Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats

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Scheer, F. A. J. L., G. J. Ter Horst, J. van der Vliet, and R. M. Buijs. Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats. Am J Physiol Heart Circ Physiol 280: H1391–H1399, 2001.—The suprachiasmatic nucleus (SCN) is the mammalian biological clock that generates the daily rhythms in physiology and behavior. Light can phase shift the rhythm of the SCN but can also acutely affect SCN activity and output, e.g., output to the pineal. Recently, multisynaptic SCN connections to other organs were also demonstrated. Moreover, they were shown to affect those organs functionally. The aim of the present study was to investigate the role of the SCN in the regulation of the heart. First, we demonstrated that heart rate (HR) in SCN-intact, but not SCN-lesioned (SCNx), male Wistar rats had a clear circadian rhythm, which was not caused by locomotor activity. Second, we demonstrated that light at night reduces HR in intact but not in SCNx rats. Finally, we demonstrated the presence of a multisynaptic autonomic connection from SCN neurons to the heart with the retrograde pseudorabies virus tracing technique. Together, these results demonstrate that the SCN affects the heart in rats and suggest that this is mediated by a neuronal mechanism.

autonomic nervous system; circadian rhythm; heart rate; masking; pseudorabies virus tracing

The suprachiasmatic nucleus (SCN), the mammalian circadian pacemaker, is strategically located in the basal hypothalamus and on top of the optic chiasm. The SCN has an endogenous circadian rhythm in neuronal activity (high during the day and low at night) both in vivo and in vitro (18, 27, 29). It is responsible for circadian rhythms in physiology and behavior (30). Light is the most important entrainment factor for the SCN, synchronizing the endogenous circadian rhythm of SCN activity and SCN output to the exogenous light-dark (LD) rhythm (23). Next to these phase-shifting effects, light also has acute effects on SCN neuronal activity (17, 18) and SCN output. Light information entering the SCN can suppress both melatonin and corticosterone, an effect that is mediated by projections from the SCN, via the autonomic nervous system to the pineal and adrenal cortex, respectively (4, 10, 13, 33). It has been suggested that the SCN modulates autonomic output to most organs of the body (2, 4). In the present study, we investigated in rats if, and how, the SCN affects the functioning of the heart.

Ambulatory heart rate (HR) monitoring shows a daily rhythm. This daily rhythm in ambulatory HR is strongly influenced by the daily rhythm in locomotor activity (LA). In humans, the direct effect of the circadian pacemaker on HR can be studied by using constant routine protocols, which demonstrates an endogenous diurnal rhythm in HR (12, 14, 26). As it is not possible to study rats during constant voluntary inactivity, indirect computational methods have generally been used to distinguish the daily rhythm in HR from the effects of activity. These computational methods indicate a day-night rhythm in HR independent of the rhythm in activity (15, 19). In the present study, we used a more direct method to determine the day-night rhythm in HR not influenced by the rhythm in LA. To be able to do this, we selected, beforehand, only those time frames at which LA had been low long enough (during periods of temporary voluntary inactivity) for HR not to be affected by LA. With the use of these selection criteria, we compared the daily rhythm in this resting HR of SCN-intact rats with that of SCN-lesioned (SCNx) rats. We hypothesized that a functional SCN generates a daily rhythm in resting HR, resulting in high resting levels during the (subjective) night and low resting levels during the (subjective) day.

As a second method to investigate the role of the SCN in HR regulation, we investigated the acute effects on HR and LA of light exposure. Because, in nocturnal rats, the high SCN neuronal activity during the (subjective) day is associated with reduced HR levels and because light during the (subjective) night stimulates SCN neuronal activity (17, 18), we hypothesized that light would reduce HR in rats. In the present study, we used exposure to light during the night in SCN-intact and SCNx animals to study SCN-dependent effects of light on HR.

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Finally, we investigated whether there is a neural connection from the SCN to the heart that would enable these day-night- and light-induced modulations of HR by the SCN. For this purpose, we employed injections of pseudorabies virus (PRV) into the heart muscle. PRV is selectively transported across multiple neuronal synapses, thus enabling the delineation of multisynaptic pathways, which may form the anatomic basis for the physiological effects of the SCN on the heart.

METHODS

Animals. For the first group of animals, 31 male Wistar rats (Harlan; Zeist, The Netherlands) were used. During the whole experiment, the rats were housed under a 12:12-h LD cycle (±100 and 5 lux, respectively; 7:00 AM lights on) (unless mentioned otherwise) in a temperature-controlled environment with ad libitum access to tap water and food (rat chow). Twenty-five rats were stereotactically operated to lesion the SCN at the age of 2 mo (see Surgery). At the age of 4 mo, six stereotactically operated rats that were arrhythmic for drinking behavior (see Surgery) and six intact rats received a telemetric HR- and core temperature-measuring device (see Surgery). After the implantation, rats were housed in solitude in sound- and light-isolated circadian cages (39 × 38 × 38 cm) in which all measurements were done. In these circadian cages, rats had access to a running wheel and were handled twice weekly. After surgery, animals recovered for at least 1 wk before HR data were used for analysis. A second and third group of rats were studied under the same experimental conditions except for the light regime. In the second group of 10 rats, we studied whether the rhythm in resting HR was endogenous in origin, thus still present in the absence of a light-dark cycle. This was done by comparing measurements during a 3-day period of LD with those during a 3-day period of constant darkness (DD). In the third group of three rats, the ability of the resting HR to free run was tested in DD (0 lux) and constant light (dim-LL; 5 lux) conditions. For the tracing study, adult male Wistar rats were housed in a temperature-controlled environment with ad libitum access to tap water and food. All experiments were conducted under approval of the Local Animal Care Committee.

Surgery. SCN lesioning was carried out under Hypnorm anesthesia (0.8 ml/kg im) at the age of 2 mo. Animals were mounted in a David Kopf stereotactic frame (tooth bar +5), and two electrode tips (0.2 mm diameter) were placed bilaterally in the SCN (Pellegrino). At each side, the tip temperature was maintained at 80°C for 1 min (lesion generator, Radiotronics).

To avoid the inclusion of too many animals with incomplete lesions in the experiment, an initial screening of SCN function was conducted. For this purpose, the absence of the day-night rhythm in drinking was determined over a period of 2 wk under LD conditions (3). This screening was started only 1 mo after surgery. If the water intake during 8 daytime hours was more than 33% of that during 24 h, the rat was considered arrhythmic in its drinking behavior and participated in the experiment. We used two further criteria to test for the completeness of the SCN lesion. In the first test, the absence of a day-night rhythm in LA, body temperature, and HR was tested in the circadian cage under LD conditions. If an animal did not show complete arrhythmicity of LA, body temperature, and HR for 2 wk, the SCN lesion was considered not functionally complete, and the data of that animal were excluded from analysis. Finally, in the second test, as a standard procedure of our group (3, 9), the anatomic completeness of the SCN lesion was checked in immersion-fixed brains using coronal vibratome sections of 50 μm (Fig. 1). The sections were stained for vasoactive intestinal peptide (VIP) and vasopressin (VP). If VIP-positive cells or fibers within the SCN region or VP-positive cells within the SCN were present, such an animal would be excluded from analysis. Only one animal was excluded from analysis on the basis of both of these criteria.

Transmitters (CTA-F40, DataScience; St. Paul, MN) were implanted in rats at the age of 4 mo according to the Data Sciences International manual (DataScience). In short, the rats were anesthetized (0.8 ml/kg im Hypnorm and 0.4 ml/kg sc Dormicium), the transmitter was secured to the inner muscle wall of the abdomen, and the two electrodes were guided subcutaneously and secured to the muscles (one rostral and one caudal to the heart). After the surgery, the rats recovered on a heating pad at 37°C and were rehydrated with 4 ml sc sterile saline. Postoperative pain was reduced with buprenorphine (1 mg/kg sc). PRV inoculation of the myocardium was performed as published before (34). In summary, the rats were inoculated in the left (n = 20) or right (n = 10) ventricular myocardium or in the right atrium (n = 10) with 2 μl of PRV solution (3 × 10⁶ pfu/ml). As a control, animals received 30 μl of the PRV solution either through a jugular vein catheter (n = 6) or directly onto the left ventricle (n = 6). The latter experiments were done to check whether unintentional spillage of virus into the bloodstream can give rise to central nervous system infections. PRV Bartha was kindly donated by Dr. T. C. Mettenleiter (Federal Research Center for Virus Diseases of Animals, Tubingen, Germany). Evan’s blue (0.2%) was added to the culture medium to allow histological verification of the injection sites.

Data acquisition and analysis. HR and core temperature data were sent by the implanted transmitter in the freely moving rat and received by a telemetry antenna (model RA1010, DataScience) that was positioned underneath the cage. The data were combined with locomotor activity data, which was detected by two infrared detectors in the circadian cage, and analyzed with the Data Quest software package (LabPro, DataScience). With the use of this software, a 10-s average of HR and core body temperature data was sampled every 4 min at a sampling frequency of 500 Hz. The LA was

Fig. 1. Coronal vibratome section of a lesion of the suprachiasmatic nucleus (SCN), illustrating how the absence of vasopressin-positive neurons was established and confirming that the lesion was complete.
stored every 4 min as the number of movements sampled continuously over that period at a frequency of ±0.8 Hz. To study the presence of a day-night rhythm in HR independent of the rhythm in LA, the “resting HR” was determined. Resting HR was defined as the mean HR determined over a 20-min period of “rest” at least 32 min after the start of rest, with rest defined as no LA level above 5. We tested whether resting HR was indeed not influenced by LA (Fig. 2) in two ways. First, we tested whether 32 min of rest was sufficient for HR to reach a stable resting level. We tested this by comparing the HR of the first, second, and third 20-min “resting period” after 32 min of rest with each other, using a one-way analysis of variance (ANOVA) for repeated measures. During the daytime, all six intact rats had sufficiently long resting periods (at least 92 min), but during the nighttime, only three rats had resting periods of sufficient duration and were used for this analysis. Second, we tested whether the resting HR was the same as HR determined in a similar way but now without any LA detection during selected 20-min periods (tested with t-test for dependent samples). This test was done for the day periods because 20-min periods without LA detection were almost absent during the night periods.

The day-night difference in resting HR under LD was tested in the first group over 6 days with a t-test for dependent samples. To determine whether a day-night rhythm in resting HR was also present in the absence of a LD cycle, this rhythm was compared between 3 days in LD and 3 days in DD in the second group. A two-way ANOVA was used to determine the influence of the circadian phase [(subjective) day or night] of the light regime (LD or DD) and the interaction between both. To test the ability of the (subjective) day-night rhythm in resting HR to free run, we determined in the third group of rats the circadian rhythm in resting HR during 10 days of free running in DD and during 10 days of free running in dim-LL. A two-way ANOVA was used to determine the influence of the circadian phase of the light regime (DD or dim-LL) and the interaction between both. If significant, an ANOVA was followed by Duncan’s post hoc test.

To study the effect of light on HR and LA, rats of the first group were exposed to a 1-h light period of day-light intensity starting 2 h after the beginning of the night period (lights off). Any changes over time in the levels of HR and LA during the hours before, during, and after light exposure were tested with a one-way ANOVA for repeated measures. Also, the levels of HR and LA in the hour before, during, and after light exposure were compared with the levels during the same circadian phase on the 6 control nights and tested with a two-way ANOVA with factor time and day(s) as repeated measures. If significance was reached for an ANOVA, Duncan’s post hoc test was used.

For the tracing study, the staining procedure was as published before (35). In short, 4 days after infection with PRV, the rats were perfused with 4% paraformaldehyde solution. The heart, brain, and spinal cord were removed, postfixed for 2 days in the same fixative, cryoprotected overnight with 30% sucrose, and cut on a cryostat microtome. Serial 40-μm coronal sections of the brain and horizontal sections of the spinal cord were collected in 0.02 M potassium phosphate-buffered saline (KPBS; pH 7.4) with azide and stored at 4°C until further immunocytochemical processing. The presence of PRV was revealed by subsequent incubation in solutions of rabbit anti-PRV (1:2,500), goat anti-rabbit IgG (1:800; Sigma), and rabbit peroxidase-antiperoxidase (1:800; Dakopatts) in KPBS containing 0.3% Triton X-100. The rabbit anti-PRV was kindly donated by Dr. J. Pol (Institute for Animal Science and Health, Lelystad, The Netherlands). Peroxidase activity was revealed with a solution consisting of 0.05% 3,3-diaminobenzidine (Sigma), 2.5% nickel ammonium sulfate, 0.04% ammonium chloride, and 0.005% H₂O₂. For the analysis, only cases with selective infection were included. We used two arguments for nonselective infection. First, those cases were excluded that showed PRV-positive cells in the nucleus ruber and in the motoneuron pools of the thoracic and cervical segments of the spinal cord (8, 35). Second, those cases were discarded in which infected cells were identified in other parts of the paraventricular nucleus (PVN) of the hypothalamus than the dorsal cap region and the ventrolateral parts of PVN, areas known to project to preganglionic autonomic centers of the brain stem and spinal cord (5, 7, 21, 31, 33, 37). The distribution of the PRV-labeled cells in the hypothalamus, brain stem, and spinal cord was studied with bright-field light microscopy.

![Fig. 2. Difference in heart rate (HR) and locomotor activity (LA) between day and night during and after a period with LA > 5. The first (left) dotted line divides activity (LA > 5; left) and rest (LA < 5; right). To the right of the second dotted line is the data as used for the analysis of a day-night rhythm in resting HR; for this only sampled HR were used at least 32 min after the last LA > 5. Both during the day and at night, the high level of HR during activity decreased during the 32 min of rest to reach a stable level thereafter. To the right of this (R), the resting level of HR and LA are compared with the levels during periods without any LA, both during the day. This comparison shows that the residual LA (≤5) during the resting periods did not influence HR. Values are means ± SE, bpm, Beats/min; a.u., arbitrary units.](http://alkaline.org/supraciasmatic-nucleus-affects-the-heart-in-rats)
RESULTS

Day-night rhythm in resting HR. The validity of the employed selection criteria for the determination of resting HR, free of the influence of LA, was demonstrated in two ways (Fig. 2): 1) by showing that HR during the first, second, and third 20-min resting period after 32 min of rest were similar both during the day ($P = 0.5, n = 6$) and, in those rats that had the required duration of resting LA, also at night ($P = 0.1, n = 3$); and 2) by showing that resting HR was similar to HR determined over 20-min periods without any LA ($P = 0.3, n = 6$). With the use of these selection criteria, we studied the day-night rhythm of HR without the influence of LA in intact and SCNx animals.

The intact rats of the first group had a significantly higher resting HR during the night than during the day ($P = 0.001, n = 5$) (Fig. 3). This day-night difference was determined in five of six intact rats because one rat did not have any resting periods of sufficient duration (minimum: 52 min) during the six night periods. There was no day-night difference in resting LA ($P = 0.92, n = 5$). The mean resting HR was 293 ± 20 beats/min during the day and 317 ± 18 beats/min during the night. Of the second group, only rats that had resting periods during both the (subjective) day and night periods in the LD and DD cycle (8 of 10 rats) were used for analysis. These rats had a clear (subjective) day-night difference in resting HR under either LD or DD ($P < 0.001$). There was no effect of light regime (LD or DD), and there was no interaction effect. There was a (subjective) day-night difference in resting HR for both the LD (28 beats/min, $P < 0.001$) and DD (18 beats/min, $P = 0.002$) condition in the same eight rats. This was similar under LD and DD conditions ($P = 0.070$). For the three rats measured under free running conditions (third group), the circadian time constant ($\tau$) was $24.42 \pm 0.15$ h during DD and $24.92 \pm 0.09$ h during dim-LL. Under these conditions, there was a significant circadian rhythm in resting HR ($P = 0.024$), but light regime (DD or dim-LL) and interaction did not have an effect (Fig. 4). During both the 10-day free run under DD conditions and the 10-day free run under dim-LL conditions, there was a significant subjective day-night difference in resting HR: 30 beats/min during the DD condition ($P = 0.017$) and 25 beats/min during the dim-LL condition ($P = 0.024$).

In the five SCNx rats under LD (first group), there were no differences in resting HR between night and day ($P = 0.4; n = 5$) (Fig. 3). In SCNx animals, the resting HR was $305 \pm 25$ beats/min during the day and $305 \pm 22$ beats/min during the night. These levels of resting HR were equal to the median resting HR in the intact rats of the same group.

Nocturnal light exposure. In intact rats, comparing the same 1 h of the circadian cycle, light reduced HR from $345 \pm 3$ beats/min during the control nights to
320 ± 14 beats/min during the 1-h nocturnal light exposure (Fig. 5, A and C). There was a significant change in HR during the period of exposure to light ($P < 0.001$). Also, there were significant light ($P = 0.002$), time ($P = 0.001$), and light/time ($P < 0.001$) effects of the light compared with the control days. HR was significantly different from 20 min after lights on until the end of light exposure, with the strongest
reduction to 294 ± 21 beats/min at 32 min after lights on. There were no effects of day, time, or day/time in the hour before or after the nocturnal light exposure when comparing the day of light exposure with the control days. Light also suppressed LA. LA was reduced from 14.7 ± 0.9 during the control nights to 6.5 ± 4.3 during the 1-h nocturnal light exposure when comparing the same 1 h of the circadian cycle. There was a significant change in LA during the exposure to light (P = 0.001), and there were significant light (P < 0.001), time (P = 0.004), and light/time (P = 0.027) effects of light compared with the control days (Fig. 5, A and C). LA was significantly different from 20 min after lights on until the end of light exposure, with the strongest reduction to 2.1 ± 2.1 at 32 min after lights on. Also for LA, there were no effects of day, time, or day/time in the hour before or after the light exposure.

In SCNx rats, comparing the same 1 h of the circadian cycle, light did not reduce HR. HR was 327 ± 4 beats/min during control nights and 334 ± 9 beats/min during the 1-h light exposure (Fig. 5, B and D). There was no change in HR over the 1-h light exposure (P = 0.60). Compared with the control nights, there were also no light (P = 0.60), time (P = 0.99), or light/time (P = 0.17) effects of light on HR. Light did not suppress LA in SCNx animals. LA was 8.6 ± 1.8 during the control nights and 8.2 ± 3.5 during the 1-h light exposure when comparing the same 1 h of the circadian cycle. There was no change in LA during the period of exposure to light (P = 0.10). There were also no significant light (P = 0.95) or time (P = 0.86) effects, but there was a light/time effect (P < 0.001) of the 1-h light exposure. There were no effects of day, time, or day/time in the hour before or after the light exposure for HR or LA.

Tracing study. Because nuclei projecting monosynaptically to the preganglionic sympathetic and parasympathetic nuclei of the heart had already been determined in previous studies, we focused our attention on the appearance of PRV labeling in the SCN and on the putative anatomic route(s) that mediate(s) SCN information to the heart. With the use of the aforementioned inclusion criteria, 6 of 20 left ventricle-injected, 4 of 10 right ventricle-injected, and 2 of 10 right atrium-injected rats showed specific labeling, and only they were used for analysis.

After inoculation of the left and right ventricle and right atrium, PRV-labeled neurons became visible in the intermediolateral cell column of the spinal cord (IML), the ventrolateral periambiguous area, and the dorsal motor nucleus (DMnX). In the hypothalamus, several nuclei contained PRV-labeled neurons, but the PVN clearly showed the most dense labeling. The labeling was localized in the dorsal cap region of the PVN and the ventrolateral parts of the PVN. Subsequently, PRV-labeled neurons became visible in the SCN, with 10–20 labeled neurons in most cases. PRV-labeled cells were distributed from rostral to caudal in the SCN and were concentrated in the dorsomedial part of the SCN (Fig. 6, A and B). All cases with SCN infection also showed PRV labeling of the dorsal cap region and ventrolateral parts of the PVN, e.g., there was no SCN labeling without labeling in the PVN.

After the control experiments with intravenous injections or deposition of virus into the thoracic cage, no infection was found in the central nervous system. In particular, possible infection of the circumventricular organs was studied, but the area postrema, the organum vasculosum of the lamina terminalis, and the subfornical organ never contained infected neurons in these control experiments. This observation agrees with similar control experiments conducted for other purposes (4).

DISCUSSION

The results in the present study demonstrated that there is a clear circadian rhythm in HR that is not caused by activity. After lesioning of the SCN, this resting HR changed to a level halfway between the day and the night level, with no detectable day-night difference. Furthermore, it was demonstrated that light at night reduces HR but only when a functional SCN is present, and a multisynaptic pathway connects the SCN with the heart. Together, these results demonstrate that the SCN affects the heart in rat and suggest that this is mediated by a neuronal mechanism.

To avoid the inclusion of too many incompletely lesioned animals in the experiment, the absence of a rhythm in drinking behavior was determined over a
period of 2 wk under LD conditions. In a previous study (2), we used a similar prescreening method. In that study, SCNx rats participated in the study if they drank between 40 and 60% during the 12-h light period. With the use of this criterion, about two in three SCNx animals were arrhythmic in drinking and, after histological analysis, about one in four animals showed a complete SCN lesion. In the present study, however, we used a stricter preselection and included rats only if they drank more than 33% during 8 h in the light period, which is equal to more than 50% over 12 h. Also, the test for arrhythmicity of drinking was done after only 1 mo after surgery in the present study. With the use of this stricter criterion, only one of six participating SCNx animals had to be excluded from analysis after histological verification.

We determined HR independent of the influence of LA and demonstrated a clear day-night rhythm in this resting HR in intact rats. This day-night rhythm in resting HR is dependent on the presence of a functional SCN, as demonstrated by the disappearance of this rhythm after lesioning of the SCN. That the clear day-night rhythm in resting HR is not caused by the LD cycle is demonstrated in the experiment carried out during DD: here, the day-night difference in resting HR is similar to that during LD conditions, as measured in the same intact rats. This shows that, although light has a strong impact on HR (the present paper), the LD cycle is not required for the daily rhythm in resting HR. That this rhythm is indeed generated by the SCN is furthermore demonstrated by the ability of the resting HR to free run under DD and dim-LL conditions. Under free running conditions of 10 days of constant 0 lux and of 10 days of constant 5 lux, the animals showed a clear (subjective) day-night difference in HR, as measured without the influence of activity. We do not attribute the daily rhythm in HR to other external masking factors than light, because all conditions were kept constant except for the handling of the rats by the experimenter, but this was done during the day period. A second argument against any external 24-h rhythmical factor generating the rhythm in resting HR is the ability of the circadian rhythm in resting HR to free run. Although we excluded the influence of behavioral LA and of external masking factors, other behavioral factors could be involved in the generation of the daily rhythm in HR. Although activity is the most influential behavioral masking factor, the sleep-wake cycle is also known to influence the HR. However, the daily rhythm in HR is still present when measured only during rapid eye movement (REM) sleep or only during non-REM sleep, resulting in a similar day-night difference in HR as found in the present study (28), indicating that the rhythm is not dependent on the sleep/wake cycle. Of the internal factors involved in the regulation of HR, the autonomic nervous system is generally accepted to be the most important. The presence of a day-night difference in resting HR in intact but not in SCNx rats thus suggests the SCN generates a daily rhythm in HR via autonomic projections.

During the daytime, the SCN has a high level of neuronal activity (18, 27, 29), whereas resting HR is low. During the nighttime, the opposite is true: the SCN has a low level of neuronal activity, and resting HR is high. These correlations might lead one to expect that SCN activity has an inhibiting influence on resting HR. However, resting HR in SCNx rats (305 beats/min, both during day and night) was halfway between that of the day (293 beats/min) and that of the night (317 beats/min) in intact rats. This suggests that the SCN has not only an inhibiting but also a stimulating influence on the heart. Similarly, LA and corticosterone are not overall increased to peak levels after lesioning of the SCN, and it is proposed that, apart from its inhibiting role, the SCN also has a stimulating influence on corticosterone secretion (11). This suggests that even during the night, when SCN neuronal firing activity is generally low, the SCN has an important modulating effect on output systems.

Next, we demonstrated that light at night reduces HR and that this is also dependent on the presence of a functional SCN. That light induces an inhibition of HR in night-active rats is to be expected, because light stimulates the SCN neuronal firing frequency (17, 18), thus mimicking the day signal of the SCN. In line with this is the finding that light at night is able to reduce corticosterone plasma concentrations in intact but not SCNx rats (4). The opposite would be expected for day-active humans, for whom light is the signal of the active period. Indeed, light stimulates resting HR (26) and resting cortisol (25) depending on the time of the day. The absence of an effect of light on HR in SCNx animals is not due to the inability of these animals to discriminate between light and dark, because they did respond to visual cues. In a previous experiment (4), by using a visual detection task in a Skinner box, we demonstrated that the animals were able to detect and respond to light. This inability of light to reduce HR in SCNx animals demonstrates that the SCN influences the regulation of the heart.

Also, LA is reduced by light at night in intact rats. But unlike what we saw with respect to the effect of light on HR in SCNx rats, light also had a (small) effect on LA in SCNx rats. This is indicated by an interaction between time and light when comparing the light-exposure night with the control nights. However, the level of LA over the 1-h light exposure was similar to that over the same time in the circadian cycle during the control nights. Furthermore, LA did not change during the 1-h light exposure. Finally, there was no effect of light on LA and no change in LA during the 1-h light exposure when compared with the control nights. Together, these data suggest that the effect of light on LA is mainly, but not completely, mediated by the SCN. The combined results of the absence of an effect of light on HR but a slight effect on LA in animals without a functional SCN suggests that there is an SCN-independent masking effect of light on LA but that the direct effect of light on HR is totally dependent on the SCN.
The acute influence of environmental changes on rhythms controlled by the pacemaker was termed masking by Aschoff (1). In circadian research, a lot of effort has been made to prevent any masking effect by light. However, just this masking by light itself can be used to demonstrate the involvement of the SCN in a particular function. For example, the masking effect of light has proven very useful for the study of the regulation of melatonin secretion by the SCN (10, 13). In the present study, we demonstrated that the masking effect of light on HR, just as on melatonin, requires the presence of the SCN. Also, we demonstrated that, for the largest part, the masking effect of light on LA requires the presence of the SCN. That the masking effect of light on activity is mainly dependent on the presence of an SCN is in agreement with a study by Redlin and Mrosovsky (22). Only by using many repeated light exposures over several days could they detect a significant SCN-independent masking effect of light on running wheel activity in SCNx and intact hamsters. Because the present study demonstrates that the masking effect of light on HR, and to a lesser degree on LA, depends on the presence of a functional SCN, we would like to stress that masking can be the result of changes in SCN output despite the general belief that masking only distorts the output of the SCN. In this way, masking effects mediated via the SCN could be used as a tool in circadian research.

The PRV labeling of SCN neurons after inoculation of the left or right ventricle of the heart demonstrated the presence of a multisynaptic pathway from the SCN to the heart. The concentration of PRV labeling in the dorsomedial part of the SCN is in agreement with a recent study (36) by Ueyama and co-workers, who demonstrated PRV labeling in the SCN after injection of the stellate ganglion, the main sympathetic ganglion innervating the heart. Because of the conservative approach to exclude false positive labeling of the SCN (using relatively short survival time and strict exclusion criteria), we found only a small number of infected cells in the SCN. It was not our goal to get a massive labeling of forebrain regions, which is usually the case when there was a significant infection of thoracic and ventral reticular cell groups, but to go for selectivity, that is, to be absolutely confident about the results. This approach may lead to underestimation of the number of participating cells in cardiovascular regulation but provides confidence about the multisynaptic projection from SCN to the myocardium (see also Ref. 4).

After myocardial inoculation, preganglionic sympathetic PRV-labeled cells were located in the IML, and preganglionic parasympathetic PRV-labeled cells were located in the ventrolateral periambiguous area and the DMnX. There are several nuclei in the hypothalamus that project directly to these preganglionic autonomic cells of the heart and also receive direct input from the SCN. In the present study, the PVN showed the most dense PRV labeling of these hypothalamic nuclei. It is also the autonomic part of the PVN that receives the strongest input from the SCN. This was demonstrated in studies combining retrograde and anterograde tracing in IML and SCN, which shows the PVN as the structure containing the highest number of closely apposed fibers from the SCN on cell bodies projecting to the IML (32, 33, 37). Together, these data suggest that the PVN is the most important relay station for the transmission of SCN influence via the brain stem and the spinal cord to the heart. This multisynaptic route from the SCN to the heart is in agreement with previous studies (4–7, 21, 24, 31, 33, 36) demonstrating that the SCN projects to the autonomic relay stations mainly via the PVN and that the SCN has a multisynaptic contact with the stellate ganglion. This multisynaptic SCN-heart pathway could provide the anatomic basis via which the SCN generates the daily rhythm in resting HR as well as passes on information of light to the heart, as found in the present study.

PRV tracing studies of different organs of the body, such as the pineal and adrenal cortex, show similar multisynaptic autonomic pathways arising from the SCN (4, 33). This suggests that the SCN has control over important organs of the body via similar autonomic routes. The information of light could thus be transmitted via the retina and retinohypothalamic tract to the SCN and then via these autonomic pathways to different important organs of the body, as is supported by recent studies (4, 10, 25, 26).

There are several neurotransmitters that might be involved in the transmission of the time-of-day and/or light signal from the SCN to the autonomic parts of the PVN. A combination of electrophysiological and physiological experiments demonstrated that the release of GABA from SCN terminals in the PVN is responsible for both the daytime- and light-induced inhibition of PVN output via the sympathetic nervous system to the pineal for the secretion of melatonin (6, 10). SCN neuronal activity is negatively correlated with the HR: SCN neuronal activity is high during the day and low during the night (18, 27, 29), whereas resting HR is low during the day and high during the night (present study); also, SCN neuronal activity is stimulated (17, 18), whereas HR is reduced (present study) by light at night. The low levels of HR during the daytime and during a 1-h light period at night, as found in the present study, both suggest that SCN neuronal activity has an inhibiting effect on HR. PVN stimulation has been demonstrated to increase HR (20), and the release of the inhibitory neurotransmitter GABA in the PVN has been demonstrated to reduce HR (6, 16). GABA release during the day and during light exposure during the night from the SCN into the PVN could thus mediate the reduction of HR.

Although the exact signaling mechanism by which the SCN affects the heart is still to be uncovered, the present study demonstrates that the time of the day and the light signal transmitted from the SCN may also reach the heart via a neuronal mechanism.

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