before they perform the criterion response on each tolerance-development trial; the subjects in the other group do not receive the drug until after they have performed the criterion response on each trial. Although the subjects in both these groups receive the same number, dose, and schedule of drug injections and the same number of opportunities to perform the criterion response, tolerance to the criterion effect is typically greater in the group that received the drug before the criterion response on each tolerance-development trial. According to the drug-effect theory, greater tolerance is observed in this group because the effect of the drug on the performance of the criterion response was repeatedly experienced [see (5,7,30)].

In one line of experiments supporting the drug-effect theory, Pinel and his colleagues (12,15,21) found that tolerance developed to the anticonvulsant effects of carbamazepine, diazepam, valproate, pentobarbital, or ethanol in amygdala-kindled rats when the drug was delivered 1 h before each of a series of convulsive stimulations, but not when it was delivered 1 h after. Similarly, tolerance to the anorexigenic effects of amphetamine (2), cocaine (31), or quipazine (27) has been shown to be facilitated by allowing the subjects to eat while drugged; tolerance to the disruptive effects of ethanol on maze and treadmill running has been shown to be facilitated by

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allowing the subjects to perform these tasks in the drugged state (3,14,29); and tolerance to the adipsic effect of scopolamine was facilitated by allowing the subjects to drink during periods of drug exposure (25) [see (5,7,30) for review]. Examples of drug tolerance whose development is dependent on the occurrence of the criterion response during the periods of drug exposure are commonly referred to as contingent drug tolerance (2).

These findings challenge the implicit assumption that drug exposure is both necessary and sufficient for the development of functional drug tolerance. Instead, these findings suggest that functional drug tolerance, like other kinds of neural adaptation, is a reaction to the disruption of co-occurring patterns of neural activity. This theoretical perspective of functional drug tolerance offers an explanation for a puzzling feature of drug tolerance: the fact that tolerance can develop to some effects of a drug while at the same time not develop to other effects of the same drug in the same subjects. The drug-effect theory suggests that tolerance will be facilitated to effects of a drug that are repeatedly manifested. The purpose of the present experiments was to provide further support for the drug-effect theory by showing that within the same subject, tolerance can develop to an effect of a drug that has been repeatedly manifested, while at the same time not develop to another effect of the same drug that has not been repeatedly manifested.

In the present study, two experimental groups of amygdala-kindled (6) rats received bidaily (one every 48 h) injections of ethanol. In each experiment, the before-and-after experimental design (13) was utilized: the rats in one experimental group received each ethanol injection 1 h before a convulsive stimulation, whereas the rats in the other experimental group received each ethanol injection 1 h after a convulsive stimulation. On the tolerance test, the rats in both of these groups, as well as those in a saline control group, received tests of ethanol's anticonvulsant, hypothermic, and ataxic effects. On the basis of the drug-effect theory, we hypothesized that tolerance to the anticonvulsant effect of ethanol would be greatest in those rats that received the convulsive stimulation in the drugged state; whereas, tolerance to the hypothermic and ataxic effects of ethanol would develop in both groups that received ethanol. These hypotheses were predicted on a fundamental difference between the anticonvulsant effect of ethanol on one hand and the ataxic and hypothermic effects of ethanol on the other: anticonvulsant drug effects can be manifested only in the presence of convulsive activity, whereas ataxic and hypothermic drug effects are invariably manifested in freely moving subjects maintained at normal room temperature.

Experiment 1 examined the anticonvulsant and hypothermic effects of ethanol. Experiment 2 not only replicated Experiment 1, but extended the findings to include ethanol's ataxic effect, and allowed for the evaluation of the interaction between stress and ethanol on the measure of hypothermia. Stress by itself can have a hyperthermic effect, but in conjunction with ethanol it can potentiate the hypothermic effect of ethanol (4,18).

## GENERAL METHOD

### *Subjects and Surgery*

The subjects were adult male, 350–450 g, Long-Evans rats obtained from Charles River (Canada). A single bipolar electrode (Plastic Products Co., MS-303-2) was implanted in each subject by conventional stereotaxic surgery under pentobarbital anesthesia (Somnotal®; Maple Leaf Food Inc.; 45 mg/kg, IP). The target was the left amygdala: 2.8 mm posterior, 5.0 mm lateral, and 8.7 mm ventral to the skull surface at bregma, with the incisor bar set at −3.3 mm [coordinates from (17)]. The electrode was held in place with stainless steel screws and dental acrylic. Each rat was allowed to recover for at least 7 days before the start of the experiment. The rats were individually housed in standard stainless steel mesh cages, with continuous access to standard rat chow and water. The colony room had controlled temperature (21°C) and lighting (lights on 0700–1900 h). All experimental manipulations occurred in the colony room at approximately the same time (±2 h) each experimental day during the light phase of the daily light : dark cycle.

### *Injections*

In both experiments, the dose of ethanol was 1.5 g/kg in a 25% v/v solution of isotonic saline. This dose had been shown to suppress kindled convulsions, and tolerance had been shown to rapidly develop to this anticonvulsant effect [e.g., (11,19,21)]. All injections of ethanol and the isotonic saline vehicle were delivered in a volume of 7.5 ml/kg, IP.

### *Kindling Phase*

After at least 7 days of postsurgical recovery, amygdala stimulation (400 μA, 60 Hz, 1 s) was delivered to each subject three times per day, five times per week for 3 weeks, with at least 2 h between consecutive stimulations. The typical response to the initial stimulations was a brief period of behavioral arrest, but by the end of the kindling phase, each stimulation elicited a stereotypical generalized clonic convulsion. The measures of convulsion severity were the duration of forelimb clonus and the convulsion class. The convulsion classes were characterized by the following [see (23,26)]: 1—facial clonus, 2—head nodding, 3—forelimb clonus, 4—rearing, 5—loss of equilibrium, and 6—multiple sequences of rearing and loss of equilibrium. Each convulsion class includes the characteristics of the preceding classes. These measures have been shown to be reliable, easy to quantify, and to respond systematically to pharmacological manipulations [e.g., (9,11,20)].

### *Experimental Treatments*

The procedures of Experiments 1 and 2 differed in several respects; however, there were many similarities. In both experiments, 48 h after the kindling phase, the following occurred: baseline phase—which established the stability of the convulsive activity in each rat; saline baseline test—which established the nondrug response to saline of each rat on the dependent measures; ethanol baseline test—which established the initial response of each rat to ethanol on the dependent measures; tolerance-development phase—in which the subjects were divided into the various treatment groups and received the appropriate treatments; and tolerance test—which was identical to the ethanol baseline test and established the final response of each rat to ethanol on the dependent measures. During the baseline phase, a bidaily (one every 48 h) schedule of convulsive stimulations was initiated and maintained for the duration of each experiment.

### *Histology*

Following each experiment, all rats were sacrificed with $CO_2$, and their brains were removed and preserved in forma-

lin. Frozen coronal sections, approximately 30 μm thick, were stained with cresyl violet and examined to confirm the location of the electrode tips.

*Statistical Analysis*

The statistical significance of the between-group differences on the tolerance test was assessed using separate one-way analyses of variance (ANOVA; $p < 0.05$) for each dependent measure, followed by Tukey post hoc tests ($p < 0.05$). Because we predicted the direction of differences between the groups, post hoc tests were one tailed. Only the data of those rats that completed the entire experiment were included in the statistical analysis.

## EXPERIMENT 1

Tolerance to ethanol's anticonvulsant and hypothermic effects was assessed in kindled rats that had received 15 bidaily injections of ethanol, each administered either 1 h before or 1 h after a convulsive stimulation. We hypothesized that tolerance to the anticonvulsant effect of ethanol would be greatest in those rats that received ethanol before each convulsive stimulation, but that tolerance to its hypothermic effect would develop in both groups that received ethanol.

*Method*

Of the 44 rats that began the study, 3 did not kindle and were not studied further.

*Baseline phase.* This phase comprised 10 bidaily stimulations, beginning 48 h following the final stimulation of the kindling phase. Rectal body temperature was measured prior to each stimulation to habituate the rats to the procedure. On the last two trials, the rats received a saline injection followed 1 h later by the measurement of body temperature and then a convulsive stimulation. This allowed the rats to habituate to the stress caused by the injection procedure.

*Saline baseline test.* This occurred 48 h after the final trial of the baseline phase. Each rat received a saline injection followed 1 h later by measurement of body temperature and then a convulsive stimulation. One rat did not meet the a priori criterion of at least 20 s of forelimb clonus and was, thus, eliminated from further study.

*Ethanol baseline test.* This was administered 48 h after the saline baseline test. It was identical to the saline baseline test except that ethanol was injected rather than saline: each rat received an ethanol injection followed 1 h later by measurement of body temperature and then a convulsive stimulation.

*Tolerance-development phase.* This commenced 48 h after the ethanol baseline test and comprised 15 bidaily trials. The rats were divided into three groups: the ethanol-before-stimulation group received ethanol 1 h before the stimulation; the ethanol-after-stimulation group received ethanol 1 h after the stimulation; and the saline-before-stimulation group received saline 1 h before the stimulation. The rats were divided into the three groups, equated for forelimb clonus durations, convulsion classes, and body temperatures on the saline baseline and drug baseline tests. During this phase, six rats were eliminated from further study because they became ill or rejected their electrode assemblies.

*Tolerance test.* This occurred 48 h after the last tolerance-development trial. It was identical to the ethanol baseline test: each rat received an ethanol injection followed 1 h later by measurements of its body temperature and then its convulsive response. Of the 44 rats that began the study, 34 completed it: ethanol-before-stimulation group ($n = 10$), ethanol-after-stimulation group ($n = 11$), and saline-before-stimulation group ($n = 13$).

*Results*

Figure 1 shows the mean forelimb clonus durations and the mean convulsion classes of the three groups on the saline baseline test, the ethanol baseline test, and the tolerance test. On the saline baseline test, the convulsive stimulation elicited generalized convulsions in all subjects. On the ethanol baseline test, there was almost total suppression of the convulsions in response to the ethanol injection. On the tolerance test, only the ethanol-before-stimulation rats displayed tolerance to ethanol's anticonvulsant effect: the ANOVAs performed on the forelimb clonus duration, $F(2, 31) = 75.6$, $p < 0.001$, and convulsion class, $F(2, 31) = 91.2$, $p < 0.001$, data were both statistically significant. Tukey post hoc tests indicated that the ethanol-before-stimulation group displayed significantly longer and more severe convulsions than the ethanol-after-

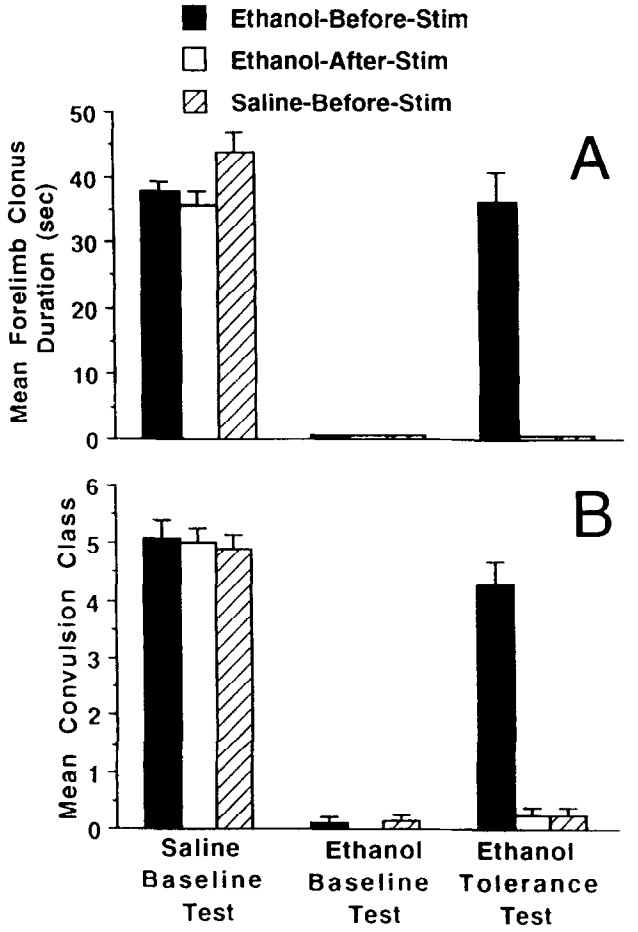


FIG. 1. The mean forelimb clonus durations (A) and mean convulsion classes (B) on the saline baseline test, the ethanol baseline test, and the tolerance test for the three groups. On the tolerance test, the ethanol-before-stimulation group displayed significantly longer forelimb clonus durations and greater convulsion classes than the ethanol-after-stimulation and saline-before-stimulation groups ($p < 0.05$); the latter two groups did not differ significantly from each other on either measure ($p > 0.05$).

stimulation and saline-before-stimulation groups ($p < 0.05$); the latter two groups did not differ significantly from one another in terms of either measure ($p > 0.05$).

Figure 2 shows the mean body temperatures of the three groups on the saline baseline test, the ethanol baseline test, and the tolerance test. There was a large drop in body temperature between the saline baseline and ethanol baseline tests in response to the ethanol injection. On the tolerance test, both the ethanol-before-stimulation and ethanol-after-stimulation groups displayed tolerance to the hypothermic effect of ethanol: the ANOVA was statistically significant, $F(2, 31) = 9.4$, $p < 0.001$. Tukey tests revealed that both the ethanol-before-stimulation and ethanol-after-stimulation groups displayed significantly higher temperatures than the saline-before-stimulation group ($p < 0.05$), and that the two ethanol groups did not differ significantly from each other ($p > 0.05$).

Histological analysis confirmed that all of the electrode tips had been positioned in or near the margins of the amygdala complex (Fig. 3).

*Discussion*

The experimental hypotheses were confirmed: tolerance to the anticonvulsant effect of ethanol was greatest in the group that received ethanol before each convulsive stimulation, whereas tolerance to the hypothermic effect of ethanol developed in both groups that received ethanol.

Although the difference was not statistically significant, the ethanol-before-stimulation rats displayed more tolerance to the hypothermic effect of ethanol than did the ethanol-after-stimulation rats (see Fig. 2)—a trend that is contrary to our expectation that the level of tolerance to the hypothermic effect would be the same in these two groups. This trend may have reflected pure statistical error, but it is possible that it reflected the confounding effects of stress. Stress can potentiate the hypothermic effect of ethanol (4,18); thus, the suggested difference could have resulted from a difference in the stressfulness of the tolerance test injection procedure for the two groups. It is possible that the injection procedure was more stressful for the ethanol-after-stimulation rats on the tolerance test because during the preceding 15 tolerance-development trials they had received their injections after the stimulation, rather than before.

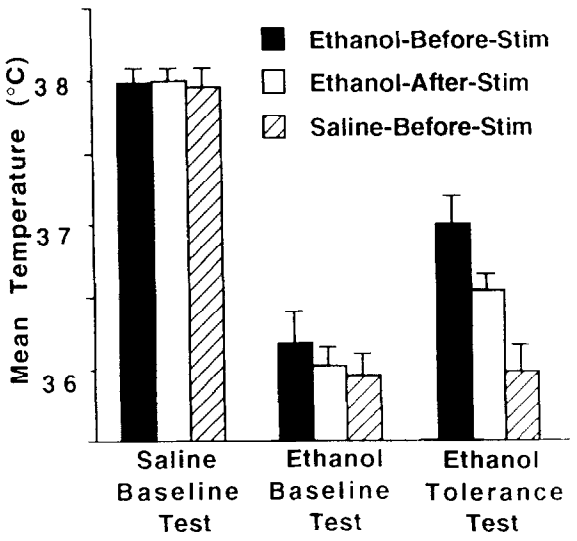


FIG. 2. The mean body temperatures on the saline baseline test, the ethanol baseline test, and the tolerance test for the three groups. On the tolerance test, the ethanol-before-stimulation and ethanol-after-stimulation groups displayed significantly higher body temperatures than the saline-before-stimulation group ($p < 0.05$); the two ethanol groups did not differ significantly from each other ($p > 0.05$).

## EXPERIMENT 2

The purpose of Experiment 2 was to provide further support for the drug-effect theory of tolerance: a) by confirming the findings of Experiment 1, b) by extending the findings of Experiment 1 to include ethanol's ataxic effect, and c) by eliminating the potential stress confound in the measurement of ethanol's hypothermic effect. The potential stress confound was eliminated by delivering injections both 1 h before and 1 h after each convulsive stimulation to all subjects during the 10 tolerance-development trials. The ethanol-before-stimulation subjects received ethanol before and saline after; the ethanol-after-stimulation subjects received saline before and ethanol after; and the saline-control subjects received saline both before and after. It was hypothesized that tolerance to the anticonvulsant effect of ethanol would be greatest in rats that received ethanol before each convulsive stimulation, and that tolerance to the hypothermic and ataxic effects of ethanol would develop in both ethanol groups.

*Method*

Of the 50 rats that began the experiment, 6 rats did not reach the baseline phase because they rejected their electrode assemblies or did not kindle.

*Baseline phase.* This phase comprised four bidaily stimulations.

*Saline baseline test.* Each rat received a saline injection followed 5 min later by the measurement of ataxia, and 1 h later by the measurement of body temperature and then a convulsive stimulation. Ataxia was assessed by placing each rat onto a flat surface and rating them on a five-point scale: 0—unconscious; 1—conscious but incapable of ambulation; 2—gross disturbance in gait, creeps along on belly, can easily push over; 3—clear but moderate disturbance in gait, stumbles, can push over; 4—slight disturbance in gait, cannot push over, less reactive than normal, body feels slack when held; 5—normal. This measure was used because pilot studies had shown it to be sensitive enough to distinguish between drug states at the doses employed in the present study. Six rats did not meet the a priori criterion of at least 20 s of forelimb clonus on the saline baseline test and were not studied further.

*Ethanol baseline test.* This was identical to the saline baseline test except that ethanol was injected rather than saline: each rat received an ethanol injection followed 5 min later by the measurement of ataxia, and 1 h later by the measurement of body temperature and then a convulsive stimulation.

*Tolerance-development phase.* Each subject received 10 bidaily tolerance-development trials. On each trial, two injections were delivered—1 h before, and 1 h after a convulsive stimulation. The rats were divided into three groups: the ethanol-before-stimulation group received ethanol before the stimulation and saline after; the ethanol-after-stimulation group received saline before and ethanol after; and the saline-control group received saline both before and after. The rats were divided into the three groups on the basis of the forelimb clonus durations, convulsion classes, body temperatures, and ataxia measures on the saline baseline and ethanol baseline

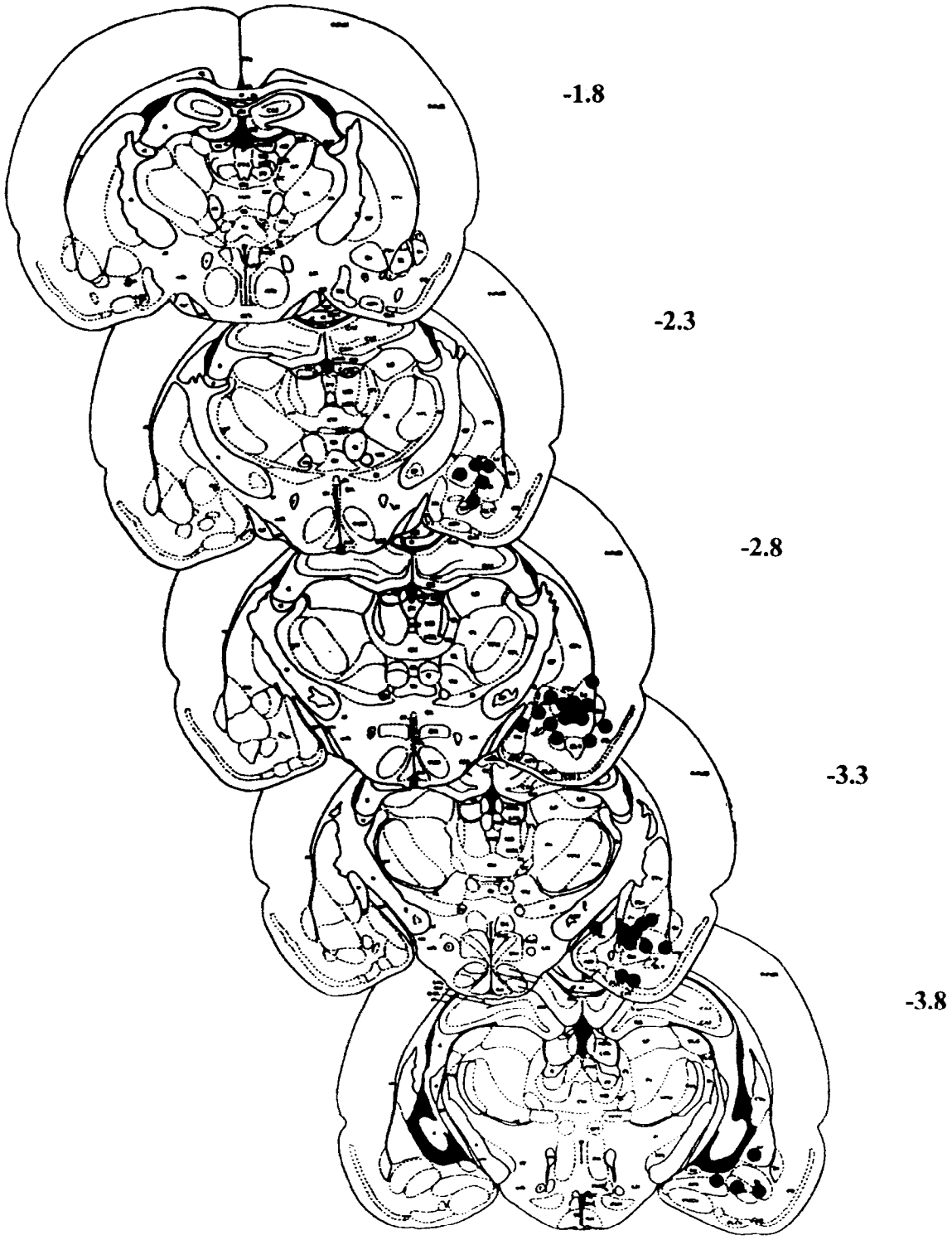


FIG. 3. Electrode placements of the 34 rats that completed Experiment 1. The numbers refer to mm posterior to bregma [coordinates from (17)].

tests. During this phase, three rats became ill and were not studied further.

*Tolerance test.* This was identical to the ethanol baseline test: every rat received an ethanol injection followed 5 min later by a test of ataxia, and 1 h later by tests of hypothermia and then anticonvulsant activity. The experimenter was blind to the experimental condition of each rat. Of the 50 rats that began the study, 35 completed it: ethanol-before-stimulation

group ($n$ = 12), ethanol-after-stimulation group ($n$ = 11), and saline-control group ($n$ = 12).

*Results*

Figure 4 illustrates the mean forelimb clonus durations and mean convulsion classes of the three groups on the saline baseline test, the ethanol baseline test, and the tolerance test. Tolerance to the anticonvulsant effect of ethanol was greatest in the ethanol-before-stimulation group: on the tolerance test, the ANOVAs were statistically significant for forelimb clonus duration, $F(2, 32) = 26.2$, $p < 0.001$, and convulsion class, $F(2, 32) = 40.2$, $p < 0.001$. Tukey tests revealed that the ethanol-before-stimulation group displayed significantly longer and more severe convulsions than the ethanol-after-stimulation and saline-control groups ($p < 0.05$); the latter two groups did not differ significantly from each other on either measure ($p > 0.05$).

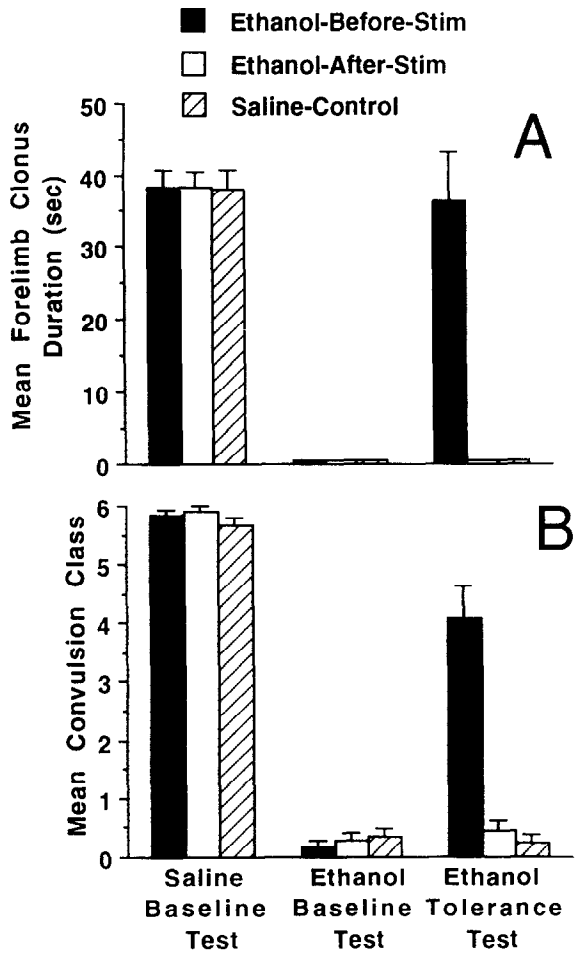


FIG. 4. The mean forelimb clonus durations (A) and mean convulsion classes (B) on the saline baseline test, the ethanol baseline test, and the tolerance test for the three groups. On the tolerance test, the ethanol-before-stimulation group displayed significantly longer forelimb clonus durations and greater convulsion classes than the ethanol-after-stimulation and saline-control groups ($p < 0.05$); the latter two groups did not differ significantly from each other on either measure ($p > 0.05$).

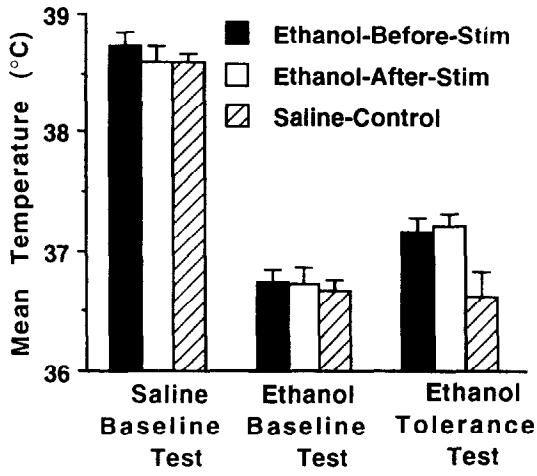


FIG. 5. The mean body temperatures on the saline baseline test, the ethanol baseline test, and the tolerance test for the three groups. On the tolerance test, the ethanol-before-stimulation and ethanol-after-stimulation groups displayed significantly higher body temperatures than the saline-control group ($p < 0.05$); the two ethanol groups did not differ significantly from each other ($p > 0.05$).

Figure 5 illustrates the mean body temperatures of the three groups on the saline baseline test, the ethanol baseline test, and the tolerance test. Significant tolerance developed to the hypothermic effect of ethanol in both ethanol groups. The ANOVA on the tolerance test hypothermia data was statistically significant, $F(2, 32) = 4.8$, $p < 0.015$, and Tukey tests

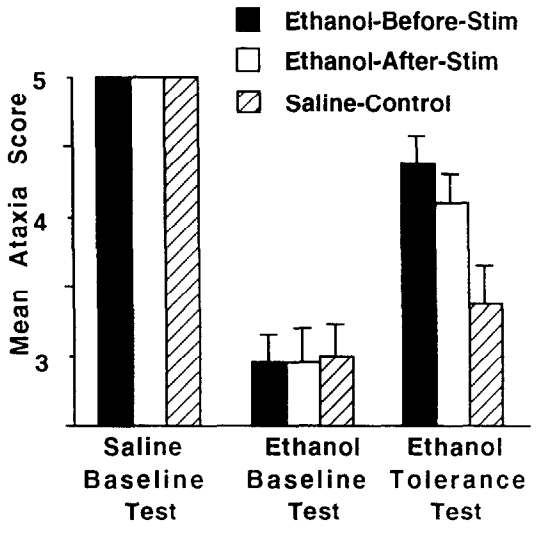


FIG. 6. The mean ataxia scores on the saline baseline test, the ethanol baseline test, and the tolerance test for the three groups. On the tolerance test, the ethanol-before-stimulation and ethanol-after-stimulation groups displayed significantly less ataxia than the saline-control group ($p < 0.05$); the two ethanol groups did not differ significantly from each other ($p > 0.05$). Ataxia was assessed using a five-point rating scale: 0—unconscious; 1—conscious but incapable of ambulation; 2—gross disturbance in gait, creeps along on belly, can easily push over; 3—clear but moderate disturbance in gait, stumbles, can push over; 4—slight disturbance in gait, cannot push over, less reactive than normal, body feels slack when held; 5—normal.

revealed that both ethanol groups differed significantly from the saline-control group ($p < 0.05$), but not from one another ($p > 0.05$).

Figure 6 shows the mean ataxia scores of the three groups on the saline baseline test, the ethanol baseline test, and the tolerance test. Significant tolerance developed to the ataxic

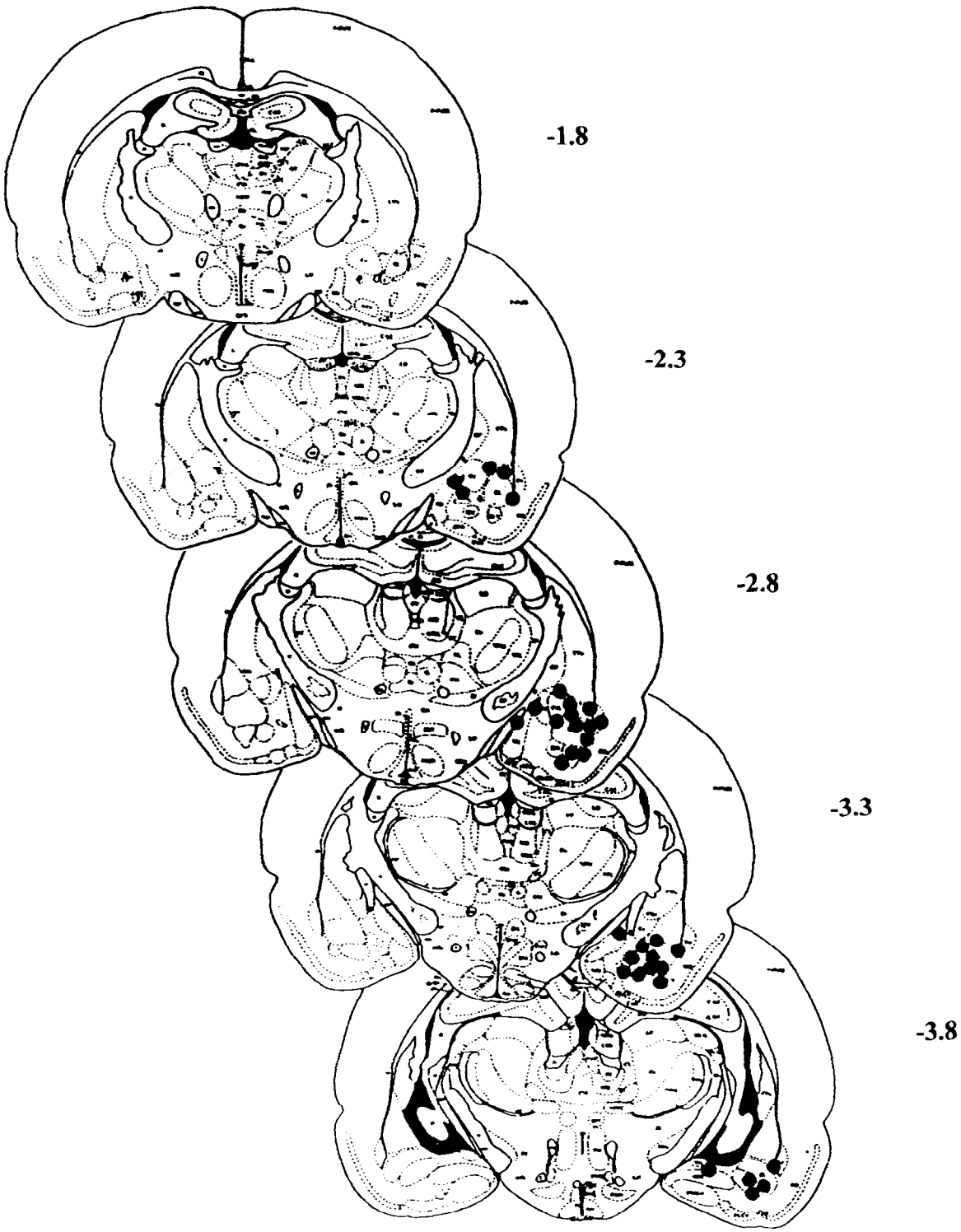


FIG. 7. Electrode placements of the 35 rats that completed Experiment 2. The numbers refer to mm posterior to bregma [coordinates from (17)].

effect of ethanol in both ethanol groups. The ANOVA on the tolerance test ataxia data was statistically significant, $F(2, 32) = 5.2$, $p < 0.011$, and Tukey tests revealed that the ethanol groups differed significantly from the saline-control group ($p < 0.05$), but not from one another ($p > 0.05$).

Histological analysis confirmed that all of the electrode tips had been positioned in or near the margins of the amygdala complex (Fig. 7).

*Discussion*

The experimental hypotheses were confirmed: tolerance to the anticonvulsant effect of ethanol was greatest in rats that received ethanol before each convulsive stimulation, whereas tolerance developed to the hypothermic and ataxic effects of ethanol in both groups that received ethanol. In Experiment 1, there was a suggestion, albeit a statistically nonsignificant one, that the mean body temperature of the ethanol-before-stimulation group was higher than that of the ethanol-after-stimulation group—the possible result of a stress confound. To eliminate the possible confounding effects of stress on the hypothermic effect of ethanol in Experiment 2, all subjects received injections both before and after each convulsive stimulation on the tolerance-development trials. As can be seen in Fig. 4, there was no suggestion of differences in the degree of tolerance to ethanol's anticonvulsant effect between the ethanol-before-stimulation and ethanol-after-stimulation groups of Experiment 2.

A comparison of the degree of tolerance to ethanol-induced hypothermia in Experiment 1 (Fig. 2) and Experiment 2 (Fig. 5) shows greater tolerance in Experiment 1. The greater tolerance in Experiment 1 than in Experiment 2 may result from the greater number of tolerance-development trials in Experiment 1 (15 vs. 10 trials). Because these were two separate experiments with numerous differences in methodology, a direct comparison of the results is not possible.

## GENERAL DISCUSSION

The results of the present experiments confirmed the experimental hypotheses. Tolerance to ethanol's anticonvulsant effect on amygdala-kindled convulsions was greatest in those rats that received ethanol before each convulsive stimulation during the tolerance-development trials (Experiments 1 and 2). In contrast, tolerance to ethanol's hypothermic (Experiments 1 and 2) and ataxic (Experiment 2) effects developed in both groups that received ethanol—the ethanol-before-stimulation and ethanol-after-stimulation groups. Thus, these results demonstrated differential tolerance to ethanol's anticonvulsant effect and to ethanol's hypothermic and ataxic effects within the same subject. Despite the same regimen of ethanol administrations and convulsive stimulations, the ethanol-before-stimulation group displayed significant tolerance to ethanol's anticonvulsant, hypothermic, and ataxic effects, whereas, the ethanol-after-stimulation group displayed significant tolerance to ethanol's hypothermic and ataxic effects only.

These results were predicted on the basis of the drug-effect theory of drug tolerance [see (22,24)]. The drug-effect theory is that functional drug tolerance is an adaptation to the disruptive effects of drugs on concurrent patterns of neural activity, rather than to drug exposure per se. The main tenet of this view is that functional tolerance to a particular effect of a drug is a reaction to the repeated expression of that drug effect. It then follows that tolerance can develop independently to the different effects of a drug within the same subject, depending on whether a particular drug effect was repeatedly expressed or not. Accordingly, we predicted that the ethanol-before-stimulation rats would display the most tolerance to the anticonvulsant effect of ethanol because they experienced the effect of ethanol on convulsive neural activity on each tolerance-development trial; and that the ethanol-after-stimulation rats would not become tolerant to the anticonvulsant effect of ethanol because they did not experience ethanol's anticonvulsant effect on each tolerance-development trial. In contrast, we predicted that both ethanol groups would become tolerant to the hypothermic and ataxic effects of ethanol because these effects would normally be experienced on each tolerance-development trial in freely moving subjects maintained at normal room temperature, whether or not convulsive stimulation was administered during drug exposure.

The present results are compatible with those of Woods and his colleagues. In one study (16), they administered ethanol to rats either before or after practice on a treadmill task. Tolerance to the ataxic effect of ethanol developed in only those rats that were allowed to practice the treadmill in the drugged state, whereas tolerance to the hypothermic effect of ethanol developed in all rats that received ethanol. In another study (8), some rats were injected with ethanol and placed in a heated environment so that the hypothermic effect of ethanol was not experienced, and others were injected and kept at room temperature so that the hypothermic effect was experienced. All rats, whether they were allowed to experience the hypothermic effect or not, were allowed to practice on the treadmill task in the drugged state. Tolerance developed to the hypothermic effect of ethanol in only those rats that experienced the hypothermic effect, whereas all rats developed tolerance to the ataxic effect of ethanol. These results, like those of the present experiments, indicate that it is the expression of the drug effect that is critical for the development of tolerance to that effect, not merely drug exposure.

In this study, tolerance developed to the ataxic effect of ethanol in both groups that received ethanol. This is in contrast to previous studies in which tolerance to the ataxic effect of ethanol measured by such tasks as treadmill or maze running (3,14,16,29) was found to be greater in those subjects that were allowed to practice the task under the influence of ethanol. In the present study, the general nonspecific ataxia was automatically experienced by all subjects injected with ethanol. By allowing the subjects to move freely in their home cages during the periods of drug exposure, they were practicing the task, and no other specific condition was necessary for this ataxic effect to be manifested. Thus, all subjects that received ethanol displayed tolerance to the ataxic effect when measured in this way. However, with tasks such as treadmill or maze running, access to the apparatus during the periods of intoxication is required for the subjects to practice. Under these conditions, tolerance develops more quickly in subjects allowed to practice the task in the drugged state.

According to the drug-effect theory, functional drug tolerance is a type of neural adaptation, and, hence, the study of drug tolerance can benefit from a consideration of this context. A case in point is the discovery that co-occurrence of activity in presynaptic and postsynaptic neurons is the fundamental causal factor in a variety of different forms of associative neural plasticity [see (1,10,28)]—this is known as Hebb's postulate for learning. The results of the present experiments and other studies of contingent drug tolerance make a similar

point: that the development of tolerance to a particular drug effect depends on the co-occurrence of the patterns of neural activity underlying the criterion response and the drug effects that disrupt them. From this perspective, it is clear that a complete understanding of the mechanisms of functional drug tolerance is unlikely to come from in vitro studies.

ACKNOWLEDGEMENTS

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## REFERENCES

1. Buonomano, D. V.; Byrne, J. H. Long-term synaptic changes produced by a cellular analog of classical conditioning in *Aplysia*. Science 249:420–423; 1990.
2. Carlton, P. L.; Wolgin, D. L. Contingent tolerance to the anorexigenic effects of amphetamine. Physiol. Behav. 7:221–223; 1971.
3. Chen, C. S. A study of the alcohol tolerance effect and an introduction of a new behavioral technique. Psychopharmacologia 12: 433–440; 1968.
4. Cunningham, C. L.; Bischof, L. L. Stress and ethanol-induced hypothermia. Physiol. Behav. 40:377–382; 1987.
5. Demellweek, C.; Goudie, A. J. Behavioral tolerance to amphetamine and other psychostimulants: The case for considering behavioral mechanisms. Psychopharmacology (Berlin) 80:287–307; 1983.
6. Goddard, G. V.; McIntyre, D. C.; Leech, C. K. A permanent change in brain function resulting from daily electrical stimulation. Exp. Neurol. 25:295–330; 1969.
7. Goudie, A. J. Behavioral techniques for assessing drug tolerance and sensitization. In: Boulton, A. A.; Baker, G. B.; Greenshaw, A. J., eds. Neuromethods, vol. 13. Psychopharmacology. Clifton, NJ: Humana Press; 1989:565–622.
8. Hjeresen, D. L.; Reed, D. R.; Woods, S. C. Tolerance to hypothermia induced by ethanol depends on specific drug effects. Psychopharmacology (Berlin) 89:45–51; 1986.
9. Kalynchuk, L. E.; Kim, C. K.; Pinel, J. P. J.; Kippin, T. E. Effect of an ascending-dose regimen on the development of tolerance to the anticonvulsant effect of diazepam. Behav. Neurosci. 108(1):1–4; 1994.
10. Kelso, S. R.; Ganong, A. H.; Brown, T. H. Hebbian synapses in hippocampus. Proc. Natl. Acad. Sci. USA 83(14):5326–5330; 1986.
11. Kim, C. K.; Dalal, S.; Pinel, J. P. J.; Weinberg, J. Prenatal ethanol exposure: Susceptibility to convulsions and ethanol's anticonvulsant effect in amygdala-kindled rats. Alcohol.: Clin. Exp. Res. 18:1506–1514; 1994.
12. Kim, C. K.; Pinel, J. P. J.; Roese, N. R. Bidirectional contingent cross tolerance between the anticonvulsant effects of pentobarbital and ethanol. Pharmacol. Biochem. Behav. 41:127–132; 1991.
13. Kumar, R.; Stolerman, J. P. Experimental and clinical aspects of drug dependence. In: Iverson, L. L.; Iverson, S. D.; Snyder, S. H., eds. Principals of behavioral pharmacology, vol. 7. New York: Plenum Press; 1977:321–367.
14. LeBlanc, A. E.; Gibbons, R. J.; Kalant, H. Behavioral augmentation of tolerance to ethanol in the rat. Psychopharmacologia 30:117–122; 1973.
15. Mana, M. J.; Kim, C. K.; Pinel, J. P. J.; Jones, C. H. Contingent tolerance to the anticonvulsant effects of carbamazepine, diazepam, and sodium valproate in kindled rats. Pharmacol. Biochem. Behav. 41:121–126; 1991.
16. Mansfield, J. G.; Benedict, R. S.; Woods, S. C. Response specificity of behaviorally augmented tolerance to ethanol supports a learning interpretation. Psychopharmacology (Berlin) 79:94–98; 1983.
17. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1982.
18. Peris, J.; Cunningham, C. L. Handling-induced enhancement of alcohol's acute physiological effects. Life Sci. 38:273–279; 1986.
19. Pinel, J. P. J.; Colborne, B.; Sigalet, J. P.; Renfrey, G. Learned tolerance to the anticonvulsant effects of alcohol in rats. Pharmacol. Biochem. Behav. 18(Suppl. 1):507–510; 1983.
20. Pinel, J. P. J.; Kim, C. K.; Mana, M. J. Contingent tolerance to the anticonvulsant effects of drugs on kindled convulsions. In: Wada, J. A., ed. Kindling. New York: Plenum Press; 1990:283–294.
21. Pinel, J. P. J.; Kim, C. K.; Paul, D. J.; Mana, M. J. Contingent tolerance and cross-tolerance to anticonvulsant drug effects: Pentobarbital and ethanol. Psychobiology 17(2):165–170; 1989.
22. Pinel, J. P. J.; Mana, M. J.; Kim, C. K. Development of tolerance to ethanol's anticonvulsant effect on kindled seizures. In: Porter, R. J.; Mattson, R. H.; Cramer, J. A.; Diamond, I., eds. Alcohol and seizures: Basic mechanisms and clinical concepts. Philadelphia: F. A. Davis; 1990:115–125.
23. Pinel, J. P. J.; Rovner, L. Experimental epileptogenesis: kindling-induced epilepsy in rats. Exp. Neurol. 58:190–202; 1978.
24. Poulos, C. X.; Cappell, H. Homeostatic theory of drug tolerance: A general model of physiological adaptation. Psychol. Rev. 98(3): 390–408; 1991.
25. Poulos, C. X.; Hinson, R. E. A homeostatic model of Pavlovian conditioning: Tolerance to scopolamine-induced adipsia. J. Exp. Psychol. [Anim. Behav.] 10:75–89; 1984.
26. Racine, R. J. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. 32:281–294; 1972.
27. Rowland, N.; Carlton, J. Different behavioral mechanisms underline tolerance to the anorectic effects of fenfluramine and quipazine. Psychopharmacology (Berlin) 81:155–157; 1983.
28. Sastry, B. R.; Goh, J. W.; Auyeung, A. Associative induction of posttetanic and long-term potentiation in $CA^1$ neurons of rat hippocampus. Science 232:988–990; 1986.
29. Wenger, J. R.; Tiffany, T. M.; Bombardier, C.; Nicholls, K.; Woods, S. C. Ethanol tolerance in the rat is learned. Science 213: 575–577; 1981.
30. Wolgin, D. L. The role of instrumental learning in behavioral tolerance to drugs. In: Goudie, A. J.; Emmett-Oglesby, M. W., eds. Psychoactive drugs: Tolerance and sensitization. Clifton, NJ: Humana Press; 1989:17–114.
31. Woolverton, W. L.; Kandel, D.; Schuster, C. R. Tolerance and cross-tolerance to cocaine and *d*-amphetamine. J. Pharmacol. Exp. Ther. 205(3):525–535; 1978.