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Pinealectomy Blocks Vernal Courtship Behavior in Red-Sided Garter Snakes

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NELSON, R. J., R. T. MASON, R. W. KROHMER AND D. CREWS. *Pinealectomy blocks vernal courtship behavior in red-sided garter snakes.* PHYSIOL BEHAV 39(2) 231–233, 1987.—Male red-sided garter snakes *(Thamnophis sirtalis parietalis)* court only on emergence from winter dormancy. The salient proximate factor responsible for the initiation of courtship behavior appears to be warm ambient temperatures subsequent to sustained exposure to cold temperatures. In the present study, autumn-captured garter snakes were pinealectomized or sham-pinealectomized 1–2 weeks prior to hibernation. After 17 weeks of maintenance in several ambient lighting and temperature conditions, all males were tested for the presence of courtship behavior. No pinealectomized animals exhibited any indications of reproductive behavior, whereas 53% of sham-pinealectomized males actively courted females. Photoperiod did not influence the prevalence of mating behavior. These results suggest that the pineal gland mediates non-photoperiodic seasonal information in the garter snake. This is the first demonstration, in any vertebrate species, that the pineal gland can directly influence reproductive behavior.

Pineal gland Reproductive behavior Garter snakes Dissociated mating patterns Hibernation
Male behavior Courtship

PINEALECTOMY blocks reproductive responsiveness to changing photoperiod [2, 11, 22, 24, 25]. Effects of the pineal gland upon reproductive behavior have been considered to be secondary to gonadal responses since reproductive behavior is typically dependent upon gonadal steroids. Male red-sided garter snakes engage in courtship behavior only upon emergence from winter dormancy [1, 8, 10]. The proximate factor responsible for the initiation of courtship behavior appears to be the onset of warm temperatures after sustained exposure to cold temperatures [3,7]. These animals are unusual in that they exhibit a dissociated reproductive pattern in which testicular recrudescence and testosterone production occur several weeks *after* all breeding activity has ceased [6,10]. Neither castration nor hypophysectomy prevents mating activities in this species [3, 5, 7]; only lesions of the preoptic area (POA) of the anterior hypothalamus interfere with courtship behavior [9,15]. The POA is involved in mediating temperature-dependent activities in this and many other vertebrate species [15,23].

We tested the hypothesis that the pineal gland of red-sided garter snakes mediates the reproductive response to ambient temperatures. The pineal gland has been demonstrated to influence thermoregulation in a number of vertebrate taxa [20,26]. Presumably, the pineal must respond, in some way, to changing temperatures. The present study tests the proposition that pinealectomized snakes are unable to process seasonal temperature information. Consequently, pinealectomized snakes should not "perceive" the changing patterns of temperature that normally trigger courtship activity.

METHOD

Snakes were collected in the Interlake region near Narcisse, Manitoba (51° N latitude) in October, 1985, and maintained in our laboratory for 4 weeks at 21°C with programmed photoperiods consisting of 14 hr of light (LD 14:10) per day. Animals were group housed (n=8) in glass aquaria (75×32×31 cm) with a substrate of wood chips. Before and after exposure to the different hibernation conditions, all animals experienced an LD 14:10 photocycle. Temperature was held constant at 23±3°C; relative humidity was maintained at 45±5%. Animals were fed chopped smelt twice weekly; vitamin supplements were added to the food. Water was continuously available for drinking and soaking. Pinealectomized (n=90) or sham-pinealectomized (n=90) snakes were then maintained for 17 weeks in LD 14:10, LD 10:14 or constant dark (DD) photoperiod at 5 or 21°C. Surgery was performed on snakes anesthetized with Sodium Brevital (1.5 mg/kg). Using a pinealectomy drill bit (Stoltz, Chicago), a 5 mm hole was centered over the junction of the parietal and occipital bones. In most cases, the pineal gland was attached to the removed bone fragment. Otherwise, the pineal was grasped at its stalk with jeweler's forceps and removed. The hole in the skull was packed with Gel-Foam®. Sham operations consisted of drilling the hole, but not re-

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moving the skull cap. After hemastasis was achieved, all snakes were placed in a heated container until they recovered from the anesthesia.

RESULTS

Regardless of photoperiod, very few snakes (31%) survived when maintained at 21°C and these experimental groups were excluded from further data analyses. Apparently, cold temperatures are necessary for autumnal survival [14]. The day after emergence from dormancy two sexually "attractive" female snakes were introduced into the males' home aquaria and the males displaying courtship behavior were recorded. The behavioral tests were conducted daily from 1000 hr to 1130 hr CST; duration of each test was 5 minutes. Courtship was assessed on a scale of 0 to 3 where 0 indicated no courtship behavior and a score of 3 indicated copulation [9]. The study was terminated 22 weeks after its onset due to a malfunction of the environmentally controlled chamber; all courtship behavior had ceased by this time.

There was no effect of photoperiod ($p>0.05$); consequently, the experimental groups were collapsed across the three day length conditions and comparisons between pinealectomized and sham-pinealectomized snakes were accomplished with Student's *t*-tests. Only sham-pinealectomized males exhibited courtship behavior (53%); this value is comparable to the percentage of individuals that exhibited courtship in previous years following artificial hibernation. The courtship behavior was intense with all courting males vigorously attempting to mate with the stimulus females for the duration of the test. None of the pinealectomized animals courted during the course of 21 days of testing. Pinealectomy had no effect on relative gonadal size ($p>0.05$); both pinealectomized and intact animals had regressed testes (i.e., <500 mg).

DISCUSSION

These results suggest a previously undescribed role of the pineal gland in mediating reproductive behavior that is not dependent upon photoperiod or gonadal steroids. Pinealectomy blocked the onset of courtship behavior usually observed in red-sided garter snakes exposed to warm ambient temperatures after chronic maintenance in the cold. Previous studies on reptilian pineal function have focused upon the role of the pineal gland in mediating photoperiodic information; removal of the pineal gland in reptiles blocks reproductive responsiveness to photoperiods [16, 24, 25]. Pinealectomized green anole lizards *(Anolis carolinensis)* become reproductively active in the non-breeding season despite exposure to inhibitory photoperiods [25]. Pinal melatonin levels in lizards are altered by photoperiod or changing ambient temperatures [26], thus suggesting that the reptilian pineal gland is part of an effector system monitoring temperature.

Information about ambient photoperiod is integrated in the hypothalamus in mammals [2,11]. The mammalian pineal gland changes the pattern of melatonin production and secretion in response to neural signals from the hypothalamus. Removal of the pineal prevents response to changing ambient conditions [2,11]. Although the mammalian pineal gland is usually considered responsive only to photoperiodic information [2,11], there are reports of pineal mediation of temperature. For example, pinealectomized Richardson's ground squirrels bar-pressed more frequently than pineal-intact control animals for cool temperatures in a hot environment [13]. Pinealectomized squirrels increased their O_2 consumption, displayed elevated body temperature and experienced greater thermal stress as compared to pineal-intact animals when unable to control the ambient temperature [13]. Field studies suggest that annual variation in temperature can affect the timing of emergence from hibernation in several species of ground squirrels (e.g., [17,18]. The mammalian pineal may be involved in the timing of annual cycles (see below). A broader view would suggest that the pineal gland serves as a neuroendocrine transducer for all seasonal information [4,26].

The hypothesis that pinealectomy interferes with the ability to respond to changing ambient conditions can be applied to the present study. Temperature information is likely integrated in the POA of the anterior hypothalamus [23]; removal of the pineal gland may disrupt an important component of the temperature effector system. Since male red-sided garter snakes require warm temperatures after sustained exposure to cold temperatures to exhibit courtship behavior [1, 3, 5–8], pinealectomy may prevent the snakes from "recognizing" the change in temperatures. The pinealectomized red-sided garter snake may be compared to the pinealectomized Syrian hamster; these hamsters remain reproductively competent in short days because, physiologically, they respond similarly to short and long photoperiods.

Alternatively, the reproductive cycle of these snakes may be timed via an endogenous circannual clock. Research on mammalian circannual rhythms has demonstrated that pinealectomy shortens the period of annual cycles [12, 19, 21, 27]. If this analogy holds for the garter snake, one would predict that the pinealectomized animals would have exhibited courtship behavior *earlier* than the control snakes. It remains possible that the pinealectomized snakes would have courted sooner, if tested at the appropriate time; however, this is unlikely since it is metabolically impossible for these snakes to engage in reproductive behavior at low ambient temperatures.

Finally, it is possible that the pineal gland is directly involved in mediating courtship behavior in this species independently from environmental factors. Perhaps a direct neural link exists between the POA and the pineal in red-sided garter snakes. It is intriguing that only POA lesions [9,15] and pinealectomy disrupt courtship behavior in these garter snakes, whereas castration and hypophysectomy do not influence reproductive behavior [3, 5, 7].

This is the first report of an effect of the pineal gland directly upon reproductive behavior in any vertebrate species independent of the gonads. Also, these data establish for the first time that the pineal mediates seasonal, non-photoperiodic cues important in timing reproduction in ectothermic vertebrates. Pinealectomy prevented the temperature-dependent vernal mating behavior in the male red-sided garter snake. Our results are consistent with the hypothesis that the pineal gland mediates seasonal temperature information in this snake species. It is possible that responsiveness to temperature preceded responsiveness to photoperiod in the evolution of pineal function. Understanding the mechanisms underlying reptilian seasonal breeding may provide insight into the evolution of the mechanisms that time seasonal reproduction in birds and mammals.

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