Phase Response Curve and Light-Induced Fos Expression in the Suprachiasmatic Nucleus and Adjacent Hypothalamus of Arvicanthis niloticus

Megan Mahoney,* Abel Bult,†‡ and Laura Smale*,†,1

*Zoology Department, †Psychology Department and Neuroscience Program, Michigan State University, East Lansing, Michigan 48824, USA, ‡Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99775-7000, USA

Abstract This article describes the phase response curve (PRC), the effect of light on Fos immunoreactivity (Fos-IR) in the suprachiasmatic nucleus (SCN), and the effect of SCN lesions on circadian rhythms in the murid rodent, Arvicanthis niloticus. In this species, all individuals are diurnal when housed without a running wheel, but running in a wheel induces a nocturnal pattern in some individuals. First, the authors characterized the PRC in animals with either the nocturnal or diurnal pattern. Both groups of animals were less affected by light during the middle of the subjective day than during the night and were phase delayed and phase advanced by pulses in the early and late subjective night, respectively. Second, the authors characterized the Fos response to light at circadian times 5, 14, or 22. Light induced an increase in Fos-IR within the SCN during the subjective night but not subjective day; this effect was especially pronounced in the ventral SCN, where retinal inputs are most concentrated, but was also evident in other regions. Both light and time influenced Fos-IR within the lower subparaventricular area. Third, SCN-lesions caused animals to become arrhythmic when housed in a light-dark cycle as well as constant darkness. In summary, Arvicanthis appear to be very similar to nocturnal rodents with respect to their PRC, temporal patterns of light-induced Fos expression in the SCN, and the effects of SCN lesions on activity rhythms.

Key words suprachiasmatic nucleus, paraventricular, circadian, Fos, Arvicanthis, light pulse

The mammalian suprachiasmatic nucleus (SCN) is the primary neural site for the generation and synchronization of circadian rhythms. SCN lesions abolish behavioral and hormonal circadian patterns, and transplants of fetal SCN tissue into SCN-lesioned animals restore a number of behavioral rhythms (Moore and Eichler, 1972; Stephan and Zucker, 1972; Ralph et al., 1990; LeSauter and Silver, 1999). Nocturnal and diurnal animals differ with respect to rhythms in rest (Kas and Edgar, 1998; Novak et al., 1999), body temperature (Refinetti, 1996), reproductive processes (Beach and Levinson, 1949; Harlan et al., 1980; Dusay, 1996; McElhinny et al., 1997), and activity. It is not yet clear how these differences are generated, but some
aspect of SCN function may be involved. The hypothalamic region immediately dorsal to the SCN, the lower subparaventricular (LSPV) zone, may also be involved in the regulation of circadian rhythms. This region receives input from both the retina (Johnson et al., 1988; Smale and Boverhof, 1999) and the SCN (Watts et al., 1987; Morin et al., 1994) and exhibits rhythms in Fos that differ in nocturnal and diurnal species (Nunez et al., 1999).

The SCN receives light information directly through the retinohypothalamic tract (Moore and Lenn, 1972) and indirectly through the geniculohypothalamic tract (Johnson et al., 1988; Card and Moore, 1989; Moga and Moore, 1997). A single pulse of light given to animals housed in constant darkness can shift the endogenous activity rhythm in a manner described by a phase response curve (PRC). The PRC has been characterized in a wide variety of nocturnal (Daan and Pittendrigh, 1976; Rea, 1992; Bult et al., 1993; Rappold and Erkert, 1994) and diurnal (Kramm, 1976; Hoban and Sulzman, 1985; Navaneethakannan and Chandrashekaran, 1986; Lee and Labyak, 1997; Kas and Edgar, 2000) animals. Light pulses during the early and late subjective night typically phase-delay and phase-advance endogenous rhythms, respectively, whereas light pulses during the subjective day generally have little effect. The PRCs of diurnal species, however, can be somewhat more variable and do not always fit this pattern (Pohl, 1982).

The proto-oncogene c-fos, which encodes the phosphoprotein Fos, may influence light-induced phase shifts. Fos is induced by light in the retinorecipient zone of the SCN (Aronin et al., 1990; Colwell and Foster, 1992; Schwartz et al., 1994). Fos protein and c-fos mRNA expression increase dramatically in the SCN of nocturnal rodents kept in constant darkness and pulsed with light during the subjective night (Aronin et al., 1990; Rusak et al., 1990; Colwell and Foster, 1992; Chambille et al., 1993; Earnest and Olschowka, 1993; Amy et al., 2000). These molecules, therefore, are photically induced only during phases at which light also phase shifts locomotor activity rhythms (Aronin et al., 1990; Kornhauser et al., 1990; Rusak et al., 1990; Rea, 1992; Earnest and Olschowka, 1993). Fos appears to play a role in the mediation of the circadian response to light (Wollnik et al., 1995) though it is not necessary for these responses (Honrado et al., 1996).

Patterns of Fos expression in the SCN appear to be more variable in diurnal compared to nocturnal rodents. In Octodon degus, the ventral SCN behaves like that of nocturnal rodents with respect to light-induced Fos expression, but in the dorsal SCN, light pulses actually decrease Fos expression during the subjective day (Krajnak et al., 1997). In the chip-munk SCN, light is almost as likely to induce Fos during the subjective day as during the subjective night, and light pulses that induce Fos do not necessarily cause phase shifts in locomotor rhythms (Abe et al., 1995). Furthermore, Fos may be expressed within different subsets of SCN cells in diurnal compared to at least some nocturnal rodents (Katona et al., 1998; Rose et al., 1999; Mahoney et al., 2000).

Research in chronobiology has focused primarily on nocturnal rodents. We therefore sought to further characterize circadian rhythms and SCN function in the diurnal rodent Arvicanthis niloticus. This murid rodent from sub-Saharan Africa has diurnal rhythms of rest (Novak et al., 1999), body temperature, mating, and general activity (McElhinny et al., 1997); these animals also have a diurnal activity pattern in the wild (Blanchong et al., 1999; Blanchong and Smale, 2000). However, when provided with a running wheel in the lab, some individuals exhibit relatively nocturnal rhythms, with a very brief bout of wheel-running just before lights-on and a much longer bout after lights-off (Blanchong et al., 1999). These “nocturnal” Arvicanthis continue to run in their wheels for 6 to 8 h after lights go out, whereas “diurnal” Arvicanthis stop running after only 1 to 2 h of darkness. When the wheels are removed, nocturnal Arvicanthis switch to a diurnal pattern of general activity. The influence of the wheel on Arvicanthis rhythms is similar to that described in some studies of the Octodon degus (Kas and Edgar, 2000). In the current article, Arvicanthis that run in their wheels predominantly during the day are referred to as “diurnal” and those with nocturnal wheel-running patterns are referred to as “nocturnal” (Blanchong et al., 1999).

In this article, we describe three studies. The first systematically characterized the effects of photic stimuli on wheel-running rhythms of nocturnal and diurnal Arvicanthis. The second study determined the effect of light pulses on Fos expression in the SCN and the region immediately dorsal to it at different phases of the circadian cycle. Our earlier work has shown that Fos is elevated in the Arvicanthis SCN during the light phase of an LD cycle (Katona et al., 1998; Nunez et al., 1999; Rose et al., 1999), but here we explicitly examined how photic stimuli influence Fos expression in the SCN of this species. Last, we examined the effect of SCN lesions on wheel-running rhythms in Arvicanthis.
MATERIALS AND METHODS

Animals

We used adult (> 60 days old) male laboratory-bred *Arvicanthis*. All animals were singly housed in polypropylene cages (34 × 30 × 16 cm), in a 12:12 light-dark (LD) cycle, and provided food (Harlan Teklad 8640 rodent diet, Harlan Teklad Laboratory, Madison, WI) and water ad libitum. A low-intensity (< 1-2 lux) red light was left on continuously.

Phase Response Curve in Diurnal and Nocturnal *Arvicanthis*

Singly housed male *Arvicanthis* (n = 20) were kept in their home cage with a 27-cm running wheel throughout the study. Wheel revolutions were recorded continuously by the Dataquest III Mini-mitter data collection system (Sun River, OR). Nocturnal and diurnal animals were selected based on the number of wheel revolutions per 15-min interval averaged over a 7-day period in a 12:12 LD cycle. A 15-min interval was designated as active if the number of wheel revolutions during the interval exceeded 5% of maximum activity in any 15-min interval. An animal was considered diurnal if it was active for at least 8 h during the light period and less than 4 h during the dark period (n = 7). An animal was considered to be nocturnal if it was active for at least 8 h during the dark period and less than 4 h during the light period (n = 7). Because we wanted to compare rhythms of diurnal and nocturnal *Arvicanthis*, only animals with unambiguous activity patterns were selected and the remaining animals with intermediate patterns were removed from the study. In diurnal *Arvicanthis*, the activity onset at the beginning of the subjective day was designated as CT 0 and was used to measure phase shifts. Among free-running nocturnal individuals, the activity bout at the beginning of the subjective day was sometimes indistinct because it merged with the major period of activity during the subjective night (Fig. 1). Therefore, it was necessary to use a different phase marker, the onset of activity at the beginning of the subjective night, which we designated as CT 12.

Animals were kept in a 12:12 LD cycle for 2½ weeks and then transferred to constant darkness (DD) for 19 weeks. When animals were kept in DD, they were given a 20-min light pulse (100-140 lux) every 2 weeks and PRCs were determined (Bult et al., 1993). The PRC was divided into 3-h intervals. If an animal received a light pulse more than once during the same 3-h interval, its average phase shift for that period was used. After the DD period, animals were transferred to LD
for 5½ weeks, then to constant light (LL) for 4 weeks, and last they were transferred back to a 12:12 LD for 4 weeks. Wheel-running activity was monitored for the entire experiment.

Before each light pulse, the average free-running period for nocturnal and diurnal Arvicanthis \( (\tau_{DD}) \) was determined using the Tau Circadian Rhythms Analysis Software (Mini-mitter, Sunriver, OR) (Bult et al., 1993). Activity profiles were determined from a 5- to 8-day period in LD (Fig. 2, Fig. 3) or DD (Fig. 3). Activity profiles for constant darkness were determined after a minimum of 2 weeks in DD and aligned to activity onset.

Fos Expression

After generating the Arvicanthis PRC, we learned that all Arvicanthis exhibit diurnal patterns of general activity and body temperature when a running wheel is not available and that running in the wheel actually induces the nocturnal pattern expressed by some individuals (Blanchong et al., 1999). We therefore restricted our analysis of the influence of light pulses on Fos expression to animals maintained without running wheels; therefore, they were all presumed to be exhibiting diurnal patterns of rhythmicity. We used a total of 47 adult male Arvicanthis. One day prior to sacrifice, animals remained in darkness after the normal dark portion of the LD cycle. For experimental animals, a white fluorescent light (550-650 lux) was turned on in the room starting at CT 5 (after 16 h of DD exposure), CT 14 (after 25 h DD exposure), or CT 22 (after 32 h DD exposure). These were times that light phase delayed (CT 14), phase advanced (CT 22), or had no effect (CT 5) on Arvicanthis’ wheel-running rhythms (see results below). Experimental animals were perfused immediately after the 1-h light pulse. Control animals were not pulsed with light, but we entered their room briefly; these animals were perfused at the same times as experimental animals. Eight animals were used per group except for CT 5/no pulse; this group had 7 animals. We use the times of sacrifice (CT 6, CT 15, and CT 23) to refer to the different groups.

At the time of sacrifice, animals were deeply anaesthetized with sodium pentobarbital (Nembutal; Abbott Laboratories, 0.5 cc/animal), then perfused transcardially with 0.01 M phosphate-buffered saline (PBS; pH 7.2, 150-200 ml/animal), followed by 150 to 200 ml of fixative (PLP; 4% paraformaldehyde, 1.4% lysine, 0.2% sodium m-periodate, in 0.1 M phosphate buffer; pH 7.4). Brains were post fixed in PLP (4 h) before being transferred to 20% sucrose in 0.1 M phosphate buffer. After 24 h in sucrose, brains were sectioned at 30 \( \mu \)m using a freezing microtome. Sections were transferred to cryoprotectant (Watson et al., 1986) and refrigerated at –20 °C until immunohistochemistry began.

Every third section was labeled to determine the pattern of Fos expression in, and above, the SCN. Free-floating tissue was incubated in 5% normal goat serum (NGS; Vector Laboratories, Burlingame, CA, in PBS with 0.3% Triton-X 100; Research Products International, Mount Prospect, IL) for 1 h at room temperature. Then tissue was incubated in primary antibody for 42 h at 4 °C (rabbit anti-Fos 1:10,000, Santa Cruz; in PBS with 0.3% Triton-X 100 and 3% NGS), followed by biotinylated secondary antibody for 1 h at room temperature (1:200, goat anti-rabbit; Vector Laboratories; in PBS with 0.3% Triton-X 100 and 3% NGS). Finally, it
was incubated in avidin-biotin complex (ABC) for 1 h at room temperature (0.9% each avidin and biotin solutions; Vector Laboratories; in PBS with 0.3% Triton-X 100). Tissue was then reacted in diaminobenzidine (0.5 mg/ml, in Trizma buffer, pH 7.2) with hydrogen peroxide (H₂O₂, 0.35 μl 30% H₂O₂/ml buffer). Between each step, tissue was rinsed three times for 10 minutes in PBS. Tissue was mounted on gelatin-coated slides, dehydrated, and coverslipped.

All sections containing SCN were examined under a light microscope (Leitz, Laborlux S, Wetzlar, Germany). For each animal, rostral, central, and caudal SCN sections were chosen and the outline of one SCN from each section was drawn using a camera lucida. In each drawing, the SCN was bisected horizontally and vertically through the center of the nucleus. Four quadrants were therefore created in each SCN drawing, and the number of Fos-immunoreactive (IR) nuclei was counted for each. These counts were used to determine the number of Fos-IR nuclei in the total SCN, as well as its ventrolateral and dorsomedial quadrants. The ventrolateral quadrant of the Arvicanthis SCN receives the densest retinal inputs, and the dorsomedial quadrant contains the highest concentration of vasopressin-containing cells (Smale and Boverhof, 1999). In three sections, we also did uni-

Figure 3. Wheel revolutions per hour for all diurnal (A, C, E) and nocturnal (B, D, F) animals; each line represents one individual’s hourly values averaged over a period of 7 consecutive days. Data represent the end of the initial (A, B), middle (C, D), and final (E, F) periods of exposure to a 12:12 light-dark cycle.
lateral counts of the number of Fos-IR nuclei in a rectangular region (215 160 µm) of the LSPV zone immediately dorsal to the SCN.

SCN Lesions

Because Arvicanthis are somewhat variable with respect to stereotaxic coordinates and SCN lesions had not been successful previously, we used multiple electrolytic lesions to ensure a complete ablation of the SCN. Adult male and female Arvicanthis were anesthetized with Nembutal (< 50 mg/kg) and methoxyflurane (Metofane, Mallinkrodt Veterinary, Oslo, Norway). Anesthetized individuals were placed in a stereotaxic apparatus with the tooth bar at -6.0 mm, and a surgical drill was used to make a 2-mm-diameter hole through the skull for electrode access. The electrode was placed at an angle of 10 degrees from the vertical, and the tip was positioned 1.4-mm lateral to bregma. Various coordinates were used to locate and lesion the SCN. SCN lesions were complete in 3 animals. For all 3 of these animals, the electrode tip was placed at four positions on each side of the midline. The electrode was placed 1.35 and 1.6 mm anterior to bregma. At the posterior position, the electrode was lowered to 6.4 and 6.7 mm ventral to the top of the skull; at the anterior position, it was lowered to 6.5 and 6.8 mm ventral to the top of the skull. A third animal, considerably larger than the other two, received lesions that were 0.05 mm more ventral. Animals with incomplete lesions served as controls (n = 8).

Electrolytic lesions (0.75 mAmp/25 sec) were produced with epoxy-coated tungsten electrodes (0.5 mm diameter). After the lesion, the incision was closed with autoclips and topical antibiotic (Nolvasan) was applied. Animals received a subcutaneous injection of 1 cc Lactated Ringer’s solution and an intramuscular injection of 0.1 cc of diluted analgesic, buprenorphine hydrochloride (Buprenex).

Animals were returned to 12:12 LD for 8 days after the surgery, then transferred to DD when periodograms indicated that they were arrhythmic (Tau Circadian Rhythms Analysis Software, Mini-mitter, Sunriver, OR). This program was also used to determine if animals were arrhythmic in DD. Cresyl-violet-stained coronal sections (40 µm) from all lesioned animals were used to determine the accuracy and extent of the stereotaxic lesions.

Statistical Evaluation

PRC data were analyzed using a two-way analysis of variance (ANOVA; SAS general linear models procedure) to evaluate the effect of phenotype (diurnal versus nocturnal), the time of light pulse, and an interaction between these two variables. Differences in the τDD between nocturnal and diurnal animals, and changes over time, were also statistically evaluated using a two-way ANOVA. When 0.05 level significance was found, pair-wise differences were tested using the Tukey Studentized Range Test with a significance level of p < 0.05. A student t-test was used to examine τLL, onset of the morning activity bout, and offset of the night activity bout after a transition from LD to LL. The number of Fos-IR nuclei was analyzed using a two-way ANOVA, with perfusion time and light treatment as independent variables (SYSTAT). When significant main effects were found, differences in the number of Fos-IR nuclei between pulsed and nonpulsed animals were compared using a Tukey Honestly Significant Difference Test (Keppel, 1982). Differences were considered significant if p < 0.05 and values are presented as mean ± SEM.

RESULTS

Phase Response Curve in Diurnal and Nocturnal Arvicanthis

Diurnal and nocturnal Arvicanthis exhibited distinctly different wheel-running patterns in a 12:12 LD cycle (Fig. 2A). The diurnal males began running in their wheels 1.21 ± 0.09 h before lights on, continued running throughout the light period, then stopped within 2 h after lights-off. Nocturnal animals had two periods of wheel-running. They began at the same time as diurnal animals but stopped just after lights-on. After this brief, but intense, bout of running, they exhibited extremely low levels of wheel-running throughout the light period and then a second, longer period of running after lights-off (Fig. 2A). This second bout began 0.21 ± 0.11 h before lights-off and lasted from 7 to 8 h. The fundamental patterns of the nocturnal and diurnal animals persisted when they were placed in DD (Fig. 2B). Figure 3 depicts the patterns of each individual at each of the three periods of exposure to an LD cycle: the initial period (Fig. 3 A,B), after DD (Fig. 3 C,D), and after LL (Fig. 3 E,F). As the
study progressed, most nocturnal individuals ran less during the dark period and eventually stopped running about 3 h after lights-off (Fig. 3F). Two nocturnal animals tended to run more during lights-on during the second period in an LD cycle (Fig. 3D), and one of these reverted to its nocturnal pattern during the last interval in an LD cycle (Fig. 3F). Diurnal animals never switched to nocturnal wheel-running patterns, but some became more crepuscular as the study progressed (Fig. 3 A,C,E).

In both groups of animals, phase delays occurred after 20-min light pulses were presented during the early subjective night and phase advances occurred after pulses were presented in the late subjective night. Examples are depicted in Figure 4. During a brief period of the subjective day, light did not induce a phase shift. Figure 5 presents the average phase shifts as a function of circadian time, for animals with nocturnal and diurnal wheel-running patterns. Although the shapes of the PRCs of diurnal and nocturnal *Arvicanthis* were similar, the curves appear to be somewhat shifted to the right in diurnal animals. The ANOVA, however, revealed no overall difference between the nocturnal and diurnal phenotypes ($F_{1,66} = 0.11$, NS). The effect of time and the Phenotype × Time interaction were significant ($F_{7,66} = 12.04$, $p < 0.0001$, and $F_{7,66} = 3.00$, $p < 0.008$, respectively) and post-hoc analyses showed a significant difference between the two groups around CT 10.5 and CT 19.5 ($p < 0.05$; Fig. 5).

The period of the free-running rhythm was slightly, but significantly, shorter among nocturnal compared to diurnal *Arvicanthis* ($F_{1,12} = 6.53$, $p < 0.025$, Fig. 6). As the study progressed, the interindividual variability in the length of the period increased in each group. Over time, the $\tau_{DD}$ of the nocturnal animals decreased while the $\tau$ of the diurnal *Arvicanthis* did not change ($\tau$ phenotype interaction: $F_{8,12} = 3.09$, $p < 0.004$). In LL, $\tau$ was also somewhat shorter in nocturnal *Arvicanthis*, but the difference was not statistically significant ($24.24 \pm 0.13$ h and $24.46 \pm 0.11$ h for nocturnal and diurnal *Arvicanthis*, respectively, $t_{12} = 1.247$, NS).

**FOS EXPRESSION**

SCN

Representative sections through the central SCN of a control animal and animals pulsed with light at the three circadian times are depicted in Figure 7. The number of Fos-IR nuclei within the whole SCN was...
significantly affected by time ($F_{2,40} = 19.74, p < 0.001$), light treatment ($F_{2,40} = 135.87, p < 0.001$), and an interaction between these two variables ($F_{2,40} = 19.27, p < 0.001$; Fig. 8). Among animals maintained in DD and not exposed to light, numbers of Fos-IR nuclei were low and unaffected by time. At CT 6, the number of Fos-IR nuclei was not influenced by light, whereas at CT 15 and CT 23, light pulses induced significant increases in Fos ($p < 0.01$). Thus, numbers of Fos-IR nuclei were significantly higher in light-pulsed animals killed during the subjective night than day (for both CT 23 vs. CT 6 and CT 15 vs. CT 6, $p < 0.01$).

Figure 9 depicts the effect of light pulses on Fos-IR in different regions of the *Arvicanthis* SCN. The basic patterns of Fos-IR in both the ventrolateral and the dorsomedial quadrants of animals exposed to light were similar to the pattern observed in the total SCN (Fig. 8). A light pulse given during the subjective night (CT 15 or CT 23) induced a significant increase in the number of Fos-IR nuclei in the rostral, central, and caudal SCN (Fig. 9).

**LSPV Zone**

Light caused a relatively small but significant increase in the number of Fos-IR nuclei in the LSPV region, dorsal to the SCN ($F_{1,41} = 11.47 p = 0.002$; Fig. 10).
A rhythm in Fos expression in this region was also apparent ($F_{2,41} = 33.74, p < 0.001$) such that animals killed during the subjective night had significantly more Fos-IR cells than animals killed during the subjective day (for both CT 23 vs. CT 6 and CT 15 vs. CT 6, $p < 0.05$; Fig. 10). This pattern was apparent in animals that were pulsed as well as animals that were not pulsed with light.

**SCN Lesions**

In 3 individuals, periodogram analysis (Tau Circadian Rhythms Analysis Software, Mini-mitter, Sunriver, OR) indicated activity rhythms were abolished by surgeries aimed at destroying the SCN (Fig. 11). Histological analysis confirmed that the SCN lesions were complete in these 3 animals (Fig. 12). Complete lesions extended from the frontal pole of the nucleus.
to the retrochiasmatic nucleus and included damage to the anterior hypothalamic area around the SCN and the optic chiasm. In all animals with incomplete lesions, rhythms persisted after surgeries.

DISCUSSION

We found that the diurnal rodent, *Arvicanthis niloticus*, is similar to nocturnal rodents with respect to a variety of features of the circadian system including the PRC, light-induced Fos-IR in the SCN, and arrhythmia following SCN lesions. Photic input in the early subjective night led to phase delays and in the late subjective night to phase advances. Light pulses in the subjective night increased Fos expression in all regions of the SCN, whereas light pulses in the subjective day had little or no effect on Fos-IR. Lesions of the SCN abolished endogenous rhythms, providing preliminary evidence that the SCN is the primary circadian pacemaker in *Arvicanthis* as it is in other nocturnal and diurnal species (Moore and Eichler, 1972; Stephan and Zucker, 1972; Sato and Kawamura, 1984; Ralph et al., 1990). However, this needs to be more rigorously tested with longer postlesion behavioral evaluations and transplant experiments.

The PRC of diurnal *Arvicanthis* appeared to be shifted by about 3 h to the right relative to the PRC of the nocturnal *Arvicanthis* (Fig. 5), raising the possibility of a difference within some feature of their light-responding mechanisms. However, phase shifts had to be determined with different phase markers for nocturnal and diurnal *Arvicanthis*. Activity onset, at the beginning of the subjective night for nocturnal *Arvicanthis* and at the beginning of the subjective day for diurnal *Arvicanthis*, had to be used. The relation-
ship of these two phase reference points to underlying circadian mechanisms may not be the same (Chandrashekaran et al., 1983), which could account for their slightly shifted PRCs. Therefore, based on the current data, we cannot be certain whether the apparent difference between the two PRCs is real. It is clear, however, that in many respects the two PRCs are extremely similar. That is, the shapes of the two PRCs are almost identical, and the maximum delays and advances are the same, as is the duration of the dead zone (Fig. 5).

Diurnal and nocturnal *Arvicanthis* differed in the length and plasticity of their free-running period in DD. The shorter free-running period of nocturnal *Arvicanthis*, as compared to diurnal individuals, may be caused by the presence of a wheel.Wheel-running has been shown to affect the variability of $\tau$ in hamsters (Pratt and Goldman, 1986) and the length of $\tau$ in rats (Yamada et al., 1988) and mice (Edgar et al., 1991). Nocturnal *Arvicanthis* run more than do diurnal ones (Blanchong et al., 1999), which may have decreased the period of the rhythm. Alternatively, the difference between nocturnal and diurnal *Arvicanthis* with respect to $\tau$ may represent an intraspecific example of “Aschoff’s Rule.” In DD, the period of the nocturnal animals was significantly shorter than in diurnal animals, but it increased in LL, which eliminated this difference. Finally, differences intrinsic to the circadian system and independent of light or wheel running may account for the different free-running periods in diurnal and nocturnal *Arvicanthis*.

The PRC of *Arvicanthis* resembles those typically seen in both nocturnal (DeCoursey, 1960; Daan and Pittendrigh, 1976; Rea, 1992; Bult et al., 1993; Rappold and Erkert, 1994) and diurnal animals (Kramm, 1976; Hoban and Sulzman, 1985; Navaneethakannan and Chandrashekar, 1986; Kas and Edgar, 2000). Common features include a nonresponsive dead zone during the subjective day and a period of phase delays and phase advances in the early and late subjective night, respectively. However, some evidence indicates that the PRCs of diurnal species may be more variable than in nocturnal species (Pohl, 1982). For example, one PRC generated for the *Octodon degus* shows phase delays during the early subjective day and phase advances in the early subjective night (Lee and Labyak, 1997). No dead zone was found in the light-induced PRC of three diurnal rodent species, *Tamiasciurus hudsonicus*, *Tamias striatus*, and *Ammospermophilus leucurus* (Kramm, 1975; Pohl, 1982). The relative variability of the PRCs in these diurnal species may reflect differing patterns of change that occurred at the multiple independent evolutionary transitions from nocturnality to diurnality.

The photic induction of Fos-IR in the SCN of *Arvicanthis* was phase dependent in that light pulses given in the subjective night induced more Fos-IR than pulses in the subjective day. The photic induction of Fos-IR in the SCN of nocturnal rodents also occurs at times when endogenous rhythms can be phase shifted by light (Aronin et al., 1990; Kornhauser et al., 1990; Rusak et al., 1990; Rea, 1992; Earnest and Olschowka, 1993). In nocturnal rats, the level of light needed to phase shift rhythms is nearly identical to the level needed to induce Fos-IR in the SCN (Kornhauser et al., 1990), and the degree of phase shifting is correlated with the amount of c-fos mRNA and Fos-IR (Travnickova et al., 1996; Beaule and Amir, 1999; Amy et al., 2000). The effects of light on Fos expression in the SCN of diurnal rodents appear to be more variable. For example, in the degus, Fos-IR is induced only during the subjective night and is actually inhibited by light during the subjective day (Krajnak et al., 1997). In the chipmunk, light induces high levels of Fos-IR in the SCN during the subjective day and night (Abe et al., 1995). By contrast, *Arvicanthis* exhibit a light-induced increase in Fos-IR within all regions of the SCN, but only at times when light also phase shifts activity rhythms. With respect to temporal patterns of light-induced Fos-IR in the SCN, *Arvicanthis* thus appear to be very similar to nocturnal species.

The distribution of light-induced Fos-IR within the SCN also appears to be similar in *Arvicanthis*, compared to at least some other rodents. A large number of Fos-IR nuclei were induced in the ventral SCN of *Arvicanthis* exposed to light pulses during the subjective night (Fig. 9). Retinal input is most heavily concentrated in this region, and the distribution of retinal projections appears to be very similar to that of light-induced Fos-IR observed in the current study (Smale and Boverhof, 1999). Similarly, in other diurnal species (e.g., degus, chipmunks) and nocturnal ones (e.g., rats, hamsters), most light-induced Fos-IR is expressed in regions where the retinal input is typically most concentrated (Earnest et al., 1990; Colwell and Foster, 1992; Chambille et al., 1993; Abe et al., 1995; Krajnak et al., 1997).

Some evidence suggests that Fos may be expressed within different subsets of SCN cells in *Arvicanthis* compared to nocturnal rodents. In *Arvicanthis* maintained on a 12:12 LD cycle, Fos-IR, which is elevated during the day, is found within vasopressin-contain-
ing cells (Katona et al., 1998; Rose et al., 1999) but not within cells containing calbindin (Mahoney et al., 2000) or gastrin-releasing peptide (Katona et al., 1998). In nocturnal rats, different patterns have been found (Earnest et al., 1993; Rose et al., 1999; Mahoney et al., 2000). Thus, despite the similarities in light-induced Fos-IR between *Arvicanthis* and other nocturnal species, it remains possible that a subset of Fos-containing cells in the *Arvicanthis* SCN may contribute to diurnality in this species.

The region immediately dorsal to the SCN receives input directly from the retina in both *Arvicanthis* and nocturnal rodents (Johnson et al., 1988; Smale and Boverhof, 1999). Fos-IR is expressed rhythmically in this region in nocturnal and diurnal rodents kept in a 12:12 LD cycle, though their patterns of expression differ (Nunez et al., 1999). The number of Fos-IR nuclei in the LSPV zone of *Rattus* peaks at zeitgeber time (ZT) 1 (ZT 0 = lights on) and is low at ZT 5, 13, and 17 (Nunez et al., 1999). By contrast, *Arvicanthis* housed in the same conditions have elevated Fos-IR in the LSPV region both at ZT 17 and at ZT 1 (Nunez et al., 1999). Data in the present study extend this finding by showing that (1) the rhythm of Fos-IR nuclei in the LSPV zone of *Arvicanthis* persisted in DD, with high levels of expression at CT 15 and CT 23 and low levels in the mid-subjective day at CT 6; (2) this pattern was exhibited in both nonpulsed and pulsed animals; and (3) light pulses increased Fos-IR in this region (Fig. 10). Light pulses similarly increased Fos-IR in the LSPV region of rats (Rusak et al., 1990), and this appears to be the case in hamsters. In pulsed hamsters, Fos increased from CT 6 (mean ± SD; 86 ± 13) to CT 13 (652 ± 100) to a peak at CT 18 (1306 ± 162) (Rea, 1992). Levels of Fos expression have not been reported for the LSPV region of nonpulsed control rats or hamsters. The magnitude of the increase in Fos-IR from subjective day to night in animals pulsed with light appears to be considerably greater in the LSPV zone of hamsters (fifteenfold increase; Rea, 1992) compared to *Arvicanthis* (twofold increase). Interestingly, electrophysiological recordings from around the SCN provide further evidence that the LSPV region may play a different role in the regulation of circadian rhythms in diurnal and nocturnal rodents. In the diurnal Siberian chipmunk, *Eutamias sibiricus*, multiple unit activity exhibited a rhythm in the SCN that was in phase with the rhythm in adjacent regions around the SCN (Sato and Kawamura, 1984). By contrast, in nocturnal rats, this rhythm was 180 degrees out of phase with the rhythm inside the SCN (Kubota et al., 1981).

In summary, the PRC and temporal patterns of Fos expression in response to light pulses appear to be the same in *Arvicanthis* and nocturnal rodents. For example, light in the early subjective night induced phase delays, whereas light in the late subjective night induced phase advances in rhythms, whereas light at both of these times induced Fos expression in the SCN. The gating mechanism that determines the SCN response to light pulses may be quite different from the mechanism that determines the relationship between the active phase of the rhythm and the LD cycle. The mechanism responsible for the latter phenomenon remains unknown, but it could involve differences in cells receiving input from the SCN, such as those in the region around the SCN (Fig. 10) (Nunez et al., 1999), or perhaps differences that have not yet been identified within some subset of SCN cells.

**ACKNOWLEDGMENTS**

We are very grateful to Cassandra Castlebury, Teresa McElhinny, Janaina Gamez, and Betty Gubik for assistance with the animals and technical support. We are also grateful to Antonio Nunez for his input as the experiments were in progress as well as comments on an earlier version of this article. We also thank Dr. Thomas Adams, Isam Qahwash, Yong-Joo Chung, Julide Celik, and Miyuki Tauchi for their extensive comments on earlier drafts. This work was supported by the NIH (grants MH#53433 and NS#07279).

**REFERENCES**


Hoban TM and Sulzman FM (1985) Light effects on circadian timing system of a diurnal primate, the squirrel monkey. Am J Physiol (Regul Integr Comp Physiol) 249:R274-R280.


