FINAL REPORT

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Temperature discrimination, behavioral thermoregulation and related measures in the rat

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SUBMITTED BY

The University of Texas

Southwestern Medical School

at

Dallas, Texas 75235

James M. Lipton, Ph.D.
Principal Investigator

Charles C. Sprague, M. D.
Dean

Robert L. Stubblefield, M.D.
Chairman, Department of Psychiatry

The reactions which allow homeotherms to regulate internal temperature can be divided into two components. One of the components, physiological thermoregulatory responses, is composed of activities which modify the rate at which heat is produced and lost from the body (e.g., shivering, sweating, panting). The other component, behavioral thermoregulatory responses, is made up of the voluntary integrated actions of the entire animal which occur when the organism is confronted with real or potential thermal stress (e.g., escape into a more temperate microclimate). In the normal lives of homeotherms these components interact and allow the animals to maintain body temperature within narrow limits. In terms of the capacity to cancel thermal threat from the environment, however, the two classes of response differ considerably. While physiological responses maintain the fine control of body temperature by counteracting relatively small changes in ambient temperature, behavioral thermoregulatory responses can counteract thermal extremes and reduce the threat to the range in which physiological thermoregulation is effective. From this point of view the behavioral responses are more powerful in offsetting thermal stress than the physiological responses. The recognition of the great power and modifiability of the regulatory behavior has made the study of its determinants of importance to basic thermoregulation and behavior theory.

Although alterations in thermoregulatory motivation produced by changes in the thermal environment are commonplace, the basic physical, neurophysiological and psychological determinants of these shifts are not well understood. Part of this lack of understanding stems from the fact that the capability for quantifying behavioral thermoregulatory motivation in animal preparations, where physiological variables could be freely manipulated, is relatively recent. Unlike physiological thermoregulation in which quantification of responses was made possible by early advances in thermometry, the development of operant conditioning techniques for precise measurement of thermoregulatory behavior is less than 20 years past its initiation. These behavior techniques were used in the present series of experiments to delineate some of the central and peripheral variables important to the control of thermoregulatory motivation. The questions attacked represented a broad front of variables believed to be important to thermoregulation: What is the role of thermosensitivity of the brain in determining behavioral thermoregulation? Is the thermoresponsiveness of the brain limited to the classic preoptic thermoregulatory region? Is the temperature of the periphery a reliable basis for the prediction of behavioral thermoregulatory responses? What do dietary alterations do to thermoregulatory motivation? Does the level of particular neural transmitters in the brain influence physiological and behavioral thermoregulation? What are the limits of temperature discrimination, a variable which is clearly a necessary antecedent to behavioral thermoregulatory responding? What role does the posterior hypothalamus, the "heat conservation center", play in thermoregulatory behavior?

Recent Progress

In the last year we have been interested in the role of central and peripheral factors in the determinants of behavioral thermoregulation. The principal groups of experiments can be categorized as follows:

1. In the search for additional influences upon thermoregulatory control several parts of the brain have been stimulated with heat and cold using rats to see if these stimulations have any influence upon thermoregulatory motivation.

2. Skin temperature of normal animals has been measured at the time a behavioral thermoregulatory response is initiated and terminated to determine whether there are specific skin temperature "limits" which control thermoregulatory behavior. In another experiment the influence of preoptic temperature upon the skin temperature thresholds for responding has been examined.

3. In view of current thinking about neural transmitter bases of thermoregulation, several types of chemicals known to be important to neural transmissions were injected into the brains of rats to see if these treatments altered physiological and behavioral thermoregulation.

4. In another series of experiments interest was focused on the posterior hypothalamus, the classic "heat production" center, to learn how thermoregulation changes when this particular region is damaged or destroyed. These experiments are continuing.

5. Since temperature discrimination is obviously an important component of behavioral thermoregulation, experiments involving this variable are continuing.

Brief summaries of these groups of experiments and implications of the results obtained follow:

1. Thermal stimulation of the brain. In the search for a basis for thermoregulation within the central nervous system information has accumulated which leaves little doubt that the preoptic-anterior hypothalamic region is important. This part of the brain is known to contain thermosensitive cells which, when locally stimulated with heat or cold, evoke both physiological and behavioral thermoregulatory responses. Because of these observations it has been suggested that the preoptic-anterior hypothalamic region acts as a central "thermostat" governing body temperature. In the present series of experiments the possibility that extra-hypothalamic thermosensitivity might contribute to the behavioral component of thermoregulation was examined. Stainless steel thermodes (concentric 18 and 21 gauge tubes, insulated with polyethylene) fitted with small thermistors were stereotaxically implanted in the brains of adult male rats. Some rats received thermodes in the preoptic-anterior hypothalamic region (PO-AH) and others were implanted with thermodes in either the medullar pontine region (M-P), the thalamus, the amygdala, the posterior hypothalamus, the dorsal hippocampus or the midbrain reticular formation. One week after surgery the animals were placed in an upright glass cylinder located in a 23°C. environment and trained to escape exogenous heat (500 W) and obtain a draft of air by depressing a lever. When the proportion of time spent escaping heat had stabilized over a series of

daily sessions thermal stimulations of the brain were begun. The routine in the stimulation sessions consisted of an initial 30 minute period in which the animal responded freely and no formal measurements were made followed by 10 minute prestimulation, stimulation and post-stimulation periods. During the thermal stimulation periods the temperature of the thermodes was controlled at 28-30°C. or 40-43°C. by regulating the temperature of the water circulated through them. The effects of the thermal stimulations were determined by comparing the time spent escaping heat in the three periods.

After the heat-escape data were obtained the investigation was carried a step further by training some of the rats to depress a lever in order to obtain heat (250 W) in a cold environment (0°C). When the proportion of time spent working for heat had stabilized thermal stimulations were carried out according to the procedures used in the heat escape sessions.

Heating the PO-AH region produced an increase, and cooling produced a decrease, in the amount of time spent escaping heat in six of the seven animals tested. In the heat-reinforcement sessions the behavioral response to thermal stimulation of the PO-AH was much the same as in earlier experiments. During local cooling the animals increased the rate of responding for heat while during local heating the proportion of time spent responding for heat decreased.

Heating and cooling the M-P region produced alterations in behavior similar to those seen when the temperature of the PO-AH region was displaced. In the heat escape experiments the rats increased the amount of time spent responding during local heating and decreased it during local cooling, of the M-P region. Analysis of the chronology of responding indicated that the stimulation-induced alterations in the behavior of some animals were consistently present within 1-2 minutes after the stimulations were initiated. To explore the possibility that the behavioral alterations were produced by secondary changes in the PO-AH temperature, a small thermistor was implanted in an animal already bearing a M-P thermode. No consistent changes in the PO-AH temperature resulted from thermal stimulation of the M-P region. Medullar-pontine thermosensitivity was examined further by training some of the rats to work for heat in the cold and testing their motivation during local thermal stimulation. All animals showed repeatable increases in responding when the M-P region was cooled and decreases when it was heated.

Three to twelve heating stimulations and a similar number of cooling stimulations carried out on each animal implanted with a thermode in either the posterior hypothalamus, thalamus, dorsal hippocampus or the mesencephalic reticular substance failed to produce systematic changes in heat escape behavior. Thermal stimulation of the basal amygdalar region produced some positive results similar to those

recorded during heating and cooling of the PO-AH and M-P regions. These results appeared to be confounded by secondary changes in the temperature of the PO-AH region, however.

These experiments demonstrate that behavioral thermoregulation is influenced by the sensitivity of the M-P region to increased and decreased temperature. The findings not only indicate that the medulla is thermosensitive but also that the thermosensitivity is important to at least one component of thermoregulation (behavioral temperature responses). If physiological and behavioral thermoregulation depend upon the same or similar receptor pools, as is generally believed, then the present results suggest that this brain region should be examined in regard to its potential role in the control of panting, sweating, shivering, etc. responses.

The findings provoke a number of questions about the relative role of the preoptic and M-P thermosensitivities in thermoregulatory control. Clearly, it is now of considerable interest to the understanding of thermoregulation to learn whether the medullar thermosensitivity merely contributes information to the preoptic controller, whether the medulla is capable of mediating thermoregulatory responses without the preoptic controller, etc. These questions are presently under study.

2. Peripheral determinants of behavioral thermoregulation. Since deep body temperature is protected against sudden variations by insulation, counteracting physiological mechanisms, etc., it is unlikely that it alone could function as a stimulus in the determination of moment to moment behavioral responding. A more promising candidate for the primary role in this determination is skin temperature change since the skin is exposed more directly to thermal stimulation and it is very sensitive to alterations in intensity and rate of heating. If a stable skin temperature is the requirement what controls behavioral regulation against thermal stimulation one would expect to find that consistent levels are maintained when the rate of heat exchange between the body and the environment are manipulated.

To clarify the role of peripheral factors controlling thermoregulatory behavior two experiments were conducted using rats trained to escape heat by holding down a lever. In both experiments the time spent responding was found to increase in a monotonic fashion as thermal intensity (heat exchange variable) was increased. Determination of the behavioral response was explored in more detail by recording dorsal skin temperature at the moment when a response was begun and when it was terminated. In a neutral environment (23°C.) a relatively stable level of skin temperature was maintained in the face of intensity alterations as a result of the changes in behavior. A qualitatively similar result was seen when the animals were tested in a hot environment (32°C). These findings are consistent with an explanation of the determination of thermoregulatory behavior in terms of a peripheral sensory basis. In a cold environment (5°C.), however,

raising intensity brought about a linear increase in the skin temperature levels maintained, which suggests that the determination of behavior in this experiment may be more complex. In further experiments the effects of central temperature manipulations upon the skin temperature "thresholds" for responding have been evaluated. Although it is still too early to make concrete statements it appears that holding the temperature of the central controller (PO-AH region) at a stable level above and below normal results in shifts in the skin temperature thresholds which are constant and closely correlated with the degree of displacement of hypothalamic temperature.

3. Brain amines and thermoregulation. The level of particular monoamines in the brain has been thought by some investigators to be the basis for physiological thermoregulation. Experiments bearing upon this theory and upon the central control of behavioral thermoregulation have been completed recently. It is clear from the results that micro-injections of a cholinomimetic agent, carbachol, into the anterior hypothalamic-preoptic region induces hyperthermia. This finding opens many questions about the generalizations from previous research by other workers where intraverticular administration techniques were used.

In the present experiments a double wall connula was implanted in the preoptic-anterior hypothalamic region of the brain in 16 rats. Following a recovery period periodic measurements of core temperature were made under the following conditions: After the animals were injected with 5mg of carbachol, saline or after sham injections hyperthermia began to develop in both the high and low concentration group (5 and 3mg carbachol) during the first 30 minutes following injection. In view of the dose dependant hypothermia it seems quite possible that there are fibers in the hypothalamus that are sensitive to cholinergic transmitter - like substances and which are intimately involved in the regulation of deep body temperature. The mechanism by which body temperature was raised after these injections (whether by shivering, chemical thermogenesis, etc.) is now under investigation.

In other experiments, not yet completed, the effects of adrenergic drugs delivered into the hypothalamus is being examined. From the early findings it appears that the drug produces hypothermia and a decrease in heat-escape responding. Injections of carbachol on the other hand produce both a rise in body temperature and an increase in heat escape responding.

4. Role of the posterior hypothalamus in temperature regulation. The posterior hypothalamus has been associated with the maintenance of body temperature in the cold. In fact, this region is popularly known as the "heat production" center and is normally described as such in both lower and upper level physiology textbooks. We now know that this oversimplification will have to be modified because the region, unlike the preoptic-anterior hypothalamic region, is "temperature blind", a fact that makes its presumed autonomy in the production of heat less likely.

The role of this region in physiological and behavioral thermoregulation continues to be of interest in this laboratory. So far it is

clear from our data that animals in which this part of the brain has been disabled are not able to regulate against cold but retain the ability to control body temperature in a hot environment. Behavioral thermoregulation seems to parallel these effects: The animals work for more heat in the cold after posterior hypothalamic lesions are made, but do not show any change in heat escape behavior. The results here are in agreement with previous work which suggested that the posterior hypothalamus is important to heat production. When the data on thermal stimulation (Section 1) is taken into account, in particular the fact that thermal stimulation of this region does not result in alterations in physiological and behavioral thermoregulatory responses, it seems likely that the posterior hypothalamus contains tracts to heat-producing effectors. If this is true, the alterations in behavioral thermoregulation can then be accounted for on the basis of the resulting lower baseline body temperature. It is interesting to note that while these lesions disrupt heat production that the sensory, motivational and response aspects of behavioral thermoregulation remain.

5. Temperature discrimination. From our experiments on temperature discrimination in the rat it is clear that these animals can accurately differentiate (70% of the time) between environmental temperatures that are 2°C. apart. This ability has been shown to hold for temperature pairs between 25 and 42°C., the range of the discrimination apparatus.

   In these experiments the animals' task was to press a pedal when the air flowing through the chamber was at a particular temperature level in order to obtain food. Pressing while the temperature was at "control" level delayed the opportunity for reinforcement. Under these conditions some rats were able to discriminate differences in the reinforced and control temperatures as small as 2°C. better than 92% of the time. These baselines will now be used to examine the role of control thermosensitivity in temperature discrimination by heating and cooling the preoptic-anterior hypothalamic region, disrupting certain sensory tracts in the brain, etc.

Previous Experiments

The work cited above was carried out during the second year of NASA funding. The following summaries are based on research completed during the first year and are discussed in more detail in the first progress report.

1. Effects of high fat diets on intake, body weight and behavioral thermoregulation against heat. Normal rats were found to increase caloric intake, body weight, and operant responding to escape heat when fed diets with high lipid content. Animals made hyperphagic through hypothalamic lesions showed characteristic changes in body weight and alterations in heat-escape responding which were related to increases and decreases in the body weight measures. The increased responding in both normal and hyperphagic rats is interpreted to be behavioral compensation for restricted body heat loss resulting from increments in depot fat insulation.

2. Effects of desalivation on behavioral thermoregulation against heat. Male and female rats made subtotally desalivate by section of the major

saliva ducts were tested in operant thermoregulation sessions. This surgery, which impairs a major thermoregulatory response in the rat, produced a rise in operant behavior to reduce heat exposure among the male animals. Through this increased responding the reduction in evaporative heat loss following desalivation was compensated somewhat, allowing post-session temperatures to be maintained at the normal pre-surgery levels. Female desalivates did not show an increase in responding. Another effect beneficial in coping with heat stress, the lowering of pre-session body temperature, emerged among both experimental and control animals in the post-operative sessions. The earlier finding of stable post-session body temperatures (38.0-39.0°C.) in normal animals when this variable is under behavioral control was also noted in the animals of this experiment.

Concluding Remarks

To summarize the entire program, the information collected has brought about a better understanding of how physiological factors such as the local temperature of particular brain structures, the level of body fat and the state of both central and peripheral organs, influence thermoregulation. Perhaps the greatest contributions are the most recent, those involving the thermosensitivity of the medulla and its contribution to the sensory, motivational and effector processes that make up behavioral thermoregulation. Other accomplishments have provided operant baseline "tools" for probing into the workings of temperature discrimination as well as basic information useful for comparing the abilities of the rat with that of other species. From another viewpoint the information collected in these experiments has added to the knowledge of the control of physiological thermoregulation since this variable was studied concomittantly in order to be able to accurately assess the effects of the manipulations of brain temperature, body fat, etc. Finally, the research has provided a wealth of techniques, expertise, equipment and procedures which will be useful in carrying the research further.

PUBLICATIONS

Lipton, J. M. Effects of high fat diets on caloric intake, body weight and heat-escape responses in normal and hyperphagic rats, J. comp. physiol. Psychol., 1969, 68, 507-515.

Lipton, J. M. and Marotto, D. R. Effects of desalivation on behavioral thermoregulation against heat, Physiol. Behav., 1969, 4, 723-727.

Avery, D. D. Hyperthermia induced by direct injections of carbachol into the anterior hypothalamus, Neuropharmacol., 1970, 9, 175-178.

Lipton, J. M., Avery, D. D. and Marotto, D. R. Determinants of behavioral thermoregulation against heat: thermal intensity and skin temperature levels, Physiol. Behav., 5, 1083-1088.

Lipton, J. M. Behavioral temperature regulation in the rat: effects of thermal stimulation of the brain, J. Physiol., Paris (in press).

Reprints of other research described in this report will be provided as they become available.

Neuropharmacology, 1970, 9, 175–178 Pergamon Press. Printed in Gt. Britain.

PRELIMINARY NOTE

HYPERTHERMIA INDUCED BY DIRECT INJECTIONS OF CARBACHOL IN THE ANTERIOR HYPOTHALAMUS*

D. D. AVERY†‡
Division of Psychology, University of Texas, Southwestern Medical School at Dallas, Dallas, Texas

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Summary—Micro-injections of carbachol in the anterior-preoptic hypothalamic area of rats induced hyperthermia. The magnitude of the effect was dose dependent and the results were related to theoretical issues which arose from previous studies in which hyperthermia was associated with intraventricular injections of acetylcholine.

MYERS and YARSH (1968) have found that injections of various concentrations of acetylcholine into the ventricular system of rats produced marked rises in colonic temperature. As pointed out by these authors, their results are difficult to interpret if one adheres to the monoamine theory of temperature regulations postulated by FELDBERG and MYERS (1964). Recently, MYERS (1968) has suggested that the hyperthermia associated with intraventricular injections of acetylcholine is not a result of functional cholinergic changes in the anterior hypothalamic "heat loss" center *per se*, but rather that the active substance diffuses, via the ventricular route, to cholinergic effector mechanisms for heat production, located caudally to the hypothalamus. To test this hypothesis a cholinomimetic agent, carbachol, was injected directly into the anterior-preoptic area of the hypothalamus.

METHODS

Sixteen male albino rats (360–500 g), under nembutal and chloral hydrate anesthesia, were implanted with double wall cannulae in the anterior-preoptic area of the hypothalamus. The cannulae were similar to those described by DECIMA and GEORGE (1964) and consisted of an outer component constructed from 22 gauge stainless steel tubing and an inner injection cannula constructed from 30 gauge stainless steel tubing. The injection cannula, when in place, extended 1 mm beyond the bottom of the outer, guide cannula. The cannulae were inserted 0·8–1·0 mm from the midline, 7·5 mm anterior to the interaural line, and 8·5 mm below the surface of the dura. Detailed histologies will appear in forthcoming papers. Throughout the experiment each animal was individually housed in a constantly illuminated, temperature controlled room (23·5±0·5°C). Carbachol (carbamylcholine chloride) was dissolved in pyrogen free normal saline in 6 mg/ml and 10 mg/ml concentrations. All injections were 0·5 μl and therefore an animal received either 3 μg or 5 μg of carbachol or control injections of 0·5 μl of normal saline. There was at least 72 hr between control and drug

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†Postdoctoral Fellow in the Department of Psychiatry at the University of Texas Southwestern Medical School at Dallas.
‡Present address: Department of Psychology, Colorado State University, Fort Collins, Colorado, 80521, U.S.A.

injections. Colonic temperature was measured with a YSI telethermometer and the probe was inserted 6 cm beyond the anal orifice. Temperatures were recorded at − 24, 0, 0·5, 1, 2, 3, 4, 5 and 24 hr with respect to injection.

RESULTS AND DISCUSSION

As shown in Fig. 1, hyperthermia began to develop in both the high and low concentration groups during the first 30 min following injection. The increases in colonic temperature became asymptotic in 2 hr for the low dosage group and in 3 hr for the high dosage

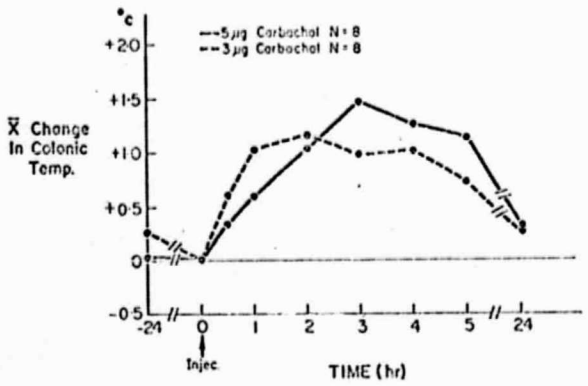

FIG. 1.

group. The maximum increases in the 5 hr post injection time ranged from 0·4 to 1·8°C in those animals receiving 3 μg of carbachol and from 1·2 to 2·4°C in those animals receiving 5 μg. Both groups were within ±0·35°C of baseline 24 hr after injection. Control application of saline alone led to a rise in colonic temperature, as did sham stimulation. However, the increases associated with control and sham injections were not nearly of the magnitude of the hyperthermia associated with applications of carbachol, and probably reflected normal temperature increases which were a result of handling the animals. Mean maximum changes in colonic temperature for experimental and control groups are expressed in Table 1.

Myers' hypothesis, that the site of the central nervous system cholinergic mechanism instrumental in producing hyperthermia is not the anterior-preoptic hypothalamic area, does not seem to be supported by these data. In view of the dose dependent hyperthermia

TABLE 1. MEAN (±S.E.) MAXIMUM CHANGES IN COLONIC TEMPERATURE DURING THE FIRST 5 hr AFTER INJECTION

Injection condition	Mean increase (°C)	S.E.
Carbachol (3 μg)	1·15	±0·22
Saline	0·49	±0·39
Sham	0·53	±0·25
Carbachol (5 μg)	1·48	±0·20
Saline	0·49	±0·25

observed in the present investigation with local application of carbachol to the "heat loss" center, it seems quite probable that there are fibers in the hypothalamus, sensitive to cholinergic substances, which are intimately involved in the regulation of deep body temperature. The increases in core temperature associated with the intraventricular injections of acetylcholine in Myers' experiments were probably a result of diffusion of the solution through the ventricular wall and into the preoptic-anterior hypothalamus.

However, any interpretation of these results must be viewed with some caution as the hyperthermic effect of intrahypothalamic injections of carbachol is not in agreement with the findings of LOMAX and JENDEN (1966), who observed hypothermia under similar conditions. There are several possible explanations for the discrepancy between the present findings and the earlier work. In the present study 3 μg and 5 μg of active substance, dissolved in 0·5 μl volumes, were applied, whereas in the previous study 1 μg/1 μl solutions were injected. These differences in concentration might account for the opposite findings of the two experiments. Another possibility is differences in injection sites. In view of MYERS' (1966) data concerning the extent of the diffusion of various dyes injected in the rat hypothalamus and the relationship between amount injected and spread of the substance, slight differences in cannula placements would not seem to be an important factor, but volumes injected would seem to be critical. Myers found the extent of diffusion of four dyes ranging in molecular weight from 229·11 to 960·83 and injected in 1·0 μl volumes to be between 1·6 and 2·2 mm with a mean of 1·9 mm. These same dyes when injected in 0·5 μl volumes diffused over an area of 0·6–1·6 mm with a mean of 1·0 mm. Thus in both the present and previous experiments the carbachol solutions could possibly have been diffused throughout the preoptic area. Additionally, in the Lomax and Jenden experiment, diffusion with 1·0 μl injections probably encompassed not only the anterior-preoptic hypothalamic area but also the medial forebrain bundle and the lateral hypothalamus, and ultimately the solution probably spread into the ventricular system.

Another important difference in the methodology of the two experiments which could account for the different results is the fact that in the present study the animals were free-moving in their home cages throughout the experiment, whereas in the previous study the animals were restricted in plastic restraining cages. GRANT (1950) observed hypothermia in rabbits following restraint. If pyrogen was injected after recovery from restraint-induced hypothermia, a large fall in colonic temperature, instead of the usual hyperthermia associated with pyrogens, occurred. Additionally, even after 7 daily restraining sessions the animals did not show signs of adapting to the procedure. It is not unlikely that a similar restraint effect on colonic temperature occurs with central applications of various substances, including carbachol.

The physiological mechanism by which the animal raised core temperature, in the present investigation e.g. shivering, could not be ascertained from visual observation. GROSSMAN (1968) has recently reported increased activity in the rat with injection of carbachol into the midbrain reticular formation, and MACPHAIL (1968) has observed both EEG and behavioral arousal with carbachol injections in the preoptic area of rats. We are currently investigating whether or not activity increases as a function of cholinergic applications to the anterior hypothalamus, and the possibility that this is the heat producing mechanism. In addition, investigations are under way in our laboratory in the hope of elucidating (1) the hypothalamic role of adrenergic, as well as cholinergic substances, in body temperature regulation, and (2) the interaction of these "transmitter" substances and ambient temperature.

REFERENCES

DECIMA, E. and GEORGE, R. (1964). A simple cannula for intracerebral injections in chronic animals. *Electroenceph. clin. Neurophysiol.* **17**: 438–439.

FELDBERG, W. and MYERS, R. D. (1964). Effects on temperature of amines injected into the cerebral ventricles. A new concept of temperature regulation. *J. Physiol.* **173**: 226–237.

GRANT, R. (1950). Emotional hypothermia in rabbits. *Am. J. Physiol.* **160**: 285–290.

GROSSMAN, S. P. (1968). Behavioral and electroencephalographic effects of micro-injections of neurohumors into the midbrain reticular formation. *Physiol. Behav.* **3**: 777–786.

LOMAX, P. and JENDEN, D. J. (1966). Hypothermia following systemic and intracerebral injection of oxotremorine in the rat. *Int. J. Neuropharmacol.* **5**: 353–359.

MACPHAIL, E. M. (1968). Effects of intracranial cholinergic stimulation in rats on drinking, EEG, and heart rate. *J. comp. physiol. Psychol.* **65**: 43–49.

MYERS, R. D. (1966). Injections of solutions into cerebral tissue: Relation between volume and diffusion. *Physiol. Behav.* **1**: 171–174.

MYERS, R. D. (1968). Temperature regulation: Neurochemical systems in the hypothalamus. In: *Hypothalamus—Anatomical, Functional and Clinical Aspects* (HAYMAKER, W., NAUTA, W. and ANDERSON, E., Eds.). Charles Thomas, Springfield.

MYERS, R. D. and YAKSH, T. L. (1968). Feeding and temperature responses in the unrestrained rat after injections of cholinergic and aminergic substances into the cerebral ventricles. *Physiol. Behav.* **3**: 917–928.

Physiology and Behavior. Vol. 4, pp. 723–727. Pergamon Press, 1969. Printed in Great Britain

Effects of Desalivation on Behavioral Thermoregulation Against Heat[1]

JAMES M. LIPTON AND DANIEL R. MAROTTO

The University of Texas Southwestern Medical School at Dallas, Dallas, Texas, U.S.A.

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LIPTON, J. M. AND D. R. MAROTTO. *Effects of desalivation on behavioral thermoregulation against heat.* PHYSIOL. BEHAV. 4 (5) 723–727, 1969.—Male and female rats made subtotally desalivate by section of the major saliva ducts were tested in operant thermoregulation sessions. This surgery, which impairs a major thermoregulatory response in the rat, produced a rise in operant behavior to reduce heat exposure among the male animals. Through this increased responding the reduction in evaporative heat loss following desalivation was compensated somewhat, allowing post-session temperatures to be maintained at the normal pre-surgery levels. Female desalivates did not show an increase in responding. Another effect beneficial in coping with heat stress, the lowering of pre-session body temperature, emerged among both experimental and control animals in the post-operative sessions. The earlier finding of stable post-session body temperatures (38.0–39.0°C) in normal animals when this variable is under behavioral control was also noted in the animals of this experiment.

Behavioral thermoregulation against heat Role of saliva spreading in thermoregulation Heat stress
Body temperature

THE QUALITATIVE observation that a number of small animals show marked salivation in response to heat has been made many times [8, 11, 13, 15]. Hainsworth [5], however, only recently showed through controlled experiments that the spreading of saliva on vascularized and furred surfaces is of critical importance to the rat in regulating body temperature against heat stress. In his experiments desalivate rats were found to show high rectal temperatures when exposed to environments of 36.0°C and above while normal animals, by augmenting evaporative cooling through increased saliva spreading, held their body temperatures within lower limits.

Homeothermic organisms confronted with changes in body heat loss or gain must counteract them if they are to regulate body temperature and survive. The reactions available can be divided into those that are primarily physiological (shivering, sweating, panting, etc.), those that are primarily behavioral (e.g., escape into a more temperate microclimate) and combinations with both physiological and behavioral components (e.g., saliva spreading). While each reaction is important individually, the interactive nature of the physiological and behavioral components allows for some interesting research. For instance, physiological thermoregulation can be studied by using a behavioral tool which is based on the fact that animals can learn to contribute to their own thermal balance by performing an arbitrary response. When the efficiency of physiological thermoregulation is reduced experimentally, this behavioral reaction often increases in a "compensatory" fashion [9, 10, 17]. The magnitude of the behavioral compensation can then be used as an index of the importance of the physiological manipulation to the animal's overall thermoregulation.

The development of behavioral compensation in the earlier experiments [9, 10, 17], combined with the importance of saliva spreading to thermoregulation, leads to the prediction that rats should show a compensatory increase in behavioral thermoregulatory responding after desalivation. This expectation is strengthened by the fact that the desalivate preparation rapidly develops other types of behavior to compensate for the loss of saliva such as an increase in prandial drinking to facilitate swallowing dry food [3]. Epstein and Milestone [2], however, were unable to find differences between desalivate and control rats in operant responding for cooling showers when environmental temperatures were increased. These authors concluded that their control animals chose to utilize the option of pressing for showers rather than spread saliva, and thus minimized any potential difference in cooling capacity between the two groups. The present experiment compared desalivate and operated control rats in an operant heat–stress situation where exogenous water was not available and convective cooling was used as reinforcement. Under these conditions saliva spreading can be used as an effective cooling response and potentially confounding prolonged reinforcement factors inherent in shower reinforcement can be avoided.

METHOD

Subjects and Operative Procedures

Five male and three female albino rats (Charles River Breeding Laboratories) were made desalivate by double liga-

[1]This research was supported by N.A.S.A. research grant 44-012-079.

tions and cutting the paratoid and submaxillary-major sublingual ducts which supply saliva to the mouth. This was accomplished by making, under ether anesthesia, a ventral midline incision in the skin of the neck through which the glands and their ducts could be located (see Greene [4] for anatomy; Vance [10] for details of a similar operation). On either side of the trachea a pair of glands in the same capsule (one submaxillary and one major sublingual) were located, along with their emerging ducts. After identification of these structures, the parallel ducts from each side were double ligated as though they were a single duct at a point near their disappearance under the digastricus muscle, and then a transection was made between the ligatures. Similar ligation and cutting of the parotid ducts (Stenson's ducts) as they traverse the lateral aspect of the masseter muscle required the sacrifice of the ramus mandibularis marginalis since it cannot be readily separated from the duct without damage. This nerve serves the lower lip as a sensory-motor branch of the VII cranial nerve and no thermoregulatory function has been attributed to it. The operative procedures stated above normally required 15–20 min after the animal was anesthetized.

The minor sublingual glands are so located (in contact with the mucous membrane of the floor of the month) that anatomical structures serving mastication and deglutition would be markedly disturbed in any attempt at ligation or removal. These glands have been found to contribute very little to total salivary flow [14] and thus would not be expected to play a large part in the evaporative cooling response in the rat. For these reasons the minor sublinguals were left intact.

Three males and two females similar in body weight and history to the desalivated animals were used as controls. These animals underwent ether anesthesia, incision, location of glands and ducts to guard against inherent differences in the ability to produce saliva, and suturing.

In the experimental rats, desalivation was assured by the increased water utilization following surgery [3, 16] and postmortem examination of ligatures, ducts and glands. Mean preoperative water intake in the desalivates was 26.1 ml. This intake reached a peak of 63.9 and levelled-off at a mean of 46.0 ml in the postoperative period. Controls average 27.3 and 29.2 ml/day in the two periods.

Except for daily testing, the animals were housed in standard cages at 23.0 ± 1.0°C (dry bulb) and allowed tap water *ad libitum*. Body weight was controlled (250 g) by restricting the diet of Purina pellets. During the recovery period, wet Purina mash was given in order to offset weight loss resulting from surgery.

Apparatus

The operant apparatus has been described in detail previously [9]. The units were composed of Plexiglas cylinders 30.5 cm high and 22.9 cm in dia. These cylinders were mounted in manifold boxes fitted with exhaust fans (60 cfm). A heat lamp (250 W) was centered above the operant chamber and was programmed to be normally-on during 1-hr sessions. A response on the bar equal to or greater than a weight of 10.6 g turned off the heat lamp and turned on the exhaust fan for a period of 10 sec, thus cooling the animal by convection. Additional responses made during this 10-sec period would not lengthen it. A matched pair of chambers located in an environment controlled at 23.0 ± 1.0°C (range) was used in this experiment. A rough index of the rate of heating under the lamp is given in Table 1. This rate was recorded from a #425 Yellow Springs thermistor probe placed in the chamber as a substitute for the animal. The amount of cooling with one reinforcement varied with the initial level of heat, being greater at higher rather than lower initial temperatures (Table 1). Humidity was measured with an electrohygrometer immediately before each session. The humidity varied between 33.0 and 64.0 per cent with 43.0 per cent being the modal level during the experiment.

Procedure

All animals were shaped to press the bar in daily sessions. Session length was initially 10 min and was then increased by 5-min increments per day until the rats were responding reliably (normally less than 5 sessions) at which point it was extended to 1 hr. Just before and immediately after a session the animals were weighed and their colonic temperatures were measured with a thermistor probe (6 cm insertion). Each animal was run at the same time every day and in the same operant chamber. The number of thermoregulatory responses per session was recorded as the basic behavioral data.

Over a month of consecutive daily sessions individual differences in responding and pre-session temperatures emerged. Some rats made more responses than others to attain the same post-session temperature. These characteristics were quite reliable within individual animals during the baseline period. Consistent differences in pre-session temperature were also noted between males and females.

After surgery the animals were allowed a week of stress-free recovery time. Following recovery the sessions were resumed for 20 additional days.

TABLE 1

HEATING RATE OF THE LAMP AND COOLING EFFECT OF ONE REINFORCEMENT

	Heating rate						
Time (sec)	0	20	40	60	80	100	120
Temp. (°C)	23.0	29.0	35.0	40.0	43.8	46.8	49.4
	Cooling with one 10-sec reinforcement						
Initial level (°C)	30	35	40	45	50		
Temp. at end of reinforcement (°C)	29.6	33.9	38.3	41.7	47.1		
Difference (°C)	−0.4	−1.1	−1.7	−3.3	−2.9		

RESULTS

Baseline Period

Saliva spreading. In similar operant thermoregulation sessions rats have been consistently noted to spread saliva throughout experiments of 120 days duration. During the training and baseline periods the rats in this experiment normally began to groom saliva within the first 10 min of the session on ears, scrotum, back, tail, etc. and continued to do so intermittently throughout the session. After desalivation, "pseudo-spreading" or grooming without saliva still occurred as noted by Hainsworth [5]. When removed from the chamber after a session the desalivate animals were warm and dry to the touch while the head, neck and underbody of the controls were usually wet. Upon return to the home cage the desalivates typically began to groom immediately, using drinking water from the Richter tubes as a substitute for saliva.

Thermoregulatory responding and colonic temperature

In the sessions before surgery individual differences in response rate and body temperature appeared. Females tended to have slightly higher mean response rates during this period, averaging 230.8 responses/hr compared with a 216.7/hr average for the males. The total number of responses theoretically possible in a session was 360 although few animals have exceeded 270/hr in this apparatus. Resting or pre-session temperatures of the females was roughly 0.5°C higher than the males (Fig. 1). Post-session temperatures of both males and females varied only slightly around 39.0°C. This stability in post-session temperature, in spite of individual differences in resting temperature and in responding, has been noted earlier [9, 10]. In the previous experiments the average post-session temperatures of normal rats, including a dark-coated hybrid variety, ranged between 38.8–39.5°C with extremely small variability within individual animals. It appears that each animal allows a characteristic level of hyperthermia which he regulates with precision by controlling response rate. It is also obvious that the regulated level does not vary greatly among animals. This stability was used as a standard to compare the effects of desalivation upon thermoregulation in the present experiment.

TABLE 2

MEAN (±S.E.) RESPONSE RATES OF THE INDIVIDUAL RATS FOR THE SESSIONS BEFORE AND AFTER SURGERY

Animal	Condition	Responses		
		Pre-op	Post-op	Change
SH-2	♂ Desal	204.8 ± 1.3	226.8 ± 2.8	↑
SH-3	♂ Desal	217.2 ± 1.9	218.1 ± 3.1	—
SH-4	♂ Desal	215.9 ± 1.6	228.0 ± 2.2	↑
SH-5	♂ Desal	205.8 ± 1.3	215.8 ± 1.5	↑
SH-6	♂ Desal	230.4 ± 1.7	239.0 ± 1.7	↑
PS-2	♀ Desal	228.4 ± 3.8	224.6 ± 2.5	—
PS-6	♀ Desal	236.5 ± 1.5	224.7 ± 1.9	↓
PS-7	♀ Desal	216.2 ± 4.11	221.2 ± 1.5	—
SH-7	♂ Con	234.2 ± 1.2	222.6 ± 1.7	↓
SH-8	♂ Con	224.2 ± 2.8	225.2 ± 2.5	—
SH-9	♂ Con	201.4 ± 3.2	198.3 ± 1.8	—
PS-4	♀ Con	218.6 ± 3.1	210.3 ± 2.7	↓
PS-16	♀ Con	254.2 ± 1.7	238.9 ± 4.7	↓

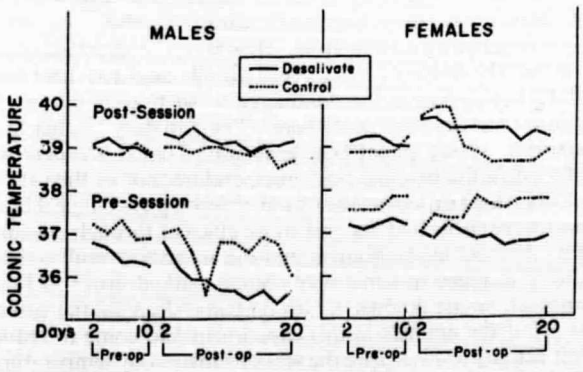

FIG. 1. Average pre- and post-session colonic temperatures for desalivate and control animals before and after surgery. Scores are means for 2-day blocks.

Postoperative Period

Males. Male desalivate rats increased responding above baseline levels soon after desalivation and continued to respond at a higher level (3.0–6.8 per cent) for the rest of the experiment (Fig. 2). This was the only group to show a sustained increase in responding after surgery, with 4 of the 5 rats participating in the increase (Table 2). Operated control males showed only a decrease followed by a modulation toward baseline in the last 10 days of the post-operative period.

Response rate and body temperature measures of both groups were intimately related. In the desalivate males post-

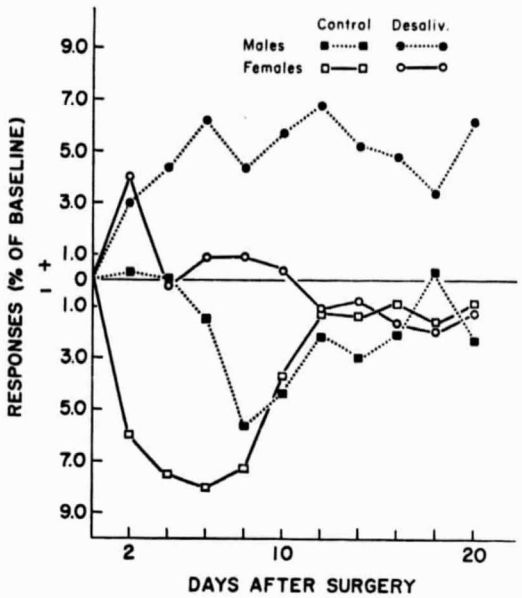

FIG. 2. Changes in behavioral thermoregulatory responding after desalivation.

session colonic temperatures immediately after surgery were slightly elevated. This elevation disappeared, however, as responding increased and pre-session temperature was reduced over sessions (Figs. 1, 2). In the control males, post-session temperature was more stable. The decrease and return toward baseline responding in this group corresponded closely with changes in pre-session temperature with the result being a relatively stable post-session temperature. It appears that the lowering of pre-session temperature can substitute, at least in part, for a reduction of the heat load through responding in determining post-session temperature.

Females. Other than a 1-day rise in responding immediately after surgery, desalivate females showed no marked changes in operant thermoregulatory behavior. The failure to increase responding after evaporative cooling had been curtailed resulted in a rise in post-session temperature to above-normal levels. One animal showed an increase from a pre-operative mean of 39.5 to a mean of 40.3°C during the first 10 days of the post-operative period. In the last days of the experiment, even though there was no change in responding, the original baseline temperature was recovered. This recovery was associated with a progressive decrement in pre-session temperature which was lower in rate and magnitude but otherwise qualitatively similar to that of the male desalivates.

For a week after sham operations, the female controls did not adhere to their previous post-session temperature standard. They showed a decrease in responding immediately after the recovery period similar to that of the male controls. The consequence of this reduced responding was a greater heat intake which was reflected in a rise in post-session colonic temperature. When responding returned to near baseline levels the post-session colonic temperature fell within the limits of the previous standard.

DISCUSSION

Three points bearing on thermoregulation in normal and desalivate rats are evident from the present data and from comparisons with earlier findings. First, the reduction of total evaporative cooling capacity by partial desalivation results in a change in behavioral thermoregulation against heat in male rats. The male rats in this experiment lessened their exposure to an external heat source and thus were able to maintain body temperature within preferred limits. In this compensatory behavior their performance was similar to that of animals whose heat-loss capacity has been attenuated by preoptic lesions [9] or through feeding high-fat diets [10]. In other respects such as the marked intake of water, the persistence of "dry grooming" and the use of drinking water as a substitute for saliva in grooming, their behavior was characteristic of desalivate rats. It appears that these animals were able to use behavior as a complement to the thermoregulatory capacity remaining to them after desalivation.

Unlike the males, female desalivates did not show a sustained increase in bar-pressing. After the first 10–12 days after surgery their responding was indistinguishable from that of their pre-surgery sessions. The reasons for this sex difference in the behavioral response to desalivation are not clear. Hainsworth [5] noted that female rats do not begin to spread saliva until the environmental temperature is above the threshold for this response in the males (32.0°C). It may be that females use methods other than evaporation for coping with relatively lower levels of heat stress. Further, desalivation may not interfere with these methods. If this is true, there would be less need for behavioral compensation, and less likelihood of a change in responding in the females. The males, on the other hand, since they use this response at lower levels of heat stress, are more affected by its debilitation and thus more likely to benefit from a behavioral compensation.

The second point concerns the emergence of lower pre-session body temperatures in both desalivate groups and in the male controls. This effect may be viewed as an important thermoregulatory strategy in itself since a greater heat exposure is required to reach high levels when the body temperature is initially below the normal range than it does when body temperature is normal. This decrease was progressive over time in the desalivates, yet 3 days after the experimental sessions were discontinued their colonic temperatures had returned to baseline levels. Two possible mechanisms of this effect are: physiological acclimation and changes in activity. Physiological acclimation could be responsible for the decrease as a normal, progressive effect of the repeated exposures to the heat stress upon hormonal, cardiovascular, and other thermoregulatory mechanisms. However, the short exposure time and the relatively small (behaviorally adjusted) heat load would not appear to be conducive to such acclimation. In addition, the decrease noted here is "anticipatory" rather than occurring during actual heat exposure. That is, the decrease is found in the baseline body temperature not in the rate of rise upon heat exposure one would expect if physiological heat-loss mechanisms had become more efficient through acclimation. Another explanation is that the animals' overall activity pattern changed in some way. Since marked drops in body temperature are known to accompany sleep in the albino rat [1], if the animals in this experiment had come to reduce their activity levels before the sessions their body temperatures would be expected to be reduced also. In an earlier heat-escape experiment [9] reductions in pre-session temperature appeared to be related to sleep just prior to the regularly

scheduled sessions. The basis for such activity changes could be solely automatic, simply as a part of the physiological acclimation or the changes could be based on the animals' learning to control voluntary activity before the sessions. Whether either acclimation in the general sense, changes in activity level, or both are responsible for the observed decreases in presession body temperature will have to be determined in future experiments.

Reduced initial body temperatures were clearly related to behavioral responding in this experiment. Most of the variation in responding with this operant technique occurs early in the session. As the session progresses, the animal's heat load increases and he is compelled to press steadily to maintain a stable body temperature. If the pre-session temperature of a particular animal is lower than usual his first few responses may be emitted somewhat later or further apart resulting in a lower total response record. The reciprocal and additive nature of responding and lowered pre-session temperature in the maintenance of body temperature were obvious from the comparisons of the two functions in both normal and desalivate rats.

The third point concerns the observation of a level of hyperthermia which was behaviorally regulated within rather narrow limits by all animals in this operant situation. As stated earlier in this paper, the level may vary slightly from one animal to another but the overall range is not great. In the present experiment the persistence of post-session temperatures of 38.8–39.0°C over many sessions, when a higher response rate could reduce this level or a lower rate increase it, is an illustration of the regulation. The durability of the behavioral regulation is reflected in the fact that animals with imposed restrictions in heat loss in the present and other [9, 10] experiments tend to increase compensatory responding to maintain previous body temperature levels. A similar regulated hyperthermia was also discovered by Epstein and Milestone [2] using an entirely different methodology. They found that normal and desalivate rats working for cooling showers in a hot environment maintained post-session temperatures around 39.0°C. It is tempting to assign the regulated level of hyperthermia seen in these experiments a "setpoint" or threshold function for the control of behavioral responses similar to that proposed in a recent theoretical model of thermoregulation [6]. The term is descriptive of the observations and provides many suggestions for further research into the factors determining the setpoint and the individual operant response.

REFERENCES

1. Abrams, R. and H. T. Hammel. Hypothalamic temperature in unanesthetized albino rats during feeding and sleeping. *Am. J. Physiol.* **206**: 641–646, 1964.
2. Epstein, A. N. and R. Milestone. Showering as a coolant for rats exposed to heat. *Science* **160**: 895–896, 1968.
3. Epstein, A. N., D. Spector, A. Samman and C. Goldblum. Exaggerated prandial drinking in the rat without salivary glands. *Nature* **201**: 1342–1343, 1964.
4. Greene, E. *Anatomy of the rat.* New York: Hafner Pub., 1955.
5. Hainsworth, F. R. Saliva spreading, activity, and body temperature regulation in the rat. *Am. J. Physiol.* **212**: 1288–1292, 1967.
6. Hammel, H. T. Regulation of internal body temperature, *Ann. Rev. Physiol.* **30**: 641–710, 1968.
7. Hardy, J. D., A. T. Milhorat and E. F. DuBois. Basal metabolism and heat loss of young women at temperatures from 22°C–35°C. *J. Nutr.* **31**: 383–404, 1941.
8. Higginbotham, A. C. and W. E. Koon. Temperature regulation in the Virginia opossum. *Am. J. Physiol.* **181**: 69–71, 1955.
9. Lipton, J. M. Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat. *Physiol. Behav.* **3**: 165–169, 1968.
10. Lipton, J. M. Effects of high-fat diets on caloric intake, body weight and heat-escape responses in normal and hyperphagic rats. *J. comp. physiol. Psychol.* 1969 (in press).
11. Prossser, L. C. and F. A. Brown, Jr. *Comparative Animal Physiology*, Phil.: Saunders, 1968, p. 268.
12. Robinson, K. W. and H. K. Lee. Animal behavior and heat regulation in hot atmospheres. *Univ. Queensland Papers Dept. Physiol.* **1**: 1–8, 1946.
13. Robinson, K. W. and P. R. Morrison. Reactions to hot atmospheres of various species of Australian marsupials and placental animals. *J. cell. comp. Physiol.* **49**: 455–478, 1957.
14. Schneyer, C. A. and L. H. Schneyer. Electrolyte levels of rat salivary secretions. *Proc. soc. exp. biol. Med.* **101**: 568–569, 1959.
15. Schmidt-Nielsen, K. *Desert Animals: Physiological Problems of Heat and Water.* London: Oxford Univ. Press, 1964.
16. Vance, W. B. Observations on the role of salivary secretions in the regulation of food and fluid intake in the white rat. *Psychol. Monogr.* **79**: 1–22, 1965.
17. Weiss, B. and V. Laties. Behavioral thermoregulation. *Science* **133**: 1338–1344, 1961.

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EFFECTS OF HIGH FAT DIETS ON CALORIC INTAKE, BODY WEIGHT, AND HEAT-ESCAPE RESPONSES IN NORMAL AND HYPERPHAGIC RATS[1]

JAMES M. LIPTON[2]

University of Texas Southwestern Medical School at Dallas

Normal rats were found to increase caloric intake, body weight, and operant responding to escape heat when fed diets with high lipid content. Animals made hyperphagic through hypothalamic lesions showed characteristic changes in body weight and alterations in heat-escape responding which were related to increases and decreases in the body weight measures. The increased responding in both normal and hyperphagic rats is interpreted to be behavioral compensation for restricted body heat loss resulting from increments in depot fat insulation.

Increasing the fat constituent of the diet has been shown to have salutary effects upon temperature maintenance in humans (Mitchell, Glickman, Lambert, Keeton, & Fahnestock, 1946), and upon survival time in rats exposed to cold environments (Dugal, 1944; Dugal & Thérien, 1947; Giaja & Gelineo, 1934). Hamilton (1963) found that a high fat diet reduced the amount of heat rats worked for when exposed to cold. In rats, diets with a high lipid content produce specific changes in food intake and assimilation which contribute to their capacity to maintain thermal balance in low ambient temperatures. One change of note is the increase in caloric intake and subsequent rise in body weight when high fat diets are substituted for the regular regimen (Corbit & Stellar, 1964; Reed, Yamaguchi, Anderson, & Mendel, 1930). Another related change concerns the assimilation and storage of fat, per se. The body composition of rats and mice becomes disproportionately greater in depot fat than in materials such as protein when dietary fat content is high (Fábry, Braun, Petrásek, Horáková, & Konopásek, 1964; Fenton & Dowling, 1953; Larsson, 1967; Reed et al., 1930). These changes result in enlargement of body-fat stores, thus increasing insulation and stored energy potentially valuable in combatting cold stress.

This insulation, of undoubtable use in animals exposed to cold, would seem to interfere with thermal balance if the animals were exposed to high temperatures. As Schmidt-Nielsen (1964) has suggested, in hot environments there is value in a thin, well-vascularized skin through which heat can be transferred to the surface and dissipated. A layer of subcutaneous fat would impede this heat flow. Since high fat diets increase insulation, heat loss would be expected to be constrained in rats fed a fat regimen. In an earlier experiment (Lipton, 1968), behavioral responding to escape heat was augmented when the physiological heat-loss capacity of rats was reduced through preoptic lesions. The following experiments were designed to see if a similar behavioral effect would accompany the feeding of high fat diets and, secondarily, to note the effects of such diets on food and water intake.

EXPERIMENT 1

In this experiment the effects of feeding high fat diets upon intake and heat-es-

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[2] Requests for reprints should be sent to James M. Lipton, Division of Psychology, University of Texas Medical School at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

TABLE 1

DAILY FOOD INTAKE, WATER INTAKE, AND URINE OUTPUT OF EXPERIMENTAL AND CONTROL RATS DURING THE THREE DIET PERIODS ($M \pm SE_m$)

Group	Diet periods		
	Base line	30% fat diet	50% fat diet
Food intake (Kcal.)			
Experimental ($n = 5$)	105.4 ± 3.4	119.9 ± 9.4	121.5 ± 8.5
Control ($n = 8$)	100.0 ± 5.0	100.2 ± 5.4	100.3 ± 12.1
Water intake (ml.)			
Experimental	43.4 ± 2.5	33.1 ± 2.1	26.6 ± 2.4
Control	36.6 ± 1.8	36.9 ± 1.2	37.3 ± 1.0
Urine volume (ml.)			
Experimental	18.9 ± 1.7	9.4 ± 1.2	4.9 ± 1.2
Control	17.7 ± 1.2	13.2 ± 1.3	20.1 ± 1.4

cape measures were examined using normal albino rats as subjects.

Method

Subjects. Thirteen adult male Charles River rats (CD strain) of about 400 gm. body weight at the beginning of the experiment were used. They were kept in metabolism cages in a constantly illuminated, temperature-controlled (22.0 ± 2.0°C. range) room on an ad-lib feeding schedule.

Diets. Three diets were used: regular ground Purina laboratory chow (4.25 kcal/gm[a]), a 30% fat diet (5.68 kcal/gm), and a 50% fat diet (6.34 kcal/gm). The fat diets were composed of 30% lard, by weight, mixed with 70% ground Purina chow and a similar 50% lard and 50% chow mixture.

Apparatus. The heat-escape apparatus has been described in detail previously (Lipton, 1968). A Plexiglas cylinder 30.5 cm. high and 22.9 cm. in diameter formed the experimental space. This cylinder was fitted into a manifold box constructed of 1.9 cm. plywood with 45.7 × 8.9 × 31.8 cm. outer dimensions.

At one end of the manifold box a fan (100 cfm) was situated to exhaust air, thus drawing in room air through the top of the chamber. Glass tubing (1.0 cm. diameter) arranged 1.0 cm. apart formed the floor. A 250-w. red bowl heat lamp mounted above a wire grid was centered over the top of the cylinder. The manipulandum was made of 1.0-cm. glass tubing fused at one end and attached to a microswitch bolted to the outside surface of the chamber. This bar extended 5.1 cm. into the chamber and rested 3.8 cm. above the floor. Approximately 10.6 gm. of force applied to the end of the bar was required to operate the switch. The heat lamp was normally on during the session; a bar press turned the lamp off and, simultaneously, the fan on for 10 sec. Additional responses made during this 10-sec. "cool" period would not lengthen the period. In this experiment two of these chambers were situated in an ambient temperature of 22.0 ± 2.0°C. (range).

Procedure. All animals were trained to escape heat in the first phase of the experiment while fed regular laboratory chow. Daily measures were taken of 23-hr. food intake, water intake, urine volume output, pre- and postsession body weight and colonic temperature, and the total number of heat-escape responses made during the 1-hr. session. Colonic temperature was measured by passing a thermistor probe 5.0 cm. beyond the anal orifice. After the animals had been responding at a stable level for at least 20 days, five controls were randomly chosen to continue on the chow diet while the remaining eight were put on the 30% fat diet regimen. After 20 days on this diet, the experimental animals were then given the 50% fat diet for an additional 10 days. Controls continued on the regular chow diet during this last phase of the experiment.

Results and Discussion

Table 1 shows the mean caloric intake of the two groups over the three diet periods. Caloric intake for the control group, fed chow throughout the experiment, did not vary significantly over the periods ($p > .10$; Wilcoxon, 1945). Rats fed the 30% fat diet decreased their gram intake of food ($p < .02$) but not enough to hold caloric intake constant. The daily caloric intake record for this group was very high in the first 10 days followed by a stabilization at a level slightly higher than base line. Similar effects were found when the 50% fat diet was introduced.

Water intake and urine volume measures did not vary significantly over the three

[a] Gross energy value recently verified through bomb calorimetry by Damon Shelton, Director, Purina Animal Research Division. Previous values, such as the 3.61 kcal/gm used by Teitelbaum (1955), Hamilton (1964), Corbit and Stellar (1964), and other workers are presumed to be calculated estimates based on approximate caloric values of the individual protein, fat, and carbohydrate components. The caloric density estimate for the fat diets used in this experiment are based on a 9.02 kcal/gm value for the lard (Armour Co.). Unfortunately, the net or metabolizable productive energy values for the chow and the fat when fed to small laboratory animals are not available at this time.

periods for the control group (Table 1). Experimental animals, on the other hand, showed a significant decrease ($p < .05$) in water intake when fed the 30% fat diet and another ($p < .05$) when the 50% fat diet was offered. These depressions in water intake were paralleled by significant decreases in urine volume ($p < .01$).

Despite the decreases in total food and water intake, the increased intake of calories by the experimental group resulted in marked gains in body weight. Figure 1 shows that the control animals increased in weight over the three periods as a result of normal growth. The experimental animals, although they weighed the same as the controls initially, gained weight at a greater rate than controls when fed diets with a high fat content.

The mean heat-escape response level (Figure 1) did not change significantly ($p > .10$) in the control group over the diet periods. The experimental animals, however, showed a significant increase when put on the 30% fat diet ($p < .05$) and another increase when offered the 50% fat regimen that did not reach the .05 level of significance. These increases were not accompanied by changes in either the pre- or postsession body temperatures. The mean presession colonic temperatures for the experimental animals were 37.4, 37.6, and 37.4°C. for the base line, 30%, and 50% fat diet periods, respectively. The mean presession colonic temperature in the controls averaged 37.0, 36.9, and 37.1°C. for the three periods. Although the means for the control rats were lower, there were no significant differences between these values and the presession temperatures of the experimental animals ($p > .10$). Mean postsession temperatures for both groups were consistently 38.8–38.9°C. in all phases of the experiment. These figures demonstrate that, in the period when the fat diets were fed, there was neither an increase in the resting nor a decrease in the postsession temperatures which might account for the

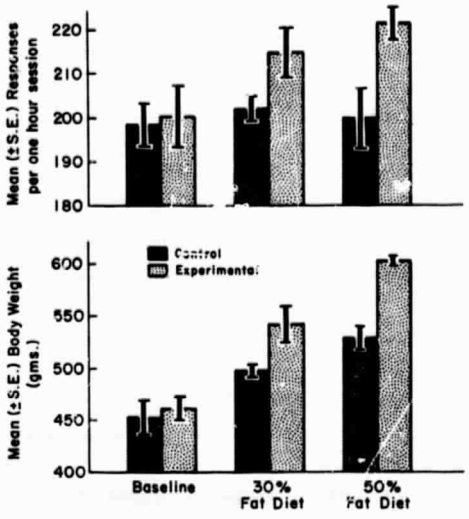

FIG. 1. Effects of high fat diets on body weight and heat-escape responding.

change in responding. The combination of a stable presession temperature and an increase in heat-escape responding which does not reduce the postsession colonic temperature below previous levels suggests that the fat diets compromise physiological thermoregulation against heat stress in some fashion.

Knowledge of the particular means through which the compromise occurs is not directly available from the data. It seems likely, however, that the increased storage of fat brought about by the intake of high fat diets plays a substantial part in curtailing temperature regulation against heat stress. In the rat, storage of fat takes place largely in subcutaneous depots and the feeding of high fat diets increases the amount of these stores (Reed et al., 1930). In the subcutaneous depots as well as in genital, intramuscular, perirenal, etc., stores, this poorly vascularized material restricts heat flow to the surface of the skin. To compensate for the reduction in heat-loss capacity the rat behaviorally lessens his exposure to heat.

A further suggestion that body composition is responsible for the response increases comes from an additional compari-

son of body weight. By the third diet period the controls, through normal growth, weighed almost the same as did the experimental animals in the second diet period, yet they did not increase their responding. This implies that something more than normal growth is necessary to produce a response increment. Disproportionate body fat, or at least some factor specific to the fat diets, appears to be responsible.

Experiment 2

From the results of the previous experiment it appears that large amounts of fat in the diet result in an elevation of behavioral thermoregulatory responding against heat stress. The increase in responding is interpreted to be a compensation for impaired heat loss as a result of the augmentation in depot fat. The purpose of this experiment was to see if these effects would be repeated in a rat well known for its elevated food intake and fat storage: the hyperphagic rat.

Method

Subjects. The subjects were 11 female Charles River rats weighing approximately 235 gm. when the experiment was begun. All animals were individually housed in standard cages with food available in spill-proof cups. Tap water was available from Richter tubes. The diets were the same as those used in Experiment 1 with the addition of Purina laboratory chow pellets. The heat-escape chambers were also the same as those used in the previous experiment.

Surgery. Bilateral electrolytic lesions were placed in the region of the ventromedial nuclei of the hypothalamus in the experimental animals. After pretreatment with atropine and chloral hydrate they were anesthetized with sodium pentobarbital and placed in a Kopf stereotaxic instrument. The coordinates were: 6.0 mm. anterior to the interaural axis, 0.7–0.8 mm. lateral to the center of the sagittal sinus, and 8.6 mm. below the surface of the cortex. A direct anodal current of 2 ma., passed through the uninsulated tip (.5 mm.) of a nichrome-alloy electrode for 10 sec., was used to produce the lesions. Control animals received the same treatment except for the delivery of current. Food was removed from the home cages for 12 hr. after the surgery.

Procedure. After the animals had been bar pressing to escape heat at a stable level for 20 or more days during which plain chow was offered, six of them received ventromedial hypothalamic lesions while five others served as operated controls. Following a 10-day recovery period these animals were reintroduced into a daily schedule of heat-escape sessions. The ground chow regimen was continued through the recovery and the 20-day postoperative periods. After the latter period all animals were offered the 30% fat diet for 20 days, as in the first experiment. This period was followed by: 10 days in which the 50% fat diet was fed; 10 additional days of ground Purina chow; 5 days of total food deprivation; and 5 days in which each animal received 5 gm. of pellets per day.

Results and Discussion

Intake and body weight. Although the control animals were eating slightly more than the experimental group prior to surgery, before the end of the postoperative period this relation was reversed (Table 2). When offered the high fat diets the control group's intake was much like that of animals fed similar diets in the first experiment. That is, the gram intake was reduced when the 30% diet was offered and reduced again during the 50% fat diet period. In the first 5 days of each of these periods gram food intake was higher than in the latter part of the same periods, resulting in "spikes" in the caloric intake record (Table 2). This effect is similar to that seen before "caloric adjustment" in animals offered a 50% fat diet by Strominger, Brobeck, and Cort (1953). The failure of intake to increase to postoperative levels when ground chow was provided following the fat diet is compatible with the findings of Strominger et al., (1953) and of Corbit and Stellar (1964).

Food intake in the rats with lesions rose steadily in the postoperative sessions and reached a peak early in the 30% fat diet period. After a short stabilization in this period intake declined progressively through the remainder of the fat diet sessions, finally leveling off at a low level when ground chow was reintroduced. Aspects of the overall intake in these animals, such as the low preference for ground chow and the early high preference for the

TABLE 2

DIFFERENTIAL EFFECTS OF DIET MANIPULATIONS ON INTAKE AND BODY WEIGHT IN HYPERPHAGICS AND NORMALS

Group	Diet periods																	
	Preop (chow)				Postop (chow)				30% fat diet				50% fat diet		Chow		0 (gm/day)	5 (gm/day)
Food intake (Kcal.)																		
Experimental (n = 5)	61.6	60.8	59.9	60.4	88.4	92.2	97.8	101.2	165.9	164.8	148.8	133.5	114.1	90.2	28.9	28.1		21.3
Control (n = 8)	64.2	68.0	65.5	65.0	75.2	77.8	81.2	76.9	91.3	75.0	75.5	75.0	90.3	82.3	49.7	54.0		21.3
Water intake (ml.)																		
Experimental	25.6	27.0	25.3	25.5	33.1	32.4	31.1	32.0	26.0	28.6	22.4	21.5	12.5	10.2	10.2	9.8	4.5	8.1
Control	25.5	28.5	25.6	25.4	34.0	35.0	32.4	31.7	17.4	18.1	16.8	16.7	13.8	14.6	22.9	23.1	20.6	20.8
Body weight (g/10)																		
Experimental	25.2	25.5	25.8	26.0	28.2	29.1	30.5	31.8	35.9	40.4	44.2	47.0	49.6	50.4	48.4	46.4	41.9	39.2
Control	25.9	26.3	27.0	27.0	27.2	27.4	27.8	27.	28.8	29.4	30.1	30.7	31.5	32.3	32.5	31.6	28.2	25.9

Note.—Scores are means for 5-day blocks.

fat diet, are common to hyperphagics in general. The only conspicuous difference was in the reduction in intake as the animals continued on the fat diets. The rate of reduction appears to be steeper than in similar rats fed diets with a lower fat content (see data of Brobeck, Tepperman, & Long, 1943; Brooks & Lambert, 1946; Teitelbaum, 1961).

Water intake in the control group decreased as fat in the diet was increased, much as in the first experiment (Table 2). When ground chow was presented again water intake increased, but not to preoperative levels. In the hyperphagic animals water intake began a general descent in the first fat diet period, which roughly paralleled the decrease in food intake for the remainder of the experiment. As noted in previous reports (Corbit & Stellar, 1964; Stevenson, 1949), the water/food ratio of the hyperphagics was lower than that of the controls for all periods after surgery. Ratios for the control group were 1.7, 1.8, 1.2, 1.1, and 1.9 for the preoperative, postoperative, 30% fat, 50% fat, and chow diet periods, respectively. Ratios for the experimental group for the same periods were 1.8, 1.4, 0.9, 0.7, and 1.5.

The hyperphagic animals showed an increased weight gain which was accentuated in the high fat diet periods (Table 2). The body weight curve contained dynamic and static phase components characteristic of the weight function of the hypothalamic hyperphagic preparation. Much weight was lost when the rats voluntarily decreased their intake of chow and when intake restrictions were imposed. Body weight in the control animals rose more slowly, stabilized during the last chow period, and decreased significantly only when they were starved and when allowed 5 gm. of pellets per day.

The body weight increases seen in hypothalamic hyperphagia are primarily the result of increases in stored fat (Bates, Nauss, Hagman, & Mayer, 1955; Hetherington & Ranson, 1940; Montemurro & Stevenson, 1960; Smith, 1927). Bates et al. (1955) found that increased fat content accounts for more than 90% of the increases in weight in animals made obese through hypothalamic lesions. Montemurro and

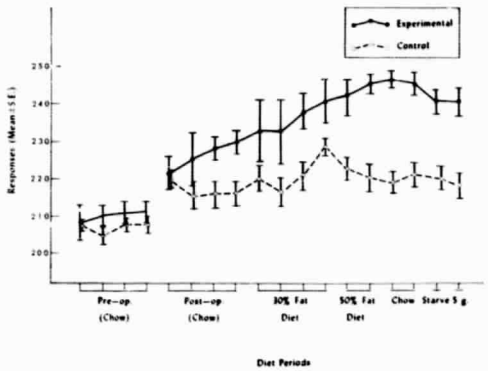

FIG. 2. Effects of hypothalamic lesions and diet manipulations upon heat-escape responding.

Stevenson (1960) noted that female hypothalamic obese rats contained approximately 240% more fat, 10% more protein, and 18% more water than controls when both groups were fed a high fat diet. It is presumed that the hyperphagics of this experiment are not different from those in the earlier experiments in that the marked increases in body weight are largely the consequence of increments in fat. The weight reduction in the later periods is also primarily fat since extra fat spares other materials, such as protein, when weight is lost (Montemurro & Stevenson, 1960).

Heat-escape responding. As indicated in Figure 2 the two groups did not differ initially in the number of heat-escape responses made in a 1-hr. session. Halfway through the postoperative period, however, the hyperphagics began to make more responses than controls and continued to respond at a higher level for the remainder of the experiment. The hyperphagic's record on this measure shows a progressive increase, a period of relative stability, followed by a decrease in the last two periods of the experiment. Comparison with Table 2 shows that the increase, stabilization, and decrease in heat-escape responding roughly correspond to changes in body weight.

Thermoregulatory responding in the control animals increased sharply during the first 5 days after the recovery period and then stabilized at a lower level for the remainder of the postoperative period. This effect has been noted previously (Lipton, 1968) and is presumed to reflect a physiological readaptation to the heat stress. The response record of the control group showed a marked increase only during the 30% fat diet period that was reliably ($p < .05$) higher than the postoperative responding.

Comparison of the heat-escape response curve of the controls (Figure 2) with their body weight figures (Table 2) indicates that the relationship between these variables is much less pronounced than that seen in the hyperphagics. The greatest responding did occur during the 30% fat diet period where weight gain was the greatest but response measures did not parallel further increases and decreases in body weight. This relatively low correspondence compared with that shown by the hyperphagics may be a consequence of the smaller amount of weight gained and lost and the much smaller fat component presumably involved in the changes.

Colonic temperature. Neither group showed marked changes in presession colonic temperature until late in the experiment when the measures decreased. The mean for the control group was 37.4°C. up to the last two diet periods when it decreased to 36.8°C. In the hyperphagic group the mean was 37.6°C. until the last three diet periods. It dropped to 37.4, 37.1, and finally, to 37.0°C. in the last chow, total deprivation, and restricted intake periods, respectively.

Postsession colonic temperature in the controls was stable around a mean of 39.4 (±0.2°C.) throughout the experiment. This average is somewhat higher than that seen in the normal animals of Experiment 1 (38.8–38.9°C.). The long-term stability of the group means results from the consistency with which individual animals adhered to characteristic levels of hyperthermia. For instance, one of the control animals maintained his mean postsession temperature at

39.2°C. and another at 39.5°C. at all periods of the experiment.

During most of the experiment the hyperphagics, like the controls, held their postsession colonic temperatures around 39.4°C. There were exceptions to this: In the first days of the postoperative period the mean temperature rose to 39.9°C.; and in the last three diet periods it dropped to 39.2°C. (last chow period) and then to 38.9°C. (starved and restricted intake periods). These results are closely related to the level of responding. As the responding increased in the postoperative period, the mean postsession colonic temperature returned to 39.4°C. and remained at this value until the last three diet periods. During these periods heat-escape responding stabilized and then decreased. The reduction in responding was not large enough to allow the postsession temperatures to be maintained at the previous level. A closer adherence to this "standard" temperature level would have resulted in a closer compliance between the body weight function and the behavioral response function.

Discussion

Increasing the lipid content of the diet fed to rats produced changes in heat-escape and intake measures in both experiments. In normal adult male rats, diets high in fat produced increases in heat-escape responding without changing pre- or postsession colonic temperatures. This effect, together with the fact that controls maintained consistent body temperatures over the experiment without a change in responding, indicates that the fat diets compromised physiological temperature regulation in some fashion.

The much younger and lighter females (Experiment 2) fed similar diets showed an increase in responding that was pronounced only during the 30% fat diet period. This discrepancy in effects between the experimental group of the first experiment and the controls of the second could be the result of many factors. The data of Reed et al. (1930) show that female rats fed a high fat diet gained less weight and had a lower proportional body fat content than males. This finding reconciles the failure of responding to increase in the females with the fat component explanation of response increases seen in the males of Experiment 1. Other factors such as differences in initial body weight (Experiment 2 females initially 200 gm. lighter, 300 gm. lighter than Experiment 1 males during respective 50% fat diet periods), differences in the sequence of the experimental procedure, differences in temperature regulation capacity accompanying reproductive cycles, and in other variables, undoubtedly contributed to the discrepancy.

In the hyperphagic animals heat-escape responding increased during the high fat diet periods. However, it had already begun to increase in the postoperative chow period, before the inception of these diets. This fact, in conjunction with the rise in body weight at about the same time, the further progressive increase in responding as body weight increased, the decline in responding when body weight decreased, and the high probability that most of the weight changes were primarily fat, suggests a body fat explanation for the changes in behavioral thermoregulation. When fat stores are increased through hypothalamic lesions, high fat diets, or both, physiological heat-loss capacity appears to be restricted. Heat production may also be increased with enlarged subcutaneous adipose tissue stores as a result of the extremely rapid rate of triglyceride turnover, activation of CoA, and esterification that are characteristic of the metabolic activity of this tissue (Cahill, 1962, p. 126), and the increased energy required to move the heavier body. To mitigate the resulting compromise in physiological temperature regulation, rats increase their heat-escape responding. Then, as fat stores are reduced, heat is lost more rapidly and responding would be expected to decrease. A reduction in responding as

weight was lost was found in the hyperphagic animals of the second experiment. The normal females did not show large increases in either weight or responding and thus a reduction in responding could not be expected.

The increased thermoregulatory responding as the hyperphagics gained weight is conceptually consistent with previous findings obtained using similar preparations in a different context. Hamilton (1963) noted that hypothalamic obese rats, when required to increase their operant responding for food reinforcement, began to extinguish at ratios above FR 32. Under the fixed ratio contingencies used in his experiment the animals were forced to exercise and consequently developed a relative hyperthermia. The progressive nature of the hyperthermia as the FR requirements were increased, the close relation between peak responding and peak body temperature, the decline in body temperature as responding began to extinguish, and the upward shift in the response function when the experiment was repeated in a 10.0°C. environment indicate that body temperature rather than food intake was more important in the motivation of these obese animals. That is, rather than maintain a constant food intake through exercise the hyperphagics chose to control body temperature.

Two points are available from the data of this experiment on food intake which are corroborative of Experiment 2 findings and interpretations. First, the obese rats developed hyperthermia when the body heat load was increased through exercise. This result was noted earlier by Han and Brobeck (1961) who found a positive connection between rate of weight gain and body temperature after exercise. Presumably, the insulative qualities of the body fat described above contributed to the hyperthermia by restricting heat loss although the heat produced in the exercising of a body of greater weight probably interacted with this insulation to compound the overall thermal effect. Second, the hyperphagics used behavioral means to reduce the threat of further hyperthermia much like those in the present research. They decreased responding for food reinforcement to avoid endogenous hyperthermia while the obese rats of Experiment 2 increased responding on a low response-cost schedule to avoid an external heat load.

REFERENCES

Bates, M., Nauss, S., Hagman, N., & Mayer, J. Fat metabolism in three forms of experimental obesity: Body composition. *American Journal of Physiology*, 1955, **180,** 301–303.

Brobeck, J. R., Tepperman, J., & Long, C. N. Experimental hypothalamic hyperphagia in the albino rat. *Yale Journal of Biology and Medicine*, 1943, **15,** 831–853.

Brooks, C. McC., & Lambert, E. F. Study of the effect of limitations of food intake and the method of feeding on the rate of weight gain during hypothalamic obesity in the albino rat. *American Journal of Physiology*, 1946, **147,** 695–707.

Cahill, G. F. Introduction to F. L. Engel's paper *Metabolic activity of adipose tissue*. In L. W. Kinsell (Ed.), *Adipose tissue as an organ*. Springfield, Ill.: Charles C Thomas, 1962.

Corbit, J., & Stellar, E. Palatability, food intake, and obesity in normal and hyperphagic rats. *Journal of Comparative and Physiological Psychology*, 1964, **58,** 63–67.

Dugal, L.-P. Resistance to extreme temperatures in connection with different diets. *Proceedings of the Fourteenth Meeting of the Associative Commission for Medical Research*, National Research Council, Canada, 1944, No. C1029.

Dugal, L.-P., & Thérien, M. Ascorbic acid and acclimatization to cold environment. *Canadian Journal of Research*, 1947, **25** E, 111–136.

Fábry, P., Braun, T., Petrásek, R., Horáková, E., & Konopásek, E. Effect of the pattern of food intake on the carcass composition of rats receiving diets with varying fat content. *Physiologia Bohemoslovenica*, 1964, **13,** 333–339.

Fenton, P., & Dowling, M. Studies on obesity: 1. Nutritional obesity in mice. *Journal of Nutrition*, 1953, **49,** 319–329.

Giaja, J., & Gelineo, S. Alimentation et resistance au froid. *Compte rendu de l'Académie des Sciences*, 1934, **198,** 2277–2278.

Hamilton, C. L. Interactions of food intake and temperature regulation in the rat. *Journal of Comparative and Physiological Psychology*, 1963, **56,** 476–488.

Hamilton, C. L. Rat's preference for high fat diets. *Journal of Comparative and Physiological Psychology*, 1964, **58,** 459–460.

Han, P. W., & Brobeck, J. R. Temperature regu-

lation of rats with hypothalamic hyperphagia. *American Journal of Physiology*, 1961, **200**, 703–706.

HETHERINGTON, A. W., & RANSON, S. W. Hypothalamic lesions and adiposity in the rat. *Anatomical Record.*, 1940, **78**, 149–172.

LARSSON, S. Factors of importance for the etiology of obesity in mice. *Acta Physiologica Scandanavica*, 1967, (Suppl. 294).

LIPTON, J. M. Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat. *Physiology & Behavior*, 1968, **3**, 165–169.

MITCHELL, H., GLICKMAN, N., LAMBERT, E., KEETON, R., & FAHNESTOCK, M. Tolerance of man to cold as affected by dietary modification: Carbohydrate versus fat and the effect of the frequency of meals. *American Journal of Physiology*, 1946, **146**, 84–96.

MONTEMURRO, D. G., & STEVENSON, J. A. F. Survival and body composition of normal and hypothalamic obese rats in acute starvation. *American Journal of Physiology*, 1960, **198**, 757–760.

REED, L., YAMAGUCHI, F., ANDERSON, W., & MENDEL, L. Factors influencing the distribution and character of adipose tissue in the rat. *Journal of Biological Chemistry*, 1930, **87**, 147–174.

SCHMIDT-NIELSEN, K. *Desert animals: Physiological problems of heat and water.* New York: Oxford Press, 1964.

SMITH, P. E. Disabilities caused by hypophysectomy and their repair. *Journal of the American Medical Association*, 1927, **88**, 158–161.

STEVENSON, J. A. F. Effects of hypothalamic lesions on water and energy metabolism in the rat. *Recent Progress in Hormone Research*, 1949, **4**, 365–394.

STROMINGER, J. L., BROBECK, J. R., & CORT, R. L. Regulation of food intake in normal rats and in rats with hypothalamic hyperphagia. *Yale Journal of Biology and Medicine*, 1953, **26**, 55–74.

TEITELBAUM, P. Sensory control of hypothalamic hyperphagia. *Journal of Comparative and Physiological Psychology*, 1955, **48**, 156–163.

TEITELBAUM, P. Disturbances in feeding and drinking behavior after hypothalamic lesions. *Nebraska Symposium on Motivation*, 1961, **9**, 39–69.

WILCOXON, F. Individual comparison by ranking methods. *Biometrics*, 1945, **1**, 80–83.

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Determinants of Behavioral Thermoregulation Against Heat: Thermal Intensity and Skin Temperature Levels[1]

JAMES M. LIPTON, D. D. AVERY[2] AND D. R. MAROTTO

The University of Texas Medical School at Dallas, Dallas, Texas, U.S.A.

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LIPTON, J. M., D. D. AVERY AND D. R. MAROTTO. *Determinants of behavioral thermoregulation against heat: Thermal intensity and skin temperature levels.* PHYSIOL. BEHAV. 5 (10) 1083–1088, 1970.—To further elucidate the factors controlling thermoregulatory behavior two experiments were conducted using rats trained to escape heat by holding down a lever. In both experiments the time spent responding was found to increase in a monotonic fashion as thermal intensity was increased. Determination of the behavioral response was explored in more detail by recording dorsal skin temperature at the moment when a response was begun and when it was terminated. In a neutral environment (23°C) a relatively stable level of skin temperature was maintained in the face of intensity alterations as a result of the changes in behavior. A qualitatively similar result was also seen when the animals were tested in a hot environment (32°C). These findings are consistent with the results of previous heat reinforcement experiments which have indicated a peripheral basis for the control of thermoregulatory behavior. In the cold (5°C), however, raising intensity brought about a linear increase in responding and in the skin temperature levels maintained, which suggests that the determination of behavior in this experiment may be more complex.

Behavioral thermoregulation Skin temperature determinants of thermoregulative behavior Heat-escape

THERMOREGULATION can be reasonably divided into two components: a physiological component made up of heat production and heat loss mechanisms and a behavioral component which has sensory, motivational and response aspects. Physiological thermoregulation controls body temperature within narrow limits although its power to counteract thermal stress is relatively small. Behavioral thermoregulation, on the other hand, exerts a less precise control but is very powerful in defending against great temperature extremes through the initiation and maintenance of various voluntary actions [5]. While in the normal life of homeotherms both components interact to assure a viable thermal level, the great power and modifiability of the behavioral component make its investigation of particular interest for theoretical and practical reasons.

Exploration of variables acting on the behavioral component of thermoregulation in the cold has implicated hypothalamic temperature [3, 7, 16, 17], peripheral temperature [8, 16, 21], reinforcement magnitude [1, 2, 4, 8, 20] and widespread receptors [2] in the determination of the response. The general appearance of a relation between responding for heat in the cold and changes in intensity or duration of thermal reinforcement in these earlier experiments prompted the investigation of similar relations in the heat-escape situation. The results of the investigation of intensity factors and the question of immediate determinants of responding led to a second experiment in which skin temperature was measured at the time a response was initiated and terminated.

EXPERIMENT 1

Weiss and Laties [21], using rats working for heat reinforcement, found that response rate decreased as the intensity of the heat was increased. Since that time similar findings have been reported by other investigators using rats [7, 8], monkeys [7], pigs [2, 4] and mice [1]. Experiment 1 was designed to explore the intensity-behavior relation in the heat escape situation for comparison with the results of these previous studies.

Method

Six male albino rats (Charles River Breeding Laboratories) maintained at 300 g body weight by restricting the diet of Purina laboratory pellets were used in this experiment. Except for testing, the animals were housed in standard cages at 23°C (dry bulb). Water was freely available in the home cages.

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[2]Postdoctoral Fellow in Psychobiology. Present address: Psychology Department, Colorado State University, Ft. Collins, Colorado.

The operant apparatus has been described in detail previously [13–15]. The animal chamber consisted of a Plexiglas cylinder set into a manifold box. The manifold box was equipped with an exhaust fan (100 cfm) which pulled ambient air through the cylinder. Both the floor and the manipulandum were constructed of glass tubing in order to reduce heat absorbtion. A 250 W red bowl heat lamp was centered over the top of the chamber. In these experiments the exhaust fan operated continuously during a session and current to the lamp was controlled through the response bar microswitch. Rate of heating was controlled by manipulating voltage to the heat lamp. Four levels of power dissipation were used: 100, 150, 200 and 250 W. Comparison of the four rates of heating a Yellow Springs No. 425 thermistor probe taped to the chamber floor is seen in Fig. 1. Note that the 75 W red-coated light bulb used as a control for photic aversion factors produced little heating effect. In the first experiment a pair of operant chambers located in an environment of 23.0 ± 0.5°C. (range) were used.

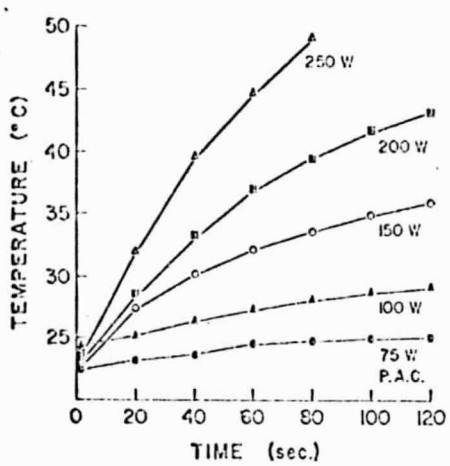

FIG. 1. Heating rates as a function of time and intensity of the infra-red bulb. P.A.C. = photic aversion control (luminous bulb).

Procedure

As in earlier experiments [13–15] the animals were trained to press a bar in order to escape heat in daily sessions of progressively greater length. When reliable responding was attained, the sessions were extended to one hour. During the shaping period and for five days after the sessions were lengthened to one hour, the heat lamp intensity was 250 W. Thereafter, one of the four intensity levels was presented each day in a random sequence until all animals had experienced all intensities three times. The control for photic aversion effects was presented to five rats for one session at the end of the series.

In the daily procedure each animal was run at the same time each day in his accustomed chamber. Immediately before and after a session the animals were weighed and their colonic temperatures were measured with a thermistor probe (6 cm insertion). The number of responses and the total time that the lamp was held off during a session were recorded automatically.

Results and Discussion

Figure 2 shows the duration that the heat lamp was held inactive by the individual animals at each level of intensity.

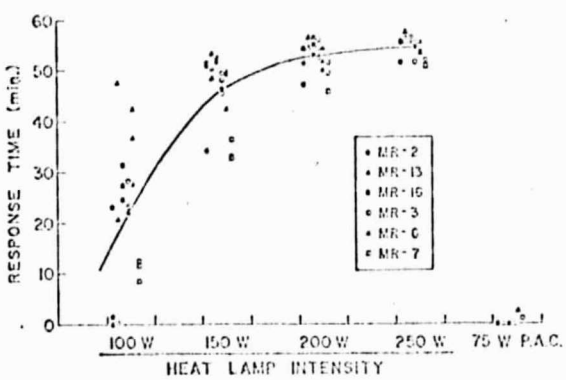

FIG. 2. Duration of bar holding to escape heat resulting from manipulations of intensity. MR plots represent scores of individual animals. P.A.C. = photic aversion control.

Two points are clear from these data: first, behavioral responding increased with increments in intensity; second, as the intensity of the lamp was increased both the inter- and intra-individual variability of behavioral thermoregulatory responding decreased. The mean response durations per session for each lamp intensity from 100 to 250 W were: 22.1, 46.2, 52.8 and 54.6 min. These scores indicate that when a thermal stimulus is increased rats tend to mitigate against the greater heat by increasing the rate of thermoregulatory responding. This finding is, appropriately, just the reverse of results seen in heat reinforcement studies where general decreases in behavioral thermoregulatory responding occurred when heat lamp intensity was increased [1, 2, 4, 7, 8, 21]. It is clear that under both contingencies, bar pressing for heat or to reduce it, thermoregulatory responding is affected by intensity in a manner which is generally salutary to the animal's thermal homeostasis.

If the rats were responding to escape increased photic stimulation one might expect results much like those obtained. However, when the brighter luminous bulb was substituted for the heat lamp the animals responded very little (Fig. 2) which indicates that thermal rather than photic aversion factors were responsible for the behavioral changes.

Another aspect of the intensity–response data of the present experiment which has some counterparts among the results on animals working for heat in the cold is the lack of proportionality between increases in responding and increases in intensity. In one of the previous heat reinforcement experiments [21] the response measures showed a lack of consistent proportionality with lamp intensity at both high and low intensity extremes. In a similar investigation [7] it was found that the decrease in working for thermal reinforcement was not inversely proportionate to the heating effect so that more heat was obtained at high intensities than at low intensities. Baldwin [1], using a strain of hairless mice pressing for heat, found that the animals obtained almost identical amounts of heat with 150 and 250 W settings of the heat lamp but considerably less at 300 W when they were allowed to control duration of reinforcement. In still another experiment, reducing the intensity of the thermal stimulus by decreasing the number of heat lamps from 12 to 6 was found to result in a disproportionately low (less than double) increase in responding in pigs [4]. Of particular interest are the recent results of Carlisle [3] which indicate that the relation between the heat obtained and the heat intensity settings is curvilinear. When

the group data were plotted using log-log coordinates, however, the function was found to be described by a straight line. For purposes of comparison, an equation was determined for a third degree polynomial model using log transformations of the intensities and of the response duration data of the present experiment. When the third degree term did not contribute significantly ($F < 1.0$, $df = 1, 69$), a second order equation was determined and the second degree term tested for its contribution. It was concluded that this equation is appropriate since the contribution of the second degree term was significant ($F > 4.2$, $df = 1, 70$) at the 0.05 level. It appears that the present data are best described by a curve rather than a straight line even when log transformations are made. This lack of linearity either with or without transformation suggests caution when comparing the size of behavioral effects noted under different intensity conditions. These findings suggest that the relatively small increase or decrease in responding which results from some treatment condition in an experiment using high intensities may be as meaningful as a large difference when lower intensities are used.

The number of responses per session increased with intensity, averaging 76.1, 95.7, 107.7 and 98.3 for the 100–250 W intensities, respectively. Average response durations showed a monotonic increase as lamp intensity was increased. The values for each intensity from 100 to 250 W were 17.4, 28.8, 29.4 and 33.6 sec. These data indicate that the general strategy for coping with progressively higher intensities was that of increasing the length of the response rather than increasing the number of shorter responses. This change in strategy is similar to that found in previous information collected on rats working for heat in a cold environment [7].

Presession colonic temperature was very stable and showed no large shifts over time (mean = 37.5°, range = 36.6–38.0°C) over all sessions. Postsession temperature, the end point often used as a measure of thermoregulation, varied only slightly around a mean of 38.1°C (range = 37.3–39.0°C). The persistence of this postsession temperature in spite of the changes in heat lamp intensity has prompted the suggestion that it may be a determinant of behavioral thermoregulatory responding [15].

EXPERIMENT 2

Since deep body temperature is protected against sudden variations by insulation, counteracting physiological mechanisms, etc., it is unlikely that it alone could function as a stimulus in the determination of moment to moment behavioral responding. A stronger candidate for the primary role in this determination is skin temperature change since the skin is exposed more directly to thermal stimulation and is very sensitive to alterations in intensity and rate of heating [12]. Empirical evidence supporting the importance of peripheral temperature in behavioral thermoregulation has been found previously by Weiss and Laties [21]. They noted that rats altered response rate with changes in heat reinforcement intensity, which resulted in a constant subcutaneous temperature being maintained. By eliminating hypothalamic temperature changes on the basis of temporal asynchrony Carlisle [7], too, has come to stress the importance of peripheral stimulation in behavioral thermoregulation against cold.

If a stable skin temperature is the requirement which controls behavioral regulation against heat one would expect to find reasonably consistent levels to be maintained when intensity is manipulated. Experiment 2 was designed to evaluate this possibility by measuring skin temperatures associated with the initiation and termination of the thermoregulatory response. Ambient temperature was varied in order to see how it affects skin temperature–behavioral response relations and to add to the response strategy information collected in the first experiment.

Method

Subjects. Five of the six rats used in the previous experiment served as subjects. The missing animal, MR-3, would not readily adapt to the skin probe and was deleted in the training sessions. Maintenance of the animals was the same as in the earlier experiment.

The operant chambers were the ones used in Experiment 1. In this case, however, they were situated in either a normal environmental temperature of 23.0 ± 0.9°C (dry bulb, range) or temperatures of 5.0 ± 0.5 or 32.0 ± 0.5°C. The latter temperatures were maintained in a Hotpack environmental chamber while the 23.0°C tests were run in a small laboratory with a less precise thermostatic control. While in previous investigations [21] skin temperature was inferred from subcutaneous measurements calibrated against recordings made on the skin of anesthetized rats, direct measurement of skin temperature in behaving animals was successfully attempted. Skin temperature was recorded from a YSI thermistor probe with reflective back (No. 425) held in contact over the shaven scapulae by a rubber band harness. Owing to the flexibility of the lead wire connected to the probe, restraint was not great. Temperature level at the time a response was initiated and terminated was recorded on a Grass recorder and/or recorded manually by an observer from a YSI Telethermometer. Responses of less than 5 sec duration were not used in the analysis since these were generally associated with grooming and saliva spreading rather than with more direct thermoregulatory behavior.

Reliable records of skin temperature are difficult to obtain even when conditions are constant [11, 19]. In the present experiment great care was taken to assure the repeatability of placement and pressure of the probe in order to surmount some of the problems. While the absolute values noted using this technique would perhaps vary from those recorded through the use of some other method of thermometry, the general relations obtained were found to be reliable among animals.

Procedure

Following Experiment 1 the animals were allowed 2–3 days without operant sessions. During this time they became adapted to the rubber band harnesses by wearing them continuously in their home cages. Behavioral thermoregulatory sessions were then resumed in the 23.0°C environment with the skin probe connected. The four heat lamp intensities used in Experiment 1 were presented once to each animal in random order over daily sessions. Following the 23° sequence, sessions were begun in the 5° environment with lamp intensity randomly varied as before. After two trials the 100 W setting was deleted because the animals would not respond to turn off the lamp at this intensity. To avoid potential effects of acclimatization to this cold exposure sessions were spaced one to two days apart. A procedural sequence similar to the 5° environmental temperature series was then conducted in the 32°C environment.

Result: 1. *Skin Temperature*

23°C *environment.* In the neutral environment three points are obvious from the skin temperature data (Fig. 3). First, there is little variability associated with the mean temperature at response initiation or termination except for some disparate scores for pressing at 100 W. Second, skin temperature measured at the time of response initiation was not completely stable across intensities but tended to increase slightly as heating rate was increased. Third, at all intensities except 100 W the mean release temperatures were about the

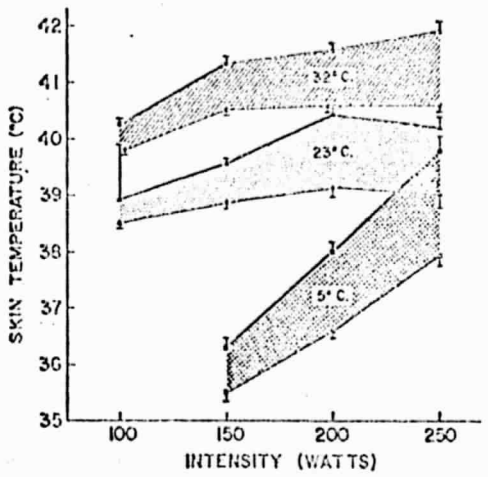

FIG. 3. Skin temperatures (mean ± S.E.) associated with response initiation and termination.

same. The average release temperatures for the 150, 200 and 250 W settings were 38.9, 39.1 and 39.0°C, respectively. For the 100 W setting the average skin temperature at release was 38.5°C. Skin temperatures were thus held within a fairly narrow range as a result of changes in behavior. The upper limit of this range was defined by the temperatures recorded at response initiation, values which were slightly affected by intensity. The lower boundary was set by the release temperatures which did not vary greatly. At the widest extremes (highest mean press temperature and lowest mean release temperature) the band was 2°C wide.

32°C *environment.* At this ambient temperature, decidedly above thermoneutrality for the rat, the overall heat load was substantially increased. Under these conditions the skin temperature associated with responding showed some parallels with results obtained in the 23°C environment. For example, low variability in skin temperature at response initiation and termination was characteristic at each of the intensity levels. Another similarity of note was the stability in mean release temperature at 150, 200 and 250 W intensities. The values for these intensities were 40.5, 40.5 and 40.6°C, respectively, indicating that the levels at release were even slightly more consistent in the 32° environment than in the normal temperature series (Fig. 3). In this high ambient temperature one might suspect physical factors to be responsible for the consistency by limiting the degree of skin cooling. However, the skin temperature at release in the 100 W series was more than 0.5° lower, ruling out the possibility that floor or constraining physical effects produced the stability by showing that temperatures lower than 40.5°C could have been reached in this environment.

A further similarity lies in the progressive increase in skin temperature associated with response initiation as heat lamp intensity was increased. While this was a trend in the 23°C environment, the effect is quite clear in the warmer ambient temperature. Skin temperature at release during the 100 W intensity sessions was some 0.7–0.8°C less than the otherwise stable level of 40.5–40.6°C and recalls a similar effect at this intensity in the neutral ambient series. This low mean release temperature and the highest mean temperature at response initiation (250 W intensity) describe limits of 39.8 and 41.9°C between which skin temperature was generally held. This band is similar to the 2.0° band seen in the neutral environment although it is displaced upward 1–2°C. It appears that in this environment, also, skin temperature was maintained within a narrow preference band as a result of changes in behavior.

5°C *environment.* At this temperature the effect of the heat lamp was reduced to the point that the two animals tested at the lowest intensity setting chose to accept the radiant heat for the duration of the session. When the intensity was increased to 150, 200 and 250 W, the skin temperature at both initiation and termination increased linearly (Fig. 3) unlike the results obtained in the warmer environments.

Result: 2. *Responses*

The curvilinear relation between mean response duration and heat lamp intensity found in Experiment 1 was repeated in the 23°C environment. However, while the basic relative positions were about the same, quantitatively the duration values were lower. This depression was, presumably, the result of the presence of the thermistor probe on the backs of the animals. On the other hand, the number of responses made per session (Table 1) was increased. Thus, while the finding of an increasing duration/response ratio with increasing intensity seen in Experiment 1 holds true here as well, the absolute size of the ratio is lower.

Surprisingly, the duration data collected in the hot environment did not differ from that gathered at ambient 23°C (Table 1). Since ceiling effects can be discounted on the basis of the total response time possible and the higher response

TABLE 1

MEAN (± S.E.) NUMBER OF RESPONSES AND TOTAL RESPONSE DURATION PER SESSION. DURATION SCORES ARE IN MINUTES

	Intensity (W)			
	100	150	200	250
		23°C		
Responses	48.3 ± 20.8	242.6 ± 45.1	149.0 ± 17.4	172.0 ± 38.4
Duration	4.1 ± 1.3	32.3 ± 1.8	45.0 ± 3.1	49.9 ± 2.0
		32°C		
Responses	61.4 ± 22.4	144.4 ± 7.4	159.0 ± 7.4	165.0 ± 28.3
Duration	6.6 ± 3.1	34.0 ± 0.9	47.2 ± 0.8	49.3 ± 0.9
		5°C		
Responses	—	128.0 ± 52.3	203.4 ± 43.4	202.0 ± 45.5
Duration	—	8.0 ± 6.0	24.1 ± 2.2	34.7 ± 1.2

durations seen in Experiment 1 it appears that the hot environment either did not increase motivation to escape heat or the cooling reinforcement was not great enough to sustain increased responding. In the cold environment, however, response durations were much lower than in either of the other temperatures. Responding to escape heat showed a linear increase with intensity in parallel with the skin temperature function.

Result: 3. *Body Temperature*

Presession colonic temperatures were quite stable around a mean of 37.4°C during all conditions of the experiment (range = 36.6–38.3°). Postsession temperatures in the neutral and hot environments were also quite consistent, generally falling between 38.8 and 39.2°C. (range = 38.0–39.8°). This behaviorally regulated slight hyperthermia has proven to be characteristic of animals working in similar heat escape [13–15] and in water-spray reinforcement [9] experiments. Body temperature did not change much during the sessions in the 5°C environment. The mean postsession temperature in this series (37.5°, range = 36.2–38.7°) was only 0.1°C greater than the presession level.

Discussion

It is clear that rats increase responding to mitigate a potentially large heat imbalance as the threat of such an imbalance is increased. This was noted in both experiments where raising the heat intensity brought about a compensatory change in escape behavior. Much the same type of behavioral change was recorded in earlier experiments when the possibility of a thermal imbalance was altered by producing lesions in the preoptic area [13], by desalivation [15], and by increasing body fat insulation [14]. Thus, with manipulation of both heat intensity and physiological thermoregulatory capacity behavior changes in the direction dictated by the drive to maintain thermal homeostasis—towards increased responding to escape heat. In this respect the results are much like the findings of previous heat reinforcement experiments [1, 2, 4, 7, 8, 21]. The thermoregulatory behavior investigated with both operant contingencies appears to be generally homeostatic in nature.

The precise sensory, motivational, and effector states which determine the occurrence of a behavioral thermoregulatory response at a particular point in time are not fully known. In the second experiment dorsal skin temperature was measured at response onset and termination to see if consistent values are associated with these events which could act as peripheral determinants of the behavior. When the animals were tested in the 23°C environment skin temperature was held within a preference range about 2°C wide. In the 32°C environment a similar, although higher, preference band was found. The relative consistency of skin temperature levels in the face of variations in thermal intensity is reminiscent of analagous information collected by Weiss and Laties [21] using animals working for heat reinforcement. These authors concluded that ". . . rats adjust reinforcement rate in accordance with reinforcement intensity to produce the end result of a constant peripheral temperature." The data gathered in the neutral and hot environments can be interpreted as illustrating direct peripheral control over behavior in a similar way. This interpretation is in keeping with previous human research which has revealed a close relationship between a particular mean skin temperature level and thermal comfort [22], and with the suggestion that discomfort provides an early motivational influence for the behavioral avoidance of thermal imbalances [10, 18]. The simple fact that the skin contains thermosensitive receptors and that it is the first organ to change state when an animal is exposed to radiant energy of the type used here obviously recommends the skin determinant explanation also.

In the 5°C environment, however, the linear rise in skin temperature limits as intensity was increased is not compatible with an explanation of response determination in terms of a drive for peripheral thermal stability. While the data collected in the neutral and hot environments seem to illustrate two definite preference bands governing behavior, the impression here is one of a more complex determination. Limitations of scope do not allow conclusions about the identity of the factors responsible for the response in this case but one important possibility can be excluded: the influence of deep body temperature. Although it has been established that core temperature can affect the affective evaluation of peripheral thermal stimulation [6], in the present experiment colonic temperature did not vary with changes in intensity and therefore could not be responsible for these results. Further investigation is required to unravel the sensory and/or motivational variables contributing to response determination in this cold environment.

REFERENCES

1. Baldwin, B. A. Behavioral thermoregulation in mice. *Physiol. Behav.* 3: 401–407, 1968.
2. Baldwin, B. A. and D. L. Ingram. Behavioral thermoregulation in pigs. *Physiol. Behav.* 2: 15–21, 1967.
3. Baldwin, B. A. and D. L. Ingram. The effect of heating and cooling the hypothalamus on behavioral thermoregulation in the pig. *J. Physiol.* 191: 375–392, 1967.
4. Baldwin, B. A. and D. L. Ingram. Factors influencing behavioral thermoregulation in the pig. *Physiol. Behav.* 3: 409–415, 1968.
5. Benzinger, T. H., C. Kitzinger and A. W. Pratt. The human thermostat. In: *Temperature, Its Measurement and Control in Science and Industry*, edited by J. D. Hardy. New York: Reinhold, 1963, Vol. 3, pp. 637–665.
6. Cabanac, M. Plaisir ou déplaisir de la sensation thermique et homeothermie. *Physiol. Behav.* 4: 359–364, 1969.
7. Carlisle, H. J. Heat intake and hypothalamic temperature during behavioral temperature regulation. *J. comp. physiol. Psychol.* 61: 388–397, 1966.
8. Carlisle, H. J. Peripheral thermal stimulation and thermoregulatory behavior. *J. comp. physiol. Psychol.* 66: 507–510, 1968.
9. Epstein, A. N. and R. Milestone. Showering as a coolant for rats exposed to heat. *Science* 160: 895–896, 1968.
10. Gagge, A. P., J. A. J. Stolwijk and J. D. Hardy. Comfort and thermal sensations and associated physiological responses at various ambient temperatures. *Envir. Res.* 1: 1–20, 1967.
11. Hardy, J. D. Method for the rapid measurement of skin temperature during exposure to intense thermal radiation. *J. appl. Physiol.* 5: 559–566, 1953.
12. Hensel, H. Physiologie der thermorezeption. *Ergebn. Physiol.* 47: 166–368, 1952.

13. Lipton, J. M. Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat. *Physiol. Behav.* 3: 165–169, 1968.
14. Lipton, J. M. Effects of high-fat diets on caloric intake, body weight and heat-escape responses in normal and hyperphagic rats. *J. comp. physiol. Psychol.* 68: 507–515, 1969.
15. Lipton, J. M. and D. R. Marotto. Effects of desalivation on behavioral thermoregulation against heat. *Physiol. Behav.* 4: 723–727, 1969.
16. Murgatroyd, D. Central and peripheral factors in behavioral thermoregulation in the rat. *Physiologist* 9: 251, 1966.
17. Satinoff, E. Behavioral thermoregulation in response to local cooling of the rat brain. *Am. J. Physiol.* 206: 1389–1394, 1964.
18. Stevens, J. C., L. E. Marks and A. P. Gagge. The quantitative assessment of thermal discomfort. *Envir. Res.* 2: 149–165, 1969.
19. Stoll, A. M. and J. D. Hardy. Study of thermocouples as skin thermometers. *J. appl. Physiol.* 2: 531–543, 1950.
20. Weiss, B. and V. G. Laties. Magnitude of reinforcement as a variable in thermoregulatory behavior. *J. comp. physiol. Psychol.* 53: 603–608, 1960.
21. Weiss, B. and V. G. Laties. Behavioral thermoregulation. *Science* 133: 1338–1344, 1961.
22. Winslow, C.-E.A., L. P. Herrington and A. P. Gagge. Relations between atmospheric condition, physiological reactions and sensations of pleasantness. *Am. J. Hyg.* 26: 103–110, 1937.