SUMMARY

A twenty-four hour rhythm is a major characteristic of the temporal profile of many mammalian species. These rhythms are regulated by a biological clock, which resides in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is a bilateral brain structure and contains about 10,000 neurons in each hemisphere. It is located immediately above the crossing of the optic nerves. The SCN generates rhythms with a period of about 24 hours, which have adaptive significance when appropriately synchronized to the environmental light-dark cycle. The SCN receives light information through the retino-hypothalamic tract (RHT). Photic cues are sensed by photosensitive ganglion cells in the inner retina containing the photopigment melanopsin. These photosensitive ganglion cells receive input from the classical rod and cone photoreceptors in the outer retina. Each class of photoreceptors has its peak sensitivity to a specific wavelength of light. The mouse retina is dichromatic and contains two classes of cone photoreceptors which are the short wavelength sensitive cones and the mid wavelength sensitive cones. The finding that mice lacking rod and cone photoreceptors as well as mice lacking melanopsin are able to entrain to an external light: dark cycle revealed that all classes of photoreceptors can regulate photoentrainment.

Chapter 2 gives an overview of the light signaling pathway from the retina to the SCN and discusses the different photoreceptors of the mouse retina and the effects of retinal illumination on photoreceptor electrical activity. The second part of this chapter describes how photic information affects the release of neurotransmitters in the SCN and how it changes SCN neuronal activity. Chapter 2 concludes with an overview of the contribution of the different photoreceptors to photoentrainment. The relative contribution of each class of photoreceptors to photoreception by the SCN was determined in chapters 3, 4 and 5. In these chapters we made use of various retinal mutant mouse models to unravel the photoreceptor origin of the response kinetics in SCN neuronal activity. Light exposure typically induces a fast transient increase in electrical impulse frequency of SCN neurons followed by a sustained component for the duration that the lights were on. It was long thought that the sustained component in SCN electrical activity is mainly dependent on melanopsin, whereas the fast response characteristics would originate from cone photoreceptors. In this thesis the degree of the contribution of the classical photoreceptors to the response kinetics of SCN neurons was investigated and the major conclusion is that classical photoreceptors can mediate sustained responses in SCN electrical activity.

In chapter 3 results of experiments are described that elucidate the effects of ultraviolet (UV) light on the circadian system and sleep. UV wavelength of 360 nm was chosen to maximally stimulate short wavelength sensitive cones. The data report an effect of UV light on SCN electrical activity that was indistinguishable from the

effect of white light. To investigate the contribution of the classical photoreceptors to the response kinetics in SCN neuronal activity induced by UV light, similar experiments were performed in mice lacking the photopigment melanopsin. Surprisingly, UV light exposure leads to similar response characteristics in SCN neuronal activity demonstrating a role for classical photoreceptors in mediating this response. Moreover, UV light still elicited an enhancement in SCN neuronal activity, when all photoreceptors except the UV-sensitive cones were desensitized using bright white light. These findings indicate a role for UV-sensitive cones in mediating this response.

The response characteristics of SCN neurons during exposure to longer wavelengths of light were investigated in chapter 4. The light-induced increase in SCN neuronal activity described in this chapter in response to both short and long wavelength light were indistinguishable in melanopsin-deficient mice compared to wild type mice. These findings elucidate a role for classical photoreceptors in irradiance detection by the SCN during exposure to both short and long wavelength light. Similar recordings in mice lacking rods and cones in their retina revealed a contribution of melanopsin to photic transmission to the SCN, especially at higher light intensities and with a relatively long response latency.

In chapter 5 the specific contribution of short and long wavelength-sensitive cone photoreceptors was determined by performing SCN *in vivo* electrophysiological recordings in mice having cones as the only functional photoreceptors in the retina. In response to relatively short light exposure (up to 1 minute), a sustained light-induced increase in SCN neuronal activity was recorded. Interestingly, the SCN electrical discharge rates decayed to baseline levels after the initial phase of light exposure. These experimental outcomes reveal an important contribution of cone photoreceptors in the initial phase of light detection, whereas cone photoreceptors are unable to generate sustained responses in SCN electrical activity for prolonged durations of light exposure. UV light induced significantly larger changes in SCN electrical activity compared to longer wavelengths of light.

The ability of cone photoreceptors in mediating the transmission of photic information to the SCN does not preclude a role for the other classes of photoreceptors in this process. Our findings indicate the likelihood that the different classes of photoreceptor fulfill additive functions to circadian photoreception. The relative contribution of melanopsin, rod and cone photoreceptors is most likely dependent on the intensity and wavelength composition of the light source.

In chapter 6 the effect of disturbed sleep and caffeine on light signaling to the SCN was investigated. Behavioral activity recordings showed that sleep deprivation in mice led to a decrease in the light-induced phase-shifting capacity of the circadian system. To unravel the mechanism causing this reduction, the effect of prolonged wakefulness on light-induced changes in SCN neuronal activity was investigated. In accordance with the effect on behavioral activity, light-induced increases in SCN

neuronal activity were attenuated after prolonged wakefulness. The attenuation of SCN electrical discharge rates were restored when the non-selective adenosine antagonist caffeine was administrated after prolonged wakefulness and prior to light exposure. Furthermore, caffeine administration enhanced period lengthening in constant light. These results suggest a role for adenosine and adenosine antagonists in modulating light sensitivity of the circadian system.

The impact of the loss of another neurotransmitter, vasoactive intestinal peptide (VIP), on photic regulation of the circadian system was investigated in chapter 7. The hypothesis was tested that VIP plays a major role in the transmission of photic information within the SCN. The light-induced changes in SCN neuronal activity did not differ in VIP mutant mice compared to wild type controls. Similarly, N-methyl-D-aspartate (NMDA) enhanced firing rates of ventral SCN neurons of both VIP mutant and wild type mice. Stimulation of the RHT was used to simulate light exposure and calcium transients were recorded. In response to light exposure and glutamate release, calcium level transients are evoked. RHT stimulation evoked calcium transients in the ventral SCN of both VIP mutant and wild type mice, but exhibited a weaker response in the dorsal SCN in the absence of VIP. Furthermore, light-induced gene expression revealed a reduction after 60 minutes of light exposure especially in the dorsal region of VIP mutant mice. Together these data show an important role for VIP in communication of photic information within the SCN, more specifically in transmission of light information from the ventral to the dorsal SCN region.

In chapter 8 the effects of behavioral activity on the circadian system were investigated. Whereas light exposure enhances SCN neuronal activity, behavioral activity leads to acute suppressions in SCN electrical activity levels. In chapter 8 the hypothesis was tested that enhanced levels of behavioral activity lead to an increase in the amplitude of the SCN electrical activity rhythm. Behavioral activity levels were enhanced by running-wheel activity to determine the influence of exercise on the SCN rhythm in electrical activity. The amplitude of the SCN rhythm in electrical activity was significantly enhanced when behavioral activity levels were increased. Exercise also leads to an increase in rhythm strength. These findings indicate an important role for exercise as a non-invasive intervention for improving the circadian system.

The studies described in this thesis are reviewed in chapter 9. The different photoreceptors in the retina each have their contribution to photoentrainment most likely depending on the light intensity and the specific wavelength of light. The experiments described in this thesis reveal an unexpected contribution of the classical photoreceptors to circadian photoreception. We also showed that photic transmission to SCN neurons can be affected downstream of the retina by sleep deprivation and caffeine administration. Furthermore, the transmission of photic information within the SCN is disrupted in the absence of vasoactive

intestinal peptide. Taken together these studies demonstrate that light input to the circadian system can be affected both in the retina as well as at the level of the SCN. Finally an important influence of exercise on enhancing the amplitude and increase the rhythm strength of the waveform in SCN electrical activity was elucidated. These findings indicate a role for exercise as a valuable intervention for deficits in circadian rhythms.