

Research report

Photic responses of suprachiasmatic area neurons in diurnal degus (*Octodon degus*) and nocturnal rats (*Rattus norvegicus*)

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Abstract

Photic sensitivity of cells in the suprachiasmatic nuclei (SCN), the principal pacemaker of the mammalian circadian system, has been documented in several species. In nocturnal rodents, the majority of photically responsive SCN cells are activated by retinal illumination. One report identified mostly photic suppressions among SCN cells in a diurnal rodent, studied under somewhat different conditions. We examined photic sensitivity of SCN cells in a predominantly diurnal rodent, the degu, studied *in vivo* under identical conditions to rats, and found that a large majority of photic SCN cells were suppressed by light. In both rats and degus, SCN cells were more responsive to light during the subjective night than during the subjective day. Light-responsive cells did not show a daily rhythm in baseline firing rates in either species, but rat SCN cells that did not respond to light were more active spontaneously during the subjective day. Light-unresponsive SCN cells in degus did not show a similar pattern. There are substantial differences in the neurophysiological activity and photic responsiveness of SCN cells in diurnal degus and nocturnal rats. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Daily behavioral and physiological rhythms in most animals are generated internally by a circadian pacemaker and synchronized to the external day–night cycle by environmental cues, especially the daily lighting cycle [66]. The suprachiasmatic nuclei (SCN) of the anterior hypothalamus function as the major pacemaker for many circadian rhythms in both nocturnal [37,52] and diurnal mammals [54,67]. Information about retinal illumination is conveyed to the SCN via a direct retinohypothalamic tract (RHT) [10,38,39,42,46,47], and an indirect geniculohypothalamic tract (GHT) formed by retinorecipient neurons in the intergeniculate leaflet and adjacent regions of the ventral lateral geniculate complex [4,15,38,41,49,65].

Responsiveness of a portion of SCN neurons in rats (*Rattus norvegicus*), Syrian hamsters (*Mesocricetus auratus*) and cats (*Felis catus*) to retinal illumination has been

documented extensively using *in vivo* single-unit, extracellular recording methods [14,31–33,55] and multiple-unit recordings [19,20]. Single-unit studies have documented that SCN neurons often show sustained, intensity-dependent changes in firing rate in response to altered retinal illumination, along with varying types of transient responses to abrupt illumination changes.

There are two opposite response types among SCN neurons showing sustained photic responses: some cells increase and others decrease firing rates as illumination intensity increases [14,32,33,55]. Similar results were found in studies *in vivo* and *in vitro* using electrical stimulation of the optic nerves instead of retinal illumination [8,43,55,58]. In the nocturnal species that have been studied, activation of SCN cells (and IGL cells [16,65]) in response to increases in illumination intensity has been the dominant response. Numerous studies have documented that such activation depends on excitatory amino acid receptors on SCN cells [2,7,25,62]. The minority of light-suppressed cells are not often considered in discussions of photic entrainment and of the role of excitatory amino acid transmission in mediating light effects on SCN cells.

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Diurnal rodents have been studied far less often in relation to photic entrainment mechanisms, and only one electrophysiological study has examined the effects of retinal illumination on firing rates of SCN neurons in a diurnal species. In that study [33], a small population of SCN neurons recorded from 13-lined ground squirrels (*Spermophilus tridecemlineatus*) also responded to retinal illumination, but the proportion of light-activated cells was very different from that observed in studies of rats and hamsters. While most studies found that 65–85% of SCN cells that responded to light or optic nerve stimulation were activated in nocturnal rodents, less than half (7/15) of photically responsive SCN neurons in squirrels were activated. In addition, the light intensities required to generate substantial firing-rate changes were much higher for the squirrels than had been reported for rats and hamsters.

These observations suggested a difference between diurnal and nocturnal rodents, implying that generalizations from previous studies to diurnal mammals might be inappropriate. There were, however, potential methodological problems in this analysis. For example, the squirrels did not tolerate urethane anesthesia that was used in the previous studies of nocturnal species, and were instead anesthetized during recordings using a barbiturate anesthetic. In addition, the squirrels were wild-caught and had experienced a very different early history of light exposure than would laboratory-bred animals. Although they were housed in the laboratory for several weeks before recordings were made, it remains possible that early exposure to bright, natural illumination might alter responsiveness of cells in the visual system.

To assess these issues in a more favorable system, we studied SCN cells in degus (*Octodon degus*). Degus are South American hystricomorph rodents (Family Octodontidae) that have been reported to be primarily diurnal both in the natural environment and in the laboratory [12,48,63]. Degus are also reported to show crepuscular modulation of activity and sleep rhythms, as well as modifications of rhythmicity in response to wheel availability [24]. Degus breed readily in the laboratory and they have been used extensively in studies of photic and non-photoc entrainment of the circadian system [12,27–29]. We undertook a direct comparison of the sensitivity of SCN neurons to retinal illumination in degus and rats.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats ($n = 29$, 220–340 g) from Charles River (St. Constant, Québec), and male degus ($n = 14$, 224–294 g), born in a colony maintained at the University of Michigan, were shipped to Dalhousie University. Animals were kept in similar, separate rooms

for at least 2 weeks under an artificial photoperiod with 12 h of light daily (LD 12:12; lights on at 0600 h Atlantic Daylight Time).

On the day of an experiment, an animal was injected intraperitoneally with an initial anesthetic dose of a 20% urethane solution (1.0–1.2 g/kg for degus; 1.2–1.4 g/kg for rats) and mounted in a stereotaxic instrument. The level of anesthesia was monitored by periodic checks of reflexes and breathing, and additional doses of 25–33% of the initial dose were given as needed throughout the recording session. Topical anesthetic was applied if needed to ear canals and incision site. Robinul (glycopyrrolate 0.2 mg/ml, Ayerst Laboratories, Canada) was administered subcutaneously (0.2 ml/animal) to reduce respiratory tract congestion during anesthesia. Rectal temperature was monitored and maintained at 37°C by a thermostatically controlled heating pad during the experiment. The eyes were treated with topical 1% atropine sulphate solution to dilate the pupils, and coated with mineral oil to prevent drying.

2.2. Electrophysiology

Extracellular single-unit discharges were recorded in and near the SCN of degus and rats using conventional amplification and time-amplitude discrimination methods with a Bak electrophysiology system, as described previously [64]. A craniotomy was performed over the region of the SCN and metal microelectrodes (Micro Probe, Gaithersburg, MD; tip diameter: 1–2 μm ; impedance: 1.8–2.2 M Ω) were aimed at the SCN using stereotaxic coordinates. In degus, these were 0.40–1.20 mm anterior to bregma and 0.65–0.80 mm lateral to midline at an angle of 4° to the vertical, and in rats, 0.60–1.10 mm posterior to bregma and 0.25–0.60 mm lateral to midline, with the incisor bar at 3.0 mm below the interaural line for both species. The electrode was lowered 8.05–10.20 mm ventrally from dura using a hydraulic drive and stepping motor (Frederick Haer).

When a neuronal discharge was isolated at the appropriate depth, the cell was assessed for photic responses to bilateral retinal illumination. This was supplied by a fiber optic system from a halogen lamp, infrared-filtered and led to the eyes through a computer-controlled shutter system. Animals were kept in darkness except for maintenance checks and for repositioning electrodes. Typically, cells were tested using 700 lx light pulses of 30–40 s duration

Table 1
Proportions of photic cells recorded in degus and rats

	Degus			Rats		
	SCN	Hypothalamus	Total	SCN	Hypothalamus	Total
Photic cells	22	10	32	27	20	47
Total cells	88	141	229	97	131	228
Photic (%)	25.0	7.1	14.0	27.8	15.3	20.6

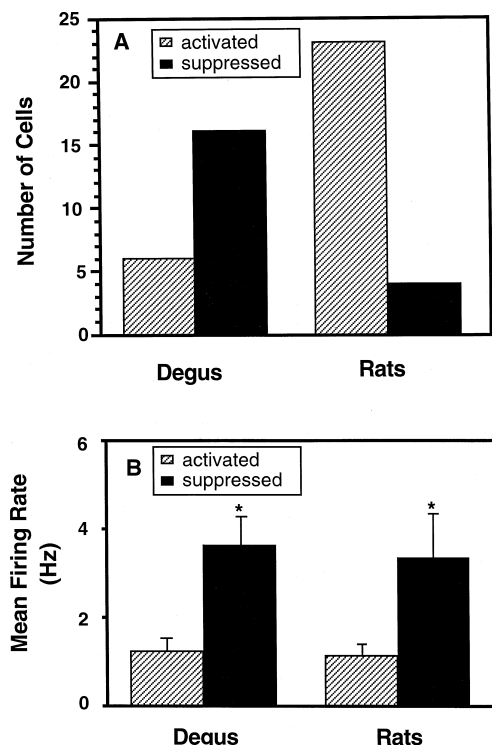


Fig. 1. (A) Number of photically responsive cells in each species that were activated or suppressed by retinal illumination. Species differences were highly significantly different ($p < 0.0001$) by Fisher's Exact Probability Test. (B) Mean (\pm S.E.M.) baseline firing rates (Hz) of light-activated and light-suppressed cells in degus and rats. * $p < 0.05$ compared with firing rate of light-activated cells.

within 2 min recording sweeps. For some trials, light intensity was increased up to 2000 lx by repositioning the fiber optics, or reduced to lower intensities by a neutral density filter. Experiments were conducted during both subjective day and subjective night from 0900 h to 2400 h.

2.3. Data analysis

Data were acquired using Pulsecount (written by Alan Hurshman) on an Apple IIe computer, or A/Dvance (written by Robert Douglas) on a StarMax computer. Peristimulus time histograms (PSTHs) were generated online and stored to disk for further analysis offline. Neuronal waveforms were stored before and after light presentation to

assess stability of the recording. Cells were classified as photically responsive if their firing rates increased more than 30%, or decreased more than 20%, from the spontaneous firing rate in darkness during three consecutive light exposure trials. The activity change during light exposure was expressed as an absolute or percentage change from dark baseline averaged over three trials. Comparisons of the results of this classification method to the results of repeated measures *t*-tests to assess significant differences in firing rates yielded $> 95\%$ concordance on a randomly selected subset of cells.

SigmaStat software was used for statistical analyses. The proportions of photic cells and the ratio of light-activated to -suppressed cells were compared between degus and rats by chi-square test or Fisher exact probability test. Unpaired *t*-tests and one-way ANOVA were used to assess the statistical reliability of differences in mean firing rates and in response amplitudes. Medians were calculated for some data sets that did not show normal distributions, and nonparametric Mann–Whitney rank-sum tests were used to compare these groups.

2.4. Histological analysis

The final position of the electrode was marked with an electrolytic lesion (3 V DC, 30 s) at the end of each experiment. The animal was then anesthetized deeply and perfused transcardially. Serial frozen sections 40 μ m thick were cut through the region of the hypothalamus, and the tissue stained with Neutral Red in order to visualize the lesion site. The positions at which both photically responsive and unresponsive cells were recorded were reconstructed relative to the marker lesion coordinates, and cells were classified on that basis as being within the SCN or in the adjacent hypothalamus.

3. Results

3.1. Photic responses

A total of 229 cells were recorded in degus, of which 88 cells were identified as being inside the SCN. A total of 32 cells met the criteria for photic sensitivity, and 22 of

Table 2
Percentage photic response types recorded in degus and rats

	Light-activated		Light-suppressed	
	Degus	Rats	Degus	Rats
SCN	27.3 (6/22)	85.2 (23/27)**	72.7 (16/22)	14.8 (4/27)
Hypothalamus	60.0 (6/10)	65.0 (13/20)	40.0 (4/10)	35.0 (7/20)
Total	37.5 (12/32)	76.6 (36/47)*	62.5 (20/32)	23.4 (11/47)

* $p < 0.01$, ** $p < 0.0001$ compared with results in degus.

these were located inside the SCN. Thus, 22/88 (25.0%) of SCN cells and 10/141 (7.1%) of extra-SCN hypothalamic cells were photically responsive. In rats, a total of 228 cells were recorded in the hypothalamus, with 97 cells inside the SCN. A total of 47 cells responded to light, of which 27 were inside the SCN. Thus, 27/97 (27.8%) of SCN cells and 20/131 (15.3%) of adjacent hypothalamic cells were light responsive (Table 1).

Of the 22 photically responsive SCN cells in degus, six (27.3%) were light-activated and 16 (72.7%) were light-suppressed. Of the 27 photically responsive SCN cells in rats, 23 (85.2%) were light-activated and four (14.8%) were light-suppressed (Fig. 1A). These differences were significant by a Fisher exact probability test ($p < 0.0001$). In degus, there were 10 photically responsive cells recorded outside the SCN, of which six (60%) were light-activated and four (40%) were light-suppressed. In rats, there were 20 light-responsive hypothalamic cells outside the SCN, of which 13 (65%) were activated and seven (35%) were suppressed (Table 2). There were no significant differences between species in patterns of response among hypothalamic cells.

Most light-responsive cells in degus were recorded during the subjective night (15/22 = 68.2%), while there was a more even distribution in rats with 12/27 (44.4%) photically responsive cells recorded during the subjective night.

Light-suppressed SCN cells had significantly higher baseline firing rates than light-activated cells. For degus and rats, the baseline firing rates (mean \pm S.E.M.) for light-activated cells were 1.24 ± 0.28 and 1.15 ± 0.24 Hz, respectively. These values were significantly lower ($p < 0.05$) than values for light-suppressed cells (3.62 ± 0.65 Hz for degus and 3.36 ± 1.00 Hz for rats) (Fig. 1B).

Because there were so few suppressed cells in rats and activated cells in degus, analyses of the amplitudes of light responses were attempted only for light-suppressed cells in

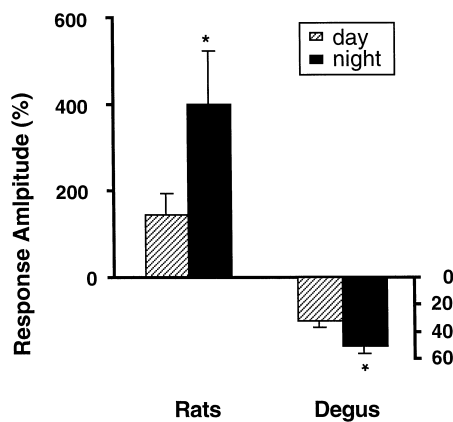


Fig. 2. Mean (\pm S.E.M.) response amplitudes (percent of change from baseline) of SCN photic cells in degus (suppression) and rats (activation) during subjective day and night. * $p < 0.05$ compared with response amplitude during subjective day.

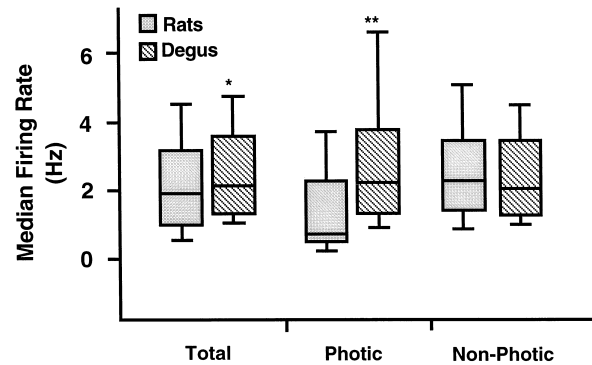


Fig. 3. Median baseline firing rates (Hz) of SCN cells in degus and rat. The boxes encompass the 25th–75th percentiles and the vertical lines indicate the 10th and 90th percentiles around the median, indicated by the horizontal line. * $p < 0.05$; ** $p < 0.01$ for comparisons of median firing rates between rats and degus.

degus and light-activated cells in rats. In response to a standard light pulse, cells in degus showed a decrease of $31.9 \pm 4.7\%$ (mean \pm S.E.M.) during the day ($n = 5$), and a decrease of $50.7 \pm 5.4\%$ during the night ($n = 11$); this difference was statistically significant ($p < 0.05$). In rats, light increased firing rates during the day by an average of $144.9 \pm 48.5\%$ ($n = 13$) and during the night by an average of $398.0 \pm 122.6\%$ ($n = 10$). This difference was also statistically significant ($p < 0.05$) (Fig. 2).

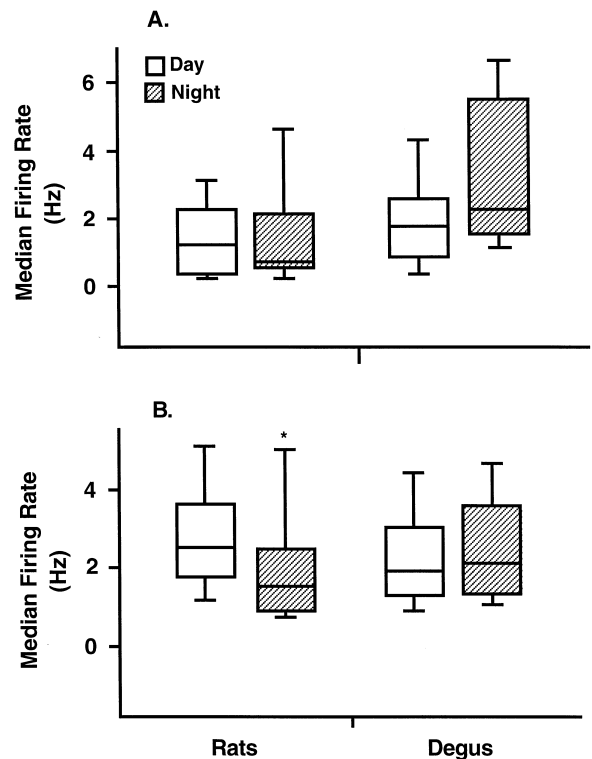


Fig. 4. Median baseline firing rates (Hz) of SCN photically responsive (A) and unresponsive (B) cells recorded during subjective day and night in degus and rats. See Fig. 3 for details. * $p < 0.05$ for comparison of daytime and nighttime firing rates.

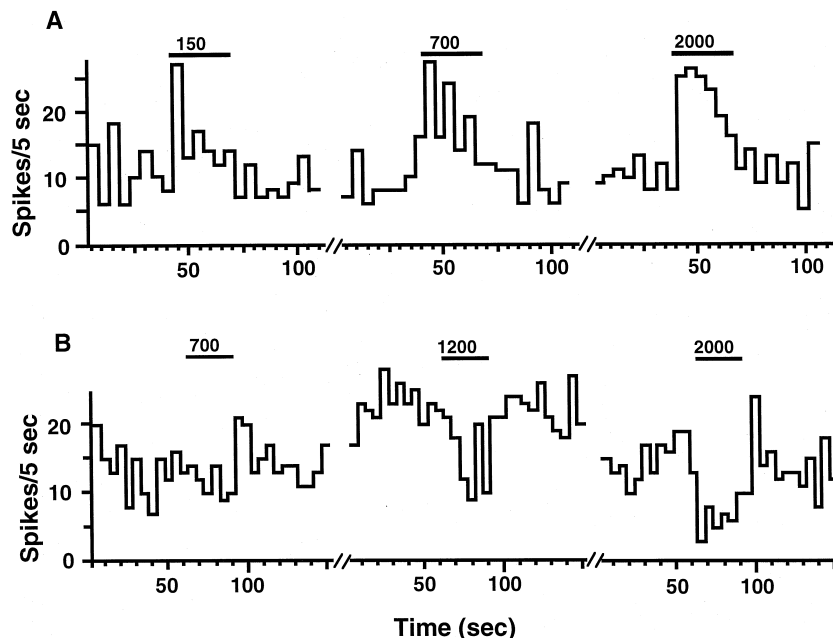


Fig. 5. Peristimulus time histograms of light-activated (A) and light-suppressed SCN cells (B) in degus. The lines at the top indicate the duration (30 s) of the light stimuli and the numbers indicate light intensity in lux. Only the highest intensity light generated sustained, robust responses.

Summed across both light-suppressed and -activated cells, there was a species difference in the absolute percentage change in firing rates induced by light in all

photically responsive cells. Rats showed significantly larger ($p < 0.001$) average changes in firing rates than degus ($228.5 \pm 55.9\%$ vs. $53.9 \pm 6.5\%$).

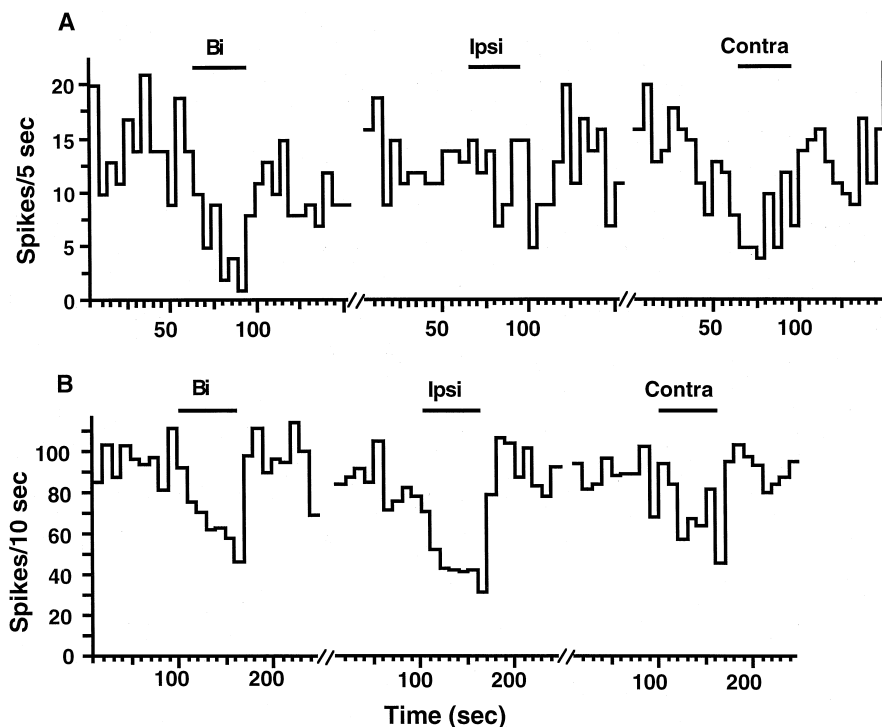


Fig. 6. Peristimulus time histograms of light-suppressed SCN cells in degus that responded only to contralateral retinal illumination (A) and that responded to both contralateral and ipsilateral retinal illumination (B). The lines at the top indicate the duration ((A) 30 s; (B) 60 s) of the light stimuli (Bi: bilateral; Ipsi: ipsilateral; Contra: contralateral retinal illumination).

3.2. Firing rates

Comparisons between species showed that the median spontaneous firing rate of all SCN cells was significantly lower ($p < 0.05$) in rats (1.88 Hz) than in degus (2.11 Hz). This difference was attributable exclusively to the population of photically responsive cells, whose median firing rates (0.70 Hz in rats and 2.20 Hz in degus) were highly significantly different ($p < .01$), while there was no species difference in median firing rates of photically unresponsive cells (2.25 Hz in rats and 2.08 Hz in degus; Fig. 3).

In degus, the baseline (dark) firing rates of photically responsive SCN cells varied from 0.25 to 10.35 Hz, with median rates during the subjective day and the subjective night of 1.78 Hz and 2.28 Hz, respectively. Although nighttime firing rates were higher, these values did not differ significantly (Fig. 4A), nor was there a significant difference between day (1.95, $n = 26$) and night (2.13, $n = 40$) baseline firing rates of non-photoc cells in degus (Fig. 4B).

In rats, the spontaneous firing rates of photically responsive cells ranged from 0.12 to 6.27 Hz, with median firing rates of 1.22 Hz in the subjective day and 0.68 Hz in the subjective night. Consistent with the results in degus, these values did not differ significantly (Fig. 4A). In contrast to degus, however, rats demonstrated a significant difference in spontaneous discharge rate among SCN cells that were not photically responsive. These cells had a median firing rate in the daytime of 2.53 Hz ($n = 46$) that was significantly higher ($p < 0.05$) than that in the subjective night (1.55 Hz; $n = 24$) (Fig. 4B).

Since photic cells in rats had lower spontaneous firing rates and since low firing rates could make detection of suppressions difficult, we assessed whether the species differences in responses could be affected by the baseline firing-rate differences. We defined a 'low' firing rate as < 2 Hz (approximately the median for both species). In degus, such low firing-rate cells comprised 42/88 (47.7%) of all cells and in rats 57/97 (58.8%). Among these cells, 5/42 (11.9%) were activated in degus, while 18/57 (31.6%) were activated in rats; 5/42 (11.9%) were suppressed in degus, while 1/57 (1.8%) were suppressed in rats. Thus, among cells with low spontaneous firing rates, there were equal numbers of light-activated and -suppressed cells in degus, even though there were overall many more light-suppressed cells in this species. By contrast, there were many more light-activated cells in rats.

These results also demonstrate that it is possible to detect suppressions in SCN cells at these low baseline rates, since 5/10 (50%) of photic degu cells with low firing rates were suppressed, while only 1/19 (~5%) of similar photic cells in rats were suppressed. Thus, the lack of suppression in rats does not seem to be related directly to low firing rates, at least by this definition. In addition, if photic suppressions were difficult to detect in rats because of their lower firing rates, this should lead to an excess of

cells being labeled 'non-photoc' rather than 'activated'. In fact, the proportions of non-photoc cells were very similar in the two species (75% rats, 72.2% degus), and among cells with firing rates < 2 Hz, 76.2% were non-photoc in degus and 66.7% were non-photoc in rats. This difference is in the opposite direction from what would be predicted if a larger number of low firing-rate cells in rats were making suppressions less detectable.

3.3. Photic sensitivity

Threshold light responses and laterality of input were tested using those cells (degus $n = 6$, rats $n = 15$) that were stable enough to be studied for long periods of time. Light intensity was varied up to 2000 lx, and cells were tested with light presentation to each eye alone as well as binocularly. In degus, one typical light-suppressed and one light-activated SCN cell were studied at several intensities. The light-activated cell showed only a transient response to a 150-lx stimulus, but a more robust sustained response to 700 lx and especially to 2000 lx (Fig. 5A). The light-suppressed cell showed little response to even 700 lx, a

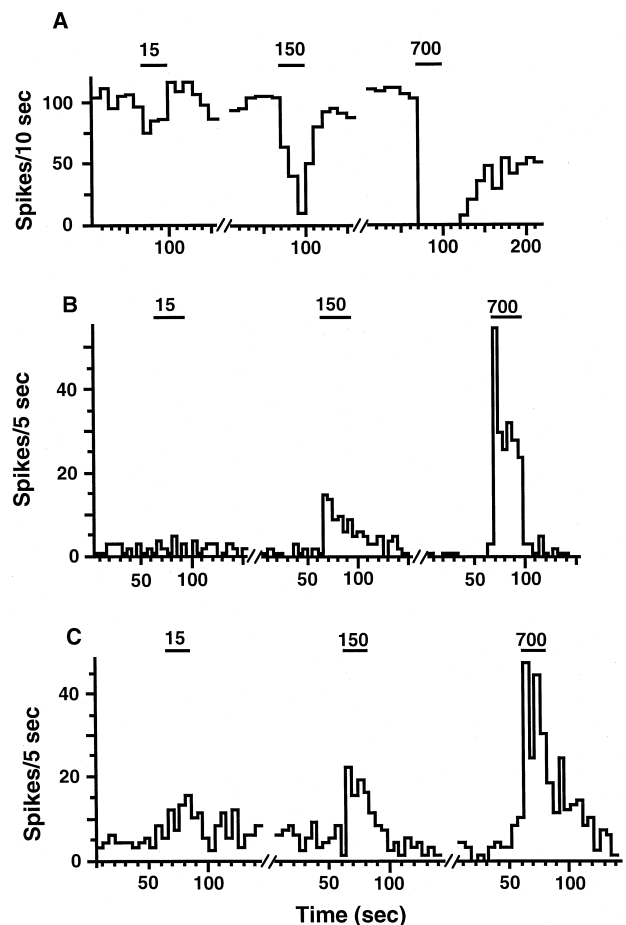


Fig. 7. Peristimulus time histograms of a light-suppressed (A) and two light-activated SCN cells (B,C) in rats. The lines at the top indicate the duration ((A, B) 30 s; (C) 20 s) of the light stimuli and the numbers indicate light intensity in lux. Responses that were sustained for the duration of the stimulus were observed at 150 lx or even 15 lx intensities (A, C).

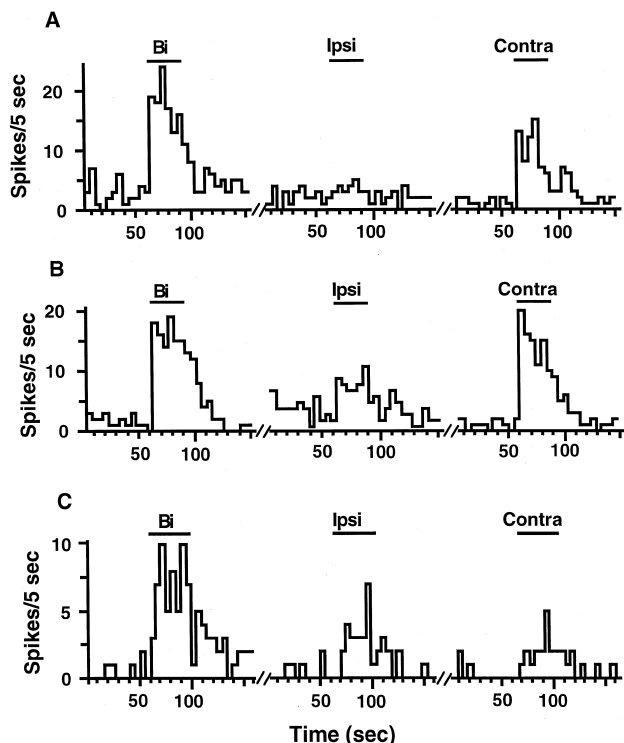


Fig. 8. Peristimulus time histograms of three light-activated SCN cells in rats that were tested for laterality of retinal light sensitivity. Photic responses could be driven only by contralateral input (A) or by either eye (B, C). The lines at the top indicate the duration ((A, B) 30 s; (C) 40 s) of the light stimuli (Bi: bilateral; Ipsi: ipsilateral; Contra: contralateral retinal input).

modest response to 1200 lx, and a sustained response only during presentation of a 2000-lx stimulus (Fig. 5B). Of two light-suppressed cells tested, one was driven almost exclusively via the contralateral eye (Fig. 6A), while the other was driven by either eye, but was somewhat more responsive to ipsilateral input (Fig. 6B).

In rats, one light-suppressed cell was tested for visual responses to a range of light intensities. Responses were intensity-dependent, but even as little as 15 lx caused a sustained decrease in firing, while a 700 lx stimulus completely suppressed cell firing (Fig. 7A). Two light-activated cells tested also showed intensity-dependent responses (Fig. 7B,C). One responded clearly to 15 lx (Fig. 7C), while both responded to 150 lx; by 700 lx both averaged firing rates > 300% of baseline rates (Fig. 7B,C). Of three light-activated cells tested for laterality of input, one was driven only contralaterally (Fig. 8A). The other two responded to illumination of either eye (Fig. 8B,C), but one was clearly more sensitive to contralateral input (Fig. 8B).

4. Discussion

4.1. SCN photic responses

There were both similarities and differences in photic responses of SCN cells in rats and degus. The proportions

of all cells sampled that met the criteria for photic responsiveness were very similar in these species (25.0% in degus and 27.8% in rats). These proportions were also similar to those found in earlier studies of rats and hamsters [32] which used urethane anesthesia, but higher than that observed in a study of 13-lined ground squirrels, using a barbiturate anesthetic [33]. These results suggest that the activity type of the animal is not related to the proportion of photically responsive SCN cells, but the type of anesthetic used in vivo might be relevant. Barbiturates are known to suppress cellular responses to photic input in other parts of the visual system [6,17,45].

Photic responses of SCN cells in rats and degus also included both activation and suppression, as had been reported for all species studied previously [14,32,33]. The most striking difference between rats and degus, however, was in the proportions of the two photic responses observed. While approximately 85% of photic SCN cells were *activated* by light in rats, nearly 75% of such cells were *suppressed* by light in degus (Fig. 1A). This observation is consistent with an earlier report of a higher proportion of light-suppressed SCN cells in the diurnal 13-lined ground squirrel than in nocturnal rodents [33].

These results suggest that the potential confounds in the previous squirrel study—the use of wild-caught animals, and the use of a different anesthetic—are probably unrelated to the observed differences in photic responsiveness of SCN neurons. The degus in this study were reared under standard laboratory lighting regimes and they were anesthetized using the same anesthetic as used in this and previous studies of nocturnal rodents. The predominance of light-suppressed over -activated SCN cells in squirrels and degus, and the opposite pattern in rats and hamsters are not obviously attributable to phylogenetic relations, since the species with similar patterns are not especially closely related. Rather, the results suggest that the types of SCN cell photic responses observed in rodents are linked to their diurnal and nocturnal activity patterns.

A limited number of tests in both species indicated that SCN neurons could be driven either binocularly or only contralaterally, but none were driven only by ipsilateral retinal input. Anatomical studies of the RHT in a variety of species have shown varying degrees of binocular input to the SCN, often with heavier contralateral projections, but there are no examples of exclusively ipsilateral projections [22,42,60]. Diurnality does not appear to be an important determinant of the degree of decussation in the RHT. In diurnal ground squirrels, projections were reported to be either entirely crossed [59] or predominantly so [33], and tracing studies in degus also indicate that there are binocular projections to the SCN in this species (unpublished observations).

Most cells were tested only with retinal illumination of 700 lx, and we investigated photic sensitivity thresholds for only a few cells. Sustained responses of some cells in degus were not evident below 2000 lx, although most cells

showed at least transient responses to 700 lx illumination. In rats, such sustained responses were often found during exposure to intensities as low as 150 lx, or even 15 lx. Thus, at least some photically responsive SCN cells appear to be more sensitive in rats than in degus. This observation is consistent with our earlier report that the threshold intensities for sustained photic responses were 0.1 lx in rats [32] and 1000 lx in a diurnal ground squirrel [33].

Although the same anesthetic was used in both species in the present study, the question remains whether response differences emerge because degus are more sensitive to urethane than rats. Through trial and error, we settled on a slightly lower initial dose of anesthetic for degus than for rats, but we also found that supplementary doses were then required somewhat sooner for degus. Thus, the differences in sensitivity with respect to anesthesia were relatively slight. It seems most likely that at least some SCN cells are considerably less sensitive to retinal illumination in degus than in rats. While earlier studies in squirrels were confounded by the use of a different anesthetic, the present results suggest the possibility that diurnal species are generally less sensitive than nocturnal species to low light intensities. This conclusion is consistent with earlier evidence that higher constant light intensities are needed to alter the periods of free-running circadian rhythms in degus [29] than in rats [3], that the phase response curves to light are different for degus and rats [29], and that low-intensity light pulses are less effective in degus than in rats at inducing c-Fos expression in SCN cells [26].

In both species, cells that were activated by light typically had lower baseline firing rates (~ 1.2 Hz) than were those that were light-suppressed (~ 3.5 Hz). Depending on their respective firing-rate ranges, this situation might not be inevitable, but it is highly likely, since light-suppressed cells would be firing at their highest rates in darkness, while light-activated cells would be firing at their lowest rates. Differences in baseline firing rates between these species, however, cannot account for the observed differences in their responses to retinal illumination. The lack of light-suppressed responses among low firing-rate cells in rats, for example, contrasts to the finding in degus that 50% of low firing-rate (< 2 Hz) photic cells were light-suppressed.

4.2. Daily rhythms in SCN cells

A well-known characteristic of SCN cells is that they exhibit circadian rhythms as assessed by a variety of methods. Thus, recordings of both multiple-unit activity and single-unit activity in rats in vivo [19,20,34,35] and in vitro [13] demonstrate higher firing rates during the subjective day than during the subjective night. This observation is also consistent with other evidence for increased metabolic activity in the SCN during the subjective day [56,57]. In this study, baseline firing rates of SCN cells in

rats were significantly higher during the day than during the night in a urethane-anesthetized in vivo preparation, but only for photically unresponsive neurons. The minority of photically responsive cells showed no significant differences in baseline firing rates during these two periods.

Thus, photically responsive SCN cells do not appear to contribute to the circadian rhythm of spontaneous firing in the rat SCN under these conditions. This observation may be related to evidence that SCN cells containing different peptides function differently with respect to peptide secretion. Thus, vasoactive intestinal polypeptide (VIP)-containing cells in the ventrolateral SCN receive direct retinal innervation [4,5] (and may be presumed to respond to retinal illumination), but they do not normally secrete this peptide rhythmically under constant conditions [18]. On the other hand, vasopressin-immunoreactive neurons, among others, are located in a dorsal SCN region that receives little if any retinal innervation [38]. Vasopressin mRNA levels are rhythmic in the SCN [61] and vasopressin is secreted rhythmically from SCN neurons under constant environmental conditions [9]. Thus, it is possible that photically unresponsive cells oscillate spontaneously in firing rate, while retinally innervated cells that respond to illumination show weak or no spontaneous firing-rate rhythms.

In degus, photically responsive ($n = 22$) and unresponsive ($n = 66$) cells had a higher median firing rate during the subjective night than during the subjective day, although these differences were not statistically reliable. Whether this difference might become significant with a larger sample is unclear, but, at a minimum, the increase in firing rates recorded in rats during the day was not observed in degus. This observation differs from a previous report in another diurnal rodent of a daytime peak of SCN multiple-unit activity recorded in freely moving animals [53]. This discrepancy may be related to the species studied. The fact that a higher daytime firing rate was observed in rats under identical recording conditions in our study suggests that the lack of rhythmicity in degu SCN cells is not attributable to the recording method or anesthetic used. Since there was actually a tendency in degus toward a difference in the opposite direction from that reported for other species, it also seems quite unlikely that a larger sample of cells would yield the expected daytime peak in firing rate in degus in vivo.

Sensitivity to light was also reported previously to vary with circadian phase in rats. Thus, more robust neurophysiological responses to light were observed during the night when baseline firing rates were lowest in in vivo preparations [19,20,35]. Cells in the SCN of urethane-anesthetized hamsters that responded to optic nerve stimulation were encountered more frequently during the night phase [8]. We found photically activated cells in the SCN of rats with equal probability during day and night, but those cells that were activated showed significantly greater increases in firing rates at night than during the day, a finding consis-

tent with results from unanesthetized rats [35]. In degus, many more light-suppressed cells were encountered during the night, and the amplitudes of their responses during the night were also significantly greater than those observed during the day. These results indicate that photically responsive SCN cells are more sensitive to retinal illumination during subjective night than during subjective day in both degus and rats. The changes in sensitivity may occur in the retina, in the SCN, or in both.

4.3. Extra-SCN neurons

As in other studies in rats, hamsters [32] and ground squirrels [33], we recorded both light-activated and light-suppressed cells outside the SCN in both degus and rats. Anatomical studies of the RHT confirm the presence of direct retinal projections to regions of the hypothalamus around the SCN, although the density of terminals is low relative to the projection reaching the SCN [30,60]. Consistent with these observations, only 7% of extra-SCN cells in degus and 15% in rats were photically responsive in this study, while 25–28% of SCN cells were responsive.

The responses of cells outside the SCN in degus were somewhat different from those of SCN cells, with 60% of 10 photically responsive hypothalamic cells being activated by light, as compared to 27% of SCN cells. Photically responsive hypothalamic cells in rats also differed from those in the SCN in that only 65% (of 20) were activated, while this value was 85% inside the SCN. Since the number of responsive hypothalamic cells is relatively small, it is unclear how reliable these differences are. Even if one were to assume that these hypothalamic cells are functionally similar to SCN cells, and therefore lumped all cells together, there would still be a significant difference between these species. Of all photically responsive cells recorded in the SCN and surrounding hypothalamus, 37.5% were activated in degus, while 76.6% were activated in rats.

4.4. Degus and rats

The major finding in this study is that most photically responsive cells recorded in and around the SCN of degus respond to retinal illumination with suppression of firing rates, while the opposite response is typical in rats. If one considers only cells within the SCN, this difference is even more striking. The findings in degus are similar to what was reported previously for diurnal ground squirrels (*S. tridecemlineatus*) [33]. Because other possible confounds were ruled out in this study, it is most parsimonious to attribute these differences to the fact that 13-lined ground squirrels and degus are diurnal while rats and hamsters are nocturnal.

Other studies have also suggested significant differences in both the anatomical and functional organization of the circadian systems of diurnal and nocturnal mammals.

Smale et al. [59] reported immunocytochemical differences between the SCN of a diurnal ground squirrel (*S. lateralis*) and that of nocturnal rodents. The squirrel SCN contains a dense cluster of leu-enkephalin-immunoreactive cells, while substance P cells are absent, in contrast to rats and hamsters [36]. Somatostatin-immunoreactive perikarya which are found in the rat and hamster SCN were also reported to be absent in another ground squirrel (*S. richardsonii*) [44]. Similarly, the SCN of two diurnal primates, squirrel monkeys and humans, show immunoreactive patterns not observed in nocturnal rodents [40].

Both patterns of phase-shifting and of immediate-early gene expression in response to light pulses have been reported to differ in diurnal and nocturnal rodents [1,26,51]. Degus also show features in their circadian systems that differ from those of nocturnal rodents, including different phase response curves to light stimuli [27,29].

While these differences may be related to diurnality and nocturnality, some caution is required. Degus are primarily diurnal when studied in cages without running wheels and have been reported to be diurnal in the wild, but their activity and sleep rhythms can also show strong crepuscular modulation [11,21,24,48]. In one study, access to a running wheel generated an activity pattern that looked more strongly nocturnal, as the dusk activity bout was extended into the night [24]. Thus, degus may be generally diurnal or crepuscular but show more flexibility in their activity patterns than highly domesticated nocturnal rodents. Such flexibility in circadian organization under different environmental conditions may be characteristic of many wild species [23,50].

Our findings indicate that extrapolation of neurophysiological results from the SCN of nocturnal rodents to other species in attempting to model how light influences circadian function may not be appropriate. These data also raise questions about the mechanisms mediating the effects of retinal illumination on SCN cells in degus, and how these may differ from the mechanisms that have been studied extensively in rats. It will be important to establish, for example, whether light-suppressed cells are driven directly or indirectly by retinal ganglion cells. Finally, these findings focus attention on the relatively small population of photically suppressed SCN cells in rats and hamsters. While these cells have received little attention, probably on the assumption that their responses are transsynaptic, the fact that they respond as do the majority of photically responsive SCN cells in degus suggests that the mechanism of their regulation deserves further investigation.

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