Behavioral thermoregulation in response to local cooling of the rat brain

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SATINOFF, EVELYN. Behavioral thermoregulation in response to local cooling of the rat brain. Am. J. Physiol. 206(6) : 1389-1394. 1964.-Local cooling of the anterior hypothalamic-preoptic area in rats, at environmental temperatures of 5 and 24 C, caused their rectal temperatures to increase as much as 3.1 C, as well as vigorous shivering. When the animals were allowed to press a bar to turn on a heat lamp directly overhead, they pressed more at both ambient temperatures when their brains were cooled than when they were not, although they worked harder in the cold. They shivered continuously during brain cooling at either temperature. The behavioral and physiological temperature regulations appeared to be complementary, since the same temperature levels were reached whether or not heat could be obtained voluntarily. Central cooling produced, in addition to the usual reflex mechanisms of increased body temperature and shivering, the behavioral motivation for heat.

The results of Magoun et al. have since been extended to other animals and to both chronic and unanesthetized preparations; they have been confirmed many times. Hemingway et al. (11) reported that diathermic heating in the anterior hypothalamus of unanesthetized dogs inhibited shivering and peripheral vasodilatation. The work of Magoun and his colleagues was repeated by Beaton et al. (5) and Eliasson and Strom (6) on monkeys and dogs, respectively. Both groups concluded that the thermosensitive area lies mainly between the anterior commissure and the optic chiasm.

Much attention has been focused on the anterior hypothalamic-preoptic region as a central temperature-sensitive device that maintains thermal homeostasis. An extensive series of experiments on chronic cats and monkeys by Ranson and his colleagues (14-16) showed that lesions in this area destroyed the adaptive response to body cooling. Magoun et al. (13) localized the heat-sensitive elements to the region between the optic chiasm and the anterior commissure. Diathermic warming of this area, that resulted in a moderate rise in temperature of the anterior hypothalamus but not of other parts of the brain, evoked in anesthetized cats the heat-loss responses of panting, sweating, and accelerated respiratory rate.

Temperature regulation hypothalamic cooling anterior hypothalamus heat reinforcement

Received for publication 16 October 1963.
1 This research was supported, in part, by National Science Foundation Grant NSF G 24306, and was conducted during the author's tenure as a Public Health Service Predoctoral Fellow.
2 This paper is based on a dissertation submitted to the Psychology Department of the University of Pennsylvania in partial fulfillment of the requirements for the Ph.D. degree.
3 A preliminary report has been published (Federation Proc. 22: 289, 1963).

Local cooling of the anterior hypothalamus also has a profound effect on thermoregulation, producing shivering, increased body temperature, and vasoconstriction at different environmental temperatures. Andersson and his co-workers (1, 3) conducted several important experiments on the effects of cooling the brains of unanesthetized goats. All centrally cooled goats developed a sustained hyperthermia, but without shivering, which lasted up to a week, until cooling was stopped, and then gradually declined. Shivering appeared only in a cold environment or when parts of the body were cooled locally. Peripheral vasoconstriction occurred in all experiments, even in a warm environment, however. Hammel et al. (8) obtained vigorous shivering and peripheral vasoconstriction in dogs kept at a neutral temperature.
environmental temperature by cooling the same hypothalamic sites where heating had previously inhibited shivering in the cold.

All of the above work has investigated physiological responses to various internal and external thermal loads, e.g., hypothalamic cooling or heating; lesions, high or low ambient temperatures. In the past few years several studies have begun to emphasize behavioral responses to the same sorts of thermal stresses (7, 12, 17-19). Weiss and Laties, in a number of experiments, have demonstrated how behavior contributes to temperature regulation. Typically they measured the latency from the time a rat was placed in a cold environment until it began pressing a lever regularly to receive a burst of heat from an infrared lamp, as well as the total amount of heat obtained by the rat. The willingness with which rats will press the bar under different experimental conditions is an indication of their motivation to keep warm.

The present study has two purposes. The first is to extend to the rat the study of the central mechanism subserving thermoregulation by the method of local cooling of the hypothalamus. The second is to discover whether stimulation of central thermal receptors, in addition to arousing physiological reflex mechanisms, also motivates rats to keep warm.

METHODS

Animals. Nine female rats of the Long-Evans strain, weighing between 250-350 g, were used. They were shaved once or twice a week, as needed, and they were maintained at 80-90% preoperative body weight, since both shaving and food deprivation increase the rats' bar-pressing behavior.

Construction of thermode. Each thermode consisted of 5 cm of 22-gauge stainless steel tubing which, after heating, was bent into a tight U shape. The arms of the thermode extending above the skull were bent away from each other slightly to facilitate connection to polyvinyl tubing. The thermode was insulated with tight-fitting polyethylene tubing from the point where it left the brain to within 1 mm of the tip, a distance of approximately 8 mm. A picture of the thermode is shown in Fig. 1.

Method of implantation. A standard dose of 0.40 ml atropine sulfate U.S.P. (concn. 0.4 mg/ml) was administered intraperitoneally to each rat and 10 min later it was anesthetized with Evipal in a dosage of 140 mg/kg. The rat was then placed in a stereotaxic instrument, its scalp incised, and a large hole drilled over the superior sagittal sinus 1 mm anterior to the bregma. Several smaller holes were drilled in other parts of the skull and jewelers' screws were screwed into them. The thermode was implanted 9 mm anterior to the interaural plane, 7.5-8 mm below the surface of the cortex and as close as possible to the superior sagittal sinus, i.e., approximately 0.5 mm lateral to the midline. The thermode was attached to the skull with dental cement, which also connected to the jewelers' screws, thus making a more secure foundation. After the operation, an intra-

muscular injection of 0.4 ml procaine penicillin (300,000 U/ml) was administered, and the rat was allowed to recuperate from the operation for at least 5 days before any training was begun.

Method of cooling. The brain was cooled by pumping cold fluid through silicone tubing into one arm of the thermode and back to a collection reservoir through the other arm. The tube began at a reservoir of 100% ethyl alcohol which was located in a bath of dry ice and acetone (kept at -60 to -70 C), and ran through a peristaltic pump, from which it led to the inflow arm of the thermode. The silicone tubing had an inner diameter of 1/36 in. and was, therefore, much larger than the 22-gauge stainless steel tubing, it was joined by a connector to a small length of 24-gauge polyvinyl chloride tubing which fit tightly over the thermode. The same type of PVC tubing led from the outflow arm of the thermode back to the reservoir. All this tubing was supported by counterweighted wires passing over pulleys; thus, the rats were able to move freely without undue strain on their heads. The temperature of the liquid passing through the thermode was measured three times. Thirty-six-gauge thermocouple wire was inserted in the connection between the PVC tubing and the inflow arm of the thermode and was connected to a Leeds-Northrop potentiometer. The temperature was found to be 18 ± 2 C, depending on the temperature of the room.

Behavioral techniques. Two identical experimental cages,

TABLE 1. Average rectal temperatures of rats whose brains were cooled in ambient temperature of 24 ± 3°C

<table>
<thead>
<tr>
<th>ANIMAL NO. OF OBSERVATIONS</th>
<th>AVERAGE RECTAL TEMPERATURE, °C</th>
<th>MAXIMUM RISE IN SINGLE SESSION, °C</th>
</tr>
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<tbody>
<tr>
<td>WT 1</td>
<td>2</td>
<td>37.3</td>
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<tr>
<td>WT 2</td>
<td>2</td>
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<td>WT 12</td>
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similar to those of Weiss and Laties (19), were used. A Plexiglas cylinder, 1 ft high and 9 in. in diameter, was mounted on a grid formed of Plexiglas rods located 1/4 in. apart. This was the test cage. A 3-in. length of Plexiglas rod, 3/8 in. in diameter, was mounted on a microswitch and served as a lever. It was located 1 in. above the grid floor and extended about 1 1/2 in. into the cage. (At first, metal cages and levers were used, but they rapidly became very cold and the rats would not approach the levers.) A 375-w, General Electric, red bulb, infrared lamp was mounted on top of the cage. This lamp was lighted whenever and for as long as the lever was depressed. Its wattage was controlled by a variable transformer, and generally it was kept at 300 w. Each rat had at least three sessions each of at least 3 hr duration with the apparatus housed in a refrigerator at 5 ± 2 C. The refrigerators had windows in their doors so that the rats could be observed. The same cage was used for each rat in all experiments.

Test of cooling technique. The test occurred at room temperature (24 ± 3 C) in the test cage but without the bar, with the rat attached to the cooling apparatus. Rectal temperature was recorded initially and after 15 min. If both readings were the same, cooling was begun. If the second reading was higher, sometimes the animal was kept in the cage until his temperature returned to normal before starting the cooling process; other times, cooling was begun immediately and the higher temperature was taken as the base temperature for that session. The second reading was never lower than the first. After 1 hr, the cooling was terminated, and 30 min later the animal's rectal temperature was taken and it was returned to its home cage. Rectal temperature was also taken after 30 and 60 min of cooling.

Tests for bar pressing for heat during local cooling. These bar-pressing tests were conducted in the test cage in a room at 24 ± 3 C and in a refrigerator at 5 ± 2 C on alternate sessions. At least 2 days elapsed between sessions. Following 10 min of access to the bar, the brain was cooled for 10 min with the rats having access to the bar. This procedure was repeated three times, yielding three 10-min intervals of no cooling followed by 10 min of brain cooling.

Measurements taken. The rats' behaviors were recorded by means of written protocols. A record of the amount of time that the heat lamp was on was obtained from a running time meter and of the pattern of responding from a cumulative recorder. The apparatus controlling the cumulative recorder was arranged so that the recorder pen moved upward steadily as long as the lamp was on. A visual record was thus obtained in addition to the numerical one furnished by the running time meter. Rectal temperatures were measured before and after each session, and irregularly at 10-min intervals during a session. They were obtained from a Yellow Springs telethermometer, model 43, connected to a Yellow Springs no. 402 rectal probe, inserted 5 cm into the anus. The telethermometer is accurate to 0.5 C.

Histology. After the tests were completed, four of the rats were killed by perfusing saline and 10% formalin through the heart, and their brains were removed, embedded in parlodion, and sectioned coronally at 40 μm. Every fifth section and every section showing signs of the thermode was stained with thionin and mounted on a slide.

RESULTS

One-hour brain cooling at 24 C ambient temperature. All animals in the neutral environment and with no opportunity to bar press for heat showed a marked rise in rectal temperature after 1 hr of brain cooling (Table 1). In three of seven cases at least 75% of the maximum elevation was achieved within 30 min of cooling. Half an hour after the cooling ceased, the rectal temperatures were within a degree of what they had been before the test.

![Figure 2](http://ajplegacy.physiology.org/) Amount of time spent bar pressing for heat at 5 ± 2 C.

![Figure 3](http://ajplegacy.physiology.org/) Comparison of amount of time spent bar pressing for heat before, during, and after brain cooling. Ambient temperature: 5 ± 2 C.
This rise in temperature during cooling and the subsequent drop afterward occurred in all animals on all tests. The maximum increase in temperature in any single session varied from 1.7 to 3.1°C for individual animals. The temperature rises were extremely consistent so that all animals on each test were within 0.3°C of their maximum.

All rats began shivering within 1.5 min after cooling began. Generally they were highly active at first, with slight shivering episodes interpolated between grooming and exploring, but soon they moved near the wall of the cage where they remained huddled in a ball for the rest of the hour. The shivering increased gradually in intensity, then reached a constant level which held throughout most of the session except near the end of the hour when it usually decreased both in intensity and in the frequency of the episodes.

**Heat reinforcement in a cold environment.** The main results of the heat reinforcement study are shown in Fig. 2. t Tests were performed on these data as well as those shown in Figs. 3 and 4, reported later. All the differences were found to be significant at the .005 level with ts of 5-30 with 4 df. On the average, the rats depressed the lever, and so kept the heat lamp on for 81 sec out of 10 min during brain cooling and only 17 sec out of 10 min when there was no cooling. In the refrigerator they shivered continuously during brain cooling, even while they held the lever down and received heat. When the cooling was stopped, the rats stopped shivering. At no time was shivering observed at 5°C after a period of brain cooling, although it was always seen at the start of the session.

After 10 min of brain cooling, temperatures were up several degrees, and after 10 min of no cooling they were reduced, but not to the precooling levels. Figure 3 compares the amount of heat received in the first three successive 10-min periods of each session: before, during, and immediately after cooling. The interesting comparison is between the pre- and postcooling periods. Before cooling, rectal temperatures were at normal levels of from 37 to 38°C. At the end of each cooling period, they had risen to higher temperatures ranging from 39 to 40.5°C. During the postcooling periods the rate of bar pressing was greatly suppressed, and the rectal temperatures had dropped to lower temperatures ranging from 38.3 to 39°C. This sequence of rising and falling temperatures was continued during the three alternations of cooling over the entire test session, as measured for one session for each rat. These data are summarized in Table 2.

**Heat reinforcement in a neutral environment.** In general, when a rat was placed in the test cage at a room temperature of 24°C, it pressed the bar a few times and then ignored it, and either explored the cage or sat quietly. When cooling began it became highly active, started to shiver violently, and after a few minutes went to the bar and held it down. Bar pressing was intermittent throughout this interval. During the next 10 min of no cooling the animal sat quietly or groomed. When brain cooling was resumed the animal again went to the bar and pressed it steadily throughout the cooling interval as shown in Fig. 4. The amount of heat for which the animals worked at room temperature during brain cooling, however, was less than in the refrigerator.

Figure 5 presents the cumulative record of bar pressing for a typical animal during two sessions, one in the cold, the other in the neutral environment. It shows clearly the phenomena just described. The two records are closely parallel, except that the rate in the neutral environment is less than in the cold. In the noncooling periods the rate in the neutral environment is essentially zero. The time spent pressing during these periods resulted from perseverence when the cooling was terminated and from, presumably, random depressions of the bar as the animal moved around the cage.

**Histology.** Four animals’ brains were studied histologically. In each case the implant was in the anterior hypothalamic area. The most anterior extent of the thermode in any animal was in the preoptic region and the most posterior extent in the anterior hypothalamic area. All four animals gave good body-temperature responses during 1 hr of brain cooling at 24°C ambient temperature.

**DISCUSSION**

There are two main findings in the present study, one primarily physiological, the other behavioral. It has been demonstrated that local cooling of the anterior hypothalamus and preoptic area of rats in a neutral environment elicits responses designed to increase heat production. The animal begins to shiver almost immediately after the onset of cooling and shivers more and more vigorously and regularly as cooling progresses. Body temperature increases by as much as 3°C and remains elevated for the duration of the stimulation, up to 1
BEHAVIORAL THERMOREGULATION IN RATS

In a cold environment, the body temperature of a normal animal generally rises only a fraction of a degree in an hour, and shivering develops at a much slower and more sporadic rate. The two response patterns seem to be essentially the same, differing only in magnitude. Presumably this difference occurs because it is easier to raise body temperature in a neutral than in a cold environment, in the sense that less heat is lost in the neutral environment and so less energy is needed to effect a given increase in body temperature. In fact, the increase in body temperature of rats cooled in a cold environment is not any greater than it is when they are cooled in a neutral environment. There seems to be a maximum increase in rectal temperatures which cannot be exceeded by increasing the flow of the coolant beyond a certain rate. Some rats achieve this maximum temperature rise within half an hour.

Such a “temperature ceiling” has been noted consistently. Hardy (9) remarked that an increase of 0.5–1.0 C in rectal temperature was the maximum observed with anterior hypothalamic cooling in dogs regardless of the temperature of the environment. Andersson et al. were able to raise the rectal temperatures of goats only 0.5–1.0 C, either by electrical stimulation of the septal area or by local cooling of the anterior hypothalamic-preoptic region (2, 4). These results were interpreted as indicating the importance of peripheral thermal inputs which limit the degree to which central stimulation has an effect.

At least two other possibilities are tenable, however. One reason that the limit of temperature increase in dogs and goats is at least 2 C lower than that obtained in rats may be that the size of the thermode used in rats was much larger relative to the size of the rat’s brain than the comparable thermode in the dog or goat brain. Thus, in the rat, a larger number of thermal receptors were excited, causing a larger thermoregulatory response.

Since experiments on local cooling have all used unilateral cooling, the observed limit could also reflect, at least in part, an antagonistic role of the noncooled side of the anterior hypothalamus. Because that side is not receiving a cold signal, it may oppose the temperature rise brought about by central stimulation resulting from cooling of the other side. If bilateral brain cooling produced a greater increase in body temperature than has been found so far, we could credit at least part of the apparent temperature ceiling to action of the opposite anterior hypothalamic area.

The rat is an excellent animal for this work because its temperature can be raised above its basal level by as large an increase as 3 C, and therefore it is easier to examine the effects of experimental manipulation. Also, the increase with fixed cooling is approximately constant and can be repeated at will, as can the shivering. In contrast, Hardy (9) reports that a stimulus that had produced excellent responses on one day with a dog would be ineffective another day. In rats a raised temperature is observed whenever the hypothalamus is cooled, and thus the rat’s responses to central stimulation are easier to observe.

The second finding is behavioral: a rat will work to receive a source of external heat when its brain is cooled, even in a neutral environment. Aside from the reflex physiological responses, such as shivering and increased body temperature, rats will make a voluntary response—bar pressing—to increase heat in the environment. The rat’s use of an arbitrary learned act in response to local brain cooling proves that the stimulus arouses the appropriate motivation, the urge to keep warm. Turning on the heat lamp is, in fact, an adaptive response to brain cooling, since it reduces the amount of heat lost to the environment. Nevertheless, the same body temperature is attained whether or not the rat can voluntarily increase the external heat. This suggests that the rat regulates the amount of heat received very carefully, since it could press for more heat during the cooling interval without burning its skin.

The almost complete cessation of pressing when the brain cooling was stopped is interesting. The animals were hyperthermic when cooling ceased. Without the strong stimulus of the cooling, they were actually over-
heated in the cold and so needed to lower their body temperature, rather than obtain more heat. When cooling was started again, their temperatures had dropped, though not to precooling levels. Occasionally, an animal began pressing in earnest toward the end of the noncooling interval. By that time the body temperature had fallen several degrees and so the cold environment was enough to start the rat working again.

At present, there is no way of knowing whether the brain temperature, the body temperature, or some interaction of the two ultimately controls an animal's motivation to work for heat. We do know from this experiment, however, that in response to hypothalamic cooling, rats exhibit not only physiological thermoregulation but also the behavioral urge to keep warm.

The author is indebted to Philip Teitelbaum for his advice and guidance throughout the course of the work.

REFERENCES