A balanced clock: network plasticity in the central mammalian clock

Olde Engberink, A.H.O.

seven

SUMMARY

Almost all living species, from unicellular to humans, have an endogenous timing system that helps them to adapt to environmental light-dark cycles. In mammals, a central circadian clock, which resides in the suprachiasmatic nucleus (SCN) of the hypothalamus, drives 24 h rhythms in physiology and behavior. The SCN is a bilateral brain structure that consists of about 10.000 neurons in each nucleus and is located on top of the optic chiasm. The SCN neuronal network uses adaptive mechanisms to synchronize the internal rhythms, that have a period length of about 24 h, to the environmental cycles of exactly 24 h. The most important environmental time cue or zeitgeber is light, received by specialized retinal ganglion cells that project directly to the SCN through the retino-hypothalamic tract. Most SCN neurons are autonomous oscillator cells that generate a rhythm in the frequency of action potentials with a peak during the middle of the day. Communication and coupling within the SCN network are important to generate a strong and coherent output signal from the SCN to the periphery, but also to synchronize the SCN to the environmental conditions. Via this manner, the SCN controls the timing of many physiological and behavioral functions and aligns these rhythms to the environmental light-dark cycle.

The SCN's ensemble electrical activity follows a sinusoidal-like pattern that peaks during the day and has a trough during the night. The amplitude of the SCN's electrical activity rhythm can be influenced by external factors, like photoperiod, and is linked to the level of synchronization within the network. For instance, after adaptation to a long photoperiod, the phases of the individual SCN neurons are more dispersed over the 24 h (less synchronized) and the ensemble output in electrical activity has a broadened waveform with a decreased amplitude. Aging is also known to affect the SCN network organization with deterioration in synchronization among the individual SCN neurons and a reduced amplitude. In this thesis, several studies have been conducted to investigate the effects of light exposure and aging on SCN network organization. Specifically, a big part of this work focused on the role of the most abundant neurotransmitter in the SCN, which is GABA, and the GABAergic excitatory/inhibitory (E/I) balance in SCN network plasticity.

The influence of light on SCN network properties and behavior has been convincingly described after adaptation to different day length. But, is reception of the full photoperiod needed for photoperiodic encoding in the SCN, or does exposure to short light pulses at the beginning and end of the day suffice? **Chapter 2** described a series of experiments that examine whether exposure to light for the full length of the day was needed to achieve cellular reorganization within the SCN network as demonstrated after entrainment to long or short photoperiod.

To study this, mice were exposed to "skeleton photoperiods" that mimicked long summer days of 16 hours or short winter days of 8 hours by two brief light exposures of 30 minutes that mark the beginning and end of the day. First, the behavioral phenotypes of entrainment to skeleton and full photoperiod were assessed using locomotor activity recordings. Behavioral entrainment to long and short days were similar under skeleton and full photoperiods, with compressed and expanded durations of the active phase under long and short photoperiods, respectively. Next, light-induced phase shifts were measured in mice after they adapted to either skeleton or full photoperiod, since previous work indicated a reduction in phase shifting capacity under long photoperiod conditions.

Again, skeleton photoperiods were sufficient to diminish the light-induced phase shifts in long photoperiod, similar to results obtained in full photoperiod.

Furthermore, to identify the level of cellular synchronization within the network, electrophysiological and bioluminescence imaging experiments were performed. The ensemble multiunit electrical activity (MUA) of SCN slices from mice entrained to skeleton long photoperiod showed a broader waveform when compared to short photoperiod. This change in waveform shape indicates a lower synchronization level in the SCN network after adaptation to skeleton long photoperiod, which is an important feature to represent and convey day length information. Cellular synchronization was also determined on the basis of the molecular rhythms in slices from mice adapted to skeleton long and short photoperiods by measuring single cell PER2::LUC expression for several consecutive cycles. These bioluminescence experiments showed a higher phase distribution of PER2 peak times in slices from mice entrained to skeleton long photoperiod, compared to skeleton short photoperiod. This confirmed that the SCN of mice adapted to skeleton long photoperiod is less synchronized than the SCN of mice adapted to skeleton short photoperiod, which was similar in mice entrained to full photoperiods.

Lastly, ex vivo calcium imaging experiments were used to study the effect of skeleton photoperiods on GABAergic activity and the E/I balance. The percentage of GABAergic inhibitory responses was significantly lower in SCN tissue from mice entrained to skeleton long compared to skeleton short photoperiod. Similar to a full long photoperiod, the E/I balance was remarkably high following a skeleton long photoperiod.

The results of **chapter 2** together showed that exposure to a skeleton photoperiod, and thus the timing of only two brief light pulses, affects the circadian clock in a similar manner as complete light exposure under a full photoperiod. This underscores the powerful, yet potentially harmful effects of even relatively short light exposures during the evening or night for example nocturnal animals.

Photoperiodic phase adjustment in the SCN network is a good example of SCN network plasticity. Research has shown that aging affects the circadian clock at different levels, among which the network level. In the following chapters we further investigated the effect of aging on several cellular, network, and behavioral properties of the circadian system. In **chapter 3**, the plasticity of the circadian clock in old mice was examined, while challenged with different light regimes. Old PER2::LUCIFERASE mice (22-28 months) and young controls were exposed to either an equinoctial (Light-Dark: LD 12:12), long (LD 16:8), or short (LD 8:16) photoperiod. The old mice showed a reduced rhythm strength and a compromised ability to adapt to short photoperiod. After these behavioral assays, PER2::LUC gene expression characteristics were measured in SCN slices of young and old mice adapted to the different photoperiods, in order to investigate seasonal encoding at the molecular level. Surprisingly, the PER2::LUC rhythms were remarkably similar in the SCN of young and old mice under the different photoperiods. Thus, photoperiodic encoding by the SCN, at least at the molecular level, is still intact after aging and the reduced ability to behaviorally adapt to different photoperiods emerges downstream from the core molecular clock.

Chapter 4 aimed to examine the effect of aging on GABAergic function, the concomitant E/I balance, and on Ca^{2+} homeostasis. In this chapter, the polarity of Ca^{2+} transients in response

to exogenous GABA stimulation was determined in SCN slices of old mice (20-24 months) and young controls and from these results, the E/I balance was established. In SCN tissue of old mice, the amount of GABAeric excitation, and concordantly the E/I balance, was higher when compared to young controls. Especially in the posterior part of the SCN, the percentage of excitatory responses increased and the percentage of inhibitory responses decreased significantly, compared to slices of young mice. Moreover, the baseline Ca^{2+} transients were higher in old SCN neurons, when compared to young suggesting an altered calcium homeostasis in the SCN of old mice. With aging, an increased E/I balance due to loss of inhibition has been demonstrated in other brain networks. The stabilization of SCN E/I ratio to a healthy range in aging could benefit SCN network properties and may also diminish age-related diseases provoked by clock dysfunction.

Thus far, the research described in this thesis, and results from other studies, suggest that the balance between GABAergic excitation and inhibition plays a role in synchronization and/or plasticity of the SCN network. However, the exact role or mechanism remains topic for future studies. Pharmacological manipulations of GABAergic signaling are important tools in both in vivo and in vitro research regarding the E/I balance. Besides drugs affecting the GABA receptor itself, manipulation of intracellular Cl^- concentration is used as this affects the amount and polarity of the ion flux through the GABA-controlled channel. In **chapter 5** experiments were conducted to investigate the role of one of the ion co-transporters (KCC2), which regulate the intracellular Cl^- concentration by removing Cl^- from the cytoplasm, on the GABAergic responses in the SCN with a newly developed blocker ML077. By the use of calcium imaging, GABA-induced single cell Ca^{2+} transients were recorded before and after blocking KCC2 with ML077 in SCN slices of mice entrained to different photoperiods (LD 12:12, LD 16:8, LD 8:16). Blocking KCC2 with ML077 caused a shift in the polarity of the GABAergic response by inducing excitatory responses in previously inhibitory responding neurons, suggesting that KCC2 is an essential component in regulating $[Cl^-]_i$ and the Cl^- equilibrium potential, thereby determining the sign of the GABAergic response. Remarkably, the percentage of excitatory cells increased with a similar degree in slices from mice adapted to long or short photoperiod and LD 12:12 condition. This indicates on the one hand that KCC2 activity is important for maintaining E/I balance under all photoperiods, but also that KCC2 can mimic the transition of network states from short to long photoperiod. The results from this chapter showed that ML077 promises to be a powerful tool in researching the effect of E/I balance on the level of synchronization in the SCN network.

Finally, the results from this thesis were discussed in **chapter 6**. Plasticity and the right balance in the circadian clock organization are needed for adaptation to environmental changes. Here, we showed for the first time that even short light exposures at the beginning and end of the day can cause network changes, including a change in the function of GABA. Artificial prolonged light duration, which is common in our modern 24 h society, has been shown to affect neurotransmitter systems in the brain and also the SCN. Additionally, this work showed that similar changes in GABAergic function are achieved by brief light exposure late in the evening. The seasonal light regime changes annually by nature. However, artificial prolonged light in the evening, and even short exposure to bright light late in the evening or at night, keeps our circadian system in a continuous mode of summer over months and years. It is still unclear how this affects our physiology and behavior,

and whether the induced changes to our clock network are still reversible. Lastly, the (prolonged) light exposure in the evening is something we impose on ourselves, however it also influences, unsolicited, the circadian clock function of nocturnal animals in their natural environment.

While light management is something that can be influenced, the effects of aging are usually irreversible. Also, aging affects to GABAergic function in the SCN network which could contribute to other age-related deficiencies in the clock machinery. Future research into pharmacological manipulation of the GABAergic E/I balance in the SCN, and its effect on the coupling mechanisms within the network, is important as it might be an interesting lead towards restoration of (age-related) deficits in circadian rhythms.