Circadian locomotor rhythms in the desert iguana

II. Effects of electrolytic lesions to the hypothalamus

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Summary. Desert iguanas, Dipsosaurus dorsalis, displaying freerunning circadian locomotor rhythms in conditions of constant darkness and temperature received electrolytic lesions to the hypothalamus. The locomotor activity of those lizards (N=9) which sustained 80% or more damage to the suprachiasmatic nucleus (SCN) became arrhythmic whereas all animals that sustained less than 35% damage to the SCN remained rhythmic, even though they sustained significant damage to nearby regions of the hypothalamus and preoptic area. These results suggest strongly that the SCN plays a role in the regulation of circadian rhythms in the desert iguana. Taken together with other evidence, they support the view that this structure is homologous to the mammalian SCN, which acts as a pacemaker in the circadian system.

Key words: Circadian - Lizard - Suprachiasmatic nucleus - Hypothalamus - Lesion

Introduction

It is perhaps surprising in view of the important role that the pineal gland plays in the circadian systems of many non-mammalian vertebrates that this organ is not centrally involved in the circadian system of the desert iguana, Dipsosaurus dorsalis (Janik and Menaker 1990). Pinealectomy has no effect on circadian locomotor rhythms, and the isolated organ-cultured pineal of Dipsosaurus does not secrete melatonin rhythmically as is characteristic of some other species of lizards and birds (Menaker and Wisner 1983; Takahashi et al. 1980; Green and Gillette 1982; Shibata et al. 1982; Drucker-Colin et al. 1984; Sawaki et al. 1984; Lehmann et al. 1987; Rosenwasser 1988). The evidence for hypothalamic involvement in the circadian system of birds is not as extensive; however, studies of the Japanese quail (Coturnix coturnix), the Java sparrow (Padda oryzivora), and the house sparrow (Passer domesticus) demonstrate that lesions to the hypothalamus can result in arrhythmic locomotor activity (Simpson and Follett 1979; Ebihara and Kawamura 1981; Takahashi and Menaker 1982). The site at which lesions are most effective in birds has yet to be fully resolved. Recent anatomical evidence indicates that the avian homologue of the mammalian SCN is located somewhat more laterally (Cassone and Moore 1987), while the lesion studies conducted in Passer and Padda suggest that the relevant circadian nucleus may be situated more medially (Ebihara and Kawamura 1981; Takahashi and Menaker 1982).

Because neither the eyes nor the pineal are essential for circadian rhythmicity in Dipsosaurus, and based on the evidence gathered in mammals and birds, we were led to the hypothesis that the site of primary circadian pacemaking activity in this lizard species is hypothalamic. This hypothesis has been tested and the results of the experiment described below support it. Further, the results suggest that a specific nuclear area within the hypothalamus is homologous to the SCN of mammals.
Materials and methods

Animals. Adult male and female desert iguanas were captured on vacant lots in Cathedral City, California under State of California permit no. 2145. They were transported to the laboratory and held in enclosures as described in Janik and Menaker (1990).

Locomotor recording. Animals were held individually in plastic activity cages which were suspended from springs. The cages were housed singly inside light-tight wooden boxes which were in turn housed in a constant temperature room at 31 °C (see Janik and Menaker 1990 for details). Each wooden box was equipped with a 4 W fluorescent light bulb which was partially covered with black electrical tape so that the light intensity at the bottom of the cage would be about 10 lux. The jiggling motion of the cage, caused by the animals' movements, was monitored by an Esterline-Angus event recorder.

Stereotaxic surgery. Lizards were removed from activity cages and placed in a freezer until immobilized (10–20 min). They were then packed in crushed ice and mounted in a Kopf 900 small animal stereotaxic instrument. A specialized pair of ear bars and a bite plate, similar to those described by Greenberg (1982), were fashioned. The bite plate and tips of the ear bars were coated with a small amount of dental impression material (Kerr Light Bodied Permlastic) to aid in securing the head. With a dental burr, a small hole was drilled through the skin and skull immediately caudal to the parietal eye. A cut was made through the dura with a 30 gauge hypodermic needle to allow passage of the lesioning electrode which consisted of a tungsten wire (0.2 mm dia.) insulated with epoxy resin. Only the flattened surface at the tip of the wire was left exposed. Lesion coordinates were determined by placing lesions in several test animals. The center of the dorsal surface of the parietal eye was used as the zero point for the coordinates: 0.0 mm lateral, 0.5 mm posterior, and 4.7 mm ventral to the parietal eye, for all animals in this study. With the electrode in place, 1.9 to 2.0 mA of direct current was passed for 8 to 11 s. The electrode was then removed and the hole in the skull was packed with Gelfoam and sealed with a layer of cyanoacrylate glue. There was at most only slight bleeding. Some of the animals were sham operated; these were treated in exactly the same manner as the lesioned animals except that no current was passed. Animals were warmed under a 60 W incandescent light bulb and when they had recovered were returned to their activity cages.

Anatomy. Since a detailed cytoarchitectonic study of the hypothalamic of Dipsosaurus is not available (but see Berk and Heath 1975), some justification is required for naming the cell group we call the SCN. First, this nucleus is topographically similar to the SCN of Tupinambis nigropunctatus and Anolis carolinensis, two lizard species whose hypothalamic anatomy has been studied in some detail (Cruce 1974; Greenberg 1982). In these two species and in Dipsosaurus, the SCN lies just dorsal to the optic chiasm and adjacent to the third ventricle in the region of transition from preoptic area to hypothalamus. In addition, these nuclei are topographically similar to the SCN of rodents. Furthermore, these nuclei receive a direct retinal projection and bind antibodies raised against arginine vasopressin and neuropeptide Y (Janik et al., unpublished). Since these characteristics are all similar to those of the mammalian SCN, it seems a reasonable, though provisional conclusion, that these nuclei represent its true reptilian homologue.

Histology. Lizards were killed by decapitation. Their brains were removed from the skull and quickly frozen with dry ice. Forty-eight μm sections in the frontal plane were cut on a cryostat, melted directly onto microscope slides and stained with thionin. The areas destroyed by the lesions were drawn and estimates of the relative lesion size were made by measuring their rostro-caudal extent and the dorso-ventral and medio-lateral extents of the lesion at their rostro-caudal midpoints. Since the SCN was found in an average of 8 sections in non-lesioned animals, the percent damage to this structure was estimated by taking the number of sections in which it could be visualized as a percentage of the total expected (8). Corrections were made for sections in which only part of the SCN was left intact. The work of Cruce (1974) and Greenberg (1982) was consulted to aid in lesion evaluation.

Experimental protocol. Lizards were transferred from group enclosures to individual activity cages maintained in constant light and temperature. Freerunning locomotor rhythms were recorded for at least 2 weeks before lesions were made. After lesioning, locomotor activity was recorded for a minimum for 6 weeks.

Results

Lesion analysis. Almost all lesions were restricted to the ventral preoptic area and hypothalamus including the periventricular preoptic nucleus (PP), medial preoptic area (MPA), supraoptic nucleus (SON), medial and periventricular anterior hypothalamic area (AHA) and the suprachiasmatic nucleus (SCN). The majority of animals also sustained extensive damage to the optic chiasm. One animal had some damage to the hypothalamic para-ventricular nucleus (PVN) and another had damage as far rostral as the diagonal band of Broca (DBB). All lesions were on midline and were, for the most part, symmetrical. Both the lateral and dorsoventral extent of the damage ranged from 125 to 450 μm, with most of the lesions in the range from 150 to 250 μm. Rostro-caudally, lesions extended farther, ranging from 400 to 1900 μm.

The relative volume of the lesions was estimated by multiplying the rostro-caudal length of the lesion by the latero- and dorso-ventral length of the lesion at the midpoint of its rostro-caudal extent. When calculated in this way, the relative lesion volume for animals that were arrhythmic after surgery was larger than that for animals remaining rhythmic (P < 0.05, Mann-Whitney-U test). Nevertheless, there was considerable overlap in the distribution of lesion size between rhythmic and arrhythmic lizards (Fig. 1A). In this respect lesion size was not a