Hypothalamic Temperature and the 22 kHz Vocalization of the Male Rat

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BLUMBERG, M. S. AND H. MOLTZ. *Hypothalamic temperature and the 22 kHz vocalization of the male rat.* PHYSIOL BEHAV **40**(5) 637–640, 1987.—Following ejaculation the male rat emits a 22 kHz vocalization that has been hypothesized to play a communicative role. We found previously that this vocalization is often accompanied by rapid and selective hypothalamic cooling, and we hypothesized that the vocalization or, more precisely, the thoracic-laryngeal maneuver underlying the vocalization, is primarily a thermoregulatory behavior. Accordingly, one prediction made herein was that heating the brain of the isolated male, through the infusion of prostaglandin E_2, would be accompanied by the vocalization. Another was that cooling the brain of the copulating male through the injection of sodium salicylate would significantly reduce the post-ejaculatory vocalization. Both predictions were confirmed.

Ultrasound PGE_2 Temperature   Rat   Sexual behavior   Sodium salicylate   Communication

WE found recently [7] that copulation in the male rat is accompanied by a significant rise in both hypothalamic temperature and deep body temperature, but that once ejaculation occurs the hypothalamus, relative to the body, undergoes rapid cooling. Often accompanying this selective hypothalamic cooling is a train of 22 kHz vocalizations that has been hypothesized to have communicative value, the "message" allegedly directed to the female partner in some instances and to male conspecifics in other instances [1,3]. Impressed, however, by the association between hypothalamic cooling and the emission of the vocalization, we suggested that the vocalization may be thermoregulatory [7]. Specifically, we thought it may function to lower the temperature of the ventral brain.

To address fully the question of whether the 22 kHz vocalization plays a role in hypothalamic thermoregulation, we need to determine whether hypothalamic temperature (T_{hy}) influences the vocalization, and conversely, whether the vocalization influences hypothalamic temperature. Here we undertook the first half of the task. We administered drugs to raise and lower hypothalamic temperature, respectively, and predicted that a higher-than-normal hypothalamic temperature would be accompanied by the vocalization even when there was no opportunity to ejaculate and that a lower-than-normal hypothalamic temperature would not be accompanied by the vocalization even after repeated ejaculations.

EXPERIMENT 1: BRAIN HYPERTHERMIA AND THE 22 kHz VOCALIZATION

Our first prediction was that a rat made to generate an elevated brain temperature will emit the vocalization when not copulating. We tested this prediction by isolating males and infusing them with prostaglandin E_2. PGE_2, when introduced directly into the ventricular system, has been shown to induce a potent hyperthermia [17,23].

METHOD

We used eight male Wistar rats, 125–165 days old at the time of surgery. These males were housed individually in standard laboratory cages under a 12L:12D lighting schedule.

Each male was anesthetized with ketamine hydrochloride (87 mg/kg) and xylazine hydrochloride (13 mg/kg). A battery-operated telemetric thermosensor (Mini-Mitter, Inc., Sunriver, OR) was implanted stereotaxically in the rostral hypothalamus using the atlas of Paxinos and Watson [15]. The coordinates were 0.6 mm posterior to bregma, 0.5 mm lateral to the midsagittal suture and 8–9 mm below the surface of the horizontal skull. In addition, a guide cannula was implanted into the cerebral aqueduct. This cannula was fitted with a screw top and with a stylet to keep it unobstructed between infusions. The cannula was implanted on the midline with coordinates of 0.8–1.1 mm anterior to the interaural line and 5.2 mm below the surface of the skull at bregma. Both the transmitter and cannula were attached to the skull using skull screws and dental cement. All observations began at least six days following surgery.

Each thermosensor was calibrated in a temperature-controlled water bath both prior to surgery and then again at the conclusion of the experiment. Signals from the thermosensors were received by an AM radio, processed to remove noise and fed into a frequency counter set to the period mode. Data from the frequency counter were expressed in a form later converted to degrees Celsius using a regression equation derived from the calibrations for each thermosensor. With this system, we were able to detect temperature changes in intervals of 0.03 to 0.06°C.

The PGE_2 (Sigma) solution was prepared by dissolving 1 mg PGE_2 in 0.1 ml of 95% ETOH. After the drug was in solution, 0.9 ml of 0.9% sterile saline was slowly added. The control solution was 90% sterile saline and 10% ETOH
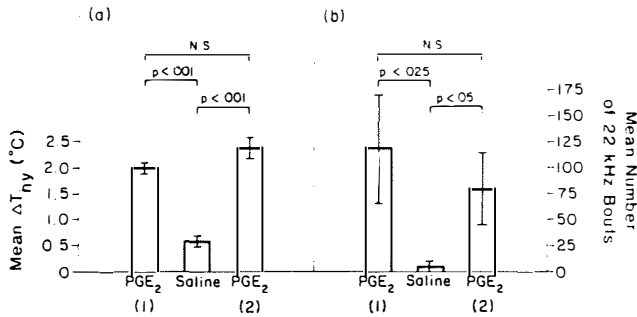
FIG. 1. (a) Mean peak changes in hypothalamic temperature and (b) mean number of 22 kHz bouts following each of two ICV infusions of PGE₂ and one ICV infusion of saline. The infusions were separated by an interval of at least two days. Vertical lines indicate ±SEM. Paired *t*-test.
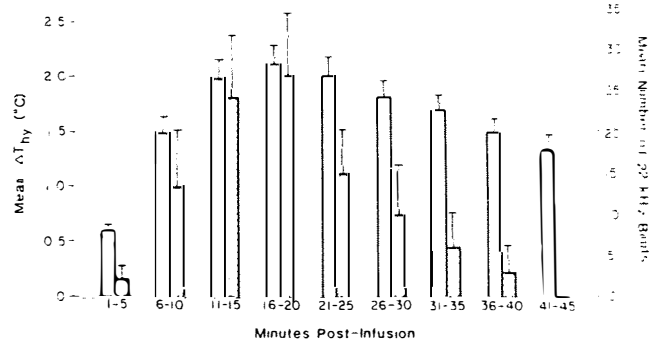

FIG. 2. Mean changes in hypothalamic temperature (clear bars) and mean number of 22 kHz bouts (hatched bars) following the ICV infusion of PGE₂. Data are for 8 animals with two tests per animal. Vertical lines indicate ±SEM.

(hereafter referred to as "saline"). Both the drug solution and the saline solution were aliquotted and then stored in a freezer until used.

All tests were conducted in the dark phase of the 12L:12D cycle. Prior to injection, each male was habituated to a semi-circular arena for at least 15 minutes. After the animal's temperature had stabilized for at least five consecutive minutes, he was taken from the arena, the top of his cannula was removed, and an injection cannula, connected by silastic tubing to a microliter syringe, was inserted into the guide cannula. 10–12 μl of solution (10–12 μg of PGE₂ or 10–12 μl of saline) was infused at the rate of 12 μl/min. Following infusion, the animal was returned to the arena.

The T_{hy} of each animal was recorded each minute for 45 minutes. Concurrently, we recorded the number of 22 kHz bouts using two Mini Bat-Detectors (QMC, Ltd., London) tuned to a range of 20–30 kHz. We defined a "bout" as the occurrence of a 22 kHz vocalization with any 5-second interval. There was, therefore, a maximum of 12 bouts/minute.

Each animal was infused on three different occasions, on two occasions with PGE₂ and on one with saline. For all but one animal, the control infusion was interposed between the two drug infusions. All infusions were separated by an interval of at least two days.

Histology was performed on the brains of three of our rats. Placement of the thermosensors within the rostral hypothalamus was confirmed for all three brains. Two of these brains were also checked and confirmed for proper cannula placement within the cerebral aqueduct.

RESULTS AND DISCUSSION

Figure 1a shows that PGE₂ had its expected hypothalamic effect: infusion of the prostaglandin elevated T_{hy} to a level significantly above that reached by the infusion of saline alone. Figure 1b shows that PGE₂ also elicited a significant increase in the number of 22 kHz vocalizations.

To determine whether the number of vocalizations emitted was related to changes in hypothalamic temperature, we combined the data of the two PGE₂ infusions for each animal and, across animals, calculated mean hypothalamic temperature and mean number of 22 kHz bouts during each 5-minute period of observation. These data are presented in Fig. 2. It is evident that the number of vocalizations emitted increased with increases in T_{hy}, reached a peak when T_{hy} was maximal and then decreased rapidly as T_{hy} slowly cooled.

Although ultrasonic "calling" can be evoked in the isolated male through the elevation of hypothalamic temperature, nonetheless the relationship between temperature and calling is highly variable. Of the 16 PGE₂ trials, 5 (31%) were "silent", and, indeed, one of our males failed to produce a single vocalization following either his first or second PGE₂ infusion. In contrast, another PGE₂ male emitted 327 bouts, or, in other words, he vocalized for 27 consecutive minutes; following ejaculation, a male rarely vocalizes for more than 5 consecutive minutes (cf. [4]). It seems reasonable to conclude that while hypothalamic heating is related to 22 kHz calling, hypothalamic heating alone is not sufficient to evoke calling.

EXPERIMENT 2: BRAIN HYPOTHERMIA AND THE 22 kHz VOCALIZATION

We now know that the 22 kHz vocalization can be evoked in the isolated male simply by elevating hypothalamic temperature. The question that next arises is whether males made to sustain a lower-than-normal hypothalamic temperature would show little or no calling, even when ejaculating normally.

Sodium salicylate has been shown to induce a significant reduction in body temperature in afebrile rats under both thermoneutral (23°C) and cold (5°C) environmental conditions [16, 20, 21]. Expecting sodium salicylate to lower hypothalamic temperature as well, we injected this aspirin-like drug into males given access to receptive females and during each of three post-ejaculatory intervals we monitored 22 kHz calling.

METHOD

We used six sexually experienced male Wistar rats, 125–135 days of age. As in Experiment 1, each male carried a telemetric thermosensor stereotaxically positioned in the rostral hypothalamus.

Sodium salicylate (Mallinckrodt) solutions were prepared by dissolving 2 mg of salicylate in 7 ml of 0.9% sterile saline. The control solution was sterile saline alone. Both solutions were aliquotted and refrigerated until used.

Testing was carried out in a semicircular arena during the dark phase of the 12L:12D cycle. After 15 minutes of habituation to the arena, each male was injected IP with either 300 mg/kg of sodium salicylate or an equivalent volume of sterile saline. Ninety minutes later, a female in natural proestrous was introduced in the arena and the male was
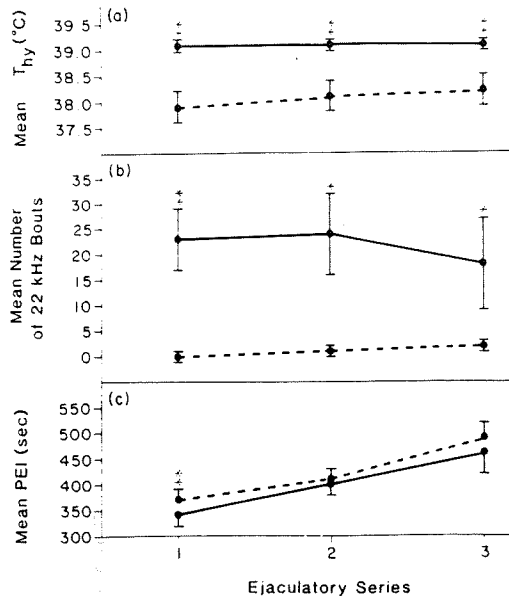
FIG. 3. (a) Mean hypothalamic temperature at ejaculation, (b) mean number of 22 kHz bouts following ejaculation and (c) mean duration of the PEI for 3 ejaculatory series following the IP injection of sodium salicylate (dashed line) and saline (solid line). The injections were separated by an interval of at least one week. Vertical lines indicate ±SEM. *$p<0.05$; **$p<0.005$. Paired t-test.

allowed to copulate freely until he ejaculated three times and reached the first intromission of the next series. We used standard scoring procedures [5] to record his behavior and the two Mini Bat-Detectors of Experiment 1 to count the number of 22 kHz bouts during each post-ejaculatory interval.

Each animal was tested twice, once following the injection of sodium salicylate and once following the injection of sterile saline. The tests were separated by an interval of at least one week and the order of injection was counterbalanced across animals. For some reason, two of our males, after being given sodium salicylate, failed to achieve an intromission within 15 minutes following the introduction of the female. These males were retested at least two days later with 200—rather than 300—mg/kg sodium salicylate and each mated to criterion.

Histology was performed on the brains of two of the animals. Proper placement of the thermosensors within the rostral hypothalamus was confirmed in both cases.

RESULTS AND DISCUSSION

Figure 3a shows that sodium salicylate produced the desired hypothermic state, which is to say that T_{hy} at ejaculation was significantly lower following the injection of sodium salicylate than following the injection of saline. Figure 3b shows that when a reduction in hypothalamic temperature is induced, 22 kHz calling following ejaculation is virtually abolished.

To underscore the effect of sodium salicylate on ultrasound production, we should mention the following comparisons. Of the 18 post-ejaculatory intervals (PEI's) that we monitored under saline and sodium salicylate, respectively, only 3 (17%) were "silent" following the injection of saline whereas 13 (72%) were "silent" following the injection of sodium salicylate. Moreover, under sodium salicylate only

one PEI (6%) yielded more than five 22 kHz bouts as compared to 14 (78%) under saline.

Finally, given the results just described and given our hypothesis concerning the selective thermolytic function of the 22 kHz vocalization, we would expect slower post-ejaculatory hypothalamic cooling under sodium salicylate than under saline. This is precisely what occurred. Post-ejaculatory hypothalamic cooling was significantly greater in the saline group than in the sodium salicylate group (Paired t-test=1.79; $p<0.05$; n=18). It seems reasonable to conclude that a radical reduction in ultrasonic calling retards hypothalamic cooling.

This is not the first report of an experimentally-induced decrease in the post-ejaculatory vocalization. Other experimenters, by lesioning the midbrain tegmentum [4], for example, or by infusing bicuciline [11], a compound that antagonizes GABA-ergic activity, have succeeded in reducing the number of 22 kHz calls. In these studies, however, a substantial reduction in the duration of the PEI was induced as well, leaving open the possibility that, short of surgically muting the animal, an overall decrease in ultrasound production can be effected only by abridging the PEI. Figure 3c shows that this is not the case: the PEI's under sodium salicylate were, on average, either the same as or significantly longer than under saline. Nonetheless, sodium salicylate, as already pointed out, virtually abolished post-ejaculatory calling.

GENERAL DISCUSSION

When we heated the hypothalamus of the isolated male through the intraventricular infusion of PGE_2, the 22 kHz vocalization was significantly increased. When we cooled the hypothalamus prior to copulation through the IP injection of sodium salicylate, the 22 kHz vocalization was virtually abolished. Our data strongly suggest that hypothalamic temperature influences ultrasonic calling.

The recognition that ultrasonic calling is influenced by hypothalamic temperature prompts the question of whether the converse is also true, that is, whether ultrasonic calling influences hypothalamic temperature. Ultrasonic calling is produced by compression of the thoracic cavity and constriction of the larynx, forcing the expulsion of air at high pressure [18,19]. This thoracic-laryGeal maneuver could cool in one or both of the following ways. First, it could produce a rise in cerebrospinal-fluid pressure and thereby "squeeze" blood out of the brain, into the nasal mucosa and then into the cavernous sinus. Freshly cooled, this blood could cool the brain, particularly the ventral brain, through conduction (cf. [8,14]). Second, the thoracic-laryngeal maneuver underlying the 22 kHz vocalization could cool the brain by forcing air from the narrow passages of the trachea and larynx into the larger spaces of the upper respiratory tract. Upon reaching these larger spaces, the compressed air would expand rapidly and, by expanding, would cool adiabatically. This cool air, passing over the surfaces of the oral and nasal mucosa, could effectively lower hypothalamic temperature. That ultrasonic calling clearly does not function to cool the body was seen previously [7] when monitoring changes in core temperature during the post-ejaculatory interval.

We should emphasize, however, that the 22 kHz vocalization does not always occur in the "normal" animal after an ejaculation (cf. [1]), nor indeed did it always occur after the infusion of PGE_2 in Experiment 1. The vocalization in brief, is manifested erratically, and for this reason we think of it as an auxiliary mechanism for brain cooling. What we think of

as fundamental involves the nasal mucosa and entails a specific vasomotor response. Here we draw on the work of Caputa et al. [9,10] on rabbits and guinea pigs. These species, when under extreme heat challenge, show a sizable dilation of nasal blood vessels. Such dilation, in promoting convective heat loss from the surface of the nasal mucosa, cools venous blood draining the nose which, upon reaching the cavernous sinus, acts to cool the hypothalamus selectively. Our hypothesis is that just such a mechanism is characteristically evoked in the rat at the time of ejaculation, when the temperature of the hypothalamus exceeds 39°C and the hypothalamus is presumably heat threatened. We suggest that it was also evoked in response to PGE_2, either because PGE_2 raised hypothalamic temperature above some critical level or because, in increasing body temperature, PGE_2 changed the ratio of hypothalamic temperature to body temperature in some critical way.

An important question remains, however: given that PGE_2 activates selected autonomic and behavioral responses to promote bodily heat *gain* [17], how could nasal vasodilation and 22 kHz calling have been activated to promote hypothalamic heat *loss*? In other words, how could heat-gain and heat-loss effectors have functioned concurrently in the same animal? That such discordance is possible, and perhaps even common, has been shown by Berek and Jessen [6] for the goat and by Graf [13] for the pigeon. In both species shivering and panting were induced simultaneously by differential manipulation of spinal cord temperature and deep body temperature. The conclusion drawn was that thermogenic and thermolytic control mechanisms are ". . . at least partially autonomous" [6].

When it was discovered that adult rodents produce ultrasonic vocalizations it was assumed that the vocalizations are emitted as messages and that the messages have meaning for conspecifics (eg., [1,3]). We would expect the call to be emitted, however, during any kind of social interaction that critically elevates physiological temperature. In this regard, it is instructive that aggression, for example, is accompanied by an increase of both brain temperature and body temperature ([2], unpublished observations) and also by the emission of ultrasonic vocalizations [22]. Moreover, it should be noted that the presence of conspecifics is not necessary to evoke the 22 kHz vocalization in the "normal" male rat; he often "calls" when alone [12]. Whether the incidence of calling in such an isolated male varies *pari passu* with circadian changes in brain temperature has not been determined.

In conclusion, we should point out that the communication hypothesis is experimentally unfalsifiable because negative results inevitably leave open the possibility that the appropriate laboratory context in which the communicative function might have been revealed was not provided. Even so, the hypothesis may be true. Indeed, both hypotheses may be true: ultrasound in rodents may have communicative value and at the same time may be an auxiliary thermoregulatory behavior. Future research, of course, will decide.

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