a serotonin depletor, has minimal effects on the postejaculatory interval (*20*) and no effect on the postejaculatory vocalization, especially in vigorous animals (*21*). (ii) Our lesions in the midbrain raphe system (an area rich in serotonergic cell bodies) did not affect this measure appreciably (*22*). Depletion of noradrenaline was correlated with deficiencies in sexual activity and unaltered postejaculatory intervals (*23*). In examining the biogenic amine pathways we found the most effective lesions were placed at the site of the dopaminergic cell bodies in the rostral midbrain. To suggest that dopamine normally inhibits male copulation is contrary to the findings of Malmnäs (*24*) who concluded, after altering catecholamine levels in the whole brain, that dopamine stimulates sexual behavior in male rats. However, several opposing neuronal systems may be involved in controlling sexual behavior, and dopamine may be a transmitter in more than one of these systems. Finally, since VML lesions probably also damage noradrenergic, serotonergic, and other pathways, the possibility of their involvement cannot be discounted (*25*).

The part of the refractory period during which vocalization appears, the absolute refractory period, is characterized by a predominance of rest behavior and sleeplike electroencephalographic activity (*4, 5*). Animals with reduced or abolished vocalization periods and reduced refractory periods showed little, if any, rest behavior; judging by overt behavior it is doubtful whether any sleeplike EEG would be evidenced in these animals. Since vocalization was absent or greatly reduced in these animals and they were behaviorally aroused, we assume that the absolute phase of the period is the one most affected and that what remains is mainly the labile, relative refractory period. The fact that a tail pinch was capable of reducing the refractory period of a late ejaculatory series to a minimal value supports our interpretation.

Regulation of the copulatory cycle is viewed as resulting from the interaction between facilitative and inhibitory processes (*4, 26*). The principal effect of the lesions described here was to abolish the profound inhibition that prevails during the refractory period. This same inhibitory process apparently does not regulate the temporal periodicity of the copulatory cycle, that is, the pacing of mount-bouts, since other parameters of sexual behavior were unaffected by the lesions.

In contrast, the massive lesions of the posterior hypothalamic-midbrain junction of Heimer and Larsson (*2*) not only reduced the refractory period but also caused a facilitation of copulatory rate and a reduction in intromission frequency. It is possible that their large lesions destroyed additional inhibitory structures or pathways, involved in the regulation of the copulatory cycle, which were not destroyed by the more circumscribed lesions that we used. A better understanding of the inhibitory mechanism (or mechanisms) that regulate male copulatory behavior will come from further analyses of this system.

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16. Lesioned animals sometimes showed transient deficits in copulatory behavior. Frequently their performance improved with time and then stabilized. Performance was considered stable and repeatable when (i) the animal did not continue to show obvious improvement from test to test and (ii) the variability among three or more consecutive tests was comparable to that in control tests in the same population, according to the judgment of the experimenter.
17. Ultrasounds were recorded with a condenser microphone constructed after the plans of J. J. G. McCue and A. Bertolini [*IEEE Trans. Sonics Ultrason.* **SU-11** (1964), p. 41] and J. D. Pye and M. Flinn [*Ultrasonics* **2**, 23 (1964)]. The microphone output was fed into a preamplifier built according to the plan of J. D. Pye [*Ultrasonics* **6**, 32 (1968)]. The resulting signal was displayed on a Tektronix oscilloscope.
18. In preoperative tests this measure did not differ significantly from the postejaculatory interval, and in no case was less than 220 seconds.
19. After long-term estrogen treatment female rats execute the complete male ejaculatory pattern. The refractory period of these females is extremely short, in some cases approaching those of the lesioned males in the present study, and they do not exhibit the postejaculatory vocalization. These females possess large pituitary tumors and substantial destruction of the basal posterior hypothalamus and midbrain [D. E. Emery and B. D. Sachs, *Science*, in press]. It is possible that the extraordinary postejaculatory behavior of these females was due to destruction of the same area of the brain as in our lesioned animals.
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† We are saddened by the death of Peter McDonald and his wife Wendy in an airline crash on 3 March 1974.

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Social Modification of Alcohol Consumption in Inbred Mice

Abstract. *The strain-specific "preference" for, or "aversion" to, an alcohol solution in a choice situation on the part of C57BL and DBA mice is believed to be under genetic control. But social rearing conditions are now shown to alter the voluntary consumption of alcohol, so that DBA weanling mice housed for 7 weeks with adult C57BL mice increase—and C57BL weanling mice housed with DBA adults decrease—their alcohol intake. Although substantial and highly significant changes in alcohol self-selection occur, strain-specific phenotypes are not reversed.*

Social facilitation, a specific type of interindividual interaction, has been defined (*1*) as ". . . any increment or decrement in an individual's behavior resulting from the presence of another organism." The exact nature or specific role of the companion in influencing behavior is, however, seldom asserted in general definitions.

In addition to man, the phenomenon has been observed in many species in a variety

Table 1. Mean body weight and mean intake of fluids (± standard error) of control (C) and experimental (E) groups of mice.

Strain	Group	*N*	Body weight (g)	Water intake (ml g^{-1} day^{-1})*	Alcohol intake (ml g^{-1} day^{-1})†	Alcohol intake (g kg^{-1} day^{-1})‡
C57BL	C	7	24.4 ± 0.8	0.100 ± 0.021	0.128 ± 0.023	12.8 ± 2.3
	E	12	24.9 ± 0.3	0.133 ± 0.012	0.065 ± 0.009	6.5 ± 0.9
DBA	C	11	20.6 ± 0.8	0.263 ± 0.017	0.011 ± 0.002	1.1 ± 0.2
	E	13	18.9 ± 0.7	0.223 ± 0.008	0.026 ± 0.007	2.6 ± 0.7

*Milliliters of water per gram of body weight per day. †Milliliters of 10 percent (wt./vol.) ethanol per gram of body weight per day. ‡Grams of ethanol per kilogram of body weight per day.

of paradigms (*2*), with the clearest effects upon feeding behaviors. For example, satiated animals can be induced to eat more solid food if unfed animals are introduced (*3*), and absolute daily food consumption increases if animals are fed in pairs or groups (*4*). Most recently, Galef and Clark (*5*) have shown that adult colony members influence food preferences and feeding sites of weanling rat pups.

Unlike feeding behavior and food preference, the influence of companions on drinking and fluid preference has received lesser attention. It has been reported that grouped rats seek water more often in the presence of shock than do isolated rats (*6*), while grouped mice either decrease their total fluid intake without exhibiting any change in their choice between water and a solution of alcohol or show a decrease in alcohol consumption, depending on the strain examined (*7*). Whether a parallelism exists between feeding and drinking behavior and food and fluid preference is not known.

The present study assessed the influence of social interaction on fluid selection in inbred mice. We hypothesized that the social interaction provided by adult colony members might be a powerful enough phenomenon to modify the well-documented strain-specific phenotype associated with voluntary alcohol consumption (*8*). The major question asked was whether social rearing conditions could alter a genetically based predisposition for alcohol selection (*9*): would "drinker" C57BL mice housed from weaning with "nondrinker" DBA mice decrease their voluntary alcohol consumption—and would "nondrinker" DBA mice housed with "drinker" C57BL mice increase their voluntary alcohol consumption?

The adult colony members were C57BL/6 and DBA/2 mice at least 18 weeks of age, born in our colony from breeder stock originally purchased from Jackson Laboratory (Bar Harbor, Maine). Two to three mice of the same sex and approximately the same age were housed together in plastic cages (19 by 29 by 14 cm) and tested for voluntary consumption of alcohol (10 percent, wt./vol., prepared from 95 percent ethanol and tap water) in a standard two-bottle choice situation where the alternative fluid was tap water. Purina lab chow was available at all times. A mean cage preference ratio (milliliters of alcohol per milliliters of total fluid per day) based on 7-day test data was computed. Only those groups of C57BL mice with a ratio above 0.60 and those DBA groups with a ratio below 0.15 were used as adult companions. Bottle leakage was estimated not to exceed 0.1 ml per day and the intake data were corrected accordingly.

At parturition, or within 1 day, all litters were culled to seven pups, and the mother and her young were left undisturbed, except for weekly bedding changes, until day 21. Tap water and chow were freely available throughout gestation and lactation. On day 21, two randomly chosen pups were housed with adults of the same strain, and two pups were housed with adults of the opposite strain. If possible, the sex of the pups and the adults was the same (*10*). Each strain, therefore, had a litter control (C) and an experimental (E) group. A total of 23 C57BL weanlings, 19 males and 4 females, and 24 DBA weanlings, 16 males and 8 females, remained with their adult companions for 7 weeks. Tap water, 10 percent (wt./vol.) alcohol, and chow were always present; fluid position was alternated weekly. Periodic measures of fluid consumption indicated that strain drinking behavior was maintained. The number of adults always exceeded the number of weanlings by at least one.

At 10 weeks of age the subjects were weighed and housed individually with a choice between water and a 10 percent (wt./vol.) alcohol solution provided for 8 days. Water and alcohol consumption were measured daily, and bottle positions were alternated at the same time; the subjects were weighed every other day.

The mean values for the various measures are shown in Table 1 (*11*). Analysis of variance for a complete orthogonal set of contrasts for the rearing and strain variables was carried out. Not unexpectedly, strain differences were highly significant (F = 14.5 to 75.0, d.f. = 1/39, P = 1.33×10^{-10} to .00048) for water, alcohol, and total fluid intake (and, of course, for preference ratio), as well as for alcohol intake expressed as grams of ethanol per kilogram of body weight per day. The body weight difference between the strains was also highly significant, although the absence of female C57BL mice probably acted to increase the significance somewhat. The effects of rearing (C versus E) were only moderately significant (F = 4.0 to 5.5, d.f. = 1/39, P = .024 to .052) for the fluid intakes, since rearing produced opposite effects in each strain.

Of the greatest interest, and import, was the highly significant (F = 13.5 to 15.9, d.f. = 1/39, P = .00028 to .00072) strain by treatment interaction for preference ratio and alcohol intake; neither body weight nor total fluid intake was affected significantly (P = .057 to .23).

The results demonstrate that strain-typical alcohol "preference" can be altered by a change in social rearing conditions. "Nondrinker" DBA mice housed with C57BL mice drank more alcohol than their controls, while "drinker" C57BL mice housed with DBA mice drank less alcohol than their controls.

The mechanisms involved in the observed modification of fluid preference are not known, but several explanations are possible: Alcohol preference may be altered as a result of strain-specific endocrine changes associated with either housing conditions or aggressive encounters (*7*); however, grouping three, or even five, animals per cage is hardly dense housing, and since both control and experimental groups were identically housed, this explanation seems unlikely. A more plausible explanation is that strain differences in aggressive behavior are accompanied by endocrine alterations in the attacked and, therefore, "stressed" animal. DBA mice are reported to be more aggressive than C57BL mice (*12*). Consequently, if animals drink alcohol to avoid stress (*13*), C57BL mice housed with DBA mice would be expected to consume more alcohol than control C57BL mice. The opposite was, of course, the case; such a reduction in alcohol intake has been reported in stressed C57BL mice, but not in RIII or C3H mice (*7*). Here, alcohol intake increased in the DBA mice: if this increase resulted from complex pituitary-adrenal-hypothalamic changes associated with stress, it would require the stressor to act differentially. We prefer a simpler hypothesis, applicable equally to each strain.

We believe the most likely explanation to be that alcohol selection was altered as a result of "peer" pressure and example, rather than as a result of housing conditions, aggressive encounters, or endocrine

changes. Since colonies of rodents tend to feed and drink at sites which adults of the colony frequent, we suggest that weanling mice "learn" to drink the solution consumed by their adult models. Alcohol intake data in these strains have been interpreted (*14*) to indicate the complex inheritance of the alcohol preference; it seems not unlikely that social pressures might operate to affect the reinforcing value of the taste of alcohol, a factor given first consideration by Fuller and Collins (*14*).

Whether critical periods exist for environmental influences to be effective in mice, as in man, is not known. Whether adult subjects with established drinking behaviors would be influenced as readily, and as substantially, as the young mice in this experiment, or whether the alteration in drinking behavior we have observed persists beyond our test period can only be answered by further experiments. The extrapolation to man of the data of such further experiments may, of course, never be warranted.

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9. The genetically based predisposition for an alcohol solution receives further support from the results of ova transfer experiments carried out in our laborato → *Nature (Lond.)* **255**, 147 (1975)]. The strain-typical preference or aversion for an alcohol solution on the part of C57BL or DBA mice remains substantially unaltered when fertilized ova are exchanged between the strains and the offsprings' self-selection is subsequently tested, emphasizing the major role of heredity and the lesser, though significant, role of maternal and environmental manipulations.
10. Some male adult mice, especially those of the C57BL strain, killed weanlings, leading to the unequal *N* of Table 1. Thus, a total of 13 weanlings of both strains were housed with adult females, rather than with males; no difference in alcohol consumption was apparent between these subjects and those housed with male adults.
11. Since male DBA mice were not significantly different from female mice in their fluid intake, the data of both sexes were pooled and treated together. There were too few ($N = 4$) female C57BL mice to be used alone, and since two of the three control female mice differed from the male control C57BL mice in their alcohol intake, the data from female C57BL mice were not used.
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* C.L.R. was supported in part by a predoctoral fellowship from the Charles and Johanna Busch Memorial Fund of Rutgers University; in partial fulfillment of the requirements for the Ph.D. degree. Present address: Department of Neuroscience, School of Medicine, University of Florida, Gainesville; postdoctoral fellow of the National Institute on Alcohol Abuse and Alcoholism.

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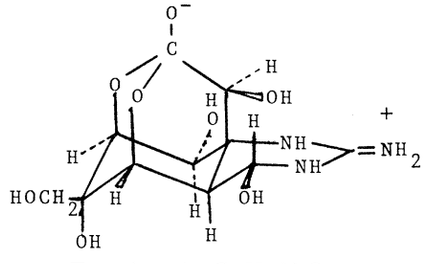
Tetrodotoxin: Occurrence in Atelopid Frogs of Costa Rica

Abstract. *The potent neurotoxin tetrodotoxin, which has previously been found in puffer fish of the order Tetraordontiformes, a goby (*Gobius criniger*), and the California newt (*Taricha torosa*), has now been identified in the skins of frogs of the genus* Atelopus *from Costa Rica.*

Tetrodotoxin (*1*), the structurally unique and pharmacologically potent neurotoxin, was first isolated from Japanese puffer fish (*2*), primarily *Spheroides rubripes*, and subsequently from the California newt, *Taricha torosa* (*3*), and other members of the genus *Taricha* (*4*). The occurrence of tetrodotoxin in two such different animals as the newt and the puffer fish was surprising in view of the fact that this compound does not appear to be a substance readily accessible via the known biogenic pathways from acetate, mevalonate, or the amino acids (*5*). More recently, tetrodotoxin has been isolated from a completely different fish, a goby (*Gobius criniger*) from Taiwan and Amami-Oshima Island (*6*). We now report that we have identified tetrodotoxin in extracts of skin from three populations of frogs of the genus *Atelopus* from Central America (*7*). We have found that aqueous extracts of skin of approximately 12 different species, subspecies or distinct populations of atelopid frogs, so far tested by us are toxic when injected intraperitoneally into mice. In four of these (Table 1) we were able to obtain enough material so that some information concerning the chemical nature of the toxin could be deduced. This was done by techniques previously described (*8*), combined with final purification with gel filtration chromatography (pyridine acetate buffer, at 5°C, on Bio-Gel P-2). Concentrates with an activity in the order of 4000 to 6000 mouse units per milligram (*9*) were obtained. The toxins were found in the skin of both males and females, but not in the viscera, muscle, or bone. In one group of male frogs it was found that the toxin was uniformly distributed in the skin dorsally, ventrally, and along the limbs. Solid tetrodotoxin ultimately separated from highly purified aqueous concentrates of the skin of *A. varius ambulatorius* (Table 1). It was identified by use of proton nuclear magnetic resonance (NMR) spectroscopy [Varian XL-100 NMR instrument in Fourier transform mode (*10*)] by direct comparison with a genuine sample. Tetrodotoxin has a unique NMR spectrum with a slightly broadened upfield doublet coupled with a sharp downfield doublet (D_2O plus CD_3COOD as solvent; δ 2.76, 5.91 ppm; J = 9.5 hz, 1H). Both signals appear in a region unencumbered by other signals and are readily recognized. Furthermore, the mass spectra of tetrodotoxin from these frogs and from puffer fish are identical (*11*). In addition, tetrodotoxin was the major and perhaps only toxic constituent in the concentrates from skin of *A. varius varius* (Table 1).



Tetrodotoxin, $C_{11}H_{17}N_3O_8$

From a population of frogs identified by Savage (*12*) as *A. chiriquiensis* (Table 1), we obtained a skin extract that proved to be a mixture of approximately 30 percent tetrodotoxin and a second major component which we have designated chiriquitoxin after the species in which it is found. After separation from tetrodotoxin by liquid-liquid chromatography (250 pounds per square inch, Bio-Gel P-2, pyridine-acetate buffer) it is clearly distinguishable from it by its NMR spectrum. The chiriquitoxin spectrum bears a certain resemblance to that of tetrodotoxin, especially with respect to the coupled upfield-downfield doublets.

We have also reexamined the previously obtained skin extracts from the Panamanian frog *A. zeteki* (*8, 9*) (Table 1) from which we had isolated a substance that had been designated atelopidtoxin and is now called zetekitoxin. We have been unable to detect either tetrodotoxin or chiriquitoxin in the *A. zeteki* extracts. We are confident that we could easily detect,