Ethanol Tolerance in the Rat Is Learned

Abstract. *Rats were trained to walk on a treadmill to avoid foot shock. The animals developed tolerance for ethanol if given subsequent practice while ethanol-intoxicated. Rats given equivalent doses of ethanol after practice did not develop tolerance, nor did saline-treated controls. These results challenge the hypothesis that mere repeated doses of ethanol are sufficient to induce tolerance. It seems that tolerance does not develop unless the response used to measure tolerance is performed while the subject is intoxicated.*

Tolerance for many psychoactive drugs seems to be due, at least in part, to learning (*1*). The one reported exception is ethanol. It has been reported that repeated ethanol administration is sufficient to lead to tolerance and that practice of a response while intoxicated merely accelerates the development of tolerance but does not increase its final level (*2*). The concept that tolerance for ethanol is caused by ethanol exposure per se has been endorsed in recent reviews (*2a, 3*), and the accelerated acquisition of tolerance caused by practice has been termed behavioral augmentation of tolerance (*2*).

The lack of congruence between the evidence against learned tolerance for ethanol and favoring learned tolerance for many other drugs motivated the present experiment. Our goal was to replicate the critical experiment (*2*) that argues against the learning interpretation of tolerance, but with an additional control group that would enable unambiguous evaluation of the hypothesis. In agreement with the literature on most other drugs, we now provide evidence that learning, not mere exposure, is a necessary determinant of tolerance for ethanol.

Subjects were 40 Long-Evans rats obtained from the colony maintained by the Department of Psychology at the University of Washington. They were approximately 100 days old, had a mean weight of 310 g, and had never been exposed to alcohol. They were housed individually in standard stainless steel hanging cages in a room with a cycle of 12 hours of light and 12 of darkness (illuminated from 0800 to 2000 hours). The animals had unrestricted access to food (Purina Rat Chow) and water throughout the experiment.

The training apparatus was an automated treadmill developed by Gibbins *et al.* (*4*) and modified by Gallaher (*5*) and Wenger *et al.* (*6*). The treadmill required a rat to walk in a straight line to avoid foot shock (0.95 mA). The treadmill chamber measured 38 cm long by 32 cm wide by 20.7 cm high. The straight line was a constantly moving (8.26 cm/sec), 6.35-cm-wide stainless steel mesh conveyor belt that ran lengthwise through the middle of, and was horizontally flush with, an electrified grid floor. Any contact by the rat with the grid floor was electronically detected and cumulated for each trial in tenths of a second. Another circuit programmed the apparatus to execute a 60-second data-acquisition trial during which the belt was moving followed by a 30-second rest period in which the belt no longer moved but the floor remained electrified. Three successive cycles composed a standard session. The animals were not handled within a session.

The treadmill apparatus was used to define and measure tolerance. This apparatus has been shown to differentiate well the degrees of intoxication produced in rats by doses of ethanol ranging from 1.6 to 2.5 g/km (*4*). Figure 1A depicts the ethanol dose-response relation for another group of 30 previously unexposed animals tested on this apparatus.

The experiment had three phases: (i) training on the treadmill, (ii) tolerance acquisition, and (iii) tolerance testing. All animals were given daily sessions on the treadmill until they were able to avoid making cumulative errors of 1 second or more over a 60-second trial on three successive days. When all the animals had met this criterion, they were randomly assigned to one of four treatment groups.

The animals in group 1 were injected intraperitoneally with ethanol (15 percent, weight to volume) in saline (0.9 percent, weight to volume) every day. Sixty minutes after the injections they were required to walk on the treadmill while intoxicated. In terms of tolerance acquisition, these animals presumably benefited both from the daily exposure to ethanol and from the daily practice while intoxicated. Group 2 animals were given practice on the treadmill every day 60 minutes before being injected with ethanol. Since these two groups received identical amounts of ethanol, any subsequent difference in tolerance could be attributed to the fact that group 1 rats were intoxicated when they practiced and group 2 rats were not. A third group was administered only saline and given practice on the treadmill 60 minutes later. Subsequent differences in tolerance between groups 2 and 3 could be attributed to ethanol exposure per se. A fourth group was treated the same as group 2 except that on injection days 4, 8, 12, 16, and 20 these animals were given ethanol before rather than after their practice on the treadmill. [Group 4 is comparable to the one used by Leblanc and colleagues (*2*) to test for the acquisition of tolerance presumably caused by exposure to ethanol per se (*7*).] Each of these tolerance-test trials, occurring every 4 days, was also an opportunity for the rat to learn tolerance.

A series of escalating doses was used to increase the difficulty of the task rela-

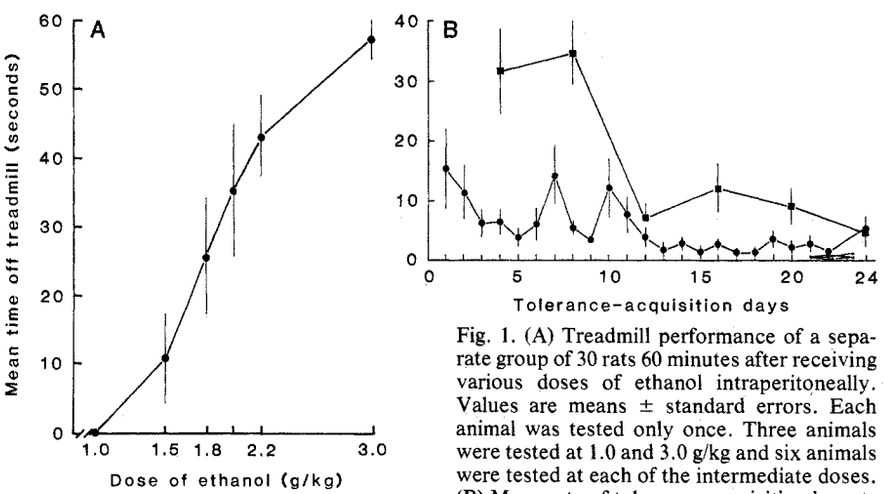

Fig. 1. (A) Treadmill performance of a separate group of 30 rats 60 minutes after receiving various doses of ethanol intraperitoneally. Values are means ± standard errors. Each animal was tested only once. Three animals were tested at 1.0 and 3.0 g/kg and six animals were tested at each of the intermediate doses. (B) Mean rate of tolerance acquisition by rats tested every day versus that of rats tested every 4 days. The vertical bars denote one standard error of the mean. Also shown are the performances of the three groups that practiced on the treadmill while not intoxicated on days 21 to 23. These performances were not significantly different from one another and were very close to the original training criterion. There were ten animals in each group.

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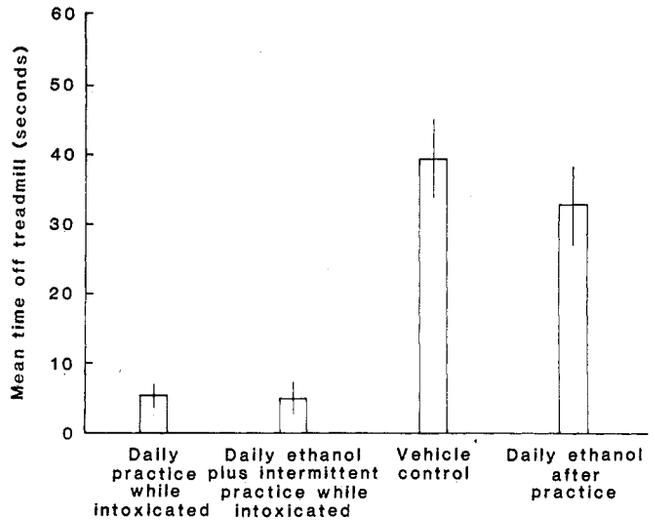

Fig. 2. Mean performance (± standard error) shown by the various groups on day 24 (2.2 g of ethanol per kilogram was injected intraperitoneally 1 hour before testing). The group sizes were adjusted to include only those rats that absorbed ethanol adequately (*9*).

tively slowly. The doses on the first 6 days were 1.6, 1.6, 1.6, 1.8, 1.8, and 2.0 g/kg. The dose was 2.2 g/kg on days 7 to 24. On day 24, the performance of the animals that were intoxicated every fourth day during practice converged with that of the animals that were intoxicated every day during practice (Fig. 1B). This indicated that the tolerance of the intermittently tested group had reached the asymptotic level of the more rapidly acquired ("behaviorally augmented") tolerance. On this day, therefore, the animals in groups 2 and 3 were given 2.2 g of ethanol per kilogram 60 minutes before being tested on the treadmill.

On day 24, blood samples were taken from the tails of all animals immediately after they finished their test session. The samples were frozen and later analyzed enzymatically to determine their ethanol content (*8*). Data for animals with blood ethanol concentrations less than 180 mg/dl were not included in the analysis (*9*). In addition, only the data from the first of the three trials of each test session were analyzed to exclude the possibility of intrasessional practice effects (*10*).

Figure 2 summarizes the results of day 24. The data were analyzed with the Cochran-Cox approximate *t*-test for unequal sample sizes and unequal variances (*11*). Performance of the rats that had been intoxicated every day during practice was not significantly different from that of the rats that had been intoxicated every fourth day during practice, nor was their blood ethanol concentration significantly different. There was also no significant difference between the performance or blood ethanol level of the rats that had always received ethanol after practice and that of the controls. This suggests that exposure to ethanol for 23 consecutive days, with no opportunity to practice on the treadmill while intoxicated, was not sufficient to induce tolerance. This group should have become tolerant according to the theory that tolerance occurs as an inevitable consequence of exposure of physiological systems to ethanol (*2*).

However, the rats that had always been given ethanol after practice were significantly more impaired ($t = 4.88$, $P < .0005$) than the rats that had usually been given ethanol after practice but that intermittently were intoxicated during practice. Moreover, this difference in performance could not have been due to differences in blood alcohol concentration, since the blood alcohol concentration of the more impaired group was actually slightly less than that of the less impaired group [this difference is not significant ($.10 < P < .20$) (*9*)].

These data support the interpretation that the tolerance reported to be a consequence of mere exposure to ethanol (*2*) is actually due to the practice given the animals every 4 days while they were being tested for the development of tolerance. All of the tolerance established over 23 days appears attributable to learning (*12*). If mere exposure to ethanol does cause tolerance, the present procedure did not detect it. Moreover, these data suggest that behavioral augmentation of tolerance (*2*) may be regarded as simply more rapidly learned tolerance resulting from increased practice while intoxicated.

This does not mean that tolerance is not mediated physiologically. On the contrary, presumably all types of tolerance, including learned tolerance, are physiologically mediated. The present data simply suggest that tolerance for ethanol, defined behaviorally, is learned as a result of practice during intoxication. This raises the question of whether learning also mediates the development of simpler, nonbehavioral forms of tolerance. It was reported recently that tolerance for ethanol, defined physiologically in terms of rectal temperature, is learned by classical conditioning (*13*). Moreover, it has been reported that tolerance develops for the effects of drugs and not for the drugs per se (*2a, 14*).

It seems reasonable to conjecture that, in general, tolerance for ethanol consists, at least in part, of learned behavioral or learned physiological adaptations to the functional demands resulting from the effects of ethanol on the organism. This may hold true for other psychoactive drugs as well. Conceivably, the principles of learning could be used to analyze tolerance and perhaps to manipulate it. This may have applications in the treatment of drug addiction.

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7. These intermittent tests of tolerance may actually have caused the tolerance observed by the earlier investigators. If so, rats administered ethanol after practice on the treadmill and tested intermittently should become tolerant. Subsequent differences in tolerance between these groups would be attributable to the intermittent tests of tolerance.
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9. Blood ethanol concentrations less than 180 mg/dl appeared to be outliers in the distribution. Such low values were considered to indicate failure to absorb the injected ethanol. The mean blood ethanol concentrations (± standard errors) and adjusted sample sizes for the various groups were as follows: group 1, 217.6 ± 3.4 mg/ml ($N = 8$); group 2, 197.3 ± 3.1 mg/ml ($N = 9$); group 3, 202.8 ± 0.4 mg/ml ($N = 10$); group 4, 209.1 ± 4.9 mg/ml ($N = 5$)..
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12. It is possible that the slight (nonsignificant) tendency towards tolerance shown by the animals given ethanol each day after practice might have resulted in tolerance if the experiment had been extended beyond 24 days. However, even if such tolerance were to have been obtained, it would not necessarily be attributable to ethanol exposure per se. There are at least two ways in which learning could mediate such tolerance. One is that the animals learn incidentally to tolerate ethanol while intoxicated in their home cages. A second possibility is some form of classical conditioning. All the injections of ethanol were given in the test room. The cues associated with this room reliably predicted the presence of ethanol and may have elicited compensatory responses through classical conditioning.
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Environmental Sex Determination: Interaction of Temperature and Genotype in a Fish

Abstract. *Sex determination in an atherinid fish, the Atlantic silverside (*Menidia menidia*), is under the control of both genotype and temperature during a specific period of larval development. The sex ratios of the progeny of different females are variable and differ in their responsiveness to temperature. This demonstrates that sex ratio in fishes that normally have separate sexes can be influenced by the environment.*

The evolution of genetic mechanisms that determine sex and the operation of natural selection on the sex ratio have long been of interest to population biologists (*1*). In many animals, the sex of offspring is determined at conception, and primary sex ratios of progeny approximate 1:1. Determination of sex by environmental factors, after conception, is a relatively rare phenomenon among gonochoristic species (those having separate sexes); this phenomenon has been found in a few invertebrates (*2*), one family of turtles, and an alligator (*3*). Although fishes probably have the most diverse array of sex-determining mechanisms and modes of sexuality of any vertebrate group (*4*), naturally occurring environmental determination of sex by factors such as temperature has not been found in any gonochoristic fishes. We now present data demonstrating that (i) sex determination in an atherinid fish, the Atlantic silverside (*Menidia menidia*), is under genetic and temperature-dependent environmental control during a critical phase of larval development, and (ii) sex ratios of progeny from different females are highly skewed, highly variable, and differ in their responsiveness to temperature.

The Atlantic silverside is a common estuarine fish of the eastern North American coast that completes its entire life cycle in 1 year (*5*). Breeding occurs on a semilunar cycle over a 2- to 3-month period during the spring, with each female producing four to five successive clutches of 200 to 2000 eggs (*6*). Our study of *Menidia* in Essex Bay and Salem Harbor, Massachusetts, revealed a consistent pattern of seasonal fluctuations in sex ratios (Fig. 1) (*5*). As juveniles of a new year class were recruited to the population in early July, proportions of females significantly exceeded 0.5 ($P < .01$); as recruitment continued, the excess of females rapidly declined. By completion of recruitment in September, the number of males either slightly (1977, 1978) or greatly (1976) exceeded that of females, and the mean lengths and weights of females were significantly greater than those of males (*5*). However, ranges in size were nearly equal, and experiments in 1978 and 1979 proved that males and females actually grow at equal rates when reared from eggs in laboratory aquariums (*7*). Furthermore, clutches of eggs taken from 6 to 10 females, fertilized by 10 to 25 males from the early, middle, and late portions of the spawning season, and reared under the prevailing photoperiod and constant warm temperatures (20° ± 1°C), produced similar proportions of females: 0.268 ($N = 123$), 0.297 ($N = 111$), and 0.273 ($N = 99$), respectively (*7*). These male-biased sex ratios focused our attention on the effect of environmental factors, specifically temperature.

In 1980, we conducted three experiments in which eggs and larvae were incubated in environmental chambers under two temperature regimes: cold fluctuating temperatures (CFT) of 11° to 19°C and warm fluctuating temperatures (WFT) of 17° to 25°C. These temperature regimes, based on data from a major spawning site in Salem Harbor, correspond to the average minimum and maximum temperatures experienced by eggs during the first 2 weeks of May (CFT) and the first 2 weeks of July (WFT). Since silversides deposit their eggs in the upper intertidal zone among vegetation,

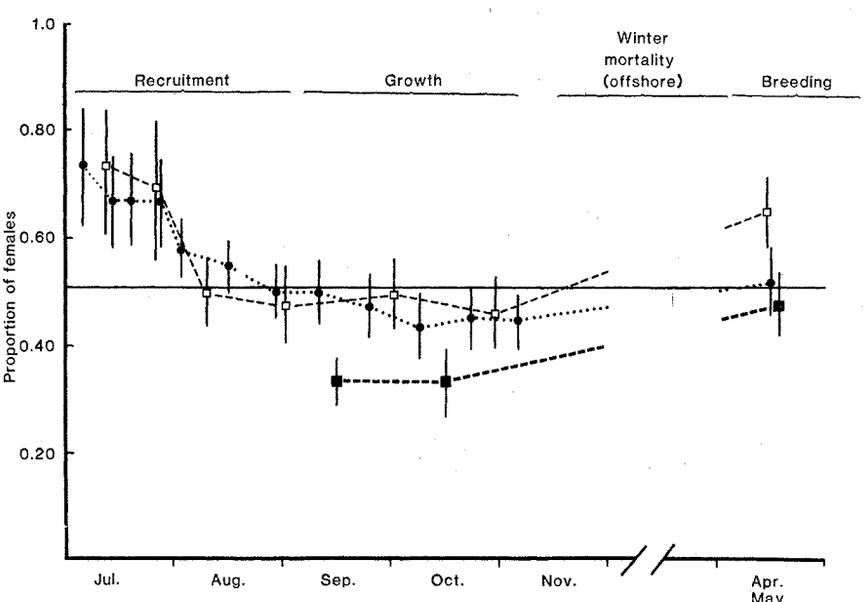

Fig. 1. Variation in sex ratios of *Menidia menidia* during major periods of its life cycle in Essex Bay, Massachusetts. No winter samples are shown because silversides winter offshore and are unavailable for capture in near-shore areas. Samples obtained in the spring are pooled because silversides suffer high winter mortality and are much less abundant afterward. Since a life cycle is completed in 1 year, each year class represents a distinct generation: (■) 1976; (●) 1977; (□) 1978. Vertical lines indicate 95 percent confidence limits based on exact probabilities (*9*). Sample sizes range from 55 to 442 (mean, 255). The horizontal solid line represents a 1:1 sex ratio.

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