Rhythmic multiunit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod

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Mrugala Maciej, Piotr Zlomanczuk, Anita Jagota, and William J. Schwartz. Rhythmic multiunit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod. Am J Physiol Regulatory Integrative Comp Physiol 278: R987–R994, 2000.—The suprachiasmatic nucleus (SCN) is an endogenous circadian pacemaker, and SCN neurons exhibit circadian rhythms of electrophysiological activity in vitro. In vivo, the functional state of the pacemaker depends on changes in day length (photoperiod), but it is not known if this property persists in SCN tissue isolated in vitro. To address this issue, we prepared brain slices from hamsters previously entrained to light-dark (LD) cycles of different photoperiods and analyzed rhythms of SCN multiunit neuronal activity using single electrodes. Rhythms in SCN slices from hamsters entrained to 8:16-, 12:12-, and 14:10-h LD cycles were characterized by peak discharge rates relatively higher during subjective day than subjective night. The mean duration of high neuronal activity was photoperiod dependent, compressed in slices from the short (8:16 and 12:12 LD) photoperiods, and decompressed (approximately doubled) in slices from the long (14:10 LD) photoperiod. In slices from all photoperiods, the mean phase of onset of high neuronal activity appeared to be anchored to subjective dawn. Our results show that the electrophysiological activity of the SCN pacemaker depends on day length, extending previous in vivo data, and demonstrate that this capacity is sustained in vitro.

STRONG EVIDENCE FROM a variety of experimental approaches indicates that the suprachiasmatic nucleus (SCN) of the hypothalamus is the site of an endogenous pacemaker that regulates circadian rhythmicity in mammals (16). Many of the behavioral, physiological, and hormonal rhythms governed by the SCN are also sensitive to seasonal changes in day length (photoperiod). For example, the rhythms of pineal N-acetyltransferase activity (responsible for the nighttime synthesis of melatonin) and of wheel-running activity (nighttime locomotion) are both photoperiodic in rodents. The durations of nocturnal melatonin production and locomotor activity are compressed during long summer days and are decompressed during short winter days (5, 12). For both rhythms, decomposition occurs gradually (over weeks) after animals are transferred from a long to a short photoperiod. These parallel changes in the waveforms of two SCN-dependent rhythms suggest that the functional state of the SCN pacemaker also depends on the photoperiod.

Support for this view has been provided by recent studies that demonstrate a photoperiodic change in the circadian rhythm of light responsiveness in the SCN of intact rats and hamsters; the duration of the photosensitive subjective night (as measured by SCN c-fos gene expression) is compressed in animals entrained to long photoperiods and is decompressed in short photoperiods (33, 34, 37). This phenomenon persists in pinealectomized animals (15, 32), but it remains unknown whether other brain regions are required for the SCN to manifest this photoperiodic property.

To address this issue, we have exploited the fact that the SCN exhibits circadian rhythms of electrophysiological activity as a brain slice in vitro. Several laboratories have reported long-term (>24 h) multiple-unit recordings using single electrodes in SCN slices from rats (2, 8, 21, 24, 35) and mice (17). SCN discharge frequency is relatively high during the subjective day [the interval when the lights would have been on during the preceding light-dark (LD) cycle], peaks near midday, and is relatively low during the subjective night (the interval when the lights would have been off). In this paper, we have analyzed rhythms of SCN multiunit neuronal activity in slices from hamsters previously entrained to LD cycles of different photoperiods. Our results show that photoperiodic changes are reflected in the electrophysiological activity of the SCN in this in vitro preparation.

METHODS

Animals. Male golden hamsters (Charles River, Kingston, NY), 21 days old at time of delivery, were housed individually in clear polycarbonate cages contained within well-ventilated, light-proof environmental cabinets isolated in an animal facility with temperature thermostatically controlled. Food and water were freely available and were replenished at irregular hours. Light was provided by 15-W cool-white fluorescent tubes mounted above the shelves and was automatically controlled by a 24-h timer; no light was present during darkness. When necessary, a single 15-W safe light with a dark red (Kodak Series 2) filter was used to allow for routine care; animals were exposed to ~30 lx maximally and <1 lx usually.
Hamsters were entrained to one of three different photoperiods: LD 8:16 for ~35 days, LD 14:10 for at least 14 days, or LD 12:12 for at least 14 days. In general, lights off was at 1500 (laboratory time), although some animals were killed after entrainment to a reversed LD 12:12 cycle (lights off at 0300). The order of death was randomized by photoperiod, and the age at death ranged between 35 and 65 days old.

Multiunit recording method. Two hours before lights off, animals were killed by decapitation (single cut by sharp blade), as approved by the University of Massachusetts Institutional Animal Care and Use Committee and the American Veterinary Medical Association, and the brains were sectioned coronally on a vibratome. The optic chiasm, SCN, and adjacent tissue were all contained in a single slice (400–500 µm), placed on a plastic netting in a chamber with a gas-liquid interface (Fine Science Tools, Foster City, CA), and superfused with Earle’s balanced salt solution (catalog no. 81100–026; Gibco-BRL) supplemented with sodium bicarbonate (26.2 mM), glucose (20 mM), penicillin (500 U/l), and streptomycin (0.5 mg/l) after membrane (0.2 µm) filtration. Flow rate was maintained at 60–80 ml/h, and the temperature was thermostatically controlled (Fine Science Tools) at 33°C (±1°C). An outer chamber provided a water bath to warm and humidify a 95% O2-5% CO2 gas mixture before it passed over the slice.

After slice equilibration for 2–4 h, a single metal electrode (90% platinum-10% iridium; resistance = 0.5 ± 0.01 MΩ; tip diameter 70–80 µm; World Precision Instruments, Sarasota, FL) was lowered 150–200 µm in the ventral SCN by a micromanipulator under direct visual guidance using an operating microscope. Multiunit discharge activity was filtered, amplified (Grass P15 AC preamplifier), and displayed on an oscilloscope (Tektronix TAS 465) to confirm that the events counted throughout the experiment represented actual spike activity (Fig. 1). The spikes were discriminated (121 Window Discriminator; World Precision Instruments) and counted by computer above a threshold (signal-to-noise ratio ~2:1) for 10 min every 30 min (48 bins/24 h) using Spike 2 software (Cambridge Electronics Design, Cambridge, UK). No attempts were made to readjust the discrimination threshold or reposition the electrode during the recording session.

Multiunit data analysis. Clear rhythms of multiunit neuronal activity were observed in slices recorded successfully for at least 24 h (Fig. 2 and results), as described previously (2, 8, 17, 21, 24, 35). Similar to these prior reports, activity was generally low during subjective night and high during subjective day, with peak levels about four to five times greater than baseline night values. Discharge rates varied between slices but usually ranged from 20 to 50 counts/s during the night to 100–200 counts/s during the day.

To compare the rhythms between slices and to assign phases of high activity onset and offset, discharge rates were first normalized for each slice (2) by dividing each half-hourly count by the peak half-hourly count for that slice over the 24-h recording interval. Normalized multiunit activity was plotted as a percent of maximum with respect to circadian time (CT, where CT 12 for all photoperiods was set as the phase that corresponded to lights off during previous entrainment; Fig. 2). For each slice, the onset of high activity was defined as that CT at which normalized activity exceeded a threshold level two SD above the baseline mean activity and remained at or above that level for at least 1 h; the offset of high activity was analogously defined as that CT at which normalized activity fell below the threshold level and remained at or below that level for at least 1 h. Baseline mean activity was calculated for each slice by averaging 10 consecutive nighttime counts (from CT 17 to CT 22 in all cases except one LD 14:10 slice, for which an earlier CT interval was used to include this slice with its unambiguous rhythm but an atypically early phase of high activity onset). A total of three slices had such highly variable baseline activities that threshold levels were too high to define an onset of high activity.

Other workers have noted that stable, long-term recordings of multiunit neuronal activity are difficult to achieve in vitro; we also encountered technical difficulties related to the stability of temperature, gas flow, fluid levels, and the tension of the plastic netting for slice support. The most common problem was an inability to maintain the recording for a full 24 h. This failure was most often due to gross slice movement and electrode displacement from the tissue (as confirmed through the microscope), with resulting loss of signal, i.e., no spikes were seen on the oscilloscope trace. Some slices
exhibited low activity levels (characteristic of subjective night) that continued flat over the entire recording session. Inspection of these recordings on the oscilloscope confirmed that spike activity persisted at a low frequency. In these slices, there was no relationship to the previous photoperiod, animal age, or the length of time between animal death and slice superfusion. The incidence of such arrhythmicity has declined, as we have gained experience with this preparation, and currently occurs in 1 out of 10 slices.

RESULTS

SCN multiunit neuronal activity rhythms appeared to be qualitatively different in slices from hamsters entrained to either short (LD 8:16) or long (LD 14:10) photoperiods. Examples of such rhythms in two individual slices from LD 8:16 are shown in Fig. 3, A and B, and the mean rhythm for a population of slices (n = 5 slices from 5 animals) is shown in Fig. 3C. In every slice, discharge rate at the onset and offset of high activity rose and fell abruptly over 1–2 h. Because the phase dispersion between the slices was small, the population rhythm resembled the rhythms in individual slices. As shown in Fig. 3C, mean neuronal activity began to increase within a few hours after subjective dawn (= CT 4, given previous entrainment to LD 8:16), rose to reach a mean peak ~6 h after dawn (CT 10), and then decreased to return to baseline within a few hours after subjective dusk (CT 12).

Examples of SCN multiunit neuronal activity rhythms in two individual slices from LD 14:10 are shown in Fig. 4, A and B, and the mean rhythm for a population of slices (n = 5 slices from 5 animals) is shown in Fig. 4C. The rhythms in LD 14:10 slices were more variable than those in LD 8:16, with prolonged levels of high neuronal activity that in some cases exhibited notched peaks. There was considerable inter-slice variability in the phases of these peaks, resulting in a population rhythm in which mean neuronal activity began to increase within a few hours after subjective dawn (= CT 22, given previous entrainment to LD 14:10), remained relatively high over the course of the entire subjective day, and then decreased to return to baseline around subjective dusk (CT 12).

To compare these data with previous reports using the multiunit method, we also recorded SCN multiunit neuronal activity rhythms in slices from hamsters entrained to LD 12:12. Examples of such rhythms in two individual slices are shown in Fig. 5, A and B, and the mean rhythm for a population of slices (n = 6 slices...
from 6 animals) is shown in Fig. 5C. The rhythms in individual LD 12:12 slices appeared to be indistinguishable from those in LD 8:16 slices. However, the phases of high activity onset and offset were variable between slices, resulting in a population rhythm that did not closely resemble the rhythm in any one individual slice.

Mean neuronal activity began to increase within a few hours after subjective dawn (= CT 0, given previous entrainment to LD 12:12), gradually rose during the early subjective day to reach a mean peak ~6 h after dawn (near midday, CT 6), and then gradually decreased during the late subjective day to return to baseline within a few hours before subjective dusk (CT 12).

Data from all three photoperiods are presented as circular distributions in Fig. 6. The mean duration of
high neuronal activity, computed from the durations of activity in individual slices, was $5.7 \pm 1.2$ h (mean ± SE) in LD 8:16 and $6.4 \pm 1.5$ h in LD 12:12 slices but doubled to $12.5 \pm 1.7$ h in LD 14:10 slices (Fig. 6A). SE values were similar among slices between the three groups, despite variability in their phases of high activity onset and offset (Fig. 6B). This indicates that individual slices with early or late phases of high neuronal activity onset also had early or late phases of high activity offset, respectively. The mean durations of high activity were significantly different between the three groups ($P = 0.016$, one-way ANOVA), and pairwise comparisons using Bonferroni t-statistics showed that the LD 14:10 mean duration was significantly greater than both the LD 8:16 ($P < 0.05$) and LD 12:12 ($P < 0.05$) mean values. For all photoperiods, the mean phase of high activity onset occurred soon after subjective dawn: 3.6 h in LD 8:16 slices, 2.4 h in LD 12:12 slices, and 1.2 h in LD 14:10 slices (Fig. 6B). On the other hand, the mean phase of high activity offset expressed an inconstant relationship to subjective dusk, following it by 1.3 h in LD 8:16 slices, preceding it by 2.6 h in LD 12:12 slices, and variably distributed around it in LD 14:10 slices.

DISCUSSION

Photoperiodism in vitro. The SCN functions as a seasonal as well as a circadian clock, and our results in vitro show that its rhythm of multiunit neuronal activity reflects prior day length. We found that the mean duration of high neuronal activity in SCN slices depends significantly on the preceding photoperiod: mean activity duration is compressed in slices from hamsters entrained to the short (LD 8:16) photoperiod and decompressed in those from the long (LD 14:10) photoperiod. On the other hand, mean durations are no different in slices from the LD 8:16 and LD 12:12 photoperiods, a result consistent with in vivo behavioral and physiological data showing that hamsters respond to LD 12:12 as a short photoperiod (see below). These in vitro electrophysiological results complement in vivo experiments that show a reciprocal photoperiodic change in the circadian rhythm of light responsiveness in the SCN (34, 37). Other properties of the multiunit neuronal activity rhythms in LD 8:16 and LD 14:10 slices correspond to additional in vivo data. When photoperiod lengthens in vivo, the duration of the SCN’s subjective night contracts asymmetrically, mostly by receding in the morning (34); in vitro, the mean duration of high neuronal activity expands asymmetrically by extending earlier into the morning. Also, in vivo, the phase of onset of nocturnal wheel running is advanced as the photoperiod lengthens (4, 6, 22, 26); similarly, in vitro, the mean phase of high activity offset occurs earlier in the LD 14:10 (and LD 12:12) than in the LD 8:16 slices, although the significance of this finding is diminished by the great variability among the LD 14:10 slices.

An interesting feature of our multiunit data is the relative stability of the relationship between the mean phase of onset of high neuronal activity and subjective dawn. Mean onset appears to be anchored to subjective dawn in different photoperiods (by our criteria, onset occurs between 1 and 4 h after dawn). This observed regularity between onset and dawn (but not laboratory clock time) provides a reassuring control that our multiunit neuronal activity rhythms are not due to some unknown, recurring laboratory artifact. Of note, independent experiments in vivo have already sug-
gested that lights on at dawn represent a critical phase point in the circadian clock's oscillatory mechanism. For example, the rhythm of pineal N-acetyltransferase activity is entrained to the LD cycle primarily by light at dawn (14). Also, gene expression in the rodent SCN (e.g., c-fos) is induced by light at dawn, regardless of the species' free-running period or whether entrainment to the LD cycle occurs primarily by phase advances or phase delays (29).

Recording SCN rhythmicity using the multiunit method. Several laboratories have established the feasibility of recording rhythms of multiunit neuronal activity from the SCN in brain slices (2, 8, 17, 21, 24, 35), but none of these studies exposed the animals to altered lighting cycles before death. Our results help to confirm the method's validity and physiological relevance by demonstrating that multiunit rhythms accurately reflect antemortem environmental conditions.

Circadian rhythms of SCN electrophysiological activity were first shown in vitro using extracellular recordings of spontaneous single-unit discharge rates from the SCN in rat brain slices (7, 9, 31). Qualitatively similar rhythms have been reported from the SCN in hamster (18, 19, 30) and mouse (17) slices. In general, these studies have been performed by sampling the spike activity of single units for short time intervals at different phases across the circadian cycle, pooling the data from a number of slices and phases, and then determining the phase of peak firing rate for the population of units as a whole. Workers using this method usually record from individual cells for a few minutes in slices kept for several hours, although there are reports of single neurons held for 14 h (9), recording sessions in individual slices lasting over 30 h (28), and single slices maintained for 3 days (25). The single-unit method is labor intensive and requires repeated electrode penetrations of the tissue. The automated multiunit method resolves these problems and removes any unintended observer bias that might occur when units are manually selected for recording. As shown by others and now by ourselves, long-term multiunit recording is a powerful approach once the technical challenges of the method are overcome.

The mean rhythm of SCN multiunit neuronal activity for the population of LD 12:12 slices closely resembles the published rhythms using the single-unit method. With either method, neuronal activity gradually rises after subjective dawn, peaks near midday, and gradually declines until subjective dusk. Importantly, the mean durations of high neuronal activity in circadian hours for the populations of slices. The mean durations of high activity were significantly different between the 3 groups (P = 0.016, one-way ANOVA), and pairwise comparisons using Bonferroni t-statistics showed that the LD 14:10 mean duration was significantly greater than both the LD 8:16 (P < 0.05) and LD 12:12 (P < 0.05) mean values (n = 5 slices in each group, except for LD 12:12 in which 6 slices were used). B: Circular distributions of rhythm onsets and offsets in slices from the 3 photoperiods. Bold portions of each scale represent subjective night (the dark phase of the previous entrainment schedule) in circadian hours. Shown are rhythm onsets (○) and offsets (●) for individual slices, with mean onsets and offsets (± SE) in CT.
tantly, our multiunit recordings reveal that the shape of this mean neuronal activity rhythm is a consequence of the variability of rhythms in individual slices, each of which differs considerably in activity onset and offset. Such interslice variability is not peculiar to hamsters, because Prosser (24) noted a similar phenomenon in her multiunit studies using rat slices. Prosser attributed the variability to gradual changes in the slice-electrode interface, resulting in tissue movement at the electrode tip. However, because our between-slice variability was substantially lessened by entraining the animals to LD 8:16, we doubt that technical, equipment, or procedural factors account for the variable rhythms in LD 12:12 slices. At present, we do not have a satisfactory biological explanation for the dependence of slice-to-slice variability on the changing photoperiod.

Implications for understanding photoperiodic timing mechanisms. Although our data show that the mean duration of high neuronal activity in SCN slices expands and contracts in relation to the preceding photoperiod, the data also show that these durations do not change in direct proportion to the changes in day length. Lengthening the light phase by 4 h from LD 8:16 to LD 12:12 causes no change in mean activity duration, whereas further lengthening by 2 h from LD 12:12 to LD 14:10 doubles the duration. This kind of step function does not appear to be a feature of the rhythm of nocturnal locomotor activity (5, 23), but it does resemble the response of the hamster reproductive system to changing photoperiods, in which a critical (minimum) day length of 12.5 h is required to maintain spermatogenesis and testes weight (4). Although study of additional photoperiods will be needed to make a persuasive case for the presence of such a photoperiodic “switch” in circadian pacemaker activity (5, 23), the presence of a photoperiodic response of the SCN to short- and long-day photoperiods would, if confirmed, provide further evidence for a role of the SCN in photoperiodic timing.

REFERENCES

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