Physalaemus pustulosus, where the bats were seen hunting during 10 percent of the observation time (*5*). They caught a total of 95 frogs. Hourly catches averaged 6.3 frogs but ranged from 2.5 to 12.1, depending on the frogs' calling behavior. We divided the choruses of *P. pustulosus* into four categories and counted the number of bat visits per capture for each category: (i) full chorus, 1.8 (68 total visits); (ii) partial chorus, 2.3 (61); (iii) few calling, 3.4 (81); and (iv) none calling, 42.7 (128). The bats were least successful when no frogs were calling [χ^2 (2) = 46.32, $P < .005$].

Captive *T. cirrhosus* responded to recorded advertisement calls from a variety of edible leptodactylid and hylid frogs. To test the hypothesis that fringe-lipped bats do not merely respond to any sound but discriminate among frog calls, we simultaneously presented the advertisement calls of an edible frog, *Hyla boulengeri*, and a poisonous toad of similar size, *Bufo typhonius*. Five bats were tested in a flight cage, with eight trials per individual (*6*). As shown in Table 1, the bats preferred the call of *H. boulengeri*. In field tests at six widely distributed sites on Barro Colorado Island, bats again preferred the *H. boulengeri* call over that of *B. typhonius* (*7*).

To ascertain the effect of prey size, we simultaneously presented the advertisement calls of *P. pustulosus* (maximum snout-to-vent length, 35 mm) and *Leptodactylus pentadactylus* (200 mm). The latter is too large to be captured by *T. cirrhosus*. The advertisement calls of these two species have approximately the same frequency range (200 to 1000 Hz). The calls were presented at the same intensity, even though *L. pentadactylus* produces a louder call. Five caged bats preferred the call of *P. pustulosus*, and bat responses in the field were similar (Table 1) (*7*).

We also sought to determine whether *T. cirrhosus* predation is affected by call repetition rate and volume (Table 2). When three fringe-lipped bats in the flight cage were simultaneously presented with *P. pustulosus* advertisement calls played at 1.6-second intervals (about normal) and 3.2-second intervals, the bats preferred the faster call rate. Three additional *T. cirrhosus* preferred *Centrolenella fleischmanni* calls played at rates of 1.6- versus 6.4-second intervals (about normal). When simultaneously presented with *P. pustulosus* advertisement calls at intensities of 74 and 78 dB SPL at 1 m, two bats both preferred the louder call.

In the absence of counterselection forces, one would expect male anurans to make themselves as conspicuous and locatable as possible when attempting to attract mates (*8*). Nevertheless, with the exception of poisonous or unusually large species such as *B. typhonius* and *L. pentadactylus*, few neotropical anurans do so. Clearly the selective advantages of sexual advertisement are balanced by the increased risk of predation. Thus we suggest that predation has had an important influence on the evolution of frog vocalizations in the Neotropics.

MERLIN D. TUTTLE
Vertebrate Division, Milwaukee Public Museum, Milwaukee, Wisconsin 53233
MICHAEL J. RYAN
Section of Neurobiology and Behavior, Langmuir Laboratory, Cornell University, Ithaca, New York 14850, and *Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Panama*

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4. *Trachops cirrhosus* were mist-netted at 11 sites and observed with a Javelin model 221 night vision scope as they responded to recorded frog calls at three sites.
5. These observations, made at Weir Pond on Lutz Stream between 1845 and 2400 hours on 17, 18, 20, 22, 23, and 27 April, totaled 14.3 hours. The average number of *P. pustulosus* in the nightly breeding chorus was 242 (range, 44 to 425). Of the 95 capture attempts believed successful, in 42 cases bats were actually seen carrying frogs; characteristic departure behavior was used to determine the result of the remaining 53 attempts. Chorus categories were defined as follows: full chorus, sustained use of complex calls (whine plus one to six chucks per call) by a majority of males in the pond; partial chorus, sustained use of complex calls by less than half of the males; few calling, sustained use of simple calls (whine only) with or without sporadic complex calls; none calling, mostly silent with no more than an occasional simple call. When fewer than 50 frogs were present, the maximum call category used was "partial chorus."
6. Calls were recorded on a Nagra IV-D tape recorder and played to the bats at 38 cm/sec on Stellavox recorders with small extension speakers. Speaker intensities were balanced at 75 dB SPL at 1 m. The flight cage was 4.5 m square and 2.3 m high and was illuminated by a single red 25-W bulb in the center. The observer sat in one corner and the bat perched in the opposite corner. One speaker was located in each of the remaining corners, about 4 m from the bat. A response was recorded if the bat passed within 1 m of a speaker within 60 seconds of call presentation. (Most responding bats flew within 30 cm of a speaker, and often landed on it.) There were no rewards for a correct choice. Speaker inputs were switched after each trial to reverse call location, and trials were repeated at intervals of 5 to 15 minutes. Bats were tested in only one set of trials per night and never twice in the same experiment. None was used in more than two experiments. The null hypothesis of no preference was tested by comparing $-2 \Sigma \ln p$ to a χ^2 distribution, d.f. = $2n$ (*9*).
7. All trials were made between 1840 and 2020 hours from 18 to 25 February and 2 to 18 April. Calls were played simultaneously at an intensity of 74 dB SPL at 1 m on Pearlcorder model D 120 microcassette tape recorders at 2.4 cm/sec. The call interval was 1.6 seconds. Speakers were placed 4 m apart and were simultaneously observed with a Javelin model 221 night vision scope from a distance of 12 m. *Trachops cirrhosus* can be identified in flight. Responses were recorded when a *T. cirrhosus* passed within 1 m of a speaker (most responding bats flew closer than 30 cm). Data were analyzed as in (*6*).
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Thyrotropin-Releasing Hormone Effects in the Central Nervous System: Dependence on Arousal State

Abstract. *Thyrotropin-releasing hormone was microinjected into the dorsal hippocampus of ground squirrels* (Citellus lateralis) *when they were at different levels of arousal, as assessed by electrophysiological and behavioral criteria. When administered to the awake animal, thyrotropin-releasing hormone produced dose-dependent decreases in body temperature accompanied by behavioral quieting and reductions in metabolic rate and electromyographic activity. The magnitude of these effects was greater when the peptide was microinjected during a period of behavioral activation. In contrast, administration of the peptide during slow wave sleep produced increased thermogenesis, an increase in electromyographic activity, and an increase in the amount of electroencephalographic desynchronization.*

Thyrotropin-releasing hormone (TRH), traditionally recognized for its ability to release thyrotropin from the pituitary gland, is now known to be widely distributed throughout the brain (*1*), and to affect several physiological and behavioral processes independent of its action on the pituitary (*2*). For example, changes occur in body temperature, respiration, blood pressure, electroencephalogram (EEG), and motor activity after central nervous system (CNS) administration of TRH in conscious animals (*3*). In addition, TRH reverses the hypother-

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mia and CNS depression induced by several CNS-acting compounds (*4*, *5*). We reported previously (*6*) that a naturally induced state of CNS depression (that is, hibernation) could be reversed by microinjection of TRH into the dorsal hippocampus of the golden-mantled ground squirrel, *Citellus lateralis*. The complete arousal from hibernation, which involved marked increases in body temperature, metabolic rate, and motor activity, was triggered by low doses of TRH (0.1 ng to 1 μg per microliter) and showed a dose-dependent latency. These results suggested that TRH participates in the control of body temperature and in the modulation of the level of arousal in the CNS.

In the study described here we investigated the effects of TRH action in the dorsal hippocampus (*7*) of euthermic (that is, not hibernating) ground squirrels. Two response patterns were observed. In sleeping animals, TRH produced changes that were similar in direction to those changes that occurred in hibernating animals. In contrast, TRH caused decreases in all parameters, including motor activity, in awake animals.

Twelve male and female ground squirrels were anesthetized with sodium pentobarbital (75 mg/kg, injected intraperitoneally) and stereotaxically implanted bilaterally with stainless steel cannula guides. The guide tubes were lowered to a position just above the dorsal hippocampus (*8*) and anchored to the skull with self-curing dental acrylic.

A miniature bead-type thermistor attached to the tip of one cannula guide was used to sense body (brain) temperature (T_b). In six of the animals, EEG and electromyogram (EMG) records were also obtained. For this purpose, cortical electrodes (0-80 stainless steel screws) were threaded into the skull overlying the occipital and parietal cortices and a bipolar electrode (multistranded stainless steel wire, 0.01 inch in diameter) was secured to the dorsal neck muscles. A reference electrode was implanted in the nasal sinus. Thermistor and electrode leads were attached to a miniature Amphenol connector, which was then mounted on the animal's skull with dental acrylic. A minimum of 1 week was allowed for recovery from surgery.

Animals were tested in a metabolic chamber through which ambient air was drawn and passed (dried) into a Beckman oxygen analyzer to obtain measurements of metabolic rate. Prior to testing, leads were attached to the skull-mounted connector and fed into a Grass polygraph for EEG and EMG recording and into a Honeywell recorder for continuous display of T_b and metabolic rate. Four states of arousal were identified on the bases of both electrographic and behavioral criteria (*9*).

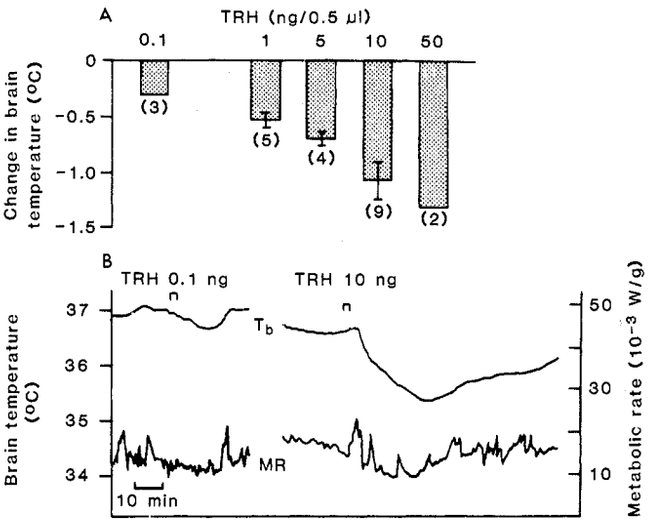

Fig. 1. (A) Dose-response histogram showing mean decrease in T_b after microinjection of TRH into the hippocampus of quiet, awake ground squirrels. The number of animals is indicated in parentheses. Brackets denote standard error of the mean. At doses of 0.1 and 50 ng, individual data points were the same. (B) Profile of decrease in T_b and metabolic rate (*MR*) in one animal after it received 0.1 and 10 ng of TRH.

Fresh solutions of TRH, dissolved in sterile isotonic saline, were microinjected in a dose range of 0.1 ng to 1 μg (0.28 p*M* to 2.8 n*M*), at *p*H 4.5 to 4.8, in a volume of 0.5 μl. The injections were delivered remotely, over a period of 1 minute, through stainless steel injection cannulae (31 gauge) connected to two Hamilton microliter syringes via lengths of polyethylene tubing. When fully inserted into the guide assembly, the injection cannulae extended 1 mm beyond the guide tips and into the substance of the dorsal hippocampus.

Isotonic saline and TRH-OH, the deamidated metabolite, were administered in control tests in the same volume and under the same conditions as experimental tests. All tests were conducted at an ambient temperature of 23° ± 2°C. Verification of cannula placement was made by light microscopic inspection of frozen sections (80 μm) stained with thionin.

When microinjected into the hippocampus of quiet, awake animals in a dose range of 0.1 to 50 ng, TRH produced dose-dependent decreases in T_b after latencies of 2 to 6 minutes (Fig. 1). The correlation coefficient obtained from linear regression analysis of the log dose-response was .69 ($P < .001$). Decreases in metabolic rate and EMG either preceded or occurred simultaneously with the fall in T_b. At higher concentrations of TRH, the dose-dependent nature of the T_b response became disrupted. Concentrations of 0.1 and 1.0 μg caused variable changes in T_b, metabolic rate, and EMG across animals, producing either a decrease, an increase, or a biphasic response pattern. Increases in the response parameters at these higher doses were associated with a general increase in motor activity.

Certain behavioral patterns were also associated with the response to TRH. In four of the twelve animals, 10 and 50 ng of TRH had a clear sedative or quieting effect (for example, postural adjustments were made to assume a resting position and cortical spindles appeared in the EEG record). In addition, sniffing and brief episodes of chewing or feeding-type motions of the mouth and forepaws occurred during the falling phase of the response in eight of the twelve animals after a dose of 10 or 50 ng. As the change in T_b approached the preinjection baseline level, grooming behavior was typically observed in these same animals.

We also administered TRH to three of these animals when they were behaviorally activated. The direction of change in T_b, metabolic rate, and EMG remained constant; however, compared to the effects of TRH administered during quiet wakefulness, the magnitude of the change in all three animals was two to six times greater when TRH was administered during episodes of intense grooming, digging, or nest building. After a 1- to 2-minute latency, T_b fell 1.9°, 1.5°, and 1.7°C in response to 0.1, 1, and 10 ng of TRH, respectively, compared to decreases of 0.3°, 0.6°, and 0.8°C during quiet wakefulness. The sedative effects produced by TRH were pronounced in all three animals; behavioral activities ceased and EMG decreased in amplitude from 250 to 300 μV to less than 50 μV within 3 minutes after the microinjection. The time course from the onset of the response to the return to baseline was 20 to 30 minutes, which was comparable to the time course of the response in these animals during quiet wakefulness.

Microinjection of 10 ng TRH into the hippocampus of four of these animals during slow wave sleep produced increases (rather than decreases) in thermogenesis, EMG, and EEG desynchronization, after a latency of 2 to 3 minutes (Table 1). Although the effects on T_b were variable, on no occasion was a decrease in T_b observed. When TRH was microinjected on a stable T_b baseline, increases in T_b occurred. When the microinjection was delivered during a progressive decline in T_b (as occurs during sleep), TRH halted the fall in T_b and a plateau ensued until the evoked increase in metabolic rate returned to the rate before injection. That an increased metabolic rate was associated with this plateau indicates that the plateau in T_b occurred as a result of increased thermogenesis.

Changes in EMG and EEG activity were transient, lasting 10 to 12 minutes in three of the animals and only 3 minutes in animal No. 7 (Fig. 2). Changes in T_b and metabolic rate occurred concomitantly and lasted 16 to 30 minutes, except in the case of animal 7. In this animal, T_b remained elevated throughout the sleep episode even though its metabolic rate had returned to preinjection baseline after 30 minutes and uninterrupted slow wave sleep had resumed 6 minutes after TRH was administered. That the changes in T_b and metabolic rate outlasted the effects of TRH on EMG and EEG activity in each animal indicates that these effects are dissociable during sleep and that the changes in T_b and metabolic rate are not simply a consequence of arousal. This finding was reported previously by Horita *et al.* (*5*) with regard to TRH effects in pentobarbital-treated rabbits.

Table 1. Changes in T_b, metabolic rate, EMG, and EEG in four ground squirrels microinjected with 10 ng of TRH during slow wave sleep and quiet wakefulness. Changes in EEG are described as desynchronization (D) or appearance of spindles (S). The latency for change in all variables was 2 to 3 minutes. Changes in metabolic rate were determined by comparing the area under the curve for the 20 minutes after injection with the 20-minute period immediately prior to injection.

Animal number	Change in			
	T_b	Metabolic rate (%)	EMG	EEG
	Effects during slow wave sleep			
7	↑ 1.0	↑ 20	↑	D*
8	†	↑ 33	↑	D
11	†	↑ 38	↑	D
12	↑ 0.6	↑ 30	↑	D
	Effects during quiet wakefulness			
7	↓ 1.8	↓ 45	↓	No change
8	↓ 1.7	↓ 47	↓	S
11	↓ 0.7	↓ 31	↓	S
12	↓ 1.0	↓ 40	↓	S

*Total desynchronization did not occur; the wave form was characterized by large amplitude slow waves mixed with lower amplitude fast activity (see Fig. 2). †T_b declining at the time of injection; a plateau in T_b occurred until the metabolic rate returned to the preinjection baseline.

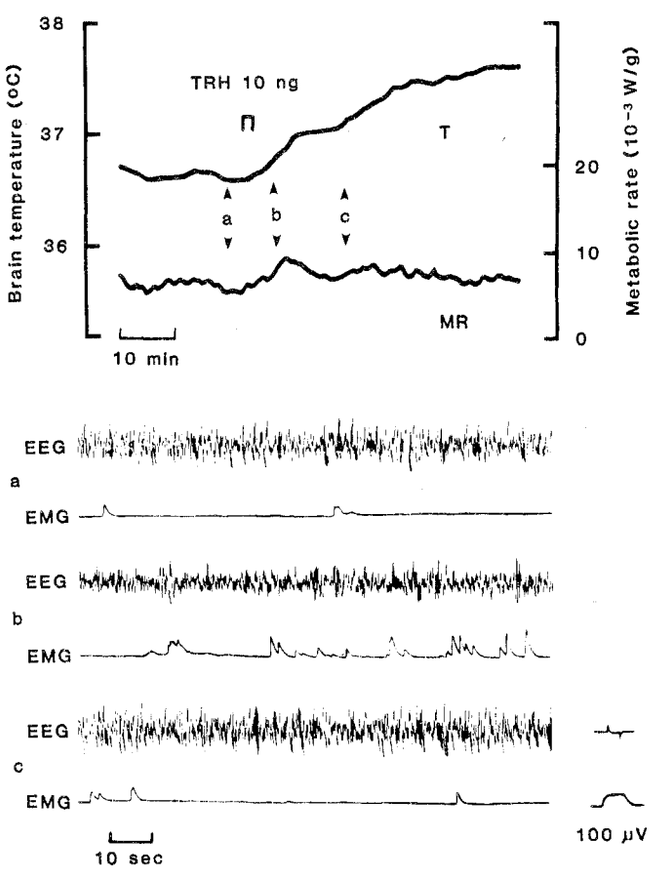

Fig. 2. Changes in T_b, metabolic rate (*MR*), EEG, and integrated EMG after intrahippocampal administration of 10 ng of TRH during slow wave sleep in animal No. 7. The EEG and EMG records are 2-minute segments taken during (a) the 2 minutes prior to microinjection, (b) 3 to 5 minutes after injection, and (c) 10 to 12 minutes after injection.

Our results show that microinjection of TRH in euthermic awake animals produces behavioral quieting and dose-dependent hypothermia and add further support to the concept that TRH participates in the central control of body temperature and is a modulator of arousal in the CNS (*10*). Our observations also show that the hippocampus, in addition to brainstem sites (*11*), can produce changes in T_b. Other work (*12*) has demonstrated a neuronal projection from the hippocampus to thermosensitive neurons in the hypothalamus, and our data suggest that this relationship may have functional significance in the regulation of body temperature.

Of interest is our finding that the action of TRH on hippocampal neurons produces effects that are state-dependent. This state dependence is apparent not only across the euthermic-hibernation states, but also within the euthermic state itself, as shown by the less dramatic, though still opposite, changes in T_b, metabolic rate, and EMG across the sleep-wake cycle. Conceivably, TRH-sensitive neurons may function in a network that serves as a homeostat for arousal in the CNS. Theoretically, this mechanism would serve to maintain a normal range of central activity by stabilizing neuronal systems against excursions to both upper and lower extremes of activity.

Although the neural basis for these state-dependent TRH effects is not

Injections of isotonic saline in one animal and 10 ng of TRH-OH in three animals were given during wakefulness. During slow wave sleep, microinjection of isotonic saline was administered in two animals. These injections caused no changes in any of the animals.

known, there are at least two possibilities. First, TRH may act on different populations of neurons in the opposing states. Although we have not recorded from hippocampus neurons in the ground squirrel, two main types of neurons have been differentiated, electrophysiologically, in the hippocampus of rats (*13*) which supports this hypothesis. Alternatively, TRH may act on the same population of neurons within the hippocampus in each state. In this case, the variable influences (associated with a change of state) arising from other CNS inputs to TRH-sensitive neuronal networks that project from the hippocampus may alter the final TRH-activated response.

TONI L. STANTON
Department of Pharmacology, Thomas Jefferson University School of Medicine, Philadelphia, Pennsylvania 19107, and *Alfred I. duPont Institute, Wilmington, Delaware 19899*
ALEXANDER L. BECKMAN
Alfred I. duPont Institute
ANDREW WINOKUR
Departments of Psychiatry and *Pharmacology, University of Pennsylvania School of Medicine, Philadelphia 19104*

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9. The four states identified were: (i) active wakefulness, characterized by desynchronized EEG, high EMG activity, and generalized motor activation; (ii) quiet wakefulness, characterized by desynchronized EEG, lowered EMG activity, and absence of overt motor activation; (iii) slow wave sleep, characterized by large amplitude slow wave EEG, low EMG, and curled sleep posture; and (iv) paradoxical sleep, characterized by low amplitude, desynchronized EEG, flat EMG, and curled sleep posture.
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13. J. Delacour, *Exp. Brain Res.* **38**, 95 (1980). The activity of one class of neurons, characterized by a bursting mode of discharge, was higher during slow wave sleep than during wakefulness and exhibited a decrease in spike frequency in response to desynchronizing stimuli presented during slow wave sleep. Some of the other class of neurons, characterized by continuous spike discharge, were more active during wakefulness and responded to desynchronizing stimuli with an increase in spike discharge.
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Female Sex Pheromone in the Skin and Circulation of a Garter Snake

Abstract. *Serums and extracts of tissues from the female garter snake* (Thamnophis sirtalis parietalis*) each act as a pheromone and elicit male courtship behavior when applied to the back of another male. Since pheromonal activity is present in yolk and liver tissue of untreated females and can be induced with estrogen treatment in the serums and livers of males, the pheromone may be associated with the circulating yolk lipoprotein, vitellogenin.*

In many vertebrates, urine, feces, and vaginal contents, as well as exocrine glandular products, function as sex attractants and serve to facilitate the location and recognition of mates (*1*). We now report an additional source for a vertebrate sex pheromone; the sex attractant pheromone of the female red-sided garter snake (*Thamnophis sirtalis parietalis*) is present in an active form in the liver and in the circulation. We also provide evidence that the pheromone reaches its active site on the skin by passing through the keratinized outer skin cells in an active process associated with courtship.

Male garter snakes use the chemosensory vomeronasal system to locate and recognize potential mates (*2–6*) by detecting an estrogen-dependent, species-specific pheromone on the skin of attractive females (*3, 6–9*). This pheromone can be left as a nonvolatile trail (*8, 10*) or transferred by contact to conspecifics (*4*); males that have come into contact with attractive females will elicit courtship from sexually active males (*3, 6, 7*).

Male *T. s. parietalis* begin courtship with a chemosensory investigation of the female's body; chemical cues are delivered by tongue flicks to the male's vomeronasal system at the Jacobson's organs and are necessary for the release of male courtship (*4, 6, 11*). Chin-rubbing behavior, in which the male rubs his chin forward and backward along the female's back, follows sex recognition and is the first unambiguous behavioral event of courtship. While being courted, females increase their apparent size by hyperventilating (*12*).

In view of the requirement for perception of a pheromone to release male courtship and the lack of any exocrine glandular structures in the dorsal skin of *Thamnophis* (*13*), we reasoned that the pheromone could be carried in the female's circulation and transported via the dermal vascular bed (*14*) to the skin, where it is actively dispersed by the female's hyperventilation during courtship. Two experiments were conducted to test this hypothesis. (i) Serums from estrogen-treated females were applied to the skin of males, and male courtship of these serum-coated males was recorded (*15*); serums from untreated and estrogen-treated males served as controls. (ii) Extracts of the skin of serum donor females were similarly tested.

Serums that elicited behavioral responses were analyzed for lipid. In addition, the source of the pheromone was examined. Since a lipid pheromone could either be released from storage sites (in the fat bodies) or newly synthesized in the liver, homogenates prepared from the liver and fat bodies of intact females were also tested for pheromonal activity by application to the backs of males. Finally, frozen sections of skin from serum donor animals were stained for lipid.

Males receiving an application of 500 μl of serum from estrogen-treated females ($N = 26$) were courted, with 15 of

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