

Research report

Neural substrates for sexual and thermoregulatory behavior in the male leopard gecko, *Eublepharis macularius*

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Abstract

The preoptic area–anterior hypothalamus (POAH) continuum is critical for the integration of environmental, physiological, and behavioral cues associated with reproduction in vertebrates. In the present study, radiofrequency lesions in the POAH abolished sexual behavior in the leopard gecko (*Eublepharis macularius*). Furthermore, results suggest a differential effect of POAH lesions on those behaviors regarded as appetitive (tail vibration and grip) and those regarded as consummatory (mounting and copulation), with consummatory behaviors being affected to a greater extent. *E. macularius* is an ectothermic vertebrate that modulates body temperature behaviorally relative to ambient temperature. In vertebrates, the POAH is also an important integrator of thermoregulation. Thus, the present study investigated whether lesions that disrupt reproductive behavior also disrupt body temperature regulation. While virtually all males displayed diurnal rhythms in thermoregulatory behavior prior to surgery, this pattern was abolished in a small proportion of animals bearing POAH lesions. Lesions that abolished thermoregulatory rhythms involved the suprachiasmatic nucleus (SCN), whereas lesions confined to the POAH, while dramatically influencing sexual behavior, did not affect thermoregulatory rhythms or temperature set point. Together, these findings identify the POAH as an important neural locus regulating sexual behavior but not thermoregulation and suggest that the SCN acts as a pacemaker controlling daily behavioral temperature regulation in this species.

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1. Introduction

The preoptic area–anterior hypothalamus (POAH) continuum is a critical brain area for the integration of environmental, physiological, and behavioral information prior to and during successful copulation in male vertebrates [2]. The POAH contains numerous steroid receptor-containing neurons and has substantial bidirectional connections with other brain areas that are involved in copulation, (e.g., medial amygdala, bed nucleus of the stria terminalis, and distinct areas in the central tegmental field) in addition to extensive

intra-hypothalamic connections [2,4]. In studies to date, lesions of this brain area abolish sexual behavior in intact, or castrated and androgen treated male vertebrates [2,16].

The purpose of the current study was to examine the role of the POAH in the regulation of male sexual and thermoregulatory behavior in the leopard gecko, *Eublepharis macularius*. Relative to mammals and birds, little is known about the neuroendocrine control of sexual behavior in squamates, a pivotal group in vertebrate evolution. Research to date suggests that, as in other vertebrates, the POAH is a major integrative area in the regulation of both reproductive and thermoregulatory behavior in various squamate species, including the red-sided garter snake (*Thamnophis sirtalis parietalis*) [7,12], green anole lizard (*Anolis carolinensis*)

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[18,25], and whiptail lizard (*Cnemidophorus inornatus* and *Cnemidophorus uniparens*) [11].

Known to contain high levels of thermosensitive neurons, the POAH defines the set point or optimal body temperature to which changes in temperature will be compared [21]. In this sense, the POAH is thought to work as a thermostat in the brain [23], with temperature being regulated by both behavioral and autonomic components [1,14,23]. Given the importance of the POAH in regulating reproductive behavior and body temperature, the present study sought to determine if this brain locus is a critical integrator of these behaviors in a lizard, an ectothermic vertebrate. Previous studies with another squamate reptile, the red-sided garter snake, suggest that the amount of damage to the POAH is correlated with the amount of disruption to both sex behavior and thermoregulation [12]. The present investigation in *E. macularius* allowed the study of the behavioral component of body temperature independent of autonomic influences.

2. Materials and methods

2.1. Animals

Animals were treated according to a research protocol approved by the university's Institutional Animal Care and Use Committee. All adult males used in this experiment were sexually naive, 1.5–2 years of age with snout-to-vent lengths between 10 and 13 cm and a weight of 70–90 g. Additionally, only males that had shown courtship and copulatory behavior in at least 5 out of 7 encounters with a receptive female were used.

2.2. Stimulus females

The females used as stimulus animals were randomly selected from breeding chamber females in the follicular stage. Females used were sexually experienced with snout-to-vent lengths of 9–12 cm and a weight of 50–80 g. Only females which showed receptivity to courtship and mating were used.

2.3. Sexual behavior

Sexual behavior in the male leopard gecko can be generally categorized into a progression of interactions with a female, from courtship to copulation. During a typical sexual encounter, a male will slowly approach the female while licking first the air or substrate, then the female [19]. This behavior is often followed by male-typical tail vibration, most likely elicited by pheromones in the female's skin [15]. Tail vibration serves as both an audible and visual display of courtship. As part of these appetitive behaviors, the male may engage in scent marking, during which the male drags his lower body on the substrate to distribute

pheromones released from the preanal pores. Males then proceed to grip the skin of the female, often beginning with her tail and lower body and progressing toward her neck. If the female is fully receptive, the body gripping is soon followed by the consummatory behaviors of mounting, intromission, and ejaculation. While a major component of mounting, the body grip is also a pivotal test of a female's receptivity to consummatory acts and is often a critical moment in deciding if mating will continue.

2.4. Gonadectomy and hormone manipulation

In experiments that deal with brain lesion effects on sexual behavior, it is important to recognize that observed deficits might be due to either direct effects of the lesion on behavior or to indirect effects such as lesion-induced declines in sex steroid hormones in the systemic circulation. For this reason, in the present experiment, the males were castrated and treated with exogenous testosterone to maintain elevated circulating concentrations of androgens. Males were castrated according to the technique described in Crews et al. [3]. At the same time, animals were implanted subcutaneously with hormone implants made of 10 mm of Silastic surgical tubing (inner diameter: 1.47 mm; outer diameter: 1.96 mm) filled with testosterone [13]. This size implant results in physiological concentrations of testosterone [20]. The ends of the tubing were sealed with Silastic adhesive, and a 1-cm piece of silk surgical thread was embedded in one of the ends to serve as an anchor after surgical implantation. Animals were allowed at least 72 h to recover in their home cages before behavioral testing began.

2.5. Housing

Throughout the duration of testing, animals were housed individually in transparent Plexiglas cages (47.5 cm long×25.7 cm wide×20.0 cm tall). Sand was used as a substrate, spread approximately 0.7 cm deep across the bottom of the enclosure. Cages were placed atop transmission receivers, which continuously collected signals from temperature transmitters in each animal. The ambient temperature in the room was maintained at 15 °C and a single 100-W ceramic heating element (CSE conical infrared, Pearlco, RAM Network: Rededa, CA) was placed to the left above each cage, remaining on constantly to establish a temperature gradient (high of 48 °C and low of 15 °C) for each animal. A hide running the length of the cage was positioned along the back wall of each enclosure, running along the entirety of the temperature gradient. A 12:12 light/dark cycle was maintained. Food and water were provided ad libitum, with water and food dishes placed at the cool end of the enclosure.

2.6. Behavior testing

All behavioral tests were conducted by introducing a receptive female into the home cage of the experimental

male. Tests were conducted between 11:00 A.M. and 5:00 P.M. Prior to each test, water/food dishes and hide were removed from the male's home cage. This was followed by a 3-min adjustment period before the female was introduced into the cage. Upon introduction of the female on the opposite side of the cage, males were allowed 5 min to achieve a grip and 5 min to achieve a mount from the time of grip (for a maximum possible time with female of 10 min). The frequency of tail vibration, grip and mount behaviors were quantified during behavioral tests. Tests were administered daily for seven consecutive days before lesion surgeries. Postoperative behavior tests were begun after at least 3 days of recovery, and were again conducted daily for seven consecutive days.

2.7. Monitoring of thermoregulatory behavior

Physiological transmitters (VitalView Series 4000 E-Mitters, Mini Mitter, Bend, OR) were surgically implanted into the abdominal cavity and anchored to the closing suture. The animals were allowed at least 1 week to recover following surgery. To monitor body temperature, animals' cages were placed on an ER-4000 receiver plate (Mini Mitter, Bend, OR) connected to a computer. The plates were situated so that animals were always within range of the receiver plate. Body temperature and heart rate were transmitted and monitored continuously. Mean body temperature was recorded every 5 min using VitalView software for a minimum of 18 days (7 days presurgery, 4 days of recovery, and 7 days post-lesion) (Minimitter, Bend, OR).

2.8. Lesion procedure

Three to 7 days after the last pre-lesion behavior test, subjects received bilateral radiofrequency lesions under cold-induced anesthesia. A platinum/iridium microelectrode, glass insulated except for the tip (250 μm exposed; Frederick Haer & Co. Bowdoinham, ME) was aimed at the POAH of nine animals using the following coordinates: 1.5 mm posteriorly from the coronal suture, 0.2 mm lateral from the midline, and 4.0 mm from the dura. A current of 25 mA was delivered for a duration of 10 s using an OWL lesion maker (Diros Technology and OWL Instruments, Toronto, Canada). The control group, consisting of six animals, received the same treatment, but lesions were not directed toward the POAH.

2.9. Histology

Following post-lesion behavioral testing, animals were sacrificed and perfused intracardially with 4% paraformaldehyde under cold-induced anesthesia. Blood samples were taken before perfusion for later radioimmunoassay (RIA) to analyze blood testosterone levels.

Subsequent to perfusion, animals' heads were placed in 4% paraformaldehyde for 24 h prior to brain extraction.

Once extracted, brains were subjected again to 4% paraformaldehyde for at least 12 h and to increasing concentrations of ethanol, followed by solutions of xylene and paraffin. Brains were then embedded in paraffin, sectioned at 6.0 μm , and stained for nuclear chromatin and Nissl substances with cresyl violet.

2.10. Statistical analysis

The percentage of tests in which tail vibration, grip, and mounting behaviors were displayed was calculated for each male before and after lesioning. Group averages were then calculated for each behavior measured. After testing groups for normal distribution and homogeneity, changes in expression of sexual behavior before and after lesioning in groups of animals receiving POAH lesions and those receiving lesions outside the POAH were analyzed using paired *t*-tests (significant when $p < 0.05$). Differences in behavior between groups of animals receiving lesions targeted to the POAH and lesions outside the POAH were analyzed using unpaired *t*-testing or the Mann–Whitney Test, where appropriate (significant when $p < 0.05$).

Body temperature rhythmicity was assessed using Clock-Lab software (Actimetrics, Evanston, IL). A chi-square periodogram Fourier analysis was conducted on temperature records from animals both pre- and post-lesion. The pattern of body temperature was considered to be diurnal if the highest peak occurred at ~ 1 cycle/day and was significant at $p < 0.01$.

3. Results

3.1. Sexual behavior

Prior to lesioning, all males exhibited similar levels of courtship and copulatory behavior. All animals found to have significant deficits in courtship and copulatory behavior were later found to have lesion damage in the POAH ($n=9$); animals with no statistically significant deficits in sexual behavior were subsequently found to have lesion damage outside the POAH ($n=6$) (Fig. 1). Mounting behavior was affected to a greater extent than either gripping or tail vibration (tail vibration: $p=0.043$; body grip: $p=0.002$; mounting: $p < 0.0001$).

3.2. Thermoregulatory behavior

One animal with a POAH lesion had a mean post-lesion body temperature greater than two standard deviations below that of the group mean. Because of this anomaly, body temperature data from this animal were not included in the analysis for thermoregulatory behavior. Virtually all (12/14) animals showed distinct diurnal rhythms in thermoregulatory behavior prior to lesion surgery, with higher mean

body temperatures during day compared to night. All animals receiving lesions outside of the POAH ($n=6$) continued to exhibit diurnal rhythms in thermoregulatory behavior following lesion surgery (Fig. 2).

Three animals with lesions of the POAH (out of 8) lost rhythms in thermoregulatory behavior following lesion surgery. In these three individuals, the diurnal rhythm was abolished and, in all three instances, lesion damage extended to the region containing the suprachiasmatic nuclei (SCN) (Figs. 2 and 3). Only those animals receiving

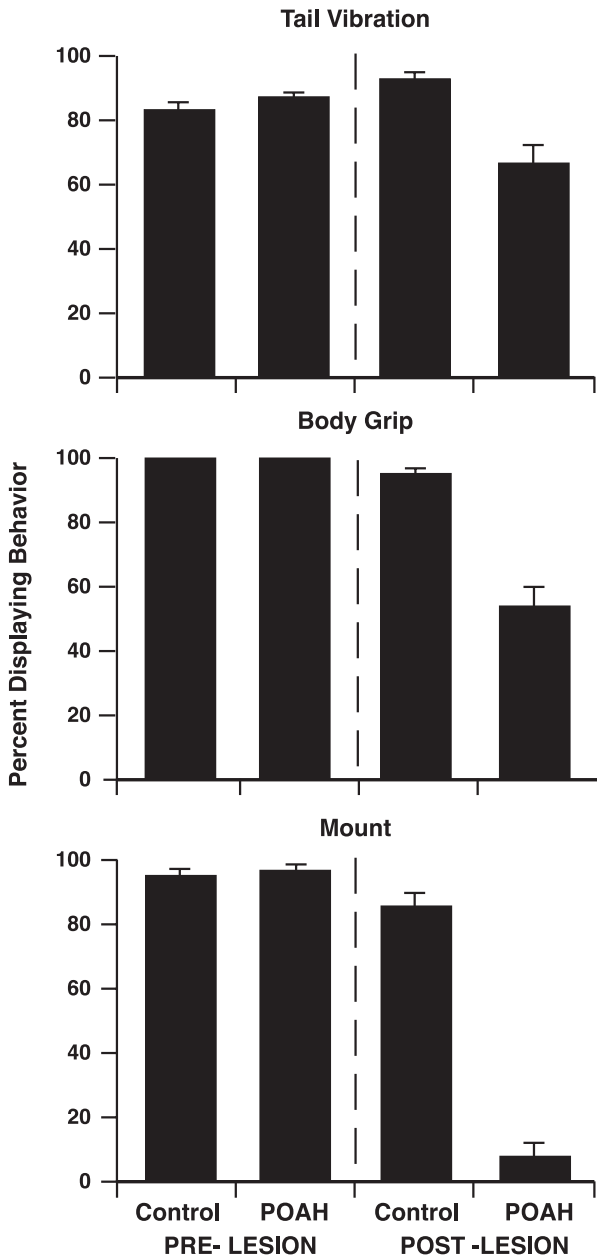


Fig. 1. Lesions located in the preoptic area–anterior hypothalamus (POAH, $n=9$) cause deficits in both appetitive (tail vibration and body grip) and consummatory behaviors (mounting) in sexually active male leopard geckos (*E. macularius*). Lesions located outside this area (Control, $n=6$) had no significant effect on any of these behaviors.

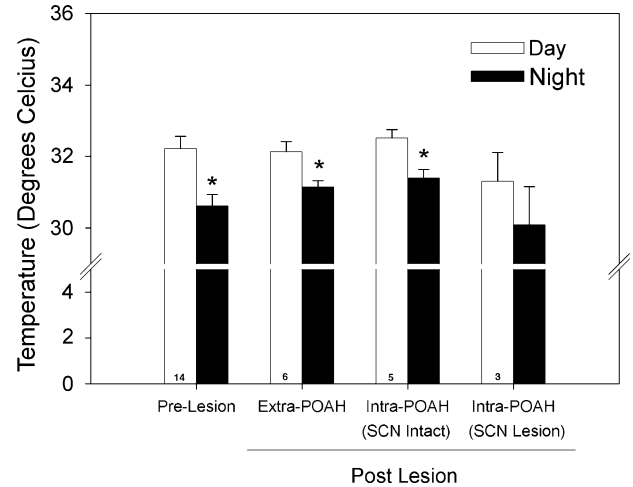


Fig. 2. Mean body temperature (plus standard error) of animals during the day (white bars) and night (dark bars). Temperature values are depicted both before (Pre-Lesion) and after (Post-Lesion) surgery. Number of animals in each group indicated by numeral in white bars. Asterisk (*) indicates significant difference in mean body temperature between day and night ($p<0.05$).

lesion damage that extended to the SCN showed abolishment of rhythmic thermoregulatory behavior.

4. Discussion

4.1. Sexual behavior

In the present study, POAH lesions produced permanent deficits in the courtship and copulatory behavior of male leopard geckos with previous sexual experience. In contrast, animals with lesions targeted outside the POAH continued to exhibit sexual behaviors with no statistical difference in behavior following lesioning. In animals that received lesions in the POAH region, there did not appear to be a correlation between the extent of POAH destroyed and the pattern of deficit in components of behavior. The slight deficit seen in components of behavior following lesion surgery within the group of animals receiving lesions outside the POAH were due primarily to two individuals (animals 430 and 634), both of whom showed extensive lesion damage in relatively broad areas of the medial cortex. However, even this deficit in behavior, in comparison to that of POAH-lesioned animals, did not approach the same magnitude of behavioral loss.

Following lesion surgery, animals receiving POAH lesions show an increasing deficit in behavior within the sequence of mating behaviors, from tail vibration, to body grip, and finally, mounting. Tail vibration and body gripping can be regarded as appetitive behaviors while mounting can be regarded as a consummatory behavior. This distinction supports earlier findings which demonstrate that sexual behavior can persist in male rats even when coital behavior has been eliminated by destruction of the POAH [5,6].

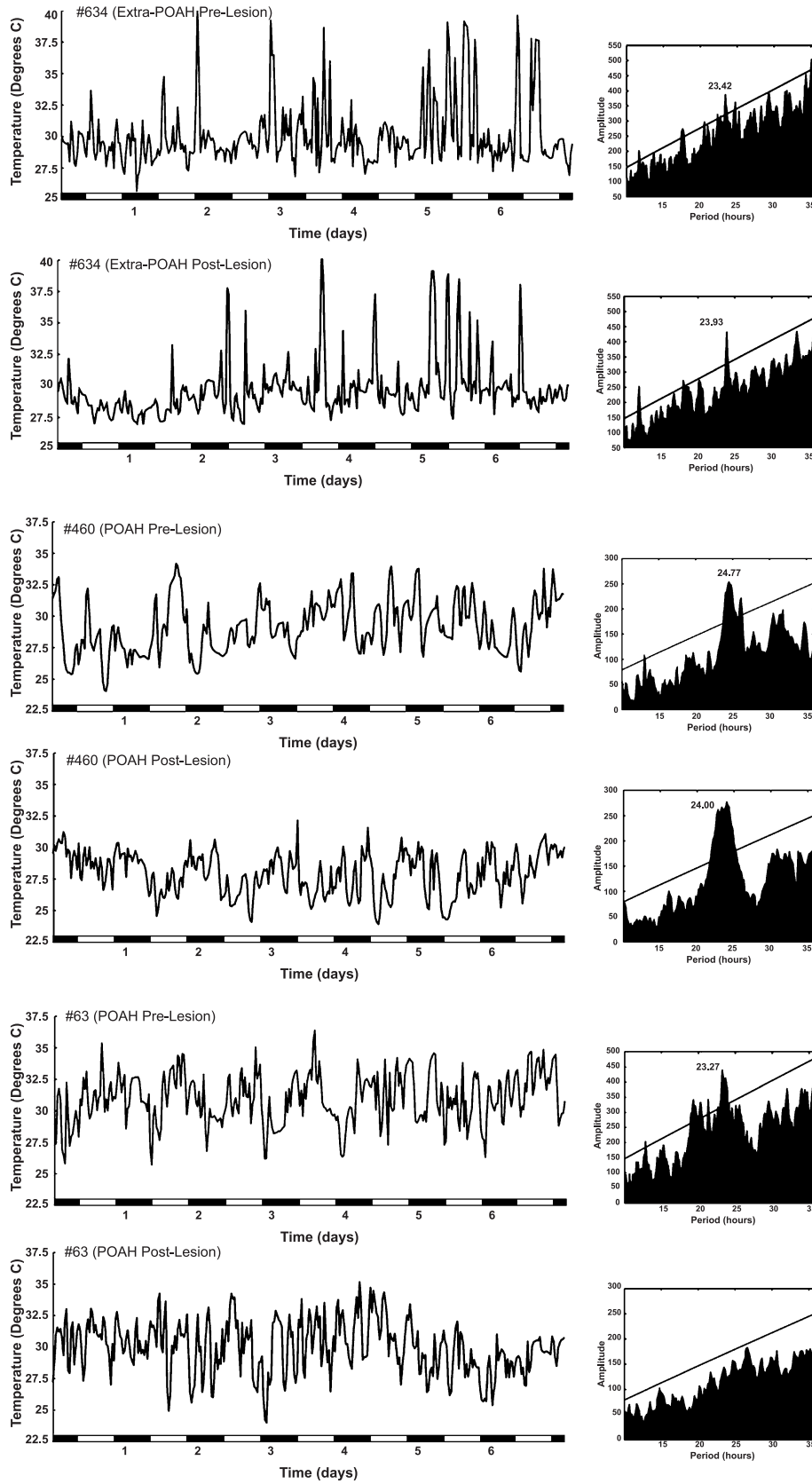


Fig. 3. Records of body temperature of animals that received lesions that: spared the POAH (top panel), was localized to the POAH (middle panel), or was localized to the POAH and extended in the region of the SCN (bottom panel). Records depict 7 days pre-lesion (top record for each animal) and post-lesion (bottom record for each animal). Spectral analyses for the data presented are shown to the right of each record. Values above peaks on each spectral analysis represent the period of the highest amplitude rhythm reaching significance ($p < 0.01$).

The persistence of appetitive behavior in animals with POAH destruction has been reported in various examinations of sexual behavior in males of other species. Male rats with neurotoxic lesions to the POAH continue to exhibit the appetitive behaviors of anogenital sniffing and climbing over stimulus females, but fail to display the consummatory behaviors involved in mating [8]. Crews et al. [3] and DeVries and Simerly [4] also delineate motivational from performance aspects of sexual behavior, finding that male rats trained to press a lever to receive access to estrous females continue to lever press following neurotoxic lesions to the POAH, yet fail to copulate with the females when they become available. In this paradigm, bar pressing results are analogous to the appetitive behaviors of anogenital sniffing and climbing over stimulus females seen in the earlier Hansen experiments [8,9].

Parallel to appetitive behaviors such as bar pushing or anogenital sniffing in the rat, tail vibration and body-gripping behaviors in the male leopard gecko also indicate motivation to mate. Analogous to results seen in rats, POAH lesions in male leopard geckos in the present studies resulted in a clear deficit in consummatory behavior, leaving appetitive behaviors comparatively less affected. This differential effect of POAH lesions on appetitive and consummatory aspects of male sexuality suggests that the POAH, while involved in the integration of motivational behaviors, has a more direct regulatory effect on a male's ability to mount, intromit, and ejaculate when mating with a female conspecific.

4.2. Thermoregulatory behavior

The POAH serves as an integrator of thermoregulatory behavior, as part of a network of inputs in the brain controlling thermoregulation [21]. According to this model of neural organization and control, the POAH, while active as an integrator of behavior in intact neural networks, may not be essential for the continuation of all behaviors associated with thermoregulation. Indeed, because multiple neural loci integrate the control of behavior, multiple regulatory effectors are available to correct errors or damage in integration [21].

Supporting this idea, studies by Satinoff and Rutstein [22] indicate that there are sufficient thermosensitive cells and integrative neurons located outside the POAH to enable rats to maintain relative body temperatures after POAH damage. In the present investigation, comparable findings were seen in leopard geckos; POAH lesions effectively disrupting consummatory sexual behavior do not result in any apparent deficit in thermoregulation. Several studies in mammals suggest that the neural substrate underlying behavioral and autonomic thermoregulatory responses are functionally and anatomically distinct [1,14,23]. Studies of ectotherms represent an opportunity to dissociate these mechanisms in vertebrates. The present results suggest that

the POAH of *E. macularius* does not contain temperature-sensitive neurons necessary for behavioral thermoregulation. Alternatively, the POAH may normally be important for temperature regulation in intact animals, while other brain loci can compensate when the POAH is destroyed. Because POAH lesions encompassing the SCN disrupt temperature regulation on a daily schedule, it remains possible that interactions between the SCN and POAH are necessary for normal temperature regulation. Further studies with lesions targeting only the SCN, to determine the specific contribution of this nucleus, are necessary to explore this possibility.

To our knowledge, the present results provide the first evidence for a role of the SCN in daily temperature regulation in leopard geckos. In most reptiles, the pineal gland is a central component regulating daily (circadian) rhythms [24]. However, in some squamate species, such as the desert iguana (*Dipsosaurus dorsalis*) and the ruin lizard (*Podarcis sicula*), removal of the SCN disrupts some circadian parameters [10,17]. The present findings suggest that the SCN may represent an important circadian pacemaker in *E. macularius*. Further studies are necessary to determine if the rhythm in temperature regulation persists in the absence of external time cues. Likewise, whether the SCN is an important regulator of temperature rhythmicity, or daily rhythmicity more generally in this species, remains to be determined.

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