best regenerate resulting from these heterologous nerve transplants was a curiously shaped outgrowth (Fig. 3A) consisting of a distal club-shaped structure which emerged from the ankle region and possessed a single digit-like protuberance on its medial surface. This response surpassed that of controls with simple amputations, but did not approach the extent of development achieved by the homologous nerve transplants.

Opossum nerve tissue evoked a positive response in 8 of 30 cases. The best regenerate resulting from homologous transplants of young opossum cerebrum is shown in Fig. 3, B and C. Figure 3B shows the animal at 24 days of age (nerve tissue implanted at 2 days and limb amputated 4 days later); after 18 days of regeneration a recognizable foot-like structure possessing the first indications of the fourth and fifth toes (Fig. 3B) could be seen. Development continued, and 20 days later (38 days regeneration) a heteromorphic foot containing the basal portions of three toes was evident (Fig. 3C). Since hindlimb development at birth (6 days before amputation) had already reached a state where all of the bones were present as cartilagenous models, the replacement of the foot and three toes must be interpreted as regeneration and not embryonic regulation.

The manner in which regeneration proceeds in the opossum hindlimb is very similar to regeneration of the metamorphosing frog hindlimb. As in hindlimb regeneration in the metamorphosing frog, histological landmarks which indicate the original plane of amputation are soon lost. Initially there exists a slight difference in tissue densities in the regenerating opossum limb, but it soon becomes impossible to detect the level of amputation. The histological aspects of regenerative phenomena in these two developing limb systems have been compared (*12*).

Two unique characteristics of the newborn opossum undoubtedly contributed to the development of regenerates: (i) absence of an immune mechanism, which prevented rejection of nerve implants; and (ii) short gestation period—a mere 12.75 days [at birth the opossum is equivalent in development to a 12-day rat embryo or a 2-month human fetus (*13*)]. Another feature of the opossum hindlimb which may participate in its ability to regenerate is the relatively "immature" state of the nerve fibers after they make their appearance in the limb. As late as 3 weeks after birth, nerves of the hindlimb are essentially in an unmyelinated condition (Fig. 4).

My studies demonstrate that young opossum limbs can regenerate when additional nervous tissue is supplied. Results of control experiments indicate that neither the trauma of simple amputation, the trauma of implantation, nor the implantation of other homoplastic tissues (for example, liver or kidney) can evoke the regenerative response which results after implantation of brain tissue. Although the opossum has afforded the opportunity to induce regeneration in young mammals, it should be pointed out that we are no closer to an understanding of the mechanism of nerve action in regeneration than before. However, we are now in a position to compare this mammalian limb regeneration with regeneration in lower vertebrates; as their similarities and differences become apparent, additional insight into the phenomenon of mammalian cellular differentiation should be gained. Hopefully, once these factors are ascertained in young opossum regenerates, the newly acquired knowledge can then be successfully applied to other mammals.

MERLE MIZELL

Department of Biology,
Tulane University,
New Orleans, Louisiana 70118

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Feeding and Core Temperature in Albino Rats: Changes Induced by Preoptic Heating and Cooling

Abstract. *At an ambient temperature of 25°C, selective cooling of the area preoptica medialis to 24° ± 1°C produced a significant decrease in food intake together with hyperthermia. Heating the same area to 43° ± 1°C resulted in the opposite effects. At an ambient temperature of 35°C, heating the area preoptica medialis to 43°C resulted in a decrease in food intake despite concomitant hypothermia.*

There is ample evidence for multiple factors affecting feeding behavior (*1*). "Glucostats," "thermostats," energy flow, psychic factors, stomach distention, and specialized receptors for various catecholamines and other hormones (*2*) all may play a role, but the overall equation for the regulation and control of food intake is yet to be written.

Since ingestion of food adds calories, a hungry homeotherm in a hot environment must choose between ingestion of fuel for metabolism and maintenance of thermal stasis; in most instances, in all mammals observed, the demand for normothermia overrides the "hunger" signal (*3*).

Temperature-sensing receptors that relay information about ambient temperature and internal body or nonbrain "core" temperature to the central nervous system exist in the periphery. There are also the temperature-sensitive neurons in the preoptic area (PO) of the diencephalon (*4*). Which of these, if any, provides the prepotent signal that determines whether the animal will eat more or less? In other words, which piece of information or contribution of bits of information govern the animal's feeding behavior?

We used male Sprague-Dawley albino rats, caged individually. During feeding test periods, each animal was placed in a box (24 by 27 by 29 mm high) with transparent plastic walls, set on a floor of galvanized mesh (*5*). Rats had free access to water in the home cage, and the amount consumed daily between trials was measured. In all feeding experiments, dry Purina chow was used. Each animal was fed for a 2-hour period, at the same time each day, in a lighted room. Data for this report were collected after food intake became stabilized.

A liquid-cooled (or heated) thermode was devised, with an exposed gold-plated silver tip 0.35 mm in diam-

eter, 0.35 mm long, and tapering to a point; the shaft was insulated with glass. With such thermodes, the temperature of the PO could be manipulated within the ranges reported in this paper, without directly changing the temperature of the area lateralis hypothalami or the nucleus ventromedialis hypothalami (*6*). One thermode was implanted stereotaxically in the preoptic area of each of eight rats. Each tip was less than 0.5 mm from the midline. Four were in the area preoptica medialis, just below the rostral end of the crossing of the commissuralis anterior and just above the most rostral portion of the chiasma opticum, designated herein as POM. Four others were placed at the rostral border of the PO, just caudal to the gyrus diagonalis; this area is designated herein as POMR. Seven of the eight locations were verified histologically at the end of the experiments. The locations in the POM group were at A9.5 ± 0.5 mm, L0.3 ± 0.2 mm, and dorsoventrally at the interaural line ± 0.5 mm, per Krieg's atlas (*7*). Those of the POMR group were the same laterally and dorsally, but 0.5 to 1.0 mm farther rostrad. In other animals, thermodes were placed in the area preoptica lateralis and in the area lateralis hypothalami. After recovery from the operation, each animal was fed daily in the box described above.

Core temperature was measured by means of a thermistor rectal probe inserted approximately 6 cm beyond the anus and read intermittently or recorded continuously during feeding sessions.

After the initial training period, food intake for each rat stabilized, at various amounts, from 15 to 22 g of dry food daily. A typical example of variability in a 15-day control period was shown by one animal whose mean food intake was 20.2 ± 1.6 g (standard deviation).

At irregular intervals, the thermode in each animal was either heated to 43° ± 1°C or cooled to 24° ± 1°C and kept at that temperature throughout the feeding period. Control periods consisted of the several days immediately before and immediately after each such manipulation.

We consistently found that an artificially induced increase in POM temperature, not exceeding 44°C, caused a significant drop in core temperature and a concomitant increase in food and water intake. Conversely, a thermode-induced decrease of 10° to 15°C in POM temperature was followed by an increase in core temperature and a concomitant decrease in food and water intake. Of all loci tested, the POM was the most responsive to both hot and cold probes, with respect to changes in core temperature and changes in food intake (Table 1). The feeding response was almost independent of the degree of water intake; significant increases in food intake were observed under the influence of heat applied to the PO even when water was withheld.

To test the relative influences of high ambient temperature (35°C), which would tend to decrease food intake, and a hot probe in the PO, which would tend to increase food intake, we subjected the rats to both high temperatures simultaneously (Table 1). Core temperature was depressed significantly ($P < .05$) but not as much as at an ambient temperature of 25°C, whereas food intake was also depressed, indeed, to the same level as when a high ambient temperature was employed with the PO thermode neutral (no hot- or cold-water perfusion).

The well-known effect of hot and cold stimuli to the PO upon core temperature (*8*) was confirmed in all these trials (Fig. 1). The figures shown in Table 1 represent means in core temperature during each 2-hour period and therefore do not reflect the maximum or minimum reached during the trial session. The maximum core temperature observed during a 2-hour exposure to a cold probe was 40.7°C; the minimum after a 2-hour exposure to a hot probe was 36.6°C. An elevation or depression of about 2°C was typical for all POM animals.

The responses of the POMR group to hot and cold probes were similar to those of the POM group, with three exceptions: (i) core temperature elevation was less (not statistically significant) in response to cold probes, although food intake decreased significantly; (ii) water intake was drastically reduced in response to the cold probe; and (iii) there was greater variability in all dependent variables measured. Nevertheless, food intake was still significantly raised and lowered ($P < .01$) by treatment with both hot and cold probes, respectively. Also, as in the case of the POM rats, when high ambient temperature (35°C) was combined with a hot (43° ± 1°C) probe, food intake dropped significantly from control values.

Experiments with probes in the lateral preoptic area and in the lateral

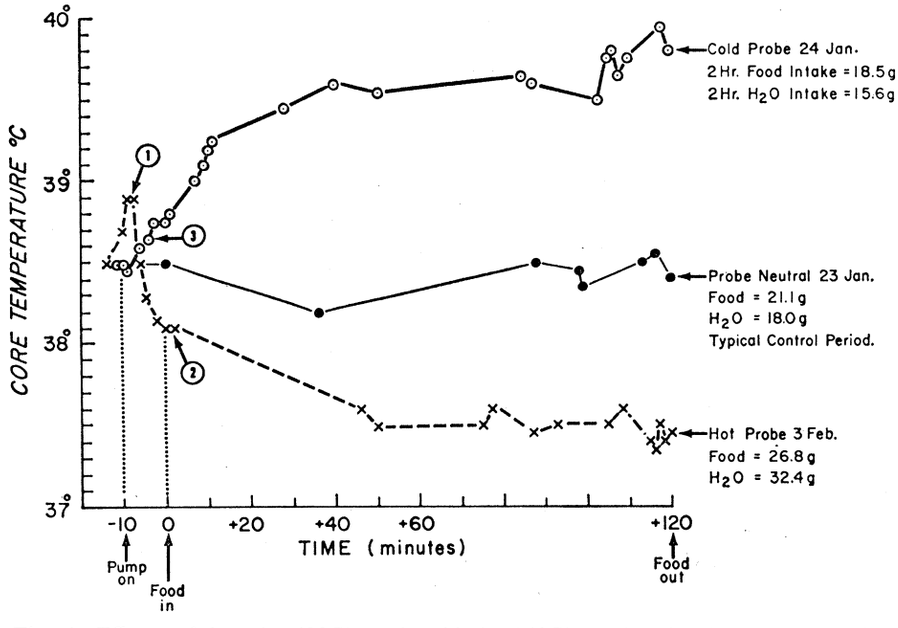

Fig. 1. Effects of hot (~ 43°C) and cold (~ 24°C) probes in the area preoptica medialis upon core temperature, food intake, and water intake. Ambient temperature was 25°C on all 3 days; the feeding period was from 3 to 5 p.m. in a lighted room; these data are from one rat. The deviations from control values shown here are not the extremes obtained for this subject, but represent typical patterns. On 3 February with the probe hot, at $t = -8$ (2 minutes after pump was on) (arrow 1), core temperature was elevated by water at room temperature (25°C) flowing through the thermode. At $t = -7$, the water in the thermode was 43° ± 1°C. At $t = 0$, on the same day (arrow 2) the animal's scrotum was distended, his face was wet, and his ears and paws were red; nevertheless, he started eating avidly when food was presented. During exposure to the cold probe, on 24 January, at $t = -4$ (6 minutes after the pump was on) (arrow 3), the rat was shivering visibly; his ears and paws were pale.

Table 1. Percentages of changes in rats with thermodes in the POM. Data from four rats are combined (for POMR group results, see text). N_2, not shown, is the sum of the number of control days for each experimental manipulation; H_0 refers to null hypothesis; N_1 is the number of experiments. Defecation rate did not change significantly under any of the experimental conditions. All probability values are two-tailed. Control values, set at 100 percent, were based on the means obtained on the days before and after each experimental manipulation. On control days the temperature was 25°C, and the probe temperatures were neutral (equal to resting brain temperatures).

Probe temperature °C (± 1°)	Ambient temperature °C (± 1°)	Mean change from control (%)	Experiments (No.)	Student's t-test	Degrees of freedom ($N_1 + N_2 - 2$)	Probability of H_0
		2-Hour food intake				
24	25	−42.9	7	2.97	38	$< .01$
43	25	+31.0	5	4.21	70	$\ll .001$
Neutral	35	−39.8	8	4.42	90	$\ll .001$
43	35	−58.0	2	5.90	25	$\ll .001$
		2-Hour water intake				
24	25	−54.9	6	3.18	35	$\ll .01$
43	25	+32.7	5	1.25	53	$> .05$*
Neutral	35	−14.6	8	0.79	74	$> .05$†
43	35	−68.0	2	2.52	22	$< .05$†
		Core temperature mean during 2-hour feeding period				
24	25	+1.8	3	5.32	15	$\ll .001$
43	25	−2.2	4	6.02	16	$\ll .001$
Neutral	35	−1.0	2	2.47	22	$> .05$
43	35	−2.0	2	5.03	22	$\ll .001$

* $P(H_0)$ was not significant, despite the large percentage increase, because of large variability. †22-Hour water intake (between feeding sessions) increased more than 200 percent at high ambient temperatures, although prandial drinking was depressed.

hypothalamus produced different responses (*6*), but the most heat-sensitive and cold-sensitive area, with respect to both core temperature responses and consistent changes in feeding behavior, was the POM.

Our experiments indicate that, with respect to the feeding response, a thermal stimulus to the brain is not equivalent to a thermal stimulus to the periphery (*9*). This is contrary to the assumption of Andersson *et al.* (*10*). However, the data from their goat experiments are subject to more than one interpretation (*11*). Although it is possible that the response of the goat to preoptic cooling and warming is different from that of the rat, it remains to be proven. We are not aware of any anatomical or physiological evidence to warrant assuming such a difference.

The observed feeding and core temperature reactions to artificial heating and cooling of the PO do not necessarily imply that PO temperature in the intact "physiological" mammal is invariably correlated either with core temperature or with feeding behavior. Although Abrams and Hammel reported, and Grossman and Rechtshaffen confirmed (*12*), a consistent rise in PO temperature with the onset of feeding, these authors and others (*13*) also have found large variations in hypothalamic temperatures that are not clearly associated with feeding behavior. Similarly, although there exists a quantitative relationship or "gain ratio" between artificially raised rostral hypothalamic temperatures and resulting depressions in body temperature (*14*), it remains to be explained why fluctuations in PO temperature in intact mammals are not always concomitant with oppositely directed core temperatures.

We conclude that, in the male albino rat, the area most sensitive to thermal stimuli in the brain is the POM; when this area is artificially heated, food intake rises and core temperature decreases; conversely, when the POM is cooled by the same means, food intake drops and core temperature increases (*9*). This pattern is specific for the POM, and insofar as tested, unique to it. High ambient temperature overrides the high temperature signal in the POM, with regard to feeding behavior; that is, under conditions of brain heating that would augment food intake at 25°C ambient temperature, under the influence of 35°C ambient temperature, food intake is depressed. Although feeding behavior is clearly related to internal and external temperatures, there is no single temperature, at any point in the body so far tested, that uniquely governs the level of food intake.

N. Herbert Spector*
John R. Brobeck
Charles L. Hamilton
Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia 19104, and *Veterans Administration Hospital, Coatesville, Pennsylvania 19320*

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9. After this report was prepared for publication, a personal communication was received from D. L. Ingram of the Institute of Animal Physiology, Babraham, Cambridge, England, part of which, with Dr. Ingram's permission, we quote: "I have been doing similar work with pigs and find like you that cooling of the preoptic region inhibits food intake and heating increases it. This is true both for *ad lib.* feeding and in an operant situation. In view of the fact that cooling the preoptic region causes a marked increase in operant responses when the reinforcement is heat I don't think it is likely that the effect seen in feeding is a none specific inhibition."
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* Present address: Department of Physiology, Medical College of Virginia, Richmond 23219.

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