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The Effect of Ethanol on Temperature Selection in the Goldfish, *Carassius auratus*

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O'CONNOR, C. S., L. I. CRAWSHAW, R. C. BEDICHEK AND J. C. CRABBE. *The effect of ethanol on temperature selection in the goldfish,* Carassius auratus. PHARMACOL BIOCHEM BEHAV **29**(2) 243–248, 1988.—The effect of ethanol on behavioral thermoregulation in the goldfish, *Carassius auratus*, was studied by adding ethanol to a horizontal aquatic temperature gradient which allowed each fish to select its preferred temperature within a range of about 9°C to 33°C. Alternating exposure to 1.0% (v/v) ethanol and water showed that fish (10 to 15 g) responded to ethanol by selecting lower temperatures. Onset and disappearance of the effect occurred within 10 min of exposure to or removal from ethanol. Fish exposed to 1.0% ethanol for 3 hr did not show acute tolerance. When fish were exposed to increasing concentrations of ethanol from 0.0% to 1.7%, the lowest concentration to elicit a response was 0.5% ethanol. The magnitude of the response plateaued at 0.7% ethanol. At this concentration and above, selected temperatures remained about 2°C below temperatures selected by controls. Because thermoregulatory responses of fish are behavioral and relatively easy to observe and quantify, goldfish offer a useful model for the study of ethanol effects on central nervous system control of thermoregulation. Ethanol produces a prompt, stable, and reproducible depression of selected temperature by lowering the thermoregulatory set point in the goldfish.

Ethanol Behavioral thermoregulation Goldfish *Carassius auratus* Temperature gradient
Ethanol tolerance Thermoregulation Ethanol hypothermia Hypothermia Thermoregulatory set point

NUMEROUS studies using a variety of animals have demonstrated that ethanol affects body temperature [7]. It has been postulated that ethanol alters the body temperature of endotherms by changing the regulated temperature [8] or by disrupting the thermoregulatory centers [9]. Ethanol could also affect body temperature by influencing peripheral effectors. At present there is disagreement about the mechanism whereby ethanol affects the thermoregulatory system [7].

Endotherms utilize a variety of autonomic effectors, such as shivering, sweating, piloerection and vasodilation, as well as behavior, to control body temperature. Ectotherms (fish, amphibians and reptiles) by and large lack effectors other than behavior. Ectotherms are useful in the study of thermoregulatory mechanisms precisely because of this lack; behavioral changes produced by a change in the central nervous system regulator of body temperature result in body temperature adjustments which are not modified by the influence of autonomic effectors. Although ectotherms do not have the autonomic effectors that endotherms possess, the neuronal mechanisms that control thermoregulatory behavior are similar throughout the vertebrate class. The anterior brainstem and peripheral thermosensors are both important in the regulation of body temperature in ectotherms [2, 6, 10], as they are in endotherms.

Fish are useful ectotherms in the study of ethanol because dosing (adding ethanol to the water) is convenient and not stressful. Goldfish in particular tolerate ethanol very well, and can be maintained for at least 5 days in 0.8% ethanol [12]. Goldfish have been used as models for studying development and loss of tolerance to ethanol intoxication [5], as well as in studies of ethanol effects on various aspects of fish behavior, such as excitability [4]. We report here the results of a series of experiments designed to evaluate the effect of

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ethanol on temperature regulation in the goldfish.

METHOD

Animals and Environment

One hundred goldfish *(Carassius auratus)* (Ozark Fisheries, Stoutland, MO), ten to fifteen grams in size, were housed, fifty to a tank, in 360 liter opaque plastic outdoor tanks exposed to the natural photoperiod. Salts (NaCl and trace KI) were added to increase water hardness to 2.8 mg/l. Fish were fed Tetra bulk tropical fish flakes every other day, but never within 30 hr of an experiment. Experimental fish that were reused were always allowed a minimum of two weeks in their home tanks between experiments.

Experimental Apparatus

Temperature selection of the fish was quantified in an aquatic temperature selection apparatus filled with water similar to that the fish were housed in. The apparatus was composed of nine adjacent, separate temperature gradients (lanes), each about 240×20 cm with a water depth of 9 cm. Submerged in both ends of every lane were heat exchangers coupled by circulating pumps to a hot or cold source. Wire mesh barriers kept the fish away from the heat exchangers. Nine pairs of thin vertical baffles, spaced equidistantly along each lane and extending about 6 cm into the lane from each side, partially divided each lane into ten chambers of equal length. The baffles maintained a discrete temperature difference between chambers, yet allowed the fish easy passage from one chamber to the next through the gap. Fish swam freely throughout their lanes. Each chamber was vigorously aerated to prevent thermal stratification. Continuous monitoring showed that the temperature within the apparatus was stable within ±0.5°C for the duration of an experiment. The complete temperature profile for each lane of the apparatus was established by measuring the temperature at the geometric center of each chamber at the end of every experiment.

All lanes were continuously and simultaneously monitored by a Panasonic WV-1854 high resolution television camera equipped with a wide angle lens. The camera signal was fed to a Panasonic NV-8050 time lapse video cassette recorder coupled to a Panasonic WV-5410 video monitor, and also to an IBM PC XT equipped with a frame grabber. Application-specific software translated fish positions into a digital color display on an IBM color monitor and an analog display on a neighboring Zenith monitor. A continuous printed trace of all fish positions, updated every five seconds, was produced by an Epson LQ-1500 dot matrix printer. A binary file containing the information describing all fish positions throughout the course of an experiment was stored on an IBM 10 Mb hard disk. The files of fish position as a function of time were readily accessible through an editing program which automatically translated fish positions into corresponding temperature values for data analysis.

Experimental Procedure

Before being used in an experiment, fish were conditioned to the gradient for about 4 hr. To begin an experiment, control and experimental fish were released, one per lane, in the temperature selection apparatus. Their behavior was observed and recorded for 45 min. Each fish was then netted and placed in a covered mesh restrainer inserted into its lane, at approximately the position of its preferred temperature. Next, the desired volume of 95% ethanol was dispensed using a VWR Dispenser 511. The total dose for each lane was divided into eleven equal portions. Each chamber received one portion. The one remaining portion was divided evenly between the two ends of each lane where the heat exchangers were located. A plastic whisk was used to mix water and ethanol in each chamber individually. After all lanes had been dosed, the restrainers were lifted gently upwards to free the fish in the same order in which they had been caged.

During the experiments, two types of non-thermoregulatory fish behavior were observed that caused data to be excluded from further analysis. The first behavior occurred when fish became too intoxicated to swim, and lost their righting reflex. The second type occurred when a fish swam to water 15°C or colder and remained motionless for 8 min or longer. This behavior was rare.

Experiment 1

To establish the effect of ethanol on temperature selection, six fish were placed in a lane containing 1% ethanol for approximately 30 min, removed to a different lane containing no ethanol for 30 min, then returned to the ethanol lane for a final 30 min.

To evaluate the stability of ethanol concentrations in the temperature selection apparatus, water samples were taken periodically from the warm, intermediate, and cold portions of the lanes at various times after ethanol had been added, and were analyzed by gas chromatography [1] to determine how much ethanol was lost due to evaporation during the experiments.

Experiment 2

To ascertain whether goldfish developed acute tolerance to the effect of ethanol on their thermoregulatory behavior, we observed the temperature selection behavior of goldfish in 1% ethanol for nearly 3 hr. Separate groups of fish were exposed to ethanol (n=5, experimental group) or water (n=4, control group) concurrently.

Experiment 3

To quantify the dose of ethanol necessary to affect the temperature selected by goldfish, the behavior of fish dosed with increasing concentrations of ethanol was examined simultaneously with that of control fish in adjacent lanes. Both control and experimental fish were placed in lanes without ethanol. Because of the large number of trials needed to establish the threshold concentration, different groups of fish were dosed in two overlapping protocols. For the lower concentrations (0.1% to 1.0%), ethanol was added in 0.1% increments to the lanes containing the experimental fish. For the higher concentrations (0.8% to 1.7%), the initial addition of ethanol to the lanes raised the concentration to 0.8%, after which ethanol was added in 0.1% increments. Data were collected from the fish for 30 min at each concentration.

Goldfish exhibit a diurnal rhythm of temperature selection [11]. We find that temperatures selected by undisturbed fish in our apparatus sometimes show variations of up to 2°C over several hours. Therefore control fish from the same population were always run concurrently with experimental fish, in adjacent lanes of the temperature selection appara-

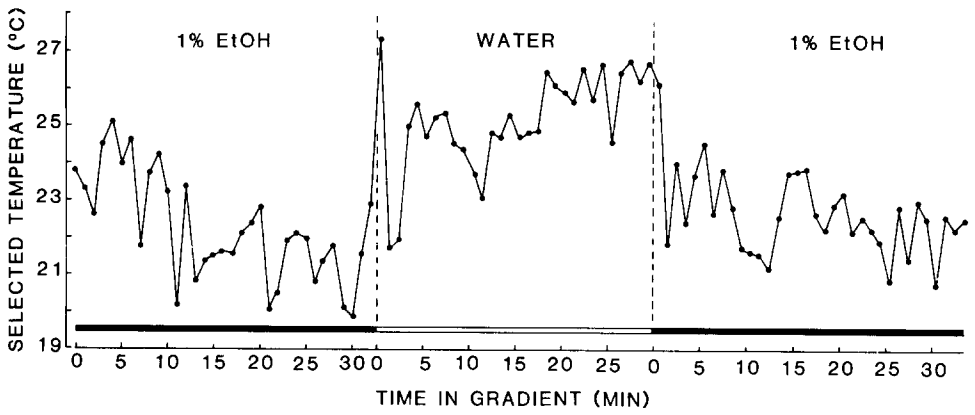

FIG. 1. Effect of alternating exposure to 1% ethanol (black bars) and water (clear bar) on the temperature selected by goldfish.

tus. To take into account the changing baseline of temperature selection, the mean temperature selected by the fish in ethanol during Experiment 3 is plotted as a difference from the mean temperature selected by the matching control fish.

Statistics

ANOVA was used to determine the effect of ethanol in Experiment 1 [14]. Differences between means were examined using the Newman-Keuls test. All measures of variability refer to standard error of the mean.

ANOVA with repeated measures was used to examine the effect of ethanol over time in Experiment 2. These fish were markedly individual in their selection of preferred temperature; background data collected before any of the fish were exposed to ethanol showed that their individual mean preference varied by as much as 5°C, though there was no difference overall in the mean temperature selected by the dosed group and the control group. Therefore, to examine the change in temperature selection behavior induced by ethanol, we chose to analyze the difference between background temperature selection and temperature selected after ethanol was added to the gradient. For ANOVA, 30 min of pre-dose background data for each fish was normalized to zero, and at 15 min intervals the change from that baseline was calculated for each fish. The values obtained were used in the ANOVA protocol.

The experimental protocol used in Experiment 3 precluded strict application of ANOVA. Therefore, we decided to test only selected data points for difference from zero using a *t*-test.

RESULTS

Experiment 1

An appropriate concentration of ethanol caused dosed goldfish to select lower temperatures than did similar fish in water. Figure 1 illustrates the basic effect, and the time course of its onset and disappearance. The first point on the curve represents the average temperature selected by the six fish used in this experiment before ethanol was added to the water. Each subsequent point represents the mean selected temperature for all fish for one minute. Standard error bars were omitted for clarity. The range of one standard error was 0.4°C to 2.32°C, with a mean of 1.16°C. The points located over the black bars represent data collected while the fish were immersed in 1% ethanol. The points located over the clear bar represent temperature selection by the same fish in water.

TABLE 1

CHANGE IN ETHANOL CONCENTRATION OVER TIME, MEASURED AT THREE LOCATIONS IN A REPRESENTATIVE LANE OF THE TEMPERATURE GRADIENT

Min. After Addition of EtOH	Percent EtOH at Location in Gradient		
	Cold	Med	Hot
10	0.87	0.87	0.94
20	0.89	0.89	0.87
30	0.89	0.88	0.88
40	0.89	—	0.87
50	0.89	0.88	0.87
60	0.90	0.88	0.86
70	0.87	0.88	0.86
120	0.90	0.86	0.85
165	0.86	0.85	0.80
265	0.82	0.81	—
380	0.79	0.78	0.78

Figure 1 clearly illustrates the prompt, reversible effect of ethanol on the temperature selection of goldfish. The effect of ethanol, though rapid, is not instantaneous. For statistical analysis, the first 15 min in ethanol or in water was discarded and the temperatures selected by fish for the second half of each bout in ethanol or in water were compared. A Newman-Keuls multiple comparison of the ordered means showed that, at $\alpha=0.01$, the means for the two bouts in ethanol were not different, but both differed significantly from the mean temperature selected by the same fish in water.

Table 1 shows the change of ethanol concentration in one lane of the gradient over time. For a period of more than 6 hr, the mean concentration changed slightly more than 0.1%, with a much more modest change occurring over the length of time covered by our experiments. The concentration of

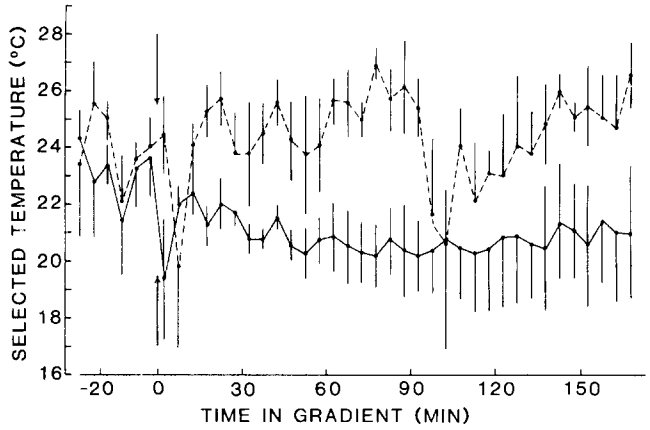

FIG. 2. Temperatures selected by goldfish before exposure to ethanol, and for nearly 3 hr in 1% ethanol (solid line) or water (dashed line).

ethanol, once equilibration had occurred, remained fairly constant along the length of the gradient. Markedly lower concentrations did not occur at the hot end (mixing may have masked losses). Temperatures at the positions sampled were 9.5°C, 22°C, and 31°C.

Experiment 2

Figure 2 shows the temperature selected by goldfish in 1% ethanol for nearly 3 hr. ANOVA showed that exposure to ethanol resulted in a significant depression of temperature selected by dosed fish, when they were compared to controls in water, $F(1,8)=11.313$, $p=0.012$. There was no significant effect of time on temperature selection, $F(10,70)=1.009$, $p=0.442$; the depression of selected temperature caused by exposure to this concentration of ethanol remained relatively constant throughout the course of this experiment.

Experiment 3

Figure 3 shows the temperature selection of goldfish in response to increasing concentrations of ethanol. Fish in water and in the lowest concentrations of ethanol selected similar temperatures. Stepwise addition of ethanol to the water revealed a narrow region of ethanol concentration in which a hypothermic effect was elicited. Using our protocol, the concentration span from beginning to full expression of the effect was about 0.5% to 0.7% ethanol, after which the depression of selected temperature remained approximately 2°C below temperatures selected by control fish until the dosed fish lost their ability to swim upright at about 1.7% ethanol. Application of t-tests at selected concentrations revealed the development of the effect. At 0.4% ($t=-0.57$, $p>0.25$) and 0.5% ($t=-1.11$, $p>0.10$) there was no significant difference between the temperatures selected by control and dosed fish. However at 0.6% ($t=-1.66$, $p<0.10$) and 0.7% ($t=2.32$, $p<0.025$) the effect of ethanol on selected temperature was significant, and remained so at the two other dose levels tested, 1.0% ($t=-3.21$, $p<0.01$) and 1.7% ($t=-2.48$, $p<0.05$).

Raising the concentration of ethanol from 0% to 0.8% in a single step resulted in immediate, full expression of the hypothermic effect; the mean temperature selected by goldfish after the large increase was the same as the temperature selected at 0.8% when the concentration had been gradually raised to that level by stepwise additions of 0.1% ethanol. A t-test at 0.8% ($t=-0.15$, $p>0.25$), 0.9% ($t=0.97$, $p>0.10$) and 1.0% ($t=0.97$, $p>0.10$) showed no difference in temperatures selected by fish undergoing the two different protocols. Therefore, temperature selection values were combined for the three overlapping concentrations, to produce the single curve shown in Fig. 3.

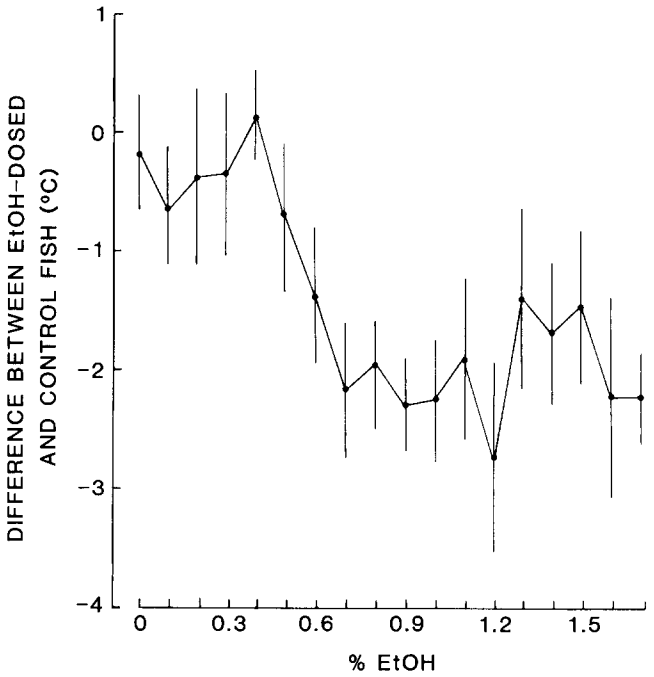

FIG. 3. Temperature selected by goldfish in increasing concentrations of ethanol, plotted as degrees of difference from temperature selected by matching control fish in water.

DISCUSSION

The effect of ethanol on the thermoregulatory behavior of rats was studied [8] by administering 1.5 g EtOH/kg IP to naive rats, and measuring the time it took the animals to escape from a radiant heat source while body temperature was falling. Core temperature was measured with a rectal probe before injection and immediately after escape. Dosed animals showed both significantly decreased escape times and lowered body temperatures at the time of escape, compared to control animals receiving saline. Higher dose levels produced ataxia, making behavioral testing impossible. These rats avoided heat despite their lowered core temperature, indicating a downward adjustment of the thermoregulatory set point by this dose of ethanol.

In another study of the effect of ethanol on body temperature in the rat [9], thermistor probes were fixed in the colon of each animal, avoiding an acute hyperthermic stress response to insertion of the rectal probe. Animals received either saline or ethanol, 2.0 or 4.0 g/kg, by intragastric gavage. Particularly at the higher dose, animals became thermolabile and could be forced into hypothermia or hyperthermia by exposing them to cold (8°C) or warm (36°C) ambient temperatures. Comparison of 4.0 g/kg ethanol and 25 mg/kg sodium pentobarbital revealed an identical effect on

body temperature and thermolability in these rats. It was concluded that ethanol acts like an anesthetic to abolish thermoregulation.

The differing conclusions reached in these two studies indicate the incomplete understanding that exists concerning the precise effects of ethanol on thermoregulatory function. It is possible that, in both studies, ethanol caused an interruption of control over physiological effectors before it rendered animals unable to behaviorally thermoregulate. If animals are prevented from using behavior to control their thermal environment, a consequent failure to thermoregulate may reflect loss of control over autonomic effectors and not an altered thermoregulatory set point. The question of whether the set point is reset or disrupted can perhaps be answered more easily with a fish. These vertebrate ectotherms can regulate their body temperature quite accurately by behavioral means [2]. Vascular, respiratory, and metabolic changes, however, have no effect on body temperature, thus simplifying the interpretation of experimental results. In the current study, an appropriate dose of ethanol caused fish to select cooler temperatures than did similar fish in water. We attribute this change in selected temperature to a decrease in the thermoregulatory set point caused by ethanol.

If the thermoregulatory system were disrupted by ethanol, rather than adjusted downward, we would expect decreased precision of temperature selection by the dosed fish. We found, however, that when fish were first exposed to ethanol they regulated with more precision than did control fish. This may be due to a sedative effect of the drug. When fish were immersed in a constant level of 1% ethanol, after about an hour their activity level began to increase, and large standard errors reflect a high activity level during the third hour in ethanol. Increased activity did not result in a change in mean selected temperature, however. Similarly treated control fish maintained a fairly constant level of activity throughout the experiment. The effect of prolonged submersion in 0.8% ethanol on the reactivity of goldfish to an aversive stimulus has been investigated [4]. The stimulus chosen was a high intensity light. Reactivity was defined as the distance a fish swam to escape the light. For the first 6 hr in ethanol, dosed fish were significantly less reactive to the light than were control fish in water. For the next 7 hr, fish in ethanol became significantly more reactive to the stimulus than were controls. The observed effect on behavior, initial depression of activity followed by increased activity, is similar to the pattern observed in our experiment. The time course is different, for reasons that are not apparent.

Acute tolerance has been defined as ". . . a change in the sensitivity of the CNS to ethanol occurring during the time that a single dose of ethanol is present in the system" [13]. In the context of Experiment 2, where fish were maintained at a stable ethanol concentration, acute tolerance would mainifest itself as an increase in the temperature preferred by fish in ethanol, after a period of depressed temperature selection. We did not observe development of acute tolerance at 1% ethanol. In addition, in Experiment 3, the mean temperature selected by goldfish after the ethanol concentration was raised from 0% to 0.8% in a single step was the same as the mean temperature selected when the concentration was raised from 0% to 0.8% in 0.1% increments over a period of 4 hr. This also indicates that the development of acute tolerance was not of major importance in these experiments. Goldfish do develop chronic tolerance to the loss of their righting reflex (overturn) in ethanol [5]. After goldfish were exposed to 0.8% (w/v) ethanol for 3 hr to 48 hr, they showed a significant increase over controls in brain concentration of ethanol at overturn when placed in 3.1% ethanol. Likewise it has been found [4] that, after fish were immersed in 0.8% ethanol for 30 hr, their reaction to an aversive stimulus was similar to that of control fish. This was interpreted as tolerance. We expect that longer exposure to ethanol will reveal tolerance to the hypothermic effect of the drug.

Dose-dependent hypothermia has been observed in mice. Mouse rectal temperatures were lowered 1.5°C to 4.0°C after doses of 1.9, 3.8, and 5.7 g ethanol/kg mouse at 24.5–25°C ambient temperature [3]. In contrast, outside of the narrow band where the response is elicited, the degree of hypothermia induced by ethanol does not appear to be dose-dependent in goldfish. However, at the highest doses of ethanol, goldfish do show increasing activity, which culminates in overturn as the fish is incapacitated by the drug. In a mammal, gradually increasing derangement of the thermoregulatory system may result in an animal whose body temperature gradually approaches ambient temperature as its physiological effector mechanisms are rendered ineffective, its set point is adjusted downwards and finally disrupted entirely, and it loses the ability to behaviorally thermoregulate. In contrast, the body temperature of a small fish is always essentially at ambient water temperature. In the fish, a similar progressive loss of control over body temperature may be evidenced by increasing activity, and the incapacitated fish floating on its side may be the equivalent of an incapacitated rat whose colonic temperature tracks ambient temperature. Ethanol may thus exert a multiphasic effect on thermoregulation, initially disrupting autonomic effectors and altering the set point, ultimately causing complete derangement of thermoregulatory capacity.

Goldfish seem to offer a useful model system for the study of ethanol effects on thermoregulation. Goldfish can utilize only behavioral responses, which are relatively easy to monitor, to change their body temperature. They tolerate ethanol very well, and respond predictably to it. The effect of ethanol is easily elicited, and onset and disappearance of the effect is rapid in these animals. This series of experiments shows that an appropriate dose of ethanol produces a prompt and reversible hypothermia in goldfish.

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