Chapter 7

Summary, conclusions and future work

In this thesis, computational models were created to investigate the biological clock in mammals, and more specifically the organization of the suprachiasmatic nuclei (SCN) and the coupling mechanisms between subpopulations of the SCN.

In mammals a circadian clock is located in the SCN. The animal can anticipate daily and seasonal rhythms in the environment because it is governed by an endogenous clock. At the molecular level, a transcriptional and translational feedback loop in SCN pacemaker cells generates an endogenous rhythm that is approximately 24 hours. To adjust to the environmental conditions, the clock must be able to synchronize, or entrain, to the daily light-dark cycle. To produce a concerted rhythmic output pattern, the clock cells within the SCN need to synchronize to each other as well as to the daily light-dark cycle. Coupling between the neurons is the mechanism to realize synchronization between the different cells.

Chapter 2 of this thesis provides an overview of different coupling mechanisms that are present in the SCN. The communication of phases between regions in the SCN may rely on different neurotransmitters, such as VIP or GABA. GABA can synchronize populations of neurons to each other, such as the dorsal and ventral region of the SCN. Within a region, VIP and gap junctions may be especially important coupling mechanisms. Thus, VIP
Network properties of the mammalian circadian clock may synchronize cells within the dorsal or ventral region, while GABA may act as a synchronizer between these regions. Gap junctions may strengthen very specific groups of neurons in their coordinated output. No doubt the real situation will be more complex than this presentation. The exact nature of these coupling mechanisms is presently a major focus of many research lines in the field of circadian rhythm research, as it has become increasingly clear that coupling of clock neurons contributes strongly to the function of the SCN pacemaker to control daily and seasonal rhythms.

Different modeling studies of the circadian timing system and the SCN in particular have already been performed. An overview of the main directions of modeling studies of the biological clock is presented in chapter 2. In the first models, the clock was modeled as a single entity using a limit cycle oscillator. One limit cycle oscillator appeared to be unable to describe all the dynamic properties of the clock, which gave rise to two-oscillator models. In these models, the two oscillators were coupled and in the interaction between the oscillators some dynamic properties of the clock could be explained. As scientific knowledge in clock research progressed, it became increasingly clear that the circadian system is a heterogeneous system containing endogenously oscillating pacemaker cells. These findings resulted in two types of models. Some models focused on the modeling of the endogenous pacemaker cell and the generation of circadian rhythms itself. These models were based on molecular findings, such as the transcriptional/translational molecular feedback loop. Other models directed their research towards the network properties of the SCN. These models focused on the heterogeneous nature of the SCN and often presumed simple oscillatory units. The network properties of the SCN appear to be just as important for the control of circadian rhythms as the endogenous generation of rhythms by pacemaker cells.

When the molecular models and the network models are combined, the resulting models may become complex which makes the models hard to understand. At present, both type of models separately seem more to function satisfactorily to answer specific questions. This thesis demonstrates that simple models at the neuronal network level can provide interesting scientific results, and that models do not need to become very complicated to
produce scientifically interesting insights. The use of simple models is justified, if the model is able to provide a satisfying answer to the research question posed. The research question should be well defined in terms of the results intended and the boundaries for which the results are valid. The model should provide an answer that is sufficient, but that is also understandable. The modeling studies are especially useful if they lead to counterintuitive results.

An example of a useful simulation model is described in chapter 3. The model was based on previous experimental results from our laboratory, where it was found that very small populations of neurons could not produce an electrical activity pattern that resembled the multiunit electrical activity pattern, in width as well as in smoothness of the pattern. In support of this it was found that electrical activity patterns for single units, which may be regarded as the smallest possible population of neurons, have peaks that are narrower than the multiunit peak. The times of maximal single unit peak activities varies strongly and are distributed over the light-dark cycle. Finally, it was also shown that, by distributing single unit patterns over the day, and computing the total sum of the activity patterns, a feasible multiunit activity pattern could be obtained. This pattern could be altered by changing the width of the distribution of neurons, in this way simulating long and short day length.

The model described above can be summarized as follows. Single unit patterns have short electrical activity patterns. By themselves, single unit patterns can not account for a pattern as broad as recorded multiunit activity patterns in rats and mice. However, when the single unit patterns are active at different times of the day, the summed activity pattern of all single unit patterns resembles the recorded multiunit patterns. The question then is: how are the single units divided over the circadian cycle, or, in other words, what distribution can be used to distribute the single units in such a way that the added activity of all single units creates a realistic multiunit pattern?

In chapter 3, this research question was used to create a simulation model in which single unit activity patterns were distributed over the circadian cycle and accumulated to produce a multiunit activity pattern. Different single unit patterns were used and each pattern could be narrowed or
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broadened. Four different distributions were tested: linear, Gaussian, bimodal and trimodal. The distributions could also be narrowed or broadened.

Using this simple simulation model, questions could be answered about how the different distributions and the changes in single unit activity patterns can alter the multiunit activity pattern. The results show that in order to obtain a realistic multiunit pattern, the single units had to be distributed in phase. It appeared also that all distributions tested can lead to a realistic multiunit pattern.

On the basis of the simulations it was predicted that changes in the width of the distribution of the single cells is necessary to account for photoperiodic encoding, even though changes in single unit activity may play an additional role. It was shown that changes in single unit patterns alone are not large enough to account for the multiunit pattern changes that are recorded in long and short day lengths. In contrast, minor changes in phase distribution between the neurons, are capable of encoding for day length.

On the basis of these findings some testable predictions are presented: if the mechanism for photoperiod encoding depends on changes in single unit patterns, the single unit activity patterns should show relatively large changes in the duration of electrical activity and the maximum frequency of the multiunit patterns should increase in long day lengths, if the distribution of the single units was kept constant. If photoperiod is encoded in the SCN by changing the distribution of neurons over the 24 h day, the frequency of the multiunit activity peak should decrease in long day lengths and single units should show a broader distribution. With this finding a way was found to distinguish between the two alternative models by simply measuring the multiunit electrical activity peak. Importantly, it was not necessary to measure the single units.

Experimental studies using extracellular electrophysiological recordings in-vitro showed that the shapes and widths of the single unit patterns in long and short days do not significantly differ. In chapter 3 these recorded single unit patterns are employed to show that changing the phase distribution between neurons can result in changes in multiunit pattern.
This simulation study is just one example of a simple model which provides interesting scientific results. These results could be obtained by a sharp focus on one specific mechanism of the SCN and by asking very specific questions about this mechanism. The model included only those mechanisms of the real system that were of importance for these specific questions. Within these well defined boundaries, the simple model could present some unsuspected and testable results.

In chapter 4 a study has been conducted to investigate the regional organization of the SCN. After a delay of 6 hours in the environmental light-dark cycle, a dissociation between the ventral and the dorsal region of the SCN was observed. At day one after the shift of the light-dark cycle, the ventral SCN immediately shifted to the new light-dark regime, while the dorsal SCN completed the shift of its phase only after 6 days. In the electrical activity patterns measured on the first day after the shift of the light-dark cycle two peaks could be identified. The electrical activity recordings were analyzed using different techniques, such as curve fitting and a subpopulation analysis. To investigate the properties of two subpopulations of neurons that are separated in time, a simple model was created. The simulations showed that only when the size of one of the populations was small and the distribution in that population narrow, a bimodal pattern could arise in the ensemble pattern. It appeared from the study that only a small number of the total population of neurons shifts immediately after a delay in the light-dark cycle. It is proposed that phase shifts are brought about by an initial rapid shift of this relatively small subpopulation of neurons within the SCN. This group is expected to be located in the ventral part of the SCN, given the fast shifts of the ventral SCN. Coupling between the shifted and the unshifted population of SCN neurons is asymmetrical, as the shifted neurons exert a strong phase shifting effect on the unshifted neurons. This causes a complete shift of the SCN which is realized after several cycles.

In future studies it should be realized that the different regions of the SCN, such as the ventral and dorsal region, may also be heterogeneous. The simulation studies that were performed indicated the existence of a small subpopulation of neurons which eventually brings about a shift in the
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complete SCN. Experimental research can direct its search towards finding
this distinct group of cells, and elucidate the coupling mechanisms which are
important within this group and with other functionally distinct groups of
neurons.

Chapter 5 describes a simple model that has been build to differentiate
between responses to phase shifts in long and short day lengths. In the field
of circadian rhythms, it is generally assumed that high amplitude rhythms
are more difficult to shift in phase than low amplitude rhythms. This
assumption originates from the theory on limit cycle oscillators, which is
often used to model either the clock as a whole or individual pacemaker
cells. Limit cycle oscillators that have high amplitudes are more difficult to
shift in phase than limit cycle oscillators with low amplitudes. Experimental
data, shown in chapter 5, showed the contradicting results that short day
electrical activity patterns which have a high amplitude, shift more than long
day patterns, with a lower amplitude. Using simple simulation studies, where
phase response curves (PRCs) were distributed according to long and short
day distributions, the experimental findings were confirmed. The results of
the simulations indicate that if neurons are more synchronized in phase, the
PRCs, or light-sensitive periods, are more compressed and overlap, resulting
in a higher number of neurons that shift at the same time in the same
direction in response to a light pulse, compared to desynchronized neurons
in long days. The highly synchronized state during short days results
consequently in a PRC with a higher amplitude, i.e. the shift in phase is
larger, for short days compared to long days. As the limit cycle theory may
be valid for individual neurons within the network, the network as an
ensemble shows different response characteristics as a function of rhythm
amplitude. The data presented in chapter 5 provides thereby a clear example
that neuronal networks are governed by different rules than single cell
oscillators and also shows the underlying explanation for this difference.

Chapter 6 provides a model to investigate the coupling mechanisms of
two regions in the SCN after a shift of the light-dark cycle. The model is
based on the ventral and the dorsal regions of the SCN, which are both
considered endogenous oscillators in our simulations. The two oscillating
regions interact with each other using non-uniform coupling mechanisms.
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The ventral region of the SCN is influenced by the external light-dark cycle according to a PRC. The coupling between the ventral and dorsal regions of the SCN is described by PRCs as well. This coupling exists of excitatory, but also inhibitory influences. The ventral and dorsal regions are self-organizing according to their coupling strengths under the influence of an external light-dark regime. The model was able to qualitatively simulate results from different experimental phase shifting studies. The results indicate that in the SCN different oscillatory regions may exist each consisting of groups of cooperating neurons with their own phase resetting characteristics. The different oscillatory regions are interacting with each other, and transmit phase information. Chapter 6 emphasizes that phase shifting properties of the SCN emerge primarily at the network level.

In our approach we used simple models to elucidate the working mechanisms of the clock network. These simple models provided evidence that different levels of organization are responsible for different properties of the clock. While the endogenous rhythms are clearly a property of single cells, properties such as entrainment, resetting, or day length encoding arise at the neuronal network level.

A first step has been taken to better understand the neuronal network properties of the SCN. This thesis shows that a simple model can provoke questions that can guide future experimental research. For example, an interesting question is about which mechanisms underly the propagation of the instantaneous effects of light on the SCN to different regions in the SCN. Future research should be directed at determining the phase response curves to light for the different regions of the SCN, and for the interaction between the regions.

While we have distinguished between a dorsal and a ventral SCN, particular subregions may be present that are essential for phase resetting. Future research should target the functional meaning of different subregions in the SCN and consider also a division of the ventral and dorsal regions in different functional subregions.

Furthermore, the present studies did not consider direct single cell interactions. Phase distributions between neurons could explain a number of properties of the clock, but for now it is unknown how the cells are able to
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organize themselves in these phase distributions. Also the communication mechanisms between cells in different regions or subregions have not been considered. The next step is to gain more knowledge about the coupling mechanisms between the neurons in the SCN in different functional groups within the SCN.

While the communication between neurons is immediate, after a shift of the light dark cycle the ventral region of the SCN shifts immediately to the new phase, but the dorsal region does not. This may be caused by the way the network is organized. However, other factors may play a role as well. A neuron may have a sensitive period for shifting its phase, which determines its PRC. The neuron also receives signals from other cells that produce a driving force for shifting its phase. The interplay between the sensitive period and the driving force is able to shift the phase of the neuron. This driving force constitutes different inputs from different cells and may differ between cell types. The driving force may differ throughout the circadian cycle and could contain an excitatory and an inhibitory period. The actual coupling signals from cell to cell should be the subject of future studies in order to identify this driving force.

Finally, we only used small and simple models to investigate questions about the network properties of the biological clock. For certain questions regarding different types of neurons cooperating in different regions in a large network of about 10,000 cells, corresponding with the real size of one SCN, simple models may not be sufficient. Larger models may be applicable to investigate these larger networks, as long as caution is taken to make the model unnecessarily complicated. These larger models are challenging for computer science studies and may demand the use of larger computers or larger networks of computers, such as grid-networks.

It is a challenge to combine different scientific disciplines. This thesis shows an example that the coordinated efforts of computer science and life sciences enrich each other and leads to scientific progress. The results that have been acquired could not have been found with separated efforts, showing that the results are more than the sum of parts.