Tongue Cooling during Drinking: A Regulator of Water Intake in Rats

Abstract. *Rats that had been deprived of water for 23½ hours were presented with water for 30 minutes per day. As the temperature of the water was increased from 12° to 36°C (body temperature), the total water intake for 30 minutes increased 71 percent. Tongue cooling during drinking appears to suppress drinking in anticipation of extracellular hydration.*

When a thirsty rat is given water, drinking subsides before extracellular fluid volume and osmolarity return to levels obtained when water is freely available (*1*). This inhibition of water intake before extracellular rehydration suggests a form of phase advance feedback in which a peripheral signal that anticipates extracellular rehydration is utilized to prevent excessive water intake. Many animals, including humans and rats, lack water-sensitive taste receptors (*2*). The absence of specific water-sensitive taste receptors implies that other receptors mediate the peripheral detection of water and the peripheral metering of water intake. The tongue contains receptors that are specifically sensitive to changes in temperature (*3*). We have demonstrated that tongue cooling during drinking inhibits drinking in anticipation of extracellular hydration.

Eight female albino rats were deprived of water for 23½ hours. Food was available freely in the home cages but not in the testing cages. Water was available 30 minutes per day and was delivered from insulated inverted bottles. Oversize glass sipper tubes were used to facilitate heat conduction between bottle and sipper tube. Ice in the bottle maintained the temperature of cold water, while an external electric heater maintained the warm temperatures. All temperatures reported were measured with a thermistor at the aperture of the sipper tube. After stabilization of daily 30-minute intake of water with room-temperature tap water, six temperatures of tap water were presented, one per day in counterbalanced order—12°, 24° (room temperature), 30°, 36° (body temperature), 42°, and 48°C (approximate pain threshold) (all ± 1°C).

Mean intakes of water for a 30-minute period for each temperature, pooled across subjects, are presented in Fig. 1. A clear relation between water consumption and water temperature is evident. When compared to the intake of water at 24°C (room temperature), the rats drank significantly less 12°C water, while consuming significantly more water at 30°, 36° (body temperature), and 42°C ($P < .05$, Mann-Whitney U test). Peak mean water intake was obtained at 36°C. At the still higher temperature of 48°C, which is reported to be the pain threshold (*4*), consumption fell significantly below peak levels ($P < .05$). The overall shape of the curve was the same for all eight rats. However, the temperature at which maximum water intake occurred varied between animals, peak water intake ranging from 30° to 42°C.

With tap water, the possibility of an interaction between the taste of the tap water, due to mineral content, and water temperature arose. There are data suggesting that maximum taste sensitivity for solutes is at body temperature, perhaps accounting for our peak intake of 36°C tap water (*5*). To test for this possibility, additional data were collected by presenting distilled water to all eight subjects. The broken line in Fig. 1 shows mean water intake of distilled water at temperatures of 12°, 24°, and 36°C. No differences between intake of tap water and distilled water were found at these temperatures. Thus, the relation between water temperature and water intake is not mediated by the taste of dissolved minerals.

Our data suggest that tongue cooling contributes to water satiation. Water below body temperature cools the tongue and signals satiation. Air licking, as a form of tongue cooling (*6*), has also been shown to temporarily inhibit water intake in rats (*7*). Water at body temperature could cool the tongue, if at all, only by evaporation. The intake of body-temperature water is therefore relatively devoid of tongue-cooling stimuli, and more sustained water intake occurs. The suppression of water consumption that does occur when the water is at body temperature (36°C) would appear to be due to factors other than tongue cooling. These other factors may include oropharyngeal pressure receptors, feedback from licking or swallowing, gastric factors, extracellular fluid volume, or osmotic changes. The decease in water intake that occurs when the water is above body temperature appears to be due to the stimulation of heat or pain receptors, or both.

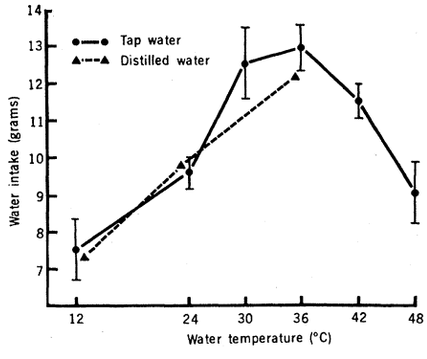

Fig. 1. Mean water intake (± standard error of the mean) as a function of water temperature ($n = 8$). Water was available for 30 minutes per day. Data for tap water and distilled water are presented.

Mendelson and Chillag (*6*) have reported that thirsty rats lick cold metal and have suggested that tongue cooling serves two functions: water detection and an immediate reward for licking. The relative rewarding effects of drinking water at different temperatures is not known, but from Mendelson and Chillag's finding one might expect that drinking cool water is more rewarding than drinking body-temperature water. Why, then, did the rats in the present study drink less of the cool water? Perhaps the greater satiating capacity of cool water more than compensates for its greater reward value in determining levels of water intake.

A precautionary implication of these data is that they can be used in studies in which water is used as a reinforcer. Typically, in such studies, water-deprived animals are given free access to water for some measured period of time each day. If this water is taken directly from the tap, water temperatures and therefore water intake will vary, daily and seasonally, thus producing undesirable fluctuations in deprivation state and performance.

Evolution has, it appears, favored the development of temperature-based water regulation. The advantage of such a mechanism to the wild rat, and perhaps to primordial man as well, is that water needs tend to correlate with the temperature of the environment, that is, they tend to be greater in warm weather than in cold weather. In addition, intemperate intake of cold water in cold weather might seriously depress body temperature.

In cold weather water at cold ambient temperature cools the tongue a

great deal, thereby suppressing water intake. In warm weather water at warm ambient temperatures cools the tongue very little. As a result, tongue cooling during drinking adjusts water intake to meet water needs.

GREGORY KAPATOS
RICHARD M. GOLD
Department of Psychology, State University of New York, Cortland

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Pregnancy Suppression by an Antiserum to the Sperm Specific Lactate Dehydrogenase

Abstract. *An antiserum induced against the isozyme of lactate dehydrogenase (LDH-X) that is unique to spermatozoa reduced significantly the number of pregnancies in mice treated at varying times after they had mated. This effect of the antiserum occurred both prior to and following implantation. The fecundity of treated animals appeared to be normal in subsequent matings.*

The sperm specific isozyme of lactate dehydrogenase (LDH-X) is the tetrameric product of a gene that is active only during the primary spermatocyte stage of spermatogenesis. A homogeneous crystalline preparation of this isozyme has been obtained from mouse testes and used to induce antibody formation in rabbits (*1*). Purity of the antigen preparation was established by disc-gel electrophoresis, analytical ultracentrifugation, and immunochemical analysis. Thus, we have available a potentially useful means of developing information on the relation between a specific, and, more importantly, a well-defined component of the sperm and the process of reproduction. Of particular interest is the area of immunoreproduction. There have been many attempts to influence fertility and to identify immunological causes of infertility by sensitization of both males and females against sperm and testes extracts (*2*). Results obtained from such studies have been variable and interpretations have been conjectural, in part, certainly, because of the complexity of the antigenic material. In this report we present evidence that a specific antiserum to a chemically defined antigen—that is, LDH-X—can reduce fertility in females.

Purification of LDH-X and induction in rabbits of antibody specific to it have been described (*1*). In our work, the complement in the antiserum was inactivated by heating at 56°C for 30 minutes before injection. For most of the experiments 6- to 8-week-old female mice were used from a random-bred Swiss Webster strain maintained in our laboratory during the past several years. In each experimental trial the same proven fertile males were used to fertilize females receiving antiserum (AS) or control serum (CS). Females were placed in a breeding cage during proestrus, and mating was evidenced by the presence of copulation plugs. Injections were made subcutaneously with 0.1 ml of serum. Each female received one injection per day during days 1 to 4, 4 to 7, 7 to 10, or 10 to 13 after coitus. Pregnancy was determined either by examining the uterus or allowing parturition to occur. All animals were caged under a 12-hour daily artificial light regimen and maintained on a diet of Purina chow with free access to water.

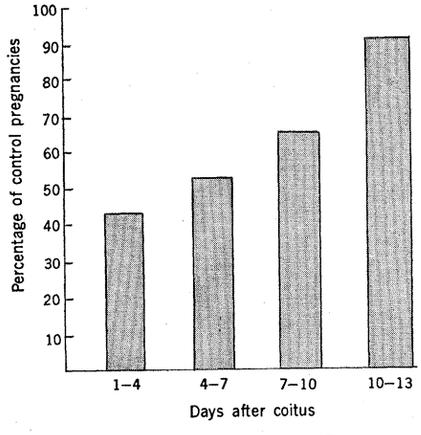

Fig. 1. Effect of antiserum to LDH-X on pregnancy rate in mice. The numbers of animals in each treatment group were as follows: days 1 to 4, 67 CS and 81 AS; days 4 to 7, 72 CS and 72 AS; days 7 to 10, 97 CS and 96 AS; and days 10 to 13, 10 CS and 10 AS.

That postcoital treatment of mice with antiserum depresses the percentage of pregnancies is shown by the data in Fig. 1. The effect of the antiserum appears to be most pronounced when injected on days 1 to 4, and nearly as effective in terminating pregnancy when used on days 4 to 7 after mating. The phenomenon appears to be all or none in a given animal, since AS-treated mice which remained pregnant delivered normal litters, comparable in size to the controls. The antiserum also suppressed pregnancy when injected on days 7 to 10 and apparently was relatively ineffective after day 10 following coitus (Fig. 1).

There are several important controls in the experimental design which are not apparent from the data in Fig. 1 and which should be noted here. Antiserums were obtained from eight rabbits immunized with the same antigen preparation. At least two different antiserum preparations were used for each of the treatment periods during these studies, and control serum was obtained from the same rabbits before their immunization. In all of the test matings, proven fertile males and females were used. There were 67 percent successful pregnancies recorded in the total of 246 control animals. In one treatment group of nine animals (day 1 to 4 after coitus), not included in Table 1, serum absorbed with antigen was injected, and 78 percent of these animals remained pregnant. This result confirms the conclusion that pregnancy suppression was due specifically to the LDH-X antibody.

Injection schedules were established in the experimental protocol to cover the pregnancy stages of oviducal passage of fertilized ova (days 1 to 4 after coitus), implantation (days 4 to 7 after coitus), and postimplantational development. From the results of these treat-