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# Contingent Tolerance to the Disruptive Effects of Alcohol on the Copulatory Behavior of Male Rats[1]

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PINEL, J. P. J., J. G. PFAUS AND B. K. CHRISTENSEN. *Contingent tolerance to the disruptive effects of alcohol on the copulatory behavior of male rats.* PHARMACOL BIOCHEM BEHAV **41**(1) 133–137, 1992.—Sexually active male rats received five 30-min copulation tests with sexually receptive females, one every 4 days. One group of rats received alcohol (1 g/kg, IP) 45 min before, and an equivalent volume of saline 45 min after, each test; a second group received saline before and alcohol after each test; and a third, control group received saline both before and after. Four days after the last of the five tolerance-development trials, each rat received an injection of alcohol (1 g/kg, IP) 45 min before a copulation test so that the development of tolerance in the three groups could be compared. Tolerance to the disruptive effects of alcohol on mount, intromission, and ejaculation latencies, and on the duration of the postejaculatory interval was found to be significantly greater in the rats injected with alcohol before each copulation test than it was in the rats in the other two groups. These results constitute the first experimental evidence that tolerance develops to the disruptive effects of alcohol on male sexual behavior, and they support the theory that tolerance is an adaptive response to the disruptive effects of drugs on concurrent patterns of neural activity, rather than to drug exposure per se.

Alcohol Sexual behavior Drug tolerance Rat Ethanol Contingent tolerance Copulation Behavioral tolerance

THE ability of alcohol to influence copulatory behavior is universally recognized but poorly understand. Despite numerous anecdotal reports that alcohol can both disrupt the copulatory behavior of healthy human subjects [e.g., (1,3)] and release it from inhibition [e.g., (14,21)] and despite numerous clinical observations that alcohol can both induce [e.g., (28,29)] and ameliorate [e.g., (26,28)] human copulatory dysfunction, the effects of alcohol on copulation have only rarely been subjected to experimental analysis. There have been no experiments on alcohol and human copulation; the few relevant human experiments have examined the effects of alcohol on indices of sexual arousal (4, 9, 27, 30, 31) or on the gratification associated with masturbation (18,19). The few relevant experiments on laboratory animals have been of limited scope; they have all focused on the effects of single alcohol injections on male copulatory behavior (7, 10–13, 23). The present experiment was the first to assess the development of tolerance to the disruptive effects of a series of alcohol injections on the copulatory behavior of male rats.

Drug tolerance whose development is facilitated by the occurrence of some experience or behavior during the periods of drug exposure has been termed contingent drug tolerance (5,24). Most demonstrations of contingent drug tolerance have used the before-and-after design (16). In before-and-after experiments, the subjects in one group receive the drug before they perform the test response on each tolerance-development trial, whereas those in the other group do not receive the drug until after they have performed the test response on each trial. Thus only the subjects in the drug-before-test group repeatedly experience the drug's effect on the test response. On the drug tolerance test, the subjects in both groups receive the drug before they perform the test response so that the degree of tolerance in each group can be compared. Because the drug-before-test and the drug-after-test subjects in before-and-after experiments are exposed to exactly the same regimen of drug injections and testing, evidence of greater tolerance in the drug-before-test subjects is evidence that experiencing the drug's effect on the test response plays a significant role in the development of tolerance.

Although there have been numerous studies of contingent drug tolerance [see (8) for a review], most have focused on the study of just four tolerance effects: tolerance to anticonvulsive drug effects [e.g., (20)], tolerance to the disruptive effects of alcohol on maze running [e.g., (6)], tolerance to the disruptive effects of alcohol on balance and motor coordination [e.g., (17)], and tolerance to the anorexigenic effects of amphetamine [e.g., (5)]. One reason why research on contingent drug tolerance has been restricted to such a small sample of drug effects is that contingent drug tolerance can be studied only if the criterion drug effect is not an inevitable consequence of drug expo-

[1]All animal husbandry, surgical procedures, testing protocols, and euthanasia conformed to the guidelines of the Canadian Council for Animal Care.
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sure, that is, only if it is possible to keep the criterion drug effect from occurring outside the test situation. The disruptive effect of alcohol on copulatory behavior is such a drug effect; it cannot occur in situations in which there is no potential sex partner.

The present experiment had two objectives. The first was to determine whether tolerance develops to the disruptive effects of repeated injections of alcohol on the copulatory behavior of sexually active male rats. The second was to determine whether such tolerance is contingent on the occurrence of copulatory activity during the periods of intoxication.

## METHOD

### *Animals and Surgery*

Male Long-Evans and female Sprague-Dawley rats were purchased from Charles River, Canada, St. Constant, Quebec. They were housed by sex in groups of five or six in standard wire mesh hanging cages in a colony room maintained at approximately 21°C on a 12:12-h light:dark cycle (lights on at 23:30). Laboratory chow and water were continuously available. The females were ovariectomized via lumbar incision under ether anesthesia several months before the experiment began. The females were rendered sexually receptive for each test by 10 μg of estradiol benzoate and 500 μg of progesterone injected 48 h and 4 h, respectively, before each test.

### *Drugs*

Ethyl alcohol (95%) was diluted with physiological saline to obtain a dose of 1 g/kg in a 25% aqueous v/v solution. An equal volume of physiological saline was administered as a control solution. Both the alcohol and saline solutions were administered intraperitoneally to the male rats either 45 min before or 45 min after each test. Estradiol benzoate and progesterone (Steraloids) were dissolved in a 0.1 ml of peanut oil and injected subcutaneously. The dose of 1 g/kg was selected on the basis of a previous dose-response study (23); it is a dose that disrupted the copulatory behavior of male rats without producing obvious ataxia. A 45 minute injection test interval was employed in that study.

### *No-Drug Baseline Phase*

Thirty male rats received 10 no-drug baseline copulation trials, one every 4 days. These tests were 30 min in duration and were conducted during the middle third of the dark phase of the light-dark cycle in 29 × 29 × 45 cm Plexiglas testing chambers that contained 5 cm of commercial bedding material. Prior to each test, each male was habituated to the testing chamber for 5 min. Then, to begin the test, a sexually receptive female was placed in the chamber with the male. The occurrence of each mount, intromission, and ejaculation was entered by a trained observer on a computerized event recorder, which subsequently calculated the following four measures: 1) mount latency (ML; time from the start of the test to the first mount), 2) intromission latency (IL; time from the start of the test until the first vaginal intromission), 3) ejaculation latency (EL; time from the first intromission to the first ejaculation), and 4) postejaculatory interval (PEI; time from the first ejaculation to the next intromission). By the tenth no-drug baseline trial, all 30 males had met a priori criteria for inclusion in the study; intromission within 25 min of the start of the session, ejaculation within 30 min of the start of the session, and reinitiation of intromission within 10 min of the first ejaculation.

Following the 10 no-drug baseline trials, the 30 male rats were given three saline baseline tests, one every 4 days beginning 4 days after the last no-drug baseline trial. These saline baseline tests were identical to the no-drug baseline trials, except that each male received two IP injections of saline, one 45 min before and one 45 min after each test. The purpose of the saline baseline tests was to habituate the rats to the injection regimen that was to be employed during the tolerance-development phase of the experiment and to measure placebo baselines. Accordingly, the volume of saline administered to each subject was matched to the volume of alcohol solution that it would subsequently receive (mean = 5.1 ml/kg).

### *Tolerance-Development Phase*

The tolerance-development phase of the experiment comprised five trials, one every 4 days. On each tolerance-development trial, the males received an IP injection 45 min before, and another 45 min after, each 30-min copulation test. Before the tolerance-development phase, each male was randomly assigned to one of three conditions. The rats in the ETOH-before-test group received alcohol (1 g/kg) 45 min before each test and saline 45 min after. The rats in the ETOH-after-test group received saline 45 min before each test and alcohol 45 min after. The rats in the saline control group received saline 45 min before each test and again 45 min after.

### *Alcohol Tolerance Test*

Four days after the last tolerance-development trial, each of the 30 males received a single IP injection of alcohol (1 g/kg) 45 min prior to a 30-min copulation test so that the level of tolerance in the three groups could be compared.

### *Statistical Analysis*

The statistical significance of between-group and within-subject differences was assessed nonparametrically, with Mann-Whitney U-tests and Sign tests, respectively. Nonparametric tests were used because the data were not normally distributed, nor were the group variances homogeneous. The level of significance was $p<0.05$, one-tailed. For the purpose of calculating mean latency scores, when a subject did not perform one of the criterion responses (e.g., mount intromission, ejaculation, or intromission after ejaculation), it was assigned a latency score equal to the highest latency for that response observed in the experiment.

## RESULTS

The results are summarized in four panels of Fig. 1. Readily apparent are the three major findings of the experiment: 1) alcohol initially disrupted copulatory behavior by increasing the duration of the mount, intromission, and ejaculation latencies, and of the postejaculatory interval; 2) tolerance developed to each of these disruptive effects; and 3) tolerance was greater in the ETOH-before-test group than in the ETOH-after-test group.

The statistical significance of the disruptive effects of alcohol on copulatory behavior was established in two ways. First, the increases observed in all four dependent measures from the last saline baseline test to the first tolerance-development trial were statistically significant in the ETOH-before-test group (Sign tests, n = 10; ML, mean = 1; IL, mean = 1; EL, mean = 0; PEI, mean = 0; all $ps<0.05$). No significant increases were observed over the same period in either the ETOH-after-test group or the saline control group ($p>0.05$). Second, the increases observed in all four measures from the last tolerance-development trial to the

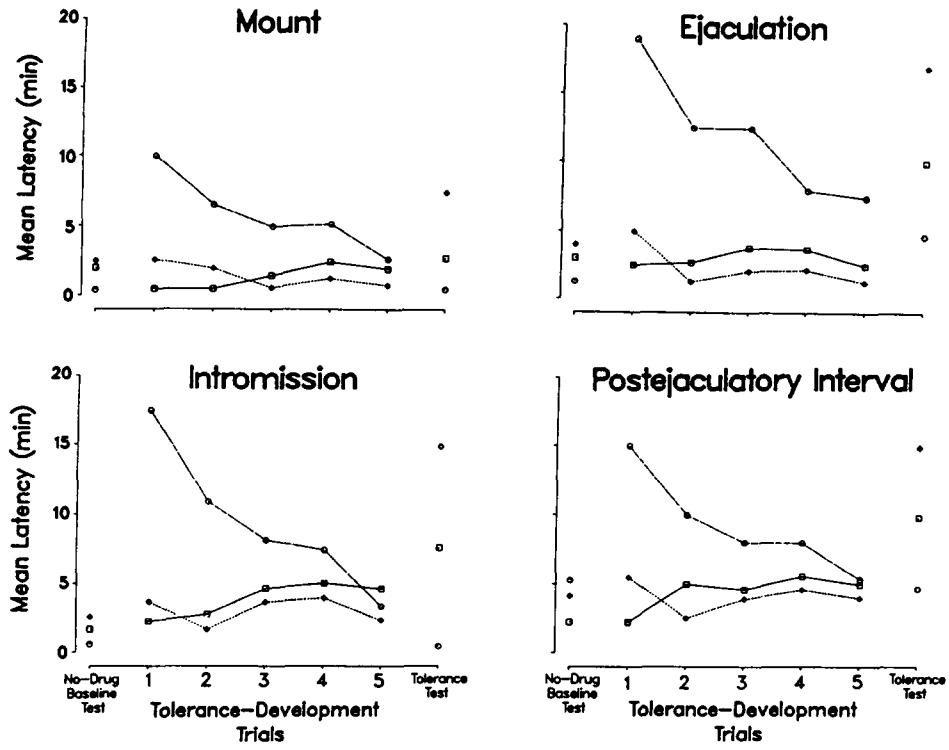


FIG. 1. Development of tolerance to the disruptive effects of 1 g/kg of alcohol on four measures of male copulatory behavior. During the tolerance-development phase of the experiment, some rats received alcohol 45 min before each copulation test (one every 4 days), some received alcohol 45 min after each copulation test, and some received saline (circles, ETOH-before-test group; squares, ETOH-after-test group; triangles, saline control group). On the tolerance test, all rats received 1 g/kg of alcohol 45 min prior to a test of copulation with a receptive female. Tolerance was significantly greater in the ETOH-before-test rats than in the ETOH-after-test rats and in the saline controls.

alcohol tolerance test in the saline control group were statistically significant (Sign tests, n = 10; ML, mean = 0; IL, mean = 0; EL, mean = 1; PEI, mean = 0; all $ps<0.05$).

The statistical significance of the tolerance effect was assessed by Sign test comparisons between the scores of the ETOH-before-test group on the first tolerance-development trial and their scores on the alcohol tolerance test. There was a significant decrease in all four measures (Sign tests, n = 10, ML, mean = 0; IL, mean = 1; EL, mean = 1; PEI, mean = 0, all $ps<0.05$). No significant decreases were observed over the same interval in the scores of the ETOH-after-test group or the saline group ($p>0.05$).

In order to assess the statistical significance of the differences among the three groups in the magnitude of their alcohol tolerance, tolerance scores were calculated by subtracting each subject's scores on the last saline baseline test from its scores on the alcohol tolerance test. Mann-Whitney U-tests conducted among these tolerance scores for each of the four measures confirmed that significantly greater tolerance developed in the ETOH-before-test rats than in either the ETOH-after-test rats (ML, U = 24; IL, U = 27; EL, U = 25; PEI, U = 19; all $ps<0.05$) or the saline control rats (ML, U = 13; IL, U = 21; EL, U = 19; PEI, U = 16; all $ps<0.05$). A significant difference between the ETOH-after-test rats and the saline control rats existed for only the mount-latency tolerance score (U = 24, $p<0.05$). The consistently significant differences between the ETOH-before-test and ETOH-after-test rats on the tolerance test support the experimental hypothesis, but it must be stressed however that the four measures are not independent.

## DISCUSSION

The results of this experiment confirm previous reports (9, 15, 27) that the acute administration of a nonanesthetic dose of alcohol disrupts the copulatory behavior of sexually active male rats; they provide the first experimental evidence that tolerance develops to the disruptive effects of alcohol on male copulatory behavior; and they demonstrate that the development of tolerance to these effects is greater in rats that have the opportunity to engage in sexual activity each time that they are intoxicated.

According to the drug-effect theory of tolerance, functional drug tolerance is an adaptation to the effects of the drug on concurrent patterns of neural activity rather than to its mere presence in the nervous system (20,24). The main prediction of the drug-effect theory is that functional tolerance will develop only to the effects of a drug on patterns of neural activity that are present during drug exposure. On the basis of this prediction, we hypothesized that male rats repeatedly experiencing the disruptive effects of alcohol on copulatory behavior would become more tolerant to these effects than would male rats receiving the same series of alcohol injections but not experiencing these effects. Accordingly, the observation of significantly greater tolerance in the ETOH-before-test rats than in the ETOH-after-test rats supports the drug-effect theory of functional tolerance.

Although tolerance was greater in the ETOH-before-test rats than in the ETOH-after-test rats, there was a suggestion of tolerance in the ETOH-after-test rats. On the alcohol tolerance test, one of the four dependent measures, mount latency, was significantly less influenced by alcohol in the ETOH-after-test rats than in the sailne control rats (see Fig. 1). There are two possible explanations for the development of a modest amount of tolerance in the ETOH-after-test condition, if indeed it did develop, neither of which is inconsistent with the drug-effect theory. First, tolerance in the ETOH-after-test subjects could have been the result of general metabolic, rather than specific functional changes, although this is unlikely given the 4-day interinjection interval. Second, contingent functional tolerance could have developed to the subtle effects of alcohol on some of the motor components of copulation (e.g., walking, rearing, clasping, or mounting) that might have regularly occurred during alcohol exposure in the ETOH-after-test rats' home cage. A modest degree of tolerance in drug-after-test conditions is not uncommon in contingent tolerance experiments (25).

Although the present experiment was the first to assess the development of tolerance to the disruptive effect of alcohol on copulatory behavior, Kumar and his associates (15,22) have examined the consequences of chronic morphine exposure on the copulatory behavior of sexually naive male rats. In their experiments, morphine (100 mg/kg) or a saline vehicle was administered daily for 5 weeks. Following a subsequent 2 weeks of abstinence, a challenge dose of morphine (30 mg/kg) was administered to all subjects prior to a 30-min test of copulatory behavior. The challenge dose of morphine eliminated the copulatory behavior of the vehicle control rats, and it reduced the number of social contacts that they made in response to sexually receptive females. Although the males that had received daily doses of morphine displayed more social contacts and copulatory attempts than did the saline-treated control males, only the increase in social contacts achieved statistical significance. These results are consistent with the drug-effect theory of functional drug tolerance. Because the rats did not have the opportunity to copulate under the influence of morphine until the end of the experiment, only a modest amount of tolerance would have been predicted on the basis of the drug-effect theory. On the other hand, because the subjects had ample opportunity to initiate social contacts with their male cage mates following morphine injections, it could have been predicted by the drug-effect theory that significant tolerance would develop to the effect of morphine on social contacts.

Although alcohol consumption is notorious for its ability to disrupt the sexual behavior of human males, there is considerable inconsistency in the magnitude and nature of its disruptive effect (1–4, 9, 14, 18, 27, 29). The present results suggest that some of this variability may be attributed to differences in the frequency with which individuals have previously engaged in sexual activity while intoxicated.

By adding to the list of demonstrated contingent drug tolerance effects, this experiment confirms that contingent tolerance is a general phenomenon. In so doing, it raises a key theoretical issue: Is all functional drug tolerance contingent? The drug effect theory of functional drug tolerance predicts that it is; it predicts that functional drug tolerance will not develop to drug effects that are not repeatedly manifested. Conversely, it predicts that the tolerance effects that develop in contingent tolerance experiments will not necessarily be restricted to those effects that are the focus of the experiment; drugs can potentially interact with all ongoing patterns of neural activity, not just those that underlie the criterion behavioral response.

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