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Temperature Preferendum of Pinealectomized Cockerels during Their Light–Dark Cycle (40566)

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The capacity for physiological adaptation has enabled organisms to survive in a varying physical environment. In the thermal environment, animals are classified as conformers (poikilotherms) if their body temperature (T_b) closely corresponds to ambient temperature (T_a) or regulators (homeotherms) if a relatively constant T_b is maintained independent of wide changes in T_a (1). The intense metabolic rate with its supportive chemical regulation distinguishes the homeothermic animal from an ectotherm which depends on modifying body temperature behaviorally (2).

Extirpation of the parapineal organ in poikilotherms has a notable influence on thermoregulatory behavior. In several species of lizards, removal or shielding of the parapineal increased the time of voluntary exposure to direct insolation (3). Accordingly, the reptilian parapineal has been described as a "photo-thermal radiation dosimeter" that limits an ectothermal animal's exposure to intense solar radiation (4). Hutchinson and Kosh (5) found that parietalectomized lizards (*Anolis carolinenis*) placed in a thermocline selected higher T_a than sham-operated controls. Impaired tolerance to heat exposure after parapineal ablation suggests that this organ participates in temperature acclimation within this species (6). Collectively, these results demonstrate that the parapineal organ of ectotherms constitutes an important component of behavioral thermoregulation.

Results of several studies have demonstrated a relation between pineal function and animal responses to the photothermal environment. In homeotherms, deviations of T_a from thermoneutrality produce cytologic (7) and metabolic (8, 9) changes in the pineal gland. Exposure of suckling rats to different photoperiods and T_a significantly modified pineal *N*-acetyltransferase activity and led to the hypothesis that both light and temperature work to regulate pineal function (10). Radiotelemetric measurement of T_b rhythms in pinealectomized female rats (11) and house sparrows (12) has shown that the pineal can influence either maintenance of T_b rhythms or the set point around which daily T_b rhythms oscillate.

The present study was designed to determine if pineal ablation alters the thermoregulatory behavior of single-comb White Leghorn (SCWL) cockerels. In particular, the T_a selection of juvenile pinealectomized cockerels was studied throughout the light–dark cycle.

Materials and methods. Day-old SCWL cockerels obtained from a commercial hatchery were distributed in unheated battery brooders and maintained in a controlled-temperature chamber on a 12L:12D lighting regimen. Chicks were started in a T_a of 35° with two subsequent decreases of 2.8° at weekly intervals. A standard chick-starter ration and water were available *ad libitum*.

Pinealectomy (PX) and sham operations (PN) were performed under Brevital sodium (Eli Lilly and Co.) anesthesia at 8 and 9 days of age in a stereotaxic unit. An additional group of unoperated birds served as controls (C). All birds were housed in a single controlled-environment chamber until exposure to a thermocline.

The thermocline apparatus was constructed using eight heating elements, mounted at the top, each controlled by an individual thermostat. At one end, the first unit was set at 20° with each successive unit set 2° higher (Fig. 1). An electric spaceheater (1650 W) was placed underneath the warm end to ensure maintenance of 35° at this end. Radiant emission from the heating elements was not in the visible spectra. The area immediately below the heating elements measured 200 × 35 cm with two sides enclosed by wire mesh. The floor of the apparatus was lined with aluminum foil and covered with 2 cm of sand. The thermocline was divided into four equal zones (50 × 35 cm), each outlined

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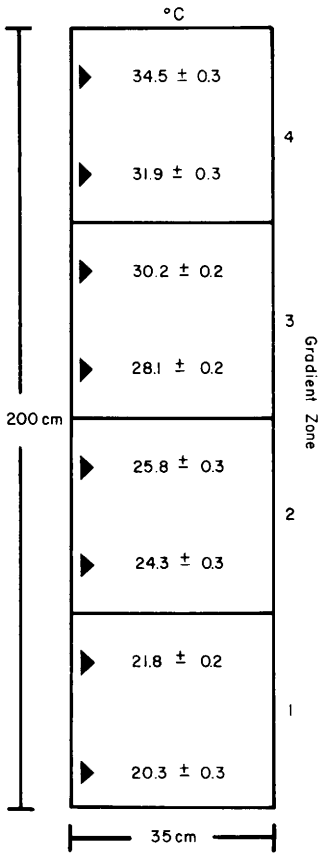

FIG. 1. Maintenance of a linear thermal gradient. Each value represents the mean ± SEM of 72 observations. Measurements were made at a height of 15 cm above the floor prior to each gradient exposure period.

by a floor marker. Two cool-white fluorescent lights were used to illuminate the apparatus during photophase. Four evenly distributed incandescent light bulbs were used to produce the daily photophase. Two fluorescent black lights were used to identify thermal zone selection during scotophase; they remained on throughout both photophase and scotophase. Maximum radiant emission from the black light was at a wavelength of 365 nm, with a spectral range from 275 to 480 nm. Light intensity was measured at 10 cm above the floor during photophase (275 fc) and scotophase (8 fc). A clear plastic tent was suspended over the entire apparatus to minimize convective disruption of the temperature gradient. The thermocline apparatus was housed in a controlled temperature chamber maintained at 18°.

Nine days following surgery cockerels were individually exposed to the thermocline in a randomized-complete-block experiment with a factorial arrangement of three surgical treatments (PX, PN, and C) and two times of day (photophase and scotophase). Photophase and scotophase were subdivided into 4-hr periods designated early (E), middle (M), and late (L). Surgical treatments were randomly assigned across days to exposure periods. During 6 days of observation, four birds from each surgical treatment were exposed to the thermocline during each E, M, and L segment of both phases. Thus, a total of 72 birds, 24 from each surgical treatment, were tested for a 2-hr period at 3 weeks of age. Each bird was exposed only once.

Prior to testing, the rectal temperature (T_r) was measured in each bird. The bird was then introduced to the cool end (20°), and after 5 min, it was gently pushed to the warm end (34°). Once positioned at the warm end, observations were made at 10-min intervals, for 100 min, through a small port in the chamber wall.

At each observation the bird's position, relative to the zone markers, was plotted on a gradient map (Fig. 1). Resultant data were submitted to analysis of variance and, where appropriate, orthogonal single-degree-of-freedom comparisons were made between surgical treatments for a given time of day.

Results. The average temperature under each heating element during the 6 days of gradient operation is presented in Fig. 1. These values were obtained from measurements made at a height of 15 cm above the floor under each heating element prior to each bird's exposure.

The average T_r of each surgical treatment prior to gradient exposure is given in Fig. 2. There was a main effect ($P < 0.01$) of pinealectomy on T_r across phase of the light–dark cycle. The PX cockerels had an average T_r (40.15°) that was 0.50 lower ($P < 0.01$) than the mean T_r of PN and C groups combined. Phase produced a main effect ($P < 0.01$) on T_r independent of surgical treatment. The average initial T_r of photophase exposed birds was 40.82° compared to 40.12° for those in the scotophase group. The PX birds had an average T_r of 40.56° for photophase while PN and C groups averaged 40.95°. During

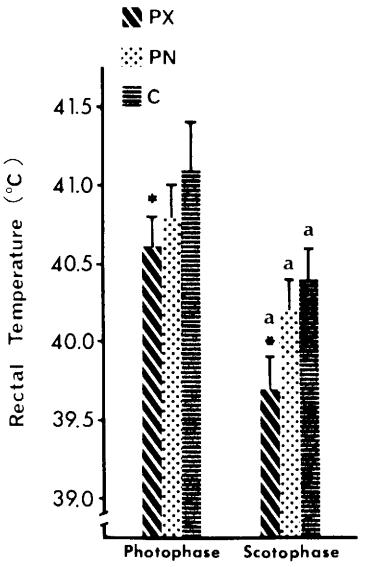

FIG. 2. Average rectal temperature of pinealectomized cockerels preceding exposure to the thermocline. Each bar represents the mean ± SEM of 12 birds within a given surgical treatment. The asterisk denotes a main effect ($P < 0.01$) of pinealectomy across phase of the light–dark cycle. The superscript "a" indicates a main effect ($P < 0.01$) of phase on the T_r of surgical treatments.

scotophase PX cockerels had an average T_r of 39.73° which was 0.6° lower than the PN and C average. No significant interaction was found in T_r between surgical treatments and time of day.

The T_a preferendum of PX cockerels is given for each segment of their light–dark cycle (Fig. 3). This figure best illustrates significant interactions between pinealectomy and photophase (E, L). The PX group selected higher ($P < 0.05$) T_a during E and L photophase and for all hours of scotophase. However, PX cockerels preferred a T_a (23.0°) similar to PN and C birds for M photophase.

The selection of warmer T_a by all groups during scotophase produced a main effect ($P < 0.01$) of phase of the light–dark cycle. Nonetheless, PX birds preferred ($P < 0.05$) 29.7° during darkness compared to an average T_a of 25.4° for PN and C cockerels. The average T_a selected by PX birds across both phases of the light–dark cycle was 28°, while PN and C groups had an overall T_a average that was 4° lower ($P < 0.01$).

Discussion. Much evidence has accumulated on pineal participation in the thermoregulatory behavior.

Selection of warmer T_a by PX cockerels in this study could be related to their relative hypothermia (Fig. 2). Others have also found irregularities in T_b following pineal ablation in birds (12) and rodents (11). The slight hypothermia demonstrated by pinealectomized rats during scotophase (13) is consonant with the observations that T_b effects observed in pinealectomized animals are ultimately related to the daily light–dark cycle. Light-regulated rhythms of melatonin (MT), norepinephrine (NE), and serotonin (5-HT) have been characterized in pineal glands from numerous homeotherms (14). It is of particular interest that these monoamines found in the pineal gland have been implicated in the central control of T_b (15–18).

The rhythmic release of MT by the avian pineal is reflected in peripheral blood (19) and the brain (20) with highest accumulation of MT in the hypothalamus. Additionally, hypothalamic content of 5-HT can be reduced by pinealectomy (21) or increased by

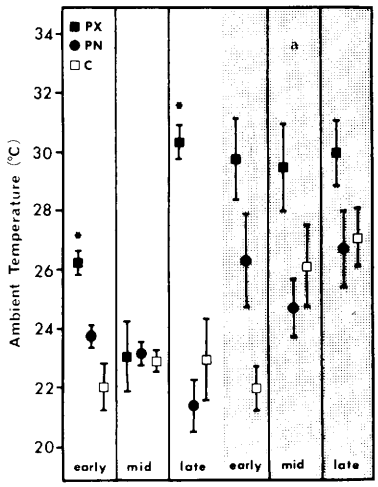

FIG. 3. Ambient temperature preferendum of pinealectomized cockerels during their light–dark cycle. Each value represents the mean ± SEM of four birds within a given surgical treatment. The 12 hr of scotophase is indicated by the stippled area. Photophase and scotophase are subdivided into three 4-hr periods designated early, middle, and late. There was a significant interaction ($P < 0.05$) between pinealectomy and ambient temperature selection during photophase. The superscript "a" indicates that all surgical treatments selected a higher average temperature during scotophase. (*) Significant difference ($P < 0.05$) between surgical treatments.

parenteral MT (22). The monoaminergic scheme of thermoregulatory control by the hypothalamus (15) involves 5-HT as a principal neurotransmitter. In birds, it is thought that 5-HT stimulates heat-productive pathways, whereas NE promotes dissipation (23). Accordingly, the hypothermia and higher T_a preferendum of PX cockerels in this study could be a reflection of the absence of the MT normally synthesized during scotophase.

We have also found (24) that pinealectomized chickens are not able to mount an adequate heat-dissipative response during scotophase. Light is known to inhibit pineal synthesis of MT and NE and to promote pineal production of 5-HT (14). However, it has not been determined to what extent pineal-synthesized 5-HT and/or MT could contribute to central thermoregulatory processes.

General adrenal and thyroid hyperactivity was reported to follow pinealectomy in several vertebrate species (25). These observations have contributed to the concept of pineal involvement in maintenance of body temperature. Quay (26) has recognized the potential of the mammalian pineal in homeostatic adjustments made to extraphotic physical variables—such as heat. The relative hypothermia and/or irregularities of homeothermy commonly noted in pinealectomized animals could possibly be explained as: (1) a change in the hypothalamic set point of T_b, (2) an absence of scotophase-dependent MT synthesis in the pineal gland, or (3) a deficit in metabolic intensity. It should be noted that—despite a lower mean T_b—PX cockerels have the ability to behaviorally select a T_a that is presumably compatible with energy economy. Our findings are consistent with the proposition that the vertebrate pineal gland has the ability to interact with the behavioral and physiological events that balance an animal's heat budget and, consequently, insure thermostasis.

Summary. Pinealectomized cockerels (PX) were individually exposed to a thermocline (20 to 34°) throughout both phases of the light–dark cycle (12L:12D). Twelve birds from each surgical treatment, including sham operated (PN) and unoperated controls (C), were singly placed in the thermocline for 100 min during photophase and scotophase in 4-hr segments designated early (E), middle (M), and late (L). Prior to placement in the thermocline, PX birds had a lower mean T_r (40.15°) than PN and C groups (40.64°). During photophase, PX birds selected a higher T_a during the E and L segments, but preferred a T_a similar to PN and C cockerels for M photophase (23°). This produced an interaction ($P < 0.05$) between pinealectomy and T_a selection during photophase. The PX group had a T_a preferendum of 29.7° for scotophase in contrast ($P < 0.05$) to 25.4° selected by PN and C birds. The average T_a preferred by PX birds across both phases of the light–dark cycle was 28° ($P < 0.05$) while on the average, PN and C groups selected 24°.

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