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A serotonin agonist phase-shifts the circadian clock in the suprachiasmatic nuclei in vitro

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The mammalian circadian pacemaker in the suprachiasmatic nuclei (SCN) receives a large serotonergic (5-HTergic) projection from the raphe nuclei. Whether the SCN pacemaker can be modulated by this afferent projection is a question of considerable theoretical and practical interest. In this study we investigated whether the 5-HT agonist, quipazine, can reset the phase of the SCN clock when it is isolated in vitro. Our results show that 1 h treatments with quipazine induce robust phase shifts in vitro, and that this effect depends upon the circadian time of treatment. We further show that the ability of quipazine to induce phase shifts is dose-dependent. These results suggest that the SCN circadian pacemaker is sensitive to 5-HTergic stimulation, and therefore that the 5-HTergic projection to the SCN may play a role in modulating the phase of the SCN clock in the intact animal.

The suprachiasmatic nuclei (SCN) contain a circadian pacemaker that controls a wide variety of physiological and behavioral rhythms in mammals[21,33]. When isolated in vitro, these nuclei continue to produce 24 h rhythms in metabolism[29], vasopressin secretion[6,12] and spontaneous electrical activity[11,13], indicating that the circadian time-keeping functions of the clock are endogenous characteristics of the SCN.

In the intact animal the SCN receive several prominent afferents. Along with direct and indirect visual afferents[22,24], they receive a large serotonergic (5-HTergic) projection from the raphe nuclei[1,31]. Previous studies of the function of this afferent projection have produced contradictory results. Some experiments have shown that disrupting the 5-HTergic input to the SCN, either through lesioning the raphe nuclei or by chemically decreasing brain 5-HT levels, can decrease the amplitude of activity or corticosterone rhythms, or change their phase of entrainment in rats and hamsters[15,30,32]. In some cases these lesions appeared to eliminate rhythmicity[3,17,34]. However, other studies have reported no effect on activity or corticosterone rhythms after raphe lesions[2,4,16]. Thus, the role of this projection in the mammalian circadian system remains unclear.

In this study we investigated whether a relatively non-specific 5-HT agonist could reset the phase of the SCN circadian pacemaker. The experiments were conducted using SCN isolated in a brain slice preparation to ensure that any effect induced by the drug was the result of local action in the SCN. If the SCN clock is sensitive to phase resetting by the 5-HTergic agent, this would strongly suggest that the 5-HTergic projection to the SCN modulates clock activity in intact animals.

For these experiments coronal brain slices (500 μm) were prepared during lights-on from adult, male Wistar rats housed in a 12:12 h light–dark cycle. Details of brain slice preparation and maintenance procedures have been reported previously[25]. Briefly, slices were placed in a brain slice dish, the outer chamber of which contained distilled water warmed to 37 °C and aerated with 95%O_2/5%CO_2. The slices were continuously perifused with warmed, oxygenated Earle's Balanced Salt Solution (Sigma) supplemented with 24.6 mM glucose and 26.2 mM sodium bicarbonate, pH 7.4. For 3 day experiments the medium was further supplemented with 0.05% gentamicin.

At a specified circadian time (CT; time referenced to the donor colony light cycle since slices were kept in constant light; thus, CT 0 refers to lights-on and CT 12 to lights-off in the colony) during the first day in vitro, perifusion was stopped and the medium inside the brain slice dish was replaced with medium supplemented with the non-specific 5-HT agonist quipazine. After 1 h the quipazine-containing medium was replaced with normal medium and perifusion was reinstated.

The spontaneous electrical activity of single SCN cells were then recorded extracellularly during the second day in vitro. For these recordings glass microelectrodes were

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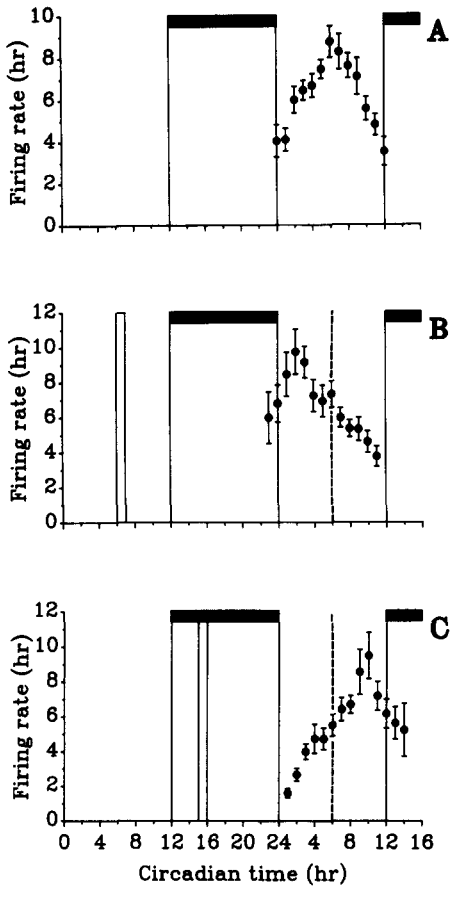


Fig. 1. Electrical activity rhythms recorded from SCN in vitro. Plotted are the 2 h means ± S.E.M. of firing rates of single SCN cells, with each plot representing a single experiment. A: control experiment with untreated slices. The peak in electrical activity occurs around CT 6 on day 2. B: slices were treated at CT 6-7 on day 1 in vitro with 10 μM quipazine. The time-of-peak on day 2 is at CT 2, 4 h earlier than in control slices. C: slices were treated at CT 15-16 on day 1 in vitro. The subsequent time-of-peak occurs at CT 10, 4 h later than in control slices. Horizontal bar = lights-off in animal colony; vertical bar = time of treatment; dotted line = mean time-of-peak in untreated slices.

filled with 3 M NaCl. Amplified signals were monitored with an oscilloscope and stored for later analysis via a data collection and analysis program (BrainWave Systems). The firing rates of single cells with signals at least twice the level of background noise were determined over 5 min intervals, and the activities of all the cells recorded during a single experiment were averaged into 2 h intervals using 1 h lags[25]. Previous studies have shown that this procedure yields a pattern of electrical activity for the population of SCN neurons that varies little between animals, and that the time of peak electrical activity in particular is a reliable marker of the phase of the SCN pacemaker[10,11]. In some experiments the SCN electrical activity was also recorded during the third day in vitro to determine whether changes seen on the day following treatment were stable, and therefore reflected a change in the phase of the underlying circadian pacemaker.

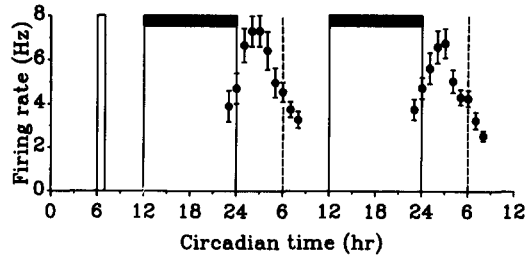


Fig. 2. Stable shift in the electrical activity rhythm induced by quipazine. Plotted are the 2 h means ± S.E.M. of firing rates of single SCN cells recorded from slices treated at CT 6-7 on day 1 in vitro with 10 μM quipazine. The subsequent peaks in electrical activity on days 2 and 3 both occur at CT 2.5, 3.5 h earlier than in untreated slices. Thus, the phase advance induced during the first cycle following treatment remains stable for an additional circadian cycle. See Fig. 1 for details.

In experiments with untreated slices the electrical activity rhythm on day 2 in vitro consistently peaked during the middle of the subjective day (Fig. 1A). Overall, the mean time-of-peak for untreated slices was CT 6.0 ± 0.32 h (n = 5). In control experiments slices were treated on day 1 in vitro at CT 6-7 with perfusion medium not supplemented with quipazine. In these experiments, the mean time-of-peak on the subsequent day occurred at CT 5.5 ± 0.71 (n = 2), indicating that the process of changing the medium does not alter the phase of the clock. However, when slices were treated at CT 6-7 on day 1 in vitro with 10 μM quipazine, the electrical activity rhythm on day 2 was altered (Fig. 1B) such that the time-of-peak occurred around CT 2, 4 h earlier than normal. The mean phase-advance induced by quipazine treatment at this time was 3.81 ± 0.14 h (n = 4). Conversely, when slices were treated with quipazine at CT 15-16 on day 1 in vitro, the subsequent peak in electrical activity occurred around CT 10 (Fig. 1C), 4 h later than normal.

The change in phase induced by quipazine treatment

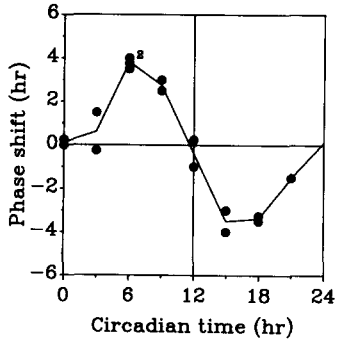


Fig. 3. Phase response curve for quipazine. The phase shifts induced by 10 μM quipazine in individual experiments are plotted according to the circadian time of treatment. Treatments during subjective daytime induced phase advances, while nighttime treatments induced phase delays. Treatments at subjective dawn and dusk had little or no effect on the phase of the electrical activity rhythm.

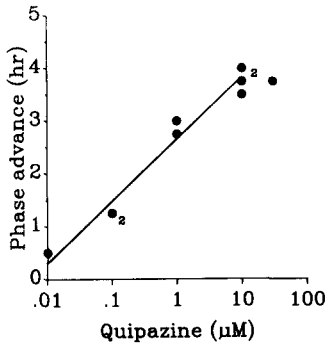


Fig. 4. Dose–response curve for quipazine. Plotted are the phase advances induced by different concentrations of quipazine applied at CT 6-7. Maximal advances of around 4 hrs were induced by 10 μM quipazine, and the ED_{50} is around 0.5 μM.

appears to be stable (Fig. 2), since the advanced time-of-peak seen on day 2 in vitro (1 day after treatment at CT 6-7) reoccurs on day 3 in vitro. Thus, the change in the time-of-peak seen after quipazine treatment does not appear to reflect transient changes in the pattern of electrical activity in the SCN, but rather a permanent shift in the phase of the underlying circadian pacemaker.

The changes induced by quipazine application at different times throughout the circadian cycle are summarized in Fig. 3. This phase response curve was generated by plotting the amount of shift induced in each experiment according to the CT of quipazine treatment. The overall pattern shows that quipazine advances the time-of-peak when applied during mid-subjective day (CTs 6 and 9), and delays the rhythm when applied during subjective night (CTs 15, 18 and 21). Little or no change is seen after quipazine treatment around subjective dawn (CTs 0 and 3) and dusk (CT 12). Thus, both the ability of quipazine to reset the SCN pacemaker and the direction of shift depends on the time of treatment.

The sensitivity of the SCN clock to resetting was assessed by determining the magnitude of phase shift induced by applying different concentrations of quipazine to SCN slices at the time of maximal phase advance (CT 6-7). The results of these experiments (Fig. 4) show that the sensitivity of the SCN clock to resetting by quipazine is dose-dependent, with an ED_{50} (dose producing half-maximal effect) of approximately 0.5 μM.

These results indicate that the SCN circadian pacemaker is sensitive to phase-resetting by quipazine. This suggests that the phase of the SCN clock can be modulated by stimulation of 5-HTergic receptors in the SCN. This sensitivity is phase-specific, indicating that the phase changes probably were not due to non-specific effects on SCN cell metabolism. Rather, quipazine appears to affect the pacemaker mechanism itself. This conclusion is further supported by previous observations that the firing rates of SCN neurons are generally inhibited by 5-HT[18,23] and by quipazine itself[20].

The sensitivity of the SCN clock to resetting by quipazine was also shown to be dose-dependent, with an ED_{50} of about 0.5 μM. This relatively low sensitivity suggests that 5-HT receptors with a high affinity for quipazine, such as the 5-HT_3 receptor, are probably not involved in these effects[28]. In addition, since quipazine appears to be largely inactive at the 5-HT_4 receptor[5], this receptor is likewise probably not involved in the current results. Rather, quipazine may act through 5-HT_1 or 5-HT_2 receptor subtypes, which have a moderate affinity for quipazine[28].

The ability of this 5-HT agonist to reset the SCN clock in vitro suggests that the 5-HTergic input to the SCN in vivo might modulate the phase of the SCN pacemaker in the intact animal. This idea is supported by studies reporting changes in the phase-angle of entrainment after raphe lesions or *p*-chlorophenylalanine injections that decrease 5-HT levels in rats and hamsters[15,30].

Evidence suggests that 5-HTergic activity in the SCN normally occurs in a fixed pattern that reflects cyclic arousal of the animal. Increases in 5-HTergic activity have been correlated with increased arousal in rats[19], and the levels of 5-HT and its primary metabolite have been shown to oscillate in the SCN, with increases occurring late in the day or during the night, parallel to the general activity pattern of these animals[9,14,26]. This suggests that the normal nocturnal increase in SCN 5-HTergic activity could alter the phase of the SCN clock on a daily basis. Interestingly, intense running-wheel activity induces phase-shifts and entrains the circadian rhythms of rodents[7,8,27]. The pattern of phase-shifts induced in vivo by behavioral activity is quite similar to those shown here to be induced in vitro by quipazine. It is therefore possible that the behaviorally-induced phase shifts are mediated by 5-HTergic input from the raphe.

In conclusion, this study has shown that the SCN circadian pacemaker can be reset in vitro by applications of the non-specific 5-HT agonist, quipazine. The pattern of phase shifts induced by quipazine is similar to the pattern of shifts induced in vivo by intense activity, suggesting that these phase changes may occur through similar mechanisms.

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