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Journal of Thermal Biology 31 (2006) 40–49

Journal of THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Effects of melatonin on thermally classified anterior hypothalamic neurons in the white-footed mouse (*Peromyscus leucopus*)

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Abstract

1. The pineal hormone melatonin influences thermoregulation in many species, but the mechanism of action is unknown.
2. We tested whether melatonin could act directly on the primary thermoregulatory control center in the preoptic area and anterior hypothalamus (PO/AH), using a white-footed mouse tissue slice preparation.
3. PO/AH neurons were classified according to their thermosensitivity, then treated with 1 μM melatonin.
4. Thirty percent of warm-sensitive and 41% of temperature-insensitive neurons showed significant changes in firing rate during melatonin treatment.
5. While there was no correlation between responses to melatonin and thermosensitivity, these results constitute the first evidence that melatonin acts directly on PO/AH thermoregulatory neurons.

Keywords: Hypothalamus; Thermoregulation; Electrophysiology; Thermosensitivity; Melatonin; Photoresponsiveness

1. Introduction

Melatonin, the primary hormone of the vertebrate pineal gland, is involved in the regulation of several body systems that exhibit circadian and seasonal variation. Production of melatonin occurs strictly during the dark phase of the light-dark cycle, allowing it to serve as an endocrine signal of day length, or photoperiod (Goldman, 2001). In mammals from the temperate zone, the melatonin signal plays an essential role in the generation of seasonal rhythms in behavioral and physiological traits (Prendergast et al., 2002). While the best studied of these rhythms is seasonal reproduction, a growing body of research highlights the importance of melatonin in the regulation of energy balance and body temperature (Heldmaier and Lynch, 1986; Saarela and Reiter, 1994; Bilbo and Nelson, 2002).

In many rodent species, short photoperiod or melatonin treatment induces a host of thermoregulatory responses (Heldmaier et al., 1989; Saarela and Reiter, 1994; Sullivan and Lynch, 1986). These can include increased nesting behavior, molt to the winter pelage, up-regulation of interscapular brown adipose tissue, and a greater incidence of spontaneous daily torpor. In addition, Djungarian hamsters (*Phodopus sungorus*) and deer mice (*Peromyscus maniculatus*), when placed in short photoperiod or given melatonin implants, show a decrease in mean T_b of 0.5–0.9 °C that is largely independent of both daily torpor and the circadian temperature rhythm (Heldmaier et al., 1989; Andrews and Belknap 1985, 1986, 1993). This seasonal decline in T_b presumably serves to reduce the energy demands of endothermy, and thus is adaptive for surviving harsh winter conditions.

Seasonal rhythms in humans are not well understood (Wehr, 2001), and there is little evidence addressing the photoperiodic regulation of body temperature in humans (but see Torii et al., 1991; Honma et al., 1992). However, melatonin is prominently involved in modulating the circadian rhythm of T_b. Blocking melatonin production attenuates the nocturnal decline in T_b by 40%, and timed melatonin administration reduces the normal daytime rise in T_b (Cagnacci et al., 1992, 1997). Other studies have demonstrated mild hypothermic effects of melatonin that

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doi:10.1016/j.jtherbio.2005.12.001

are independent of circadian rhythm, both in humans (Satoh and Mishima, 2001; Strassman et al., 1991) and other vertebrates (Heldmaier and Lynch, 1986; Saarela and Reiter, 1994; Varghese and Pati, 1996).

Depending on the organism and the specific conditions, melatonin may act on a variety of target tissues to alter T_b. Peripheral sites of action have been suggested (Prunet-Marcassus et al., 2001; Viswanathan et al., 1990; Delagrange and Jockers, 2003) but the primary site is thought to be the central thermostat located in the preoptic area and anterior hypothalamus (PO/AH). This region integrates local thermal information with input from cutaneous and deep body thermoreceptors to establish and defend a set-point of T_b (Boulant, 1998). Evidence for a set-point adjustment by melatonin is not conclusive, but Cagnacci and colleagues have argued that the precise and consistent hypothermic effect (0.3–0.4 °C) suggests the involvement of central thermoregulatory centers (Cagnacci et al., 1997). More recently, Aizawa and colleagues reported that melatonin administration lowered the threshold for the activation of autonomic heat-loss mechanisms (sweating and cutaneous vasodilation), implying a downward shift in the set-point (Aizawa et al., 2002).

What sort of neural mechanisms could underlie this set-point shift? A well-supported model of hypothalamic temperature regulation involves the activity of two basic types of neurons within the PO/AH: warm-sensitive neurons that fire in proportion to local and peripheral temperature, and temperature-insensitive neurons that do not respond to temperature (Hammel, 1965; Boulant, 1998; Griffin et al., 2001). These two populations of neurons are thought to provide antagonistic synaptic input to heat loss and heat production effector neurons, such that a thermal challenge will trigger the appropriate autonomic and behavioral responses to maintain the set-point. The model also suggests that an exogenous or endogenous substance (i.e., a pyrogen or hormone) can shift the set-point by preferentially exciting or inhibiting the activity of either population of cells (Boulant, 1998; Ranels and Griffin, 2003).

The goal of the present study was to determine if melatonin can have a direct effect on neural firing in the PO/AH, and if neuronal responses to melatonin are consistent with an adjustment of the thermal set-point. Since most studies have demonstrated a hypothermic effect of the hormone, we predicted that melatonin would preferentially excite warm-sensitive neurons and/or inhibit temperature-insensitive neurons, which according to the model would result in a decrease in the set-point. To test this hypothesis, we recorded from single PO/AH neurons in tissue slices from *P. leucopus*. This species is known to have robust photoperiodic responses, and to express melatonin receptors in the PO/AH (Weaver et al., 1990; Heideman et al., 1999a).

Because our animal colony is derived from a natural population, we were also interested in possible within-species variation in neuronal sensitivity to melatonin and temperature. We addressed this question by comparing responses in two selected lines of mice that differ in reproductive responsiveness to short photoperiod (Heideman et al., 1999b). Our laboratory previously reported that these lines differ in the amount of 2-[^{125}I]iodomelatonin (I-MEL) binding in the medial preoptic area (MPOA) and bed nucleus of the stria terminalis (BNST; Heideman et al., 1999a), suggesting that variation in photoresponsiveness is partly caused by differences in melatonin receptor density or affinity in these areas. Thus, we predicted that the excitation-inhibition trend described above would be stronger in mice from the photoresponsive line than in mice from the non-photoresponsive line.

2. Materials and methods

2.1. Animals

Sixty-four adult female *P. leucopus*, aged 72–146 days at the time of recording experiments, were used in this study. Mice were taken from the F6 to F9 generations of either a reproductively photoresponsive line (RM; $N = 29$) or a non-photoresponsive line (NRM; $N = 35$; Heideman et al., 1999b). Mice were weaned at age 22 ± 1 d to individual polyethylene cages with wire tops and 3 cm pine shavings. Food (LM-485, Harlan Teklad, Madison, WI) and tap water were provided ad lib, and temperature was kept at 23 ± 3 °C. At age 70 ± 10 d, the ovaries were removed under deep isoflurane anesthesia, in order to control for the effects of circulating sex steroids on temperature regulation (Hessemer and Brück, 1985; Stachenfeld et al., 2000) and on the density of melatonin receptors (Morgan et al., 1994; Clemens et al., 2001; Seltzer et al., 1992). Reproductive status was assessed during the ovariectomy procedure in a subset of animals from both lines. This confirmed that the great majority of RM were reproductively inhibited by short photoperiod, whereas most NRM were reproductively well-developed. Animals were allowed at least 5 d to recover from surgery before experiments. All procedures were approved by the Animal Care and Use Committee of the College of William and Mary.

2.2. Photoperiod and circadian timing of experiments

Several studies have reported an increase in melatonin binding following pinealectomy or when animals are kept in short photoperiod, indicating an inverse relationship between melatonin exposure and receptor density (Gauer et al., 1993; Recio et al., 1996; Dardente et al., 2003). To examine possible effects of photoperiod on the sensitivity of PO/AH neurons to melatonin, approximately half of the mice were transferred within 2 d of birth to a short day photoperiod (L8:D16; lights on at 0800 h; SD), while the other half remained in a long day photoperiod (L16:D8; lights on at 0400 h; LD). In addition, all animals were sacrificed within 1 h of the onset of the dark phase (SD mice: 1500–1600 h clock time, 0700–0800 circadian time (CT); LD mice: 1900–2000 h clock time, 1500–1600 CT), in order to minimize potential circadian variation in melato-

nin receptor levels (Masson-Pevet et al., 2000; Schuster et al., 2001; Brooks and Cassone, 1992; Zisapel et al., 1998). This also ensured that the experimental melatonin treatment occurred during the subjective night, when melatonin levels would have been high in the intact animal.

2.3. Tissue slice recordings

Prior to each recording session, a single mouse was anesthetized with isoflurane and rapidly decapitated. The brain was quickly removed and 2–3 coronal tissue slices (400 μm thick) containing the PO/AH were sectioned with a vibratome. Slices were transferred to an interface-recording chamber (Kelso et al., 1983) and allowed to equilibrate for at least 90 min. The chamber was continuously perfused (1.5 ml/min) with artificial cerebrospinal fluid (aCSF) containing (in mM) 5 KCl, 124 NaCl, 1.3 $MgSO_4$, 25 NaHCO, 1.24 KH_2PO_4, 10 glucose, and 2.4 $CaCl_2$. The aCSF was saturated with 95% O_2–5% CO_2, and heated to 36 °C by a Peltier device.

Extracellular single-unit recordings were made using glass microelectrodes (tip diameter < 1 μm) filled with 3 M NaCl. Recordings were amplified (FHC Xcell-3), displayed on a personal computer with AxoScope software (Axon Instruments, Inc.), and stored on digital tape for offline analysis. The temperature of the perfusion medium was monitored via a small thermocouple positioned just below the slices. Using a stereomicroscope, the electrode was visually placed within the PO/AH, as delimited rostrally by the organum vasculosum of the lamina terminalis (OVLT), dorsally by the fornix or anterior commissure, and caudally by the separation of the optic tracts (see Fig. 2). Recording locations were arbitrarily selected within this region, although care was taken to avoid the suprachiasmatic nuclei (SCN), paraventricular nuclei (PVN), arcuate nucleus, and diagonal band of Broca.

Once a neuron with a stable firing rate was isolated, the temperature of the medium was varied 2–3 °C above and below the baseline of 36 °C (rate = 1 °C/min) to determine neuronal thermosensitivity. After temperature and firing rate returned to baseline, the perfusate was switched to aCSF containing 1 μM melatonin. Melatonin treatment was continued until a change in firing rate (impulses s^{-1}) of greater than 15% occurred and persisted for at least 2 min, or if 10–15 min elapsed with no response. Exposure to melatonin was then followed by a washout period (perfusion with normal aCSF) of at least 10 min. In the event of a significant change in firing rate (>15%), the washout period was extended until the firing rate returned to near-baseline levels, or for up to 1 h if firing rate failed to return to baseline.

2.4. Histological processing and recording site verification

Following each recording session, the location of the electrode was visually confirmed (Dean and Boulant, 1989; Burgoon and Boulant, 2001) and marked on a slice map derived from an atlas of the rat brain (Paxinos and Watson, 1998). Tissue slices were fixed in 10% formalin for at least 3 h, then in 30% formalin–sucrose for at least 1 h. Slices were then re-sectioned to 50 μm, mounted on gelatin-coated slides, and processed with Giemsa to label cell bodies. This allowed the identification of well-defined cell groups with a light microscope, providing landmarks with which to compare the visually confirmed electrode location. Assignment of neuronal location was verified by examining the 50 μm stained section corresponding to the depth of the electrode tip (noted during each experiment).

Recording site location was defined using a rat brain atlas because the sole available *Peromyscus* atlas (Eleftheriou and Zolovick, 1965) does not provide sufficient spatial detail of the PO/AH. Relative size and position of hypothalamic structures in *P. leucopus* appears more similar to the rat than the laboratory mouse (Avigdor et al., 2005); however, no homology of cell groups is assumed.

2.5. Data analysis

For each neuron, thermosensitivity was determined by a linear regression of firing rate as a function of temperature over a range of at least 2.5 °C. A regression coefficient of at least 0.8 impulses $°C^{-1}\,s^{-1}$ was required to classify a neuron as warm-sensitive (Boulant and Hardy, 1974; Griffin et al., 2001; Ranels and Griffin, 2003); all other neurons were classified as temperature-insensitive.

Mean firing rate was measured over three 1-min segments: immediately before melatonin treatment ('Baseline'), during the peak of a response to melatonin ('Mel'), and at the end of the washout period ('Washout'). A response was deemed significant only if the change in mean firing rate (impulses s^{-1}) between Baseline and Mel segments was at least 15%, and was significant at $p < 0.05$ (Student's *t*-test). Data during washout was used as a qualitative assessment of whether the firing rate returned to near-baseline levels.

Contingency tables were constructed to test for nonrandom associations between melatonin response (increase or decrease in firing rate) and three independent measures: thermosensitivity class (warm-sensitive vs. temperature-insensitive), mouse line (NRM vs. RM), and photoperiod (SD vs. LD). The overall percentage of melatonin-sensitive neurons (collapsing across increases and decreases in firing rate) was also examined with respect to the three independent measures. The significance of each comparison was evaluated with a two-tailed Fisher's Exact test, at a significance level of 0.05. Statistical tests were performed using SPSS and SigmaPlot (SPSS, Inc.) software.

3. Results

3.1. Neuronal thermosensitivity and baseline activity

Of 96 neurons, 37 (38.5%) were classified as warm-sensitive and 59 (61.5%) were temperature-insensitive, consistent with previous reports in the laboratory rat (Dean and Boulant, 1989; Griffin et al., 2001). Mean

baseline firing rate was 7.27 ± 0.75 (SEM) impulses s^{-1} for warm-sensitive neurons and 3.71 ± 0.33 impulses s^{-1} for temperature-insensitive neurons. Thermosensitivity coefficient ranged from 0.80 to 5.75 impulses $s^{-1}\,°C^{-1}$ for warm-sensitive neurons, and -0.35–0.74 impulses $s^{-1}\,°C^{-1}$ for temperature-insensitive neurons.

3.2. Responses to melatonin

Fig. 1(a–d) shows firing rate plotted against time for 4 representative neurons, and the responses of all neurons are summarized in Table 1. Changes in firing rate were often modest (Fig. 1a), but could be quite large (b and c); the neuron in (d) did not respond to melatonin. Response latencies ranged from about 2 min (a and c) to as long as 13 min (b). Firing rates during washout typically did not return completely to baseline (a and b), but 60% of melatonin-sensitive neurons showed at least a partial recovery after melatonin was washed out of the perfusion bath.

There was a slight tendency for melatonin to excite warm-sensitive neurons and inhibit temperature-insensitive

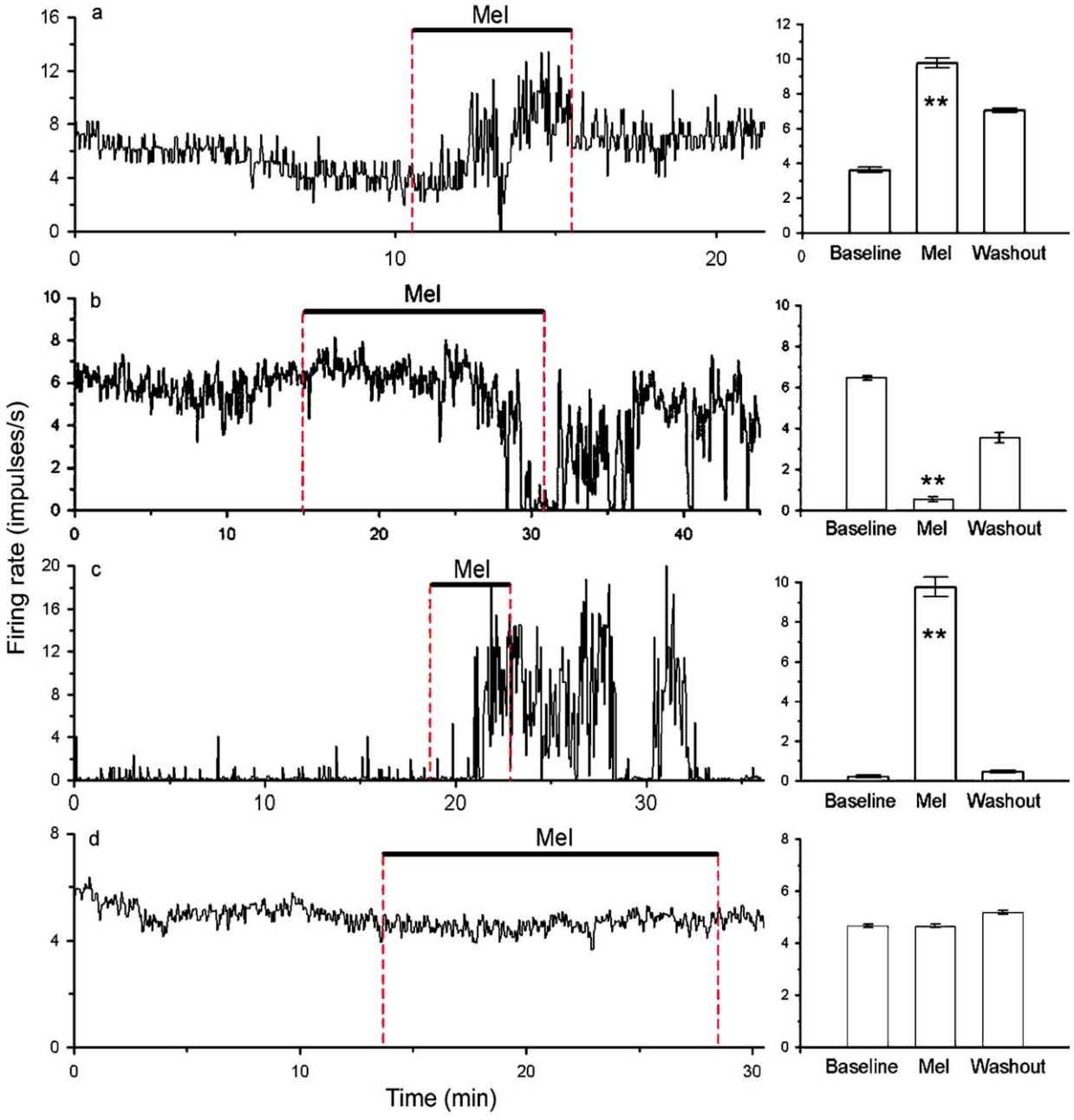

Fig. 1. Firing rate traces for 4 representative neurons (a–d). Treatment bars and dashed lines indicate time of melatonin treatment. Bar graphs for each neuron show mean firing rate (±SEM) during one-minute segments before (Baseline), during (Mel), and after (Washout) melatonin treatment. **, firing rate during Mel was significantly different from Baseline ($p < 0.0001$).

Table 1
Effects of melatonin on the firing rates of PO/AH neurons, separated by neuronal thermosensitivity, mouse line, and photoperiod

	N	No response	Increase in firing rate	Decrease in firing rate
All neurons	96	61 (64%)	17 (18%)	18 (19%)
Thermosensitivity				
Warm-sensitive	37	26 (70%)	8 (22%)	3 (8%)
Temperature-insensitive	59	35 (59%)	9 (15%)	15 (25%)
Mouse line				
Photoresponsive (RM)	46	31 (67%)	7 (15%)	8 (17%)
Non-photoresponsive (NRM)	50	30 (60%)	10 (20%)	10 (20%)
Photoperiod				
Short photoperiod (SD)	48	33 (69%)	9 (19%)	6 (13%)
Long photoperiod (LD)	48	28 (58%)	8 (17%)	12 (25%)

neurons (Table 1, *Thermosensitivity*). However, a Fisher's Exact test on the relationship between thermosensitivity classification and melatonin response (2×2 contingency table) revealed this trend to be nonsignificant ($p = 0.075$). We also analyzed the correlation between melatonin response and thermosensitivity within each mouse line (data not shown). In RM, melatonin tended to excite warm-sensitive neurons and inhibit temperature-insensitive neurons (Fisher's Exact test, $p = 0.04$), whereas no such trend was seen in NRM ($p > 0.9$). However, a Bonferroni correction for multiple comparisons ($\alpha = 0.025$) renders this result nonsignificant. Independent of thermosensitivity, there was no significant association between melatonin response and mouse line ($p > 0.9$) or photoperiod ($p = 0.31$; Table 1).

Collapsing across increases and decreases in firing rate, a greater percentage of neurons were sensitive to melatonin in NRM (40%) as compared to RM (33%), but this difference was not significant ($p = 0.53$). Similarly, LD mice showed a greater percentage of neurons than SD mice (42% vs. 31%), but the difference did not reach significance ($p = 0.40$). The greater percentage of LD mice showing a decrease in firing rate with melatonin treatment (25% vs. 13%) was also nonsignificant ($p = 0.19$).

3.3. Neuron location and melatonin response

Fig. 2 shows the anatomical distribution of recording sites. Colored symbols represent a significant response to melatonin (increase or decrease in firing rate; see legend), while black symbols represent no response. Shapes denote neuronal thermosensitivity. For descriptive purposes (see Methods), map locations were visually assigned to one of 4 sub-regions within the PO/AH: the medial preoptic area (MPOA), ventromedial preoptic area (VMPO), anterior hypothalamic area (AHA), and within or near the medial/posteriolateral bed nucleus of the stria terminalis (BNST), including the striohypothalamic nucleus (StHy). Again, these designations are for spatial reference only, and may not represent homologous nuclei in this species.

Melatonin-sensitive neurons located more ventrally (i.e., the VMPO) tended to show an increase in firing rate (5 increases vs. 1 decrease). Conversely, neurons located more dorsally (BNST/StHy) were more often inhibited by melatonin, but sample sizes are too small to make strong conclusions from these results. Recording sites located within the MPOA resulted in fewer melatonin-sensitive neurons (30%) than other regions (42%), but this difference was not significant ($p = 0.21$). Apart from these minor differences, no systematic pattern was apparent in the spatial distribution of responsive neurons, and no single nucleus or region stands out as being particularly sensitive to melatonin.

3.4. Lack of circadian effects

Because of the potential for circadian variation in melatonin receptor levels, one might expect the number or magnitude of responses to vary with the circadian time of melatonin treatment. These comparisons are shown in Figs. 3 and 4. Here, CT is defined as the time after lights-on (and thus represents different clock times for the two photoperiod groups). Previous studies have reported a peak in melatonin binding toward the end of the light phase (CT 10–13; Brooks and Cassone, 1992; Zisapel et al., 1998). Surprisingly, we found no such trend in either the percent change in firing rate ((Baseline−Mel)/Baseline × 100; Fig. 3) or the prevalence of melatonin-sensitive neurons (Fig. 4). A similar analysis using clock time instead of CT also failed to reveal an overt effect (not shown).

4. Discussion

The role of the pineal in thermoregulation has been studied for decades (reviewed by Heldmaier and Lynch, 1986; Saarela and Reiter, 1994; Ralph et al., 1979), but the physiological basis for the action of melatonin on body temperature remains poorly understood. The present study is the first to examine the effects of melatonin on neural firing in the PO/AH, a region vital to the control of body

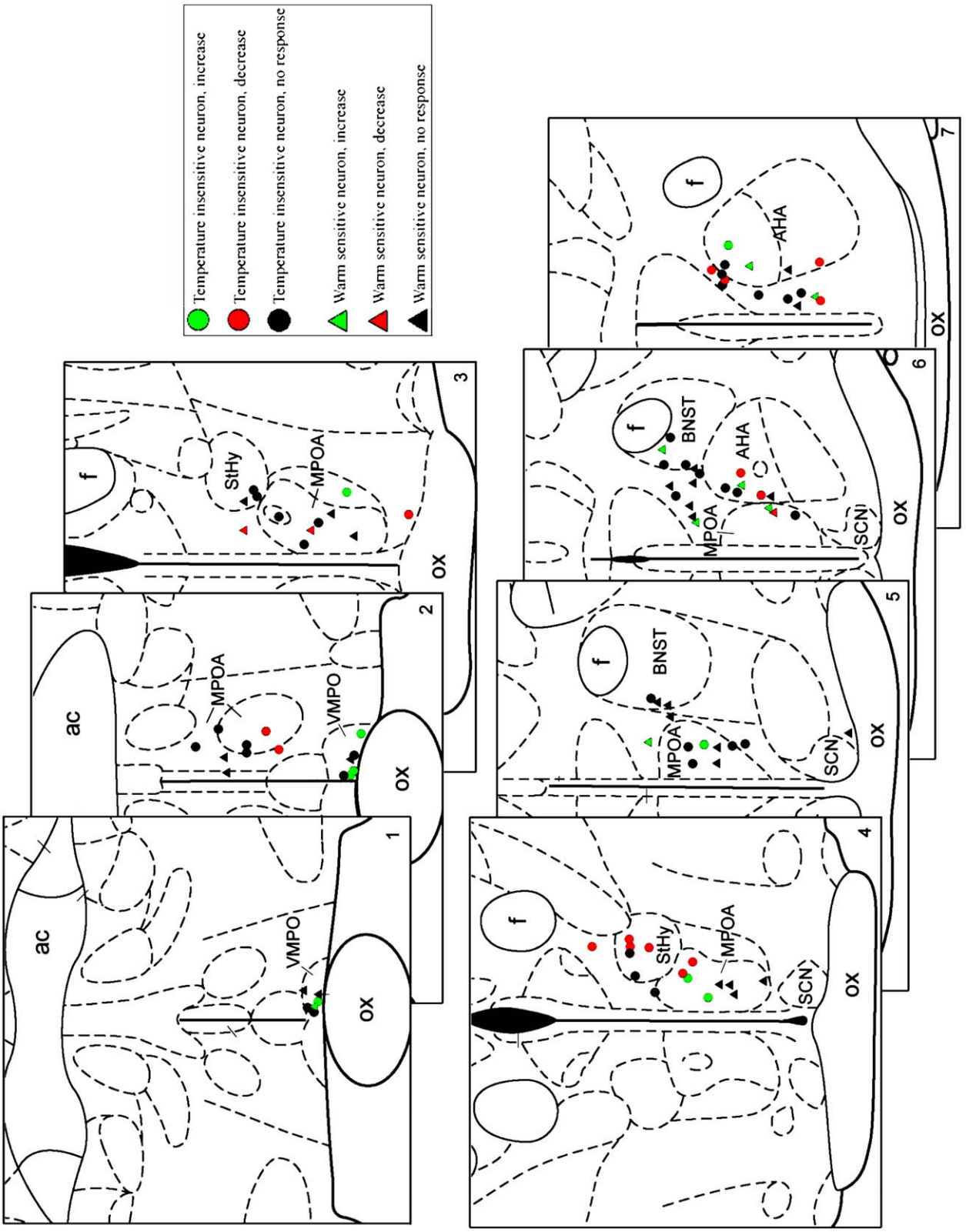

Fig. 2. Map of recording sites in coronal hypothalamic sections from *P. leucopus* (see legend). Sections are numbered in order from anterior to posterior, and all recording sites are represented on the right side for display purposes. Slice maps were taken from an atlas of the rat brain (Paxinos and Watson, 1998; see Methods). ac, anterior commissure; AHA, anterior hypothalamic area; BNST, bed nuc. stria terminalis; f, fornix; MPOA, medial preoptic area; ox, optic chiasm; SCN, suprachiasmatic nuc.; StHy, striohypothalamic nuc.; VMPO, ventromedial preoptic area.

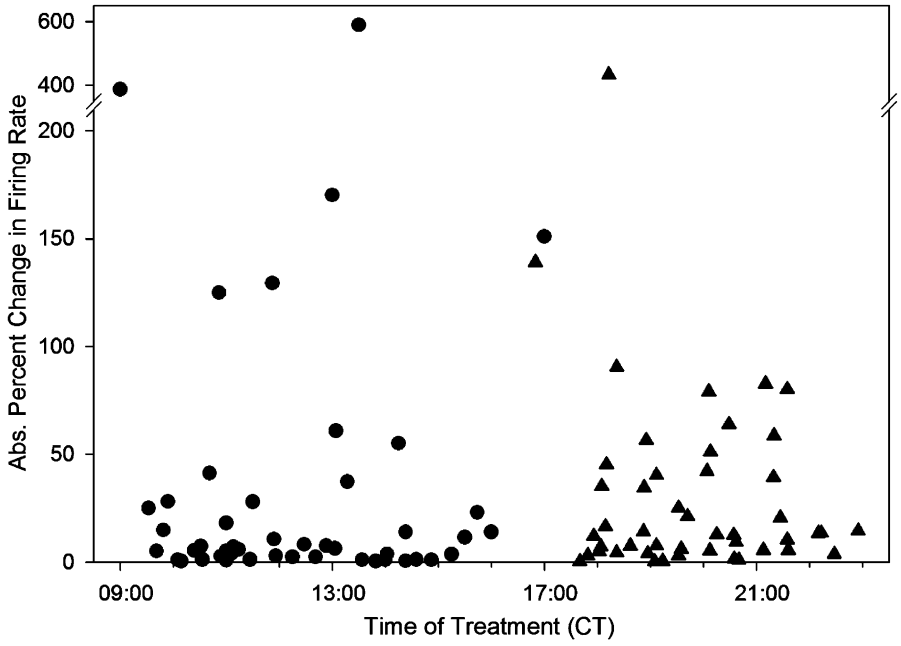

Fig. 3. Percent change in firing rate (absolute value) for all neurons ($N = 96$) plotted against the time (CT) of melatonin treatment. Circles = SD mice; triangles = LD mice.

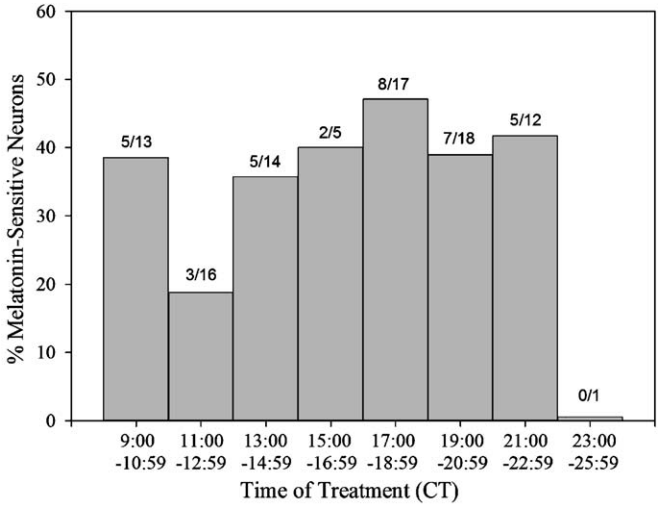

Fig. 4. Histogram depicting the proportion of neurons that showed a significant response to melatonin (with sample sizes above each bar) vs. time of treatment (CT), in 2 h bins.

temperature in endotherms. Thirty-six percent of neurons in our sample, including both warm-sensitive (30%) and temperature-insensitive neurons (41%), showed a significant response to treatment with 1 μM melatonin. Melatonin-sensitive neurons were located throughout the PO/AH, showing no strong clustering in any particular nucleus. Despite the reported peak in melatonin binding in late afternoon or early evening (Brooks and Cassone, 1992; Zisapel et al., 1998), we found no relationship between CT and the number or average magnitude of responses to melatonin.

The reported hypothermic effect of melatonin in vivo (e.g., Andrews and Belknap, 1985, 1986; Heldmaier et al., 1989; Satoh and Mishima, 2001; Cagnacci et al., 1997) predicts a specific pattern of responses in the PO/AH with respect to neuronal thermosensitivity. According to a leading model of hypothalamic set-point control initially proposed by Hammel (1965), the T_b set-point is regulated by the relative activity of warm-sensitive and temperature-insensitive neurons in the PO/AH. The model proposes that autonomic mechanisms for heat loss (i.e., cutaneous vasodilation, evaporative heat loss) are driven by a population of effector neurons, which in turn are synaptically excited by warm-sensitive neurons and inhibited by temperature-insensitive neurons. Conversely, heat-production effector neurons are thought to be inhibited by warm-sensitive neurons and excited by temperature-insensitive neurons (Boulant, 1991). Therefore, a neuromodulatory substance can induce a downward shift in the set-point by selectively increasing the firing rate of warm-sensitive neurons and/or decreasing that of temperature-insensitive neurons (Boulant, 1998; Ranels and Griffin, 2003). We hypothesized that melatonin acts in this way to produce the mild hypothermic effects seen in humans and other mammals.

The results show a modest but nonsignificant trend in support of the hypothesis. Of 11 warm-sensitive neurons that responded to melatonin, 8 (73%) showed a significant increase in firing rate, while 3 (27%) showed a decrease in firing rate. Twenty-four temperature-insensitive neurons responded to melatonin, with 9 (37%) showing an increase and 15 (63%) a decrease in firing rate (Fisher's Exact test: $p = 0.075$; Table 1). The statistical power of this compar-

ison is difficult to determine, as we had no a priori estimate of effect size. A conservative estimate, however, indicates that we may not have had sufficient sample size to detect a significant trend if one existed. Thus, further studies are needed to verify this negative result.

Irrespective of the correlation with thermosensitivity, many neurons in this thermoregulatory region clearly show robust responses to melatonin in vitro. What is the physiological significance of these responses? Thermoregulatory adjustments triggered by melatonin in vivo may require the involvement of multiple neural and endocrine systems over a period of days or weeks. Our recording experiments can give only a snapshot of the acute effects of the hormone on neural firing, and thus are unable to measure the long-term changes in the activity of PO/AH neurons that may be important for the regulation of seasonal changes in body temperature. It is also possible that the effects of melatonin on T_b are not produced by altering the hypothalamic set-point, but instead are mediated by other structures such as the thyroid gland, brown adipose tissue (BAT), or vasculature. Melatonin can regulate TSH release from the pars tuberalis of the pituitary (Sakamoto et al., 2000), and a thyroid-mediated metabolic adjustment is thought to underlie the melatonin-induced hyperthermia in rats (Padmavathamma and Joshi, 1994). Binding sites for melatonin have been observed in BAT, and treatment of brown adipocytes with melatonin-induced differential mitochondrial gene expression (Prunet-Marcassus et al., 2001). Other studies have found an effect of melatonin on vasomotor tone (Geary et al., 1997; Masana et al., 2002), and melatonin receptors are prevalent in arterial beds involved in thermoregulation (Viswanathan et al., 1990).

There are, however, several lines of evidence supporting a direct action of melatonin on thermoregulatory centers in the hypothalamus. Studies by Cagnacci and colleagues have explored the role of melatonin in modulating the circadian T_b rhythm (Cagnacci et al., 1992, 1997). They found that blocking melatonin production with a β-adrenergic antagonist blunted the nighttime decrease in T_b by 40%. The daytime rise in T_b was similarly attenuated by oral administration of melatonin (2.5 mg). This effect was highly consistent and reproducible, implicating a central thermoregulatory mechanism. Importantly, these and other studies (Satoh and Mishima, 2001; Strassman et al., 1991) seem to require doses of melatonin that produce blood concentrations much greater than the physiological range, inviting the criticism that endogenous melatonin in humans may not be prominently involved in temperature regulation. Nevertheless, it has been argued that a physiological action of the hormone is still probable, since a large dose of melatonin administered peripherally may be required to achieve physiological concentrations in the brain and cerebrospinal fluid (Cagnacci et al., 1997).

Recent findings by Aizawa et al. (2002) are consistent with a centrally mediated thermoregulatory response to melatonin. Oral administration of melatonin (3 mg) during the daytime decreased tympanic temperature by about 0.12 °C. Subjects were then partially immersed in a hot water bath, and the tympanic temperature was measured at the time of onset of cutaneous vasodilation and sweating of the forearm. Compared with placebo, both measures of autonomic thermoregulatory threshold were reduced in the subjects who had taken melatonin (Aizawa et al., 2002). This result suggests that the melatonin-induced hypothermia in humans is at least partly due to a downward shift in the set-point, a process likely to be implemented by neurons in the PO/AH.

Bilbo, Nelson, and colleagues have shown that short photoperiod or melatonin treatment can alter the duration of fever in Siberian hamsters (Bilbo et al., 2002; Bilbo and Nelson, 2002), apparently to conserve energy during winter. The essential role of PO/AH neurons in fever is well known (Boulant, 1991; Ranels and Griffin, 2003), suggesting that this particular adaptive effect of melatonin may be mediated by neural responses in the PO/AH such as those we observed. It should be noted, however, that attenuation of fever duration required a long-term exposure to melatonin or short days, limiting the conclusions we can draw from a comparison with our study.

Since non-shivering thermogenesis is the primary mechanism for heat production in rodents, it is important to consider the effects of photoperiod and melatonin on the size and thermogenic capacity of BAT deposits. Both the size and thermogenic capacity of BAT deposits (as measured by the amount and oxidative capacity of BAT mitochondria) increases in short photoperiods or after melatonin injections in several rodent species, including the white-footed mouse (Saarela and Reiter, 1994; Heldmaier et al., 1989; Reilly et al., in Prep.). These changes in BAT thermogenesis likely result from greater peripheral sympathetic activation, which may be initiated by circuits in the medial preoptic area (Morrison, 2004). In fact, white-footed mice with melatonin-beeswax pellets implanted only in the anterior hypothalamus showed an increase in interscapular BAT mass, as well as increased nesting behavior (Glass and Lynch, 1982), further implicating this region in seasonal temperature regulation.

If melatonin acts on the PO/AH to induce seasonal thermoregulatory changes, then the relationship between neuronal thermosensitivity and response to melatonin can be a measure of the thermoregulatory responsiveness to photoperiod. Populations of white-footed mice show extensive individual variation in reproductive responsiveness to photoperiod (Heideman and Bronson, 1991; Heideman et al., 1999b), and we were interested in whether this variation extends to the neural sensitivity to melatonin and temperature. We found that RM and not NRM showed a correlation between thermosensitivity and melatonin response (data not shown), but the effect was not significant when correcting for multiple comparisons. This may indicate that selection for reproductive responsiveness to photoperiod does not induce parallel changes in

seasonal thermoregulation, but conclusive evidence will require more detailed in vivo and in vitro experiments.

Studies exploring the neural mechanisms of photoperiodic adjustments are lacking, especially in the realm of thermoregulation. Complicating the situation is the fact that melatonin can directly adjust SCN-driven circadian rhythms, including the rhythm in body temperature. Circadian regulation of T_b is thought to be distinct from the evolutionarily newer homeostatic system (Refinetti, 1998), and some effects of melatonin on T_b (e.g., Cagnacci et al., 1997) could be mediated by the SCN, which contains abundant melatonin receptors. Nevertheless, our results indicate that melatonin can have robust effects on neurons outside the SCN that are likely to be involved in homeostatic thermoregulatory processes. Additional experiments are needed to evaluate the functional significance of the observed responses, and also to explore further the effects of melatonin on T_b in intact *P. leucopus* and other photoperiodic species.

Acknowledgments

The authors would like to thank Charles Finneran and Khrystel King for animal care, Elizabeth Wallis for histological work, and their comments on the manuscript. Supported by National Science Foundation Grant IBN-9983624 to John D. Griffin, IBN-9875866 to Paul D. Heideman, and in part by a Howard Hughes Medical Institute grant through the Undergraduate Biological Science Program to the College of William and Mary.

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