Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards

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THE CORE OF THE CIRCADIAN SYSTEM of vertebrates resides in three structures of diencephalic origin: the pineal gland, the retinas, and the suprachiasmatic nuclei (SCN) of the hypothalamus (26). In our model animal the Ruin lizard Podarcis sicula (family Lacertidae), each of these structures (pineal, retinas, and SCN) was shown to participate in the control of circadian rhythms of locomotor activity (27). Either pinealectomy or bilateral removal of the retinas (henceforth referred to as retineclectomy) induces changes in the free-running period of locomotor rhythms in constant conditions (8). However, the role of melatonin and the pineal in controlling circadian locomotor behavior was shown to be mainly limited to summer (2, 16, 17). Despite the importance ascribed to the pineal and retinas in the circadian organization of birds and lizards, behavioral rhythmicity was found to persist in Ruin lizards in constant temperature and constant darkness (DD) after combination of pinealectomy and retineclectomy in the same individual animal (8, 25). Complete bilateral lesions to the SCN invariably abolish circadian locomotor rhythmicity of Ruin lizards, but they are incapable of restoring rhythmicity in subjects previously rendered arrhythmic by complete bilateral SCN lesions (2). Altogether, these data support the view that the SCN of the Ruin lizard may contain the primary circadian pacemaker that drives locomotor rhythms. Lesions of at least 80% of the SCN were shown to abolish circadian locomotor rhythmicity in the Desert iguana Dipsosaurus dorsalis (20). This suggests a primary role of the SCN also in D. dorsalis.

Whether the SCN of the Ruin lizard, the Desert iguana, or other nonmammalian species contain circadian oscillators is at present unknown. In mammals, namely rodents, the SCN were shown to contain circadian oscillators. In rats, for instance, circadian firing rhythms were recorded from populations of SCN neurons in vitro (13, 36). The mammalian SCN were recently shown to be a circadian multioscillator system, because SCN neurons (clock cells) of Syrian hamsters (Mesocricetus auratus) in the same in vitro culture were found to express circadian rhythms of widely different phases and different period lengths, and, furthermore, the circadian period of locomotor rhythms (i.e., in vivo) was shown to be determined by the mean period that arises from the coupling of SCN neurons with diverse circadian periods (24, 44). Whereas in hamsters a complete SCN lesion (SCN-X) eliminates locomotor rhythmicity, unilateral SCN lesion (USCN-X) induces period changes (6, 29, 33). Such period changes are explained by the multioscillatory nature of the SCN themselves, because after USCN-X the mean period of the remaining SCN neurons (clock cells) is expected to be different from the mean period derived from the whole ensemble of clock cells contained within the bilaterally intact SCN.
One of the basic properties of the circadian pacemaker consists of entraining the rhythms it drives with the 24-h light-dark (LD) cycle of the external day (1). The circadian pacemaker within the SCN of mammals fulfills the requirement above, as mammalian SCN are known to mediate photic entrainment of circadian rhythms (32, 45). Gene expression of the immediate-early gene c-fos in the ventrolateral mammalian SCN is associated with entrainment to light, because c-fos is induced only at those circadian phases when light has phase-shifting effects on circadian rhythms (15, 23, 34). Whether the SCN of nonmammalian vertebrates play a role in the process of entrainment of circadian rhythms to light is at present unknown.

The present study was aimed at further characterizing the role of the SCN in the circadian organization of Ruin lizards. Three different experiments were carried out. We tested whether the SCN play a role in entrainment of circadian locomotor rhythms to light. Accordingly, we examined the effects of the exposure to 12:12-h LD cycles on the locomotor behavior of lizards with SCN lesions. We also asked whether light causes an increase of Fos-like immunoreactivity (Fos-LI) in the SCN. For this purpose, at several sampling points around the clock, we quantified induction of Fos-LI in SCN cells of lizards maintained on either a 12:12-h LD cycle or in DD. Furthermore, we tested whether USCN-X affect locomotor rhythms of lizards in DD and constant temperature. If behavioral rhythmicity persists in Ruin lizards after USCN-X and this surgery mainly induces period changes (as it does in hamsters), these data would support the existence of a strong functional similarity between the SCN of lizards and the SCN of mammals.

**MATERIALS AND METHODS**

**Animals and Locomotor Recording**

Ruin lizards (Podarcis sicula campestris; De Betta 1,857; adult males only, 6.5- to 8-cm snout-vent length) from the area of Ferrara (Italy) were used. After capture, each lizard was carried to the lab and immediately put into an individual tilt cage (30 x 15 x 11 cm) for locomotor recording. Tilt cages were placed inside environmental chambers kept at a constant temperature of 29 ± 0.5°C and connected to a computer-based data-acquisition system (DataQuest III, MiniMitter, Sunriver, OR) for monitoring locomotor activity. During experiments, lizards were kept under 12:12-h LD cycles (Lc: fluorescent light of 900 lx at the level of the head of lizards; D: 0 lx) or in DD (0 lx). Food (Tenebrio molitor larvae) and water were supplied twice a week.

**Surgery**

All surgeries were always performed under bright light, during the lizard’s subjective day (0–12 h after activity onset). For anesthesia, the lizards were first cooled in a refrigerator (1–4°C) for 30–50 min until they were immobilized. They were then packed in crushed ice and mounted in a Kopf 900 small stereotaxic instrument. For electrolytic lesions, an electrode, which consisted of a platinum-iridium wire (Ø = 0.05 mm) insulated with teflon (outer Ø = 0.075 mm) (Advent Research Materials, UK), was used. Bilateral electrolytic lesions to the SCN were performed as described in Ref. 2. For unilateral lesions, coordinates were 0.0 mm lateral and 0.8 mm anterior to the center of the parietal eye and 1.75 mm ventral to the surface of the telencephalon. Unilateral electrolytic lesions to the SCN were performed as described in Ref. 2. For unilateral lesions, coordinates were 0.2 mm lateral and 0.8 mm anterior to the center of the parietal eye and 1.75 mm ventral to the surface of the telencephalon. The direct current supplied to perform bilateral SCN lesions was 0.6 mA for 10 s, and to perform USCN-X, it was 0.6 mA for 8 s. Some of the lizards were sham operated (Sham); these lizards were treated in exactly the same manner as the lesioned animals, except that no current was passed.

**Post surgery Histology**

The lizards were deeply anesthesized and perfused through the cardiac ventricle with 0.1 M phosphate buffer (PBS, pH = 7.4) followed by 4% paraformaldehyde in PBS. Brains were removed and postfixed at 4°C overnight in the same fixative. After postfixation, brains were embedded in paraffin wax and cut in 7-μm coronal sections on a microtome. Sections were stained with Mayer’s hematoxylin solution, or cresyl violet acetate, and examined to determine location and extent of the lesions.

**Fos Immunocytochemistry**

The lizards were deeply anesthesized and perfused through the cardiac ventricle with PBS followed by 4% paraformaldehyde in PBS. For time points falling in the dark, lizards were anesthetized under dim red light and perfused while wearing a light tight hood that covered their heads and necks. Brains were removed and postfixed at 4°C overnight in the same fixative. After postfixation, brains were embedded in paraffin wax and cut in 7-μm coronal sections on a microtome. Sections were reacted for Fos-LI with the use of a standard avidin-biotin immunohistochemical protocol. After dehydrating and hydrating through xylenses and graded ethanol series, tissue sections were preincubated in 2% hydrogen peroxide for 15 min to quench endogenous peroxidase activity. After two washes in 0.1% Triton X-100 in PBS (PBS-TX; Sigma Chemicals), sections were incubated at room temperature in 10% normal goat serum (NGS; Vector Labs) in PBS-TX for 30 min. After being rinsed in PBS, sections were incubated in rabbit anti-Fos antiserum (1:300; Santa Cruz, CA) with 1.5% NGS in PBS-TX for 48 h at 4°C. Sections were then rinsed in PBS and incubated in biotinylated goat antirabbit antiserum (1:200; Vector Labs) with 2.5% NGS in PBS-TX for 30 min. After the incubation in the secondary antibody, we followed the procedures described in the protocol of the Elite ABC Vectastain kit, with the use of diamobenzidine in 0.1 M Tris buffer (pH = 7.2) reacted with hydrogen peroxide (SK-4100; Vector Labs). All sections were counterstained with Mayer’s hematoxylin solution, dehydrated, and coverslipped. To minimize variability, we used a standardized immunohistochemical protocol, which kept incubation times constant and used a single batch of antiserum and other reagents. Two control series were treated as described above, except that the primary and secondary antibodies were deleted; no reaction product was observed in these sections.

**Experimental Design**

**Behavioral experiments. BILATERAL SCN-X (DD-LD TEST).** Lizards were allowed to freerun in DD for 4 wk and then subjected to Sham lesions (n = 2) or to bilateral SCN lesions (n = 12). Thirty-five days after surgery lizards were transferred from DD to a 12:12-h LD cycle (LD1: lights on at 0800). After 3 wk, the LD cycle was shifted (LD2: lights on at 1400) and locomotor activity was recorded for 2–3 wk thereafter.
USCN-X (DD TEST). Lizards were allowed to freerun in DD for 2–3 wk. After this period of time, lizards were subjected either to Sham lesions (n = 5) or USCN-X lesions (n = 14). Locomotor activity in DD was further recorded for 6–10 wk after surgery.

Fos-LI experiments. In intact lizards, we found that activity onsets in 12:12-h LD cycles mainly occur around lights-on (ZT0), and activity onsets in the first (and second) day in DD after release from 12:12-h LD cycles occur around the phase of projected lights-on (CT0: subjective dawn) (representative example in Fig. 6A). Furthermore, because the free-running period during the first days in DD does not shift from 24 h, ZTs and CTs mark the same phases and can be used to identify the same time points of Fos sampling between the LD and DD test described below.

LD TEST. Lizards (n = 46) were maintained 2 wk on a 12:12-h LD cycle and then perfused at 14 time points (3–4 lizards at each ZT) of the LD cycle, as indicated in Fig. 6B (top).

DD TEST. Lizards (n = 29) were maintained 2 wk on a 12:12-h LD cycle and then allowed to freerun in DD. After 48 h in DD, lizards were perfused at eight different time points (3–4 lizards at each CT), as indicated in Fig. 6B (bottom). Because we found a peak of Fos-LI shortly after ZT0 in the LD cycle, we tested for a possible endogenous peak in DD in the early subjective day by adding two further sampling points between those at CT0 and CT4.

Data evaluations. Circadian parameters. Estimates of the free-running period (τ) and length of circadian activity (α) were made for a 10-day segment just before surgery and before the end of locomotor recording. τ was measured by means of the eye-fitting method and by means of χ²-periodogram analysis (30, 38). α was estimated to the nearest 0.5 h by measuring the interval between an eye-fitted straight line connecting the onsets and another connecting the offsets of activity for each 10-day segment (30). Presence of circadian periodicities in the locomotor activity of SCN-X lizards was tested by means of χ²-periodogram analysis.

Electrolytic lesions. Brain sections of lesioned lizards were examined under a light microscope and drawn onto paper to determine location and completeness of lesions and to compare brain lesions among different individuals (Fig. 1). Drawings were made without knowledge of the animals’ locomotor behavior.

Cell counts and statistical analysis. Fos-LI was quantified by cell counts. Fos-LI was considered as “not detectable” when staining was absent or when the intensity of staining was too low for being distinguished unequivocally from the background. For each animal, three sections through the central SCN with the heaviest label were selected and Fos-LI cells were counted. Two observers counted labeled cells independently and without knowledge of treatment conditions, and their totals were then averaged for each brain. The scores were then analyzed with the use of a one-way analysis of variance followed by Newman-Keuls post hoc test for multiple comparisons. Differences were considered significant when P < 0.05.

RESULTS

Bilateral SCN-X

Analysis of lesions. Representative examples of different SCN lesions are drawn in Fig. 1. Five of twelve lizards sustained an almost complete lesion to both the SCN (SCN-X), whereas brain structures outside the SCN received no damage. Another three lizards sustained an incomplete lesion to both the SCN (SCN-Xi). In two of these SCN-Xi lizards, the area between the ventral border of the third ventricle and the dorsal border of the optic chiasm (OC) was partially damaged. In SCN-X and SCN-Xi lizards, neither the OC nor the nuclei periventricularis hypothalamy (PH) were lesioned, and lesions were quite small, ranging from 180 to 210 μm rostrocaudally, 150 to 180 μm laterally, and 60 to 80 μm dorsoventrally. This volume slightly exceeds that occupied by both SCN. In two lizards, no damage to the SCN was present, and lesions were restricted to 10–40% of both PH (PH-X). In the remaining two lizards, lesions were restricted to 85–95% of both optic nerves at the level of the OC (OC-X). OC-X included both retinohypothalamic tracts (RHT), which cross the OC to reach the contralateral SCN (3). In OC-X lizards, all brain structures remained intact with the exception of the optic nerve fibers (representative photomicrograph in Fig. 2B).

Behavioral Results

Sham lizards. Sham lesions (n = 2) did not affect the locomotor behavior in DD and did not prevent entrainment of the activity rhythm to the LD cycle (not shown).
SCN-X lizards. The five lizards that received a complete lesion to both SCN became arrhythmic in DD and constant temperature, and when exposed to LD cycles, they remained arrhythmic (Fig. 3). Arrhythmicity, as judged from visual inspection of the records, was confirmed by χ² periodogram analysis.

SCN-Xi lizards. Under DD conditions, incomplete lesions induced different behavioral effects such as arrhythmicity, splitting of the locomotor rhythm in two activity components, or period changes (Fig. 4). When exposed to a LD cycle, SCN-Xi lizards showed a 24-h rhythm. A 6-h shift of the LD cycle resulted in entrainment to the new schedule after several transient cycles (Fig. 4, top).

OC-X and PH-X lizards. In constant conditions, lizards remained rhythmic after either OC-X or PH-X. PH-X and OC-X did not prevent entrainment of the activity rhythm to 24-h LD cycles. Entrainment of the activity rhythm of an OC-X lizard to a shifted LD schedule is shown in Fig. 2C.

USCN-X

Analysis of lesions. Nine of 14 lizards subjected to unilateral electrolytic lesion sustained complete unilateral damage to the SCN (USCN-X), whereas brain structures outside the SCN received no damage (n = 6) or marginal unilateral damage to the PH (n = 3). USCN-X were very small, ranging from 150 to 180 μm rostrocaudally, 100 to 130 μm laterally, and 50 to 70 μm dorsoventrally. This volume slightly exceeds that occupied by a single SCN. In one lizard, the PH was unilaterally lesioned, whereas the SCN received no damage. In the remaining lizards, either the damage to the SCN was marginal (1 lizard) or the histological result was unclear (3 lizards), and their behavioral results were not considered.

Behavioral Results

USCN-X lizards. USCN-X surgery induced dramatic changes in τ (5 lengthening and 4 shortening) and in α (6 lengthening and 3 shortening) (Fig. 5). The average absolute change in τ was 0.53 h (±0.16 h SE). The average absolute change in α was 2.42 h (±0.57 h SE). Sham surgery (n = 5) did not affect the locomotor activity rhythm, as the average absolute change in τ was 0.1 h (±0.04 h SE) and the average absolute change in α was 0.6 h (±0.04 h SE). The absolute change in τ in USCN-X lizards was significantly greater than that observed in Sham lizards (T = 21.5, P < 0.04, Mann-Whitney U test). The absolute change in α in USCN-X lizards was significantly greater than that observed in Sham lizards (T = 20.5, P < 0.03, Mann-Whitney U test). In one lizard that sustained...
unilateral PH-X instead of USCN-X, locomotor rhythmicity continued completely undisturbed after surgery (Fig. 5, top right).

Fos-LI Experiments

**LD test.** Fos-LI was clearly observable within the SCN cells of lizards exposed to a 12:12-h LD cycle (Figs. 6 and 7). The number of Fos-LI cells in the SCN changed significantly as a function of time of day ($F = 293.6, P < 0.001$). The number of Fos-LI cells started to increase significantly 0.75 h after ZT0 ($P < 0.01$). Cell numbers were highest 1.25 h after ZT0 ($P < 0.01$) and started to decrease significantly 1.5–1.75 h after ZT0 ($P < 0.01$). By 4 h after ZT0, Fos-LI expression was...
undetectable and remained so throughout the rest of the day and the night.

**DD test.** In DD (sampling started after 48 h of DD), no Fos-LI expression was detected within the SCN cells, whatever the CT (Fig. 6). Hence, the daily rhythm in Fos-LI expression we found in SCN cells in the LD test is a direct consequence of environmental lighting conditions.

Fig. 4. Locomotor records of 3 SCN-Xi lizards (#01, #02, and #11). Postsurgery histology of lizards #01 and #11 is shown in Fig. 1. Locomotor records were double-plotted on a 48-h time scale to aid in interpretation. In DD, SCN-Xi induced 1) splitting of the activity rhythm into 2 components, which freeran with different periods (lizard #01); best-fitted solid lines drawn through onsets of activity allow visualization of the expression of 2 different free-running periods during splitting; 2) lengthening of the free-running period of 0.7 h (lizard #02, periodogram D); and 3) arrhythmicity (lizard #11, periodogram F). Exposure of SCN-Xi lizards to the LD1 cycle induced appearance of a 24-h rhythm (periodograms B, E, G). Note that a 6-h shift from LD1 to LD2 cycle resulted in entrainment of the activity rhythm to the new LD schedule after several transient cycles (SCN-Xi lizards #01 and #02).
DISCUSSION

Several aspects of the present results led us to conclude, for the first time in a nonmammalian vertebrate, that the SCN play a central role in mediating entrainment of circadian locomotor rhythms to 24-h LD cycles. First of all, lizards became arrhythmic in response to SCN-X under constant temperature and DD. This confirms the results of previous studies, showing that bilateral lesions damaging 80% or more of the SCN abolish circadian locomotor rhythmicity of Ruin lizards in DD (27). The present data further showed that SCN-X lizards do not react to the administration of a 24-h LD cycle; they remain arrhythmic all the time (Fig. 3). On the other hand, remnants of SCN tissue in SCN-Xi lizards were sufficient to warrant entrainment to 24-h LD cycles (Fig. 4). The 24-h LD cycles actually entrained the activity rhythm of SCN-Xi lizards and did not merely cause masking of the underlying oscillation because 1) after a 6-h shift of the LD cycle, the activity rhythm did not follow the phase shift instantaneously, but instead it went through several transient cycles (Fig. 4, top); 2) steady-state entrainment to

Fig. 5. Locomotor records of 3 lizards that received either USCN-X or a lesion to the nuclei periventricularis hypothalami (PH-X), while free running in DD. USCN-X induced lengthening (lizard #08) or shortening (lizard #03) of the free-running period of locomotor rhythms. Unilateral PH-X (lizard #07) left locomotor rhythmicity completely undisturbed. Bottom: photomicrograph of a coronal brain section taken from USCN-X lizard #03, showing unilateral ablation of the right SCN. Arrowhead points to the left SCN, which remained intact. Note the area of OC just ventral to the SCN, which remained intact. Section is stained with Mayer's hematoxylin solution (×150). Postsurgery histology of USCN-X lizard #08 is drawn in Fig. 1.
the LD cycle after DD was achieved after a series of transients (Fig. 4, top right) (1). Furthermore, the present results indicate that Sham surgery and brain lesions outside the SCN (OC-X, PH-X) do not prevent entrainment of circadian locomotor rhythms to 24-h LD cycles (Fig. 2). The dramatic behavioral differences in response to 24-h LD cycles between SCN-X (no reaction) and SCN-Xi lizards (entrainment) convincingly show a central role of the Ruin lizard SCN in photic entrainment (Figs. 3 and 4). It is also remarkable that in SCN-X lizards, the intact extrahypothalamic components of the circadian system—pineal, retinas, and the parietal eye—that are known in several nonmammalian species to contain both photoreceptors and circadian oscillators are neither capable of restoring rhythmicity nor mediating photic entrainment (27, 41).

Previous findings in Ruin lizards showed a similar role of the SCN with respect to another potential zeitgeber, namely melatonin. Daily injections of exogenous melatonin entrain locomotor rhythms of lizards with SCN-Xi, whereas the same treatment fails to restore rhythmicity in individuals previously rendered arrhythmic by SCN-X (2). Taken together, all these results concur in proving that the SCN of Ruin lizards are essential both to maintain circadian locomotor rhythmicity and to mediate entrainment of these rhythms to different zeitgebers (light and melatonin), i.e., the SCN of Ruin lizards contain a primary pacemaker that drives locomotor rhythms.

Other experiments examined whether light causes Fos-LI expression within cells of the SCN. Under a 12:12-h LD cycle, the SCN of Ruin lizards express a daily rhythm in Fos-LI expression. Fos-LI expression within SCN cells was found to peak ~1 h after ZT0 and to decrease thereafter. If the rhythm in Fos-LI expression was circadian in nature, such a rhythm should have persisted in lizards kept in DD. However, we have shown that in DD, Fos-LI expression was undetectable. Therefore, the rhythm seen in LD cycles is a direct consequence of environmental lighting conditions and is not a reflection of an endogenous rhythmicity. The daily rhythm of Fos-LI expression in the SCN of Ruin lizards is similar to that observed in the SCN of rodents. In fact, in either diurnal (Arvicanthis niloticus) or nocturnal (mouse, rat) rodents maintained in a 12:12-h LD cycle, Fos-LI expression peaks shortly after ZT0 (5, 21). Besides P. sicula, photic induction of Fos-LI expression was tested so far in two further nonmammalian species, namely the quail (Coturnix coturnix japonica) and the starling (Sturnus vulgaris) (22). These data are difficult to interpret, however, because in both quail and starlings, photic induction of Fos-LI expression is absent in the medial SCN, which are well known in birds to play a central role for maintenance of circadian rhythmicity, but is present in the visual SCN, whose function in the system has not been clarified (7, 22, 37, 40).

As the behavioral data gathered in the present study showed involvement of the SCN of Ruin lizards in photic entrainment of circadian rhythms, it is important here to have functional evidence at the cellular level that light is capable of inducing Fos expression in the lizard SCN. On the other hand, direct evidence that

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**A**

LD 12:12

DD

B

ZT 0 4 8 12 16 20 24

LD 12:12 Sample time Fos response

DD Sample time Fos response

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**Fig. 6.** Fos-like immunoreactivity (Fos-LI) experiments in LD and DD conditions. **A**: the locomotor record is a representative example of the fact that activity onsets of intact Ruin lizards in 12:12-h LD cycle mainly occur around lights-on (ZT0) and activity onsets in the first 2–3 days in DD after release from 12:12-h LD cycle occur around the phase of the projected lights-on (CT0: subjective dawn). The best-fitted solid line drawn through activity onsets shows persistence of a 24-h period during the first days in DD. ZT and CT mark the same phases and are used to identify the same time points of Fos sampling between LD and DD test. **B**: schematic diagram illustrating schedule of sampling and presence/absence of Fos-LI expression within SCN cells of lizards in a 12:12-h LD cycle or after 48 h of exposure to DD. The phases in which Fos levels were sampled are indicated by vertical lines. The presence of Fos-LI expression in the SCN is indicated by a (+), whereas the lack of Fos-LI expression is indicated by a (-). **Top**: in 12:12-h LD cycle, we found a peak of Fos-LI expression shortly after ZT0. We therefore tested for a possible endogenous peak in the early subjective day in DD by adding 2 further sampling points between CT0 and CT4 (bottom). However, in DD, Fos-LI expression was undetectable.
light induction of Fos expression in the SCN is part of the photic entrainment pathway is still lacking in Ruin lizards. To test this, one should examine whether light induces Fos-LI expression in SCN cells only at those circadian phases when light elicits phase shifts of circadian locomotor rhythms. In other words, intensity of Fos expression in the SCN and dimension of phase shifts in behavior should correlate across the phase-response curve (PRC) to light. This was shown to be true in nocturnal rodents (15, 23, 34). As light pulses must be short in duration (~1 h in Ruin lizards) to detect Fos expression, it may be taken into account that the only described PRC in a lizard (the Iguanid lizard *Sceloporus occidentalis*) uses 6-h long light pulses to elicit phase shifts in behavior (43). In any case, before testing the role of Fos in photic entrainment of Ruin lizards, it may be worth examining whether light pulses shorter than 1 h can elicit phase shifts of their activity rhythm.

Further experiments examined whether USCN-X affect locomotor rhythms in DD. Rhythmicity always persisted in response to USCN-X, and this surgery was found to induce period changes (Fig. 5). The behavioral results of USCN-X in Ruin lizards are similar to those observed in mammals. In the Syrian hamster (*M. auratus*), USCN-X induce period changes in constant conditions (6, 29). Such period changes are readily explained by the existence of a multioscillatory circadian system within the SCN: single SCN neurons of hamsters in the same in vitro culture express circadian rhythms of different period lengths, and the circadian period of locomotor rhythms (i.e., in vivo) is determined by the mean period that arises from the coupling of SCN neurons with diverse circadian periods (24, 44). Period changes in response to USCN-X in hamsters reflect the fact that the mean period of the remaining SCN neurons is different from the mean period derived from the whole ensemble of clock cells contained within the bilaterally intact SCN. We do not know whether the SCN of Ruin lizards contain circadian oscillators. However, the period changes in response to USCN-X in hamsters and the splitting of activity rhythm in two components free running with different periods in a SCN-Xi lizard support the existence of a multioscillatory circadian system within the SCN (Figs. 4 and 5).

Data gathered in both the Desert iguana *D. dorsalis* and the Ruin lizard support the view that the SCN of lizards are homologous to the SCN of mammals. The SCN of the Desert iguana and the Ruin lizard are topographically similar to the SCN of mammals and
receive a direct retinal projection (3, 19). As in mammals, the SCN of the Desert iguana bind antibodies raised against neuropeptide Y (19). As in mammals, in both the Desert iguana and the Ruin lizard, SCN-X abolish circadian locomotor rhythmicity in constant conditions (20, 27, 33, 39). Support for homology comes from further evidence in Ruin lizards, indicating a strong functional similarity between SCN of lizards and SCN of mammals: 1) USC-N induce period changes (6, 29, present results); 2) daily melatonin injections do not restore rhythmicity in animals that became arrhythmic in response to SCN-X (2, 4); 3) light increases Fos-LI expression in the SCN (5, 15, 21, 23, 34, present results); and 4) the SCN are crucially involved in mediating entrainment of locomotor rhythms to light (for review, see Ref. 32; present results). It must be pointed out, however, that in Ruin lizards but not in the mammals: 1) besides the SCN, the pineal and the retinas also participate in the control of behavioral circadian rhythmicity in DD (8); 2) besides the retinas, extraretinal photoreceptors can also mediate photic entrainment of behavioral rhythms (9). Regarding the first issue, it is clear that in Ruin lizards, pineal, pineal retina, and retinal retina in DD induce period changes, whereas in mammals the same (or similar) surgeries do not affect behavioral rhythmicity (8, 31). Nevertheless, the importance of either the pineal or retinas in the circadian organization of Ruin lizards is reduced by the evidence that locomotor rhythms in DD persist after combination of pineal-ectomy and retina-ectomy in the same individual (8). Several lines of evidence suggest that pineal and retinas of Ruin lizards are respectively hormonal (pineal melatonin) and neural (RHT) modulators of a circadian pacemaker in the SCN, with the role of the pineal even limited to summer (2, 10, 16, 17, 27). That locomotor rhythms of OC-X lizards (whose RHT was sectioned) still entrain to LD cycles (Fig. 2C) confirms previous data showing persistent capability of photic entrainment after retinectomy (9). In mammals, however, no photic entrainment occurs after blinding (14, 35, 45).

Perspectives

The present results suggest a strong functional similarity between the SCN of lizards and the SCN of mammals in circadian organization. However, whereas in the mammals photic entrainment is mediated exclusively by photoreceptors contained in the lateral eyes, in lizards, as in other nonmammalian vertebrates, photic entrainment is mediated by several classes of photoreceptors (pineal, parietal eye, retinas, and deep brain photoreceptors) (11, 12, 18, 42). The existence of extraretinal photoreceptors that lie deep in the brain is also firmly established in Ruin lizards (9). Furthermore, an opsin was recently cloned from the brain of Ruin lizards (28). Given the essential role played by the SCN in Ruin lizards and the nonessential role by the RHT in photic entrainment, future work should establish both the precise location(s) of deep brain photoreceptors involved in photic entrainment and the pathway(s) from those photoreceptors to the lizard SCN.