How to stop a biological clock

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How to stop a biological clock: Point of singularity

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To the memory of Arthur Winfree, who thought and worked on the point of singularity of biological rhythms profoundly. He died on the 5th of November 2002 of brain cancer

Great are the works of the Lord,
studied by all who delight in them.

Psalm 111

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Preface

An older version of this book was written by the author WE[1]. In march 2004 he was asked by Charlotte Helfrich-Förster, to give a lecture for the Graduate College 'Sensory photoreceptors in natural and artificial systems' at the University of Regensburg about the point of singularity in biological rhythms. He used his preparations to write the first version with two goals: Firstly, in the short time of a lecture one cannot mention all details, which are, however, partly important to better understand the field. This should be made up by showing also the illustrations shown in the lecture and further ones. On the other hand it was noticed that it is a fascinating topic which might be of interest to others too.

There is already some literature on this field with which we can't and won't compete with. They are mainly papers and books by Arthur T. Winfree, a colleague of the author WE during his stay as a guest of Colin S. Pittendrigh at the Department of Biology at the Princeton University in New Jersey, USA. Art, how he was called, worked at that time on his PhD thesis. WE got to know him as an excellent and gifted scientist, but estimated him also highly personally. He learned a lot from him and his (self)critical way to do science. It was a very sad news when on the 8th of November 2002 a letter of his son Eric arrived:

From: Erik Winfree <EmailErikWinfree>

Subject: Re: Art Winfree

Dear Friends of Art Winfree,

We regret to inform you that at approximately 9pm, Tuesday, Nov 5th, Art passed away after many months of fighting a brain cancer that was diagnosed in April. He died at home in peace, attended to lovingly by his devoted wife, Ji-Yun, with his father C. Van and his brother Charlie present. We know that his life was made richer by his interaction with all of you. There will be a gathering for family members this weekend, in Tucson, to mourn his passing.

The Family

P.S. It is impossible to contact all of Art's friends who would want to know. We wish all Art's dear friends to help us spread the news of Art's passing. If you each would kindly pass this email on to others, that you know would have wanted to know, it would be greatly appreciated by his wife and family.

[1] http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-380xxx07 und http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-380xxx17

In a special edition of the Journal of Theoretical Biology he and his scientific work was commemorated in obituaries (Tyson and Glass, 2004).

Here are the books, which Arthur Winfree wrote about the topic. They are highly recommendable, whereby the two last one are quite pretentious: Winfree 1987b, 2004, 2001, 1983, 1987a. Some of the illustrations we used are from Winfree 1987a, 1983. A short introduction in limit cycles by Lakin-Thomas 1995 is advisable.

The new edition and publication of this book has several reasons. First of all during this time several new studies were done and the results published, about which we will report. Furthermore we thought it adequate to include also some simulations of rhythmic processes, which shall demonstrate the rhythmic behavior of organisms and their reactions towards environmental factors such as light and temperature. One of the authors (KHW) has much experience in this area and will deal with the basics and practical procedures in a separate chapter.

While writing this book, we noticed that it would be a good idea to write a book about *Models for biological Rhythms* with further and more detailed explanations, information and examples. We will refer to this book at the appropriate places. Its publication will, however, take some time.

1. Introduction in Biological rhythms: Flower clock *Kalanchoe*

Rhythms are wide spread among organisms. First of all, they might be brought about by the rhythmic structure of the environment such as the day-night- and the annual rhythm, but also by the lunar cycle. Many of these daily, annual and lunar rhythms exist also as *endogenous* rhythms inside the organisms; they were so to speak internalized and occur therefore also under conditions, where they are shielded from the external rhythms. However, they then deviate slightly from the exact period lengths of the 24-hour rhythm, from the 12 months of the annual rhythm or from the about 30 days of the lunar rhythm.

There are furthermore rhythms in organisms, which are longer than annual rhythms (example: the flowering of various bamboo species, see *Engelmann, Rhythms of Life - An introduction using selected topics and* examples http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37998). Or they are shorter than daily rhythms (examples: respiration rhythm, heart beat, see chapter 4). Extremely short rhythms are found in the firing of nerve cells and in the metabolism.

Rhythms are not always useful and strategies exist to avoid them. One of it is the *dead beat strategy* which avoids overshooting. It is used for instance, to suppress or avoid oscillations in technology such as in motors, machines, bridges, buildings and plays an important role especially in control circuits (Pedersen and Johnsson, 1994; Isermann, 1987).

In this book rhythms are presented such as daily rhythms, which are surely of advantage for the organisms showing them; otherwise they would not have persisted during evolution. That a perturbation by e.g. a light pulse at a certain phase of the oscillation can induce arrhythmia was surprising and occurs rarely under natural conditions[1]. The possibility, to bring the oscillation to a halt, can, however, be used, to obtain a deeper insight in the way such rhythms function. We will begin with an example, which shall show the reader this property.

One of the authors (WE) studied during his PhD work under Bünning at the

[1] an exception is the sudden cardiac death, see page 68; to avoid it, care has to be taken, to restore the heart beat as fast as possible).

Botanical Institute of the University of Tübingen, whether and how in the Crassulacea *Kalanchoe blossfeldiana* the daily clock is connected with the photoperiodic induction of flower formation. There were already a number of studies in the research group of Harder in Göttingen, especially by Bünsow. In Tübingen it was the director of the Max Planck Institute for Biology, Melchers, who made with his coworkers important experiments with *Kalanchoe*. There were, however, still open questions, especially in respect to the effect of light pulses (red and far red) during the dark period.

Kalanchoe blossfeldiana is a plant endemic to Madagascar. It grows at dry places and blooms after the rainy season, a time at which the days are shorter than the nights. In Europe the plant blooms therefore during the winter time and is sold by gardeners and flower shops because of its many red flowers around Christmas time.

Plants which flower during the season with short days (long nights) are called shortday plants. *Kalanchoe blossfeldiana* belongs to them. It is a photoperiodically reacting plant.

The flowers open their petals during the day and close them during the night. Flowers cut off from the plant are able to continue the petal movement for one to two weeks under proper laboratory conditions. Even under weak green light (which is like darkness for the flowers) the rhythmic opening and closing continues. However, the length of an oscillation (*period length*) is not any more 24 h, but only 22 to 23 h. This speaks for an internal rhythm, which is entrained by the light-dark cycle of the day to 24 h. The petal movement can be recorded by different methods and serves as a hand of the internal clock (*circadian* clock). Light influences the course of the movement, and under certain conditions it can even prevent the rhythm of the petals. More about it in the following chapter.

1.1. Petal movement of *Kalanchoe*

The daily movement of the petals of *Kalanchoe blossfeldiana* is shown as a time lapse movie in KalanchoeRhythm. Figure 1.1 shows a plant in bloom. Cut off flowers are mounted in holes of a lid covering a plastic cuvette filled with sugar water (figure 1.1 right). The petals also move rhythmically under weak green light instead of a light-dark-change. For the flowers this particular light acts as darkness, and they continue to move up to fourteen days. The clock, which controls this movement, is, however, faster than 24 h, namely 22 to 23 h per cycle (figure 1.2) at temperatures between 13 and 30^o C (Oltmanns, 1954).

The movement can be recorded by taking shots of the flowers from above with a video-camera every hour (figure 1.3).

With an image analysis program the size of the flowers can be determined. If

Figure 1.1.: Flowering *Kalanchoe* plant. The small flowers open and close their petals daily. Right: If single flowers are cut off from a plant and mounted in holes of the lid of a plastic cuvette filled with sugar solution, the petals will continue to move in a daily cycling. This can be recorded, as shown in figure 1.3. Substances can be added to the water in order to test, whether they influence the rhythm.

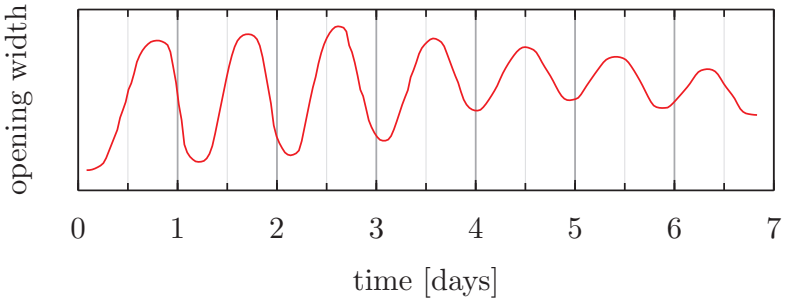


Figure 1.2.: *Kalanchoe* flowers open and close (opening width at the y-axis) also under constant conditions (weak green light, constant temperature, time in days). The period length of the oscillations amounts to 22 to 23 h.

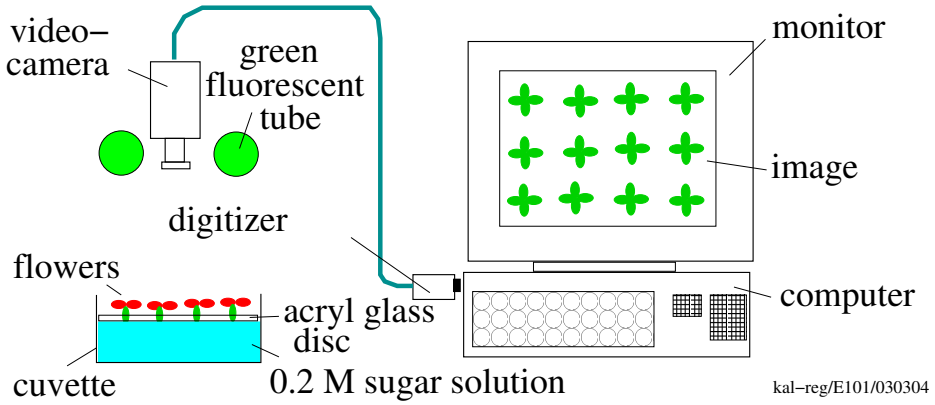


Figure 1.3.: The petal movement is recorded with a video-camera (data are digitized) and analyzed with an image analysis program. Green light as safelight (does not influence the petal movement). The flowers in the holes of an acryl glass disk are above a 0.2 molar succhrose solution in a plastic cuvette (right part of figure 1.1) and can be seen on the monitor of the computer.

the flowers are closed, they appear small if seen from above; open flowers appear much larger. The data can be stored for each flower in a file and plotted against time as a diagram (figure 1.2). The resulting oscillations are quite harmonic and can be analyzed directly without smoothing and other methods. Amplitude, period length and phase position of the curves are used to characterize the oscillations. The period length of the rhythm is only slightly affected by temperature (see figure 1.4). Light pulses shift the rhythm (see figure 1.5), as shown in the following.

1.2. Phase response curves and arrhythmia

The periodic opening movement of the *Kalanchoe* petals can be influenced by light pulses (see figure 1.5). Weak light pulses shift the rhythm only slightly, strong light pulses more strongly. A larger number of experiments were performed, in which the light pulses were administered at different phases of the cycle and the duration of the illumination varied. The effect of the light pulses can be illustrated in phase response curves (see figure 1.6). Depending on the strength of the light pulses two types of response curves result, *weak* and *strong* ones. In the case of the weak type the shift is less pronounced as compared to the shift in the strong type and around the midnight point there are just small shifts in the case of a weak pulse, whereas strong light pulses at this time exert their maximal effects.

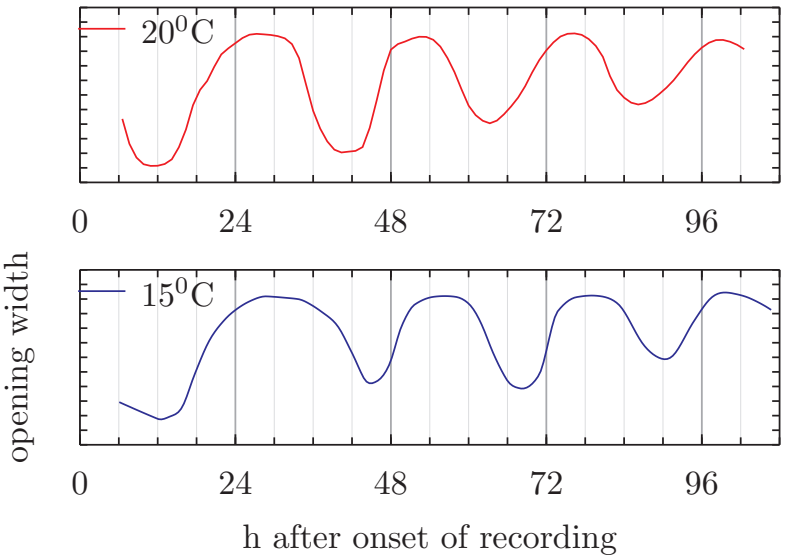


Figure 1.4.: Examples for curves of the petal movement of individual *Kalanchoe* flowers under constant conditions of temperature (top, red: 20 °C, bottom, blue: 15 °C) and weak green light. Opening width (y-axis) plotted against time [h, x-axis]. The period length is only marginally affected by the temperature (22-23 h).

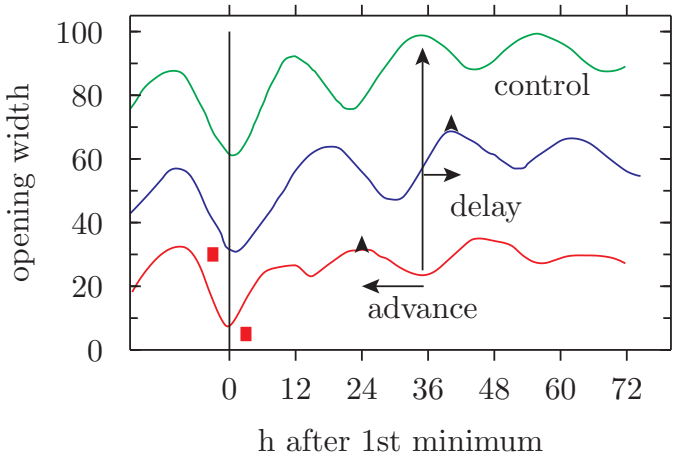


Figure 1.5.: The *Kalanchoe* petal movement rhythm was phase shifted with a light pulse (red square) before (blue curve in middle) and after (red curve at bottom) complete closure of the petals (0 on x-axis). The rhythm is either delayed (middle, horizontal arrow to the right) or advanced (bottom, arrow to the left). The large black vertical arrow shows the position of maximum of the control (green curve).

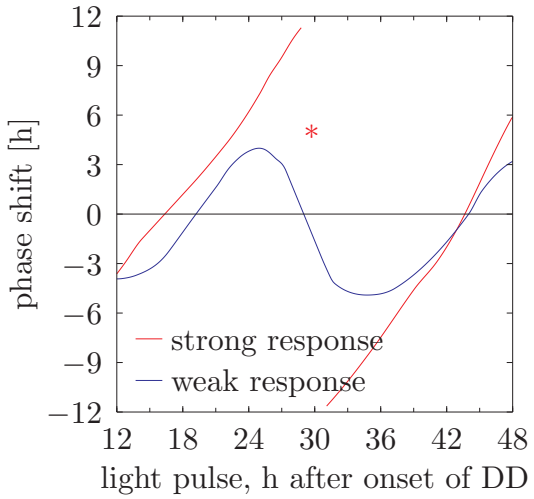


Figure 1.6.: Phase response curve of the circadian petal movement of *Kalanchoe* to 10 min (blue, weak reaction) respectively 3 h light pulse (red, strong reaction), administered at different times after onset of 'continuous darkness (weak green light). Delays of the rhythm in respect to control plotted upward, advances downward (in h). A light pulse with a critical strength (between those giving a weak and a those giving a strong response) administered at the time marked by a red star would induce arrhythmia (see figure 1.7).

Interestingly a light pulse given just between a weak and a strong one clears out the rhythm (figure 1.7). In this way arrhythmia is induced. A second light

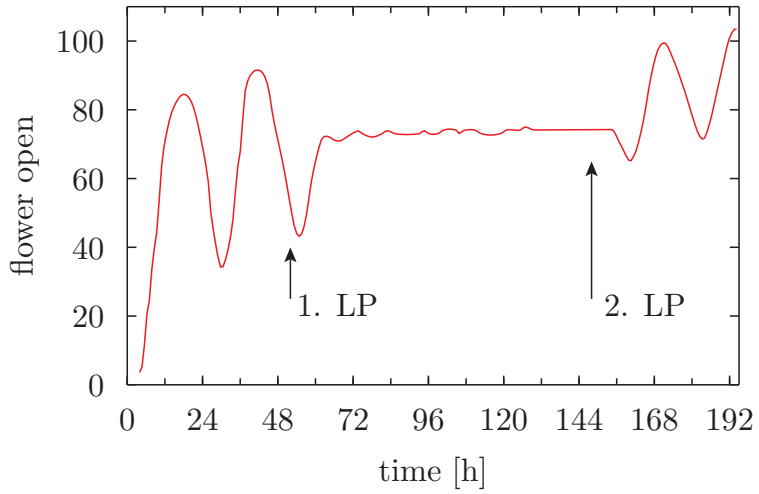


Figure 1.7.: A special red light pulse (LP) of 230μWcm^{-2} and 130 min duration induces arrhythmia in *Kalanchoe* flowers, if given at 30 h or, as shown here, at 52 h (briefly before the 2nd minimum, short arrow) after onset of continuous darkness. A second light pulse (long arrow) induces the rhythm again.

pulse restarts the oscillation again.

1.3. The point of singularity in *Kalanchoe*

If the results of the light pulse experiments were plotted in such a way, that the old phase is shown on the x axis and the time of the maximum after the pulse (the new phase) on the y axis, figure 1.8 results, whereby the values after long pulses are shown as small weak (o) and the values after the short ones as strong thick points (●). A time crystal with a spiral staircase like structure can be imagined. The spindle of the stairs corresponds to the singular conditions, under which no oscillations are found any more.

We have to ask our self, whether the *cells* of the flowers, the swelling and shrinking of which are responsible for the petal movement, are indeed arrhythmic or whether they have just lost their mutual synchronization? That can be tested in the following way:

If all cells are arrhythmic, a relatively short light pulse would re-initiate their rhythms and all of them would oscillate in the same phase. If, however, the cells oscillate in desynchrony with each other, that is, in different phases, a stronger light pulse is required as in cells, which are all arrhythmic.

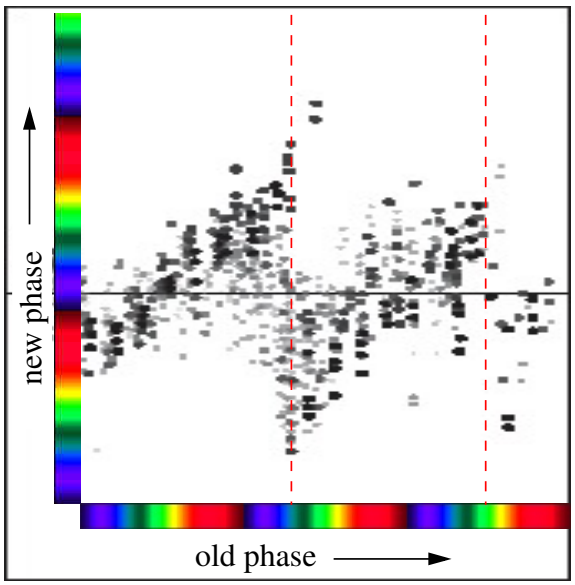


Figure 1.8.: On the x-axis old phase is plotted (for three cycles, color coded), at which light pulses of different length were administered. The resulting shifts of the rhythm are shown on the y-axis as new phase (again color coded). The thick points in the foreground represent the effect of short light pulses, the smallest one in the background the effect of longer light pulses. One can see, that the points form a spindle which, seen from above, mirror the singular point. See also the three dimensional diagram for the respiration rhythm of a cat in figure 6.5.

It was found that a stimulus of only a fourth of the duration of the singular light pulse was able to re-initiate the rhythmic movement of the petals. Such a light pulse would not suffice to synchronize cells which were all oscillating in different phases. *The cells are thus arrhythmic*, and the flowers have lost their rhythm of opening and closing the petals by illumination with the singular stimulus.

A further test was to measure under the microscope the diameter of the cells of the petals after the critical illumination. If the cells would still oscillate, but not in synchrony, their diameters should vary. They were, however, of uniform size.

1.4. Fiddling around with the point of singularity

In *Kalanchoe* a longer light pulse is needed (4 h) to annihilate the oscillations in the first cycle after onset of continuous darkness as compared to the following cycles (2 h) (figure 1.9).

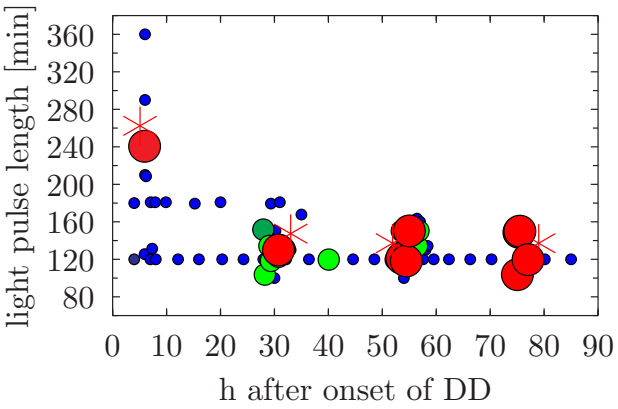


Figure 1.9.: To induce arrhythmia in *Kalanchoe* flowers, a light pulse must be given in the first cycle after onset of darkness, which is twice as long (240 min) as the one in the following cycle (130 min). The position of the singular point (*, see section 3.2) and the duration of the light pulses (y-axis), which induce arrhythmia, are given. After Engelmann et al (1978).

Can arrhythmia be induced in *Kalanchoe* also with a shorter, but stronger light pulse or with a longer, but weaker pulse, that is, reciprocity is valid? A number of experiments were performed, in which the time of application, the duration and the strength of the light pulse (figure 1.10) were varied and the degree of damping determined.

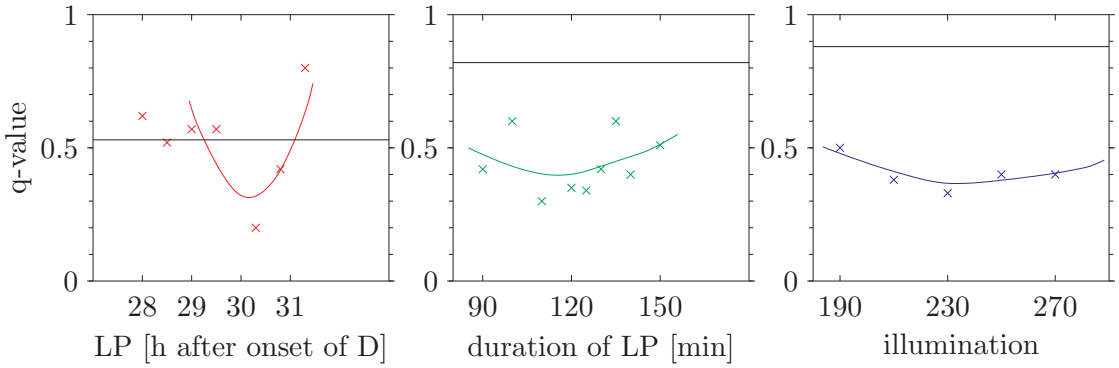


Figure 1.10.: Right: Damping was highest (low q-value), if the light pulse was administered between the 30th and the 31st h after onset of darkness (right part of figure). Middle: The duration of the light pulse can be varied between 100 and 140 min and still lead to arrhythmia. Left: Arrhythmia can be induced with intensities of the red light between 200 and 270 μWcm^{-2}. Horizontal line: Corresponding control values. AfterEngelmann et al (1978).

In further experiments the irradiance and duration was varied. The results are shown in figure 1.11.

The results differ from the expectations if reciprocity (i.e. the product of light intensity and duration determines the response) would hold (red curve in figure 1.12). A logarithmic transformation of the light signal to the clock would change the expected (red) curve in the figure to the experimentally found one (blue). It is therefore likely, that some kind of logarithmic change in the light signal from the photoreceptor to the clock takes place (see Engelmann et al 1978.

1.5. Arrhythmia at different temperatures

The light signal, which extinguishes the rhythm, has to be received by photoreceptors and transferred to the oscillator. The involved processes are probably temperature dependent. They would run faster at higher temperatures as compared to lower ones. This can be tested in an elegant way, if the rhythm-extinguishing light pulse is offered at higher or lower temperatures instead of the usual 22.5 °C. If the pulse is given at the same time as the control pulse at 22.5 °C, it is expected to arrive earlier at the oscillator at higher temperatures (27.5 °C). At that time the oscillator is, however, not yet in the critical phase, where arrhythmia is induced. This is indeed found experimentally (figure 1.13, Engelmann and Heilemann 1981). The pulse has to be given half an hour later as usual. At a lower temperature (6 °C) it takes longer, until the light signal

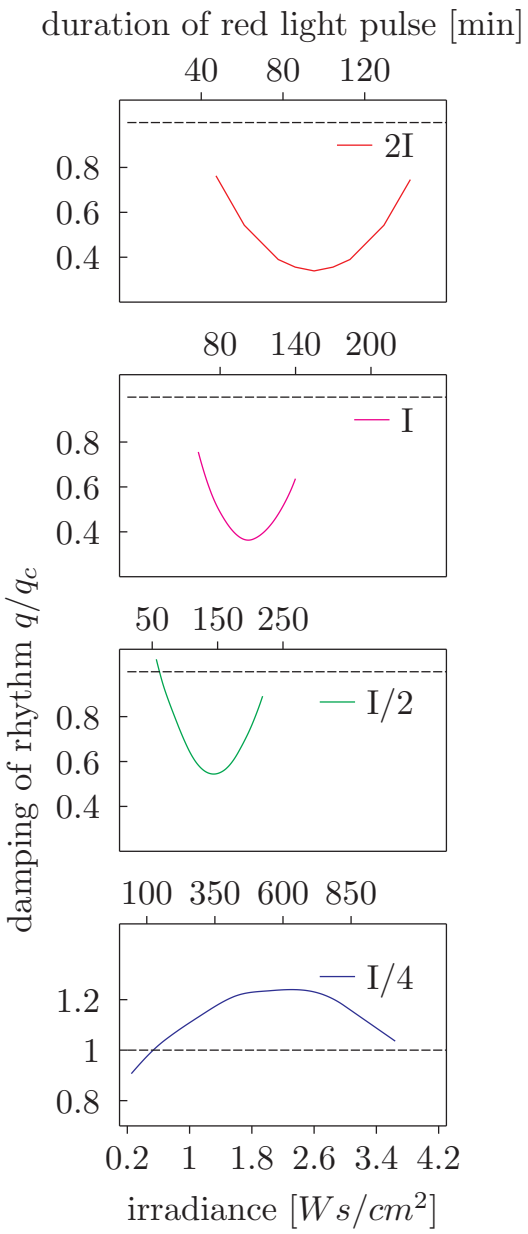


Figure 1.11: To test whether reciprocity holds the standard irradiance of the critical light pulse (I) was doubled (2I, top) or reduced to half (I/2) or a fourth (I/4, bottom) and the duration of the light pulse varied (x2-axis). At 2I 100 min lead to the strongest damping (q/q_c, where q_c is the corresponding value of the controls) as compared to 120 min at the standard irradiance I. 150 min is the corresponding value for I/2. At the I/4 irradiance no arrhythmia is induced (the values are higher than $q = 0.9$). From Engelmann et al 1978.

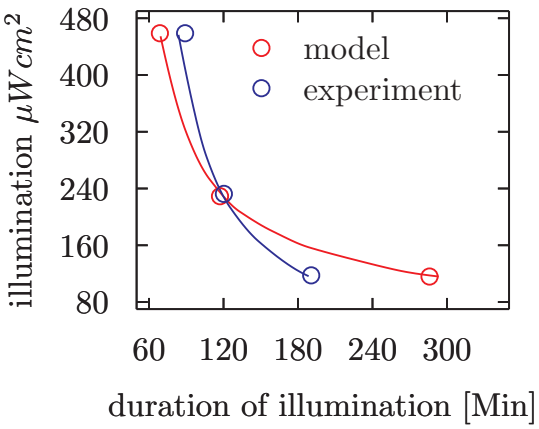


Figure 1.12.: If reciprocity would hold for inducing arrhythmia in the *Kalanchoe* petal movement rhythm (that is, the product of intensity and duration has to be constant), the red curve would be expected. The experiments show, however, a curve (blue) which deviates from it. If the signal is transformed logarithmically on its way to the clock, the expected (red) curve would change to the blue one. From Engelmann et al (1978).

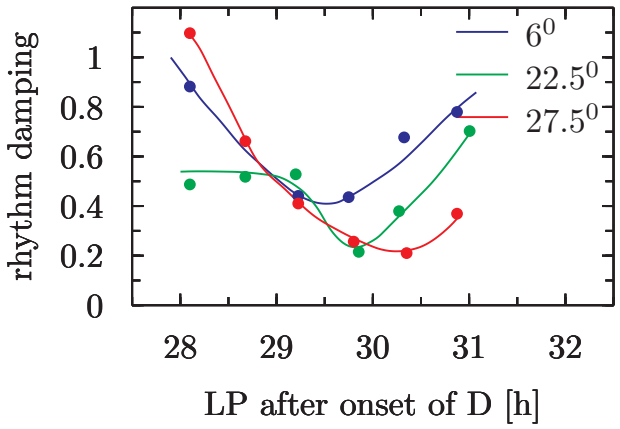


Figure 1.13.: A singular light pulse must be applied half an hour later at a higher temperature (27.5 °C) than at the normal 22.5 °C temperature, in order to induce arrhythmia. At a lower temperature (6 °C) the critical time lies 15 min earlier as compared to the normal 22.5 °C temperature, in order to dampen the rhythm of the petal movement. From Engelmann and Heilemann 1981.

has reached the oscillator. The critical phase has then passed already. The light pulse must, therefore, be given earlier as usual, in order to induce arrhythmia. This was experimentally found (figure 1.13). It can be shown, that temperature influences the light signal on its way to the oscillator, the oscillator and as well the signal from the oscillator to the rhythmic process (turgor changes, figure 1.14, Engelmann and Heilemann 1981).

1.6. What can be done with the point of singularity?

Arrhythmia and singularities are interesting properties of biological clocks and can help us to understand the structure and function of these clocks. But are these properties useful also for more down to earth things? This section and the next chapter will show, that it is not just of academic value.

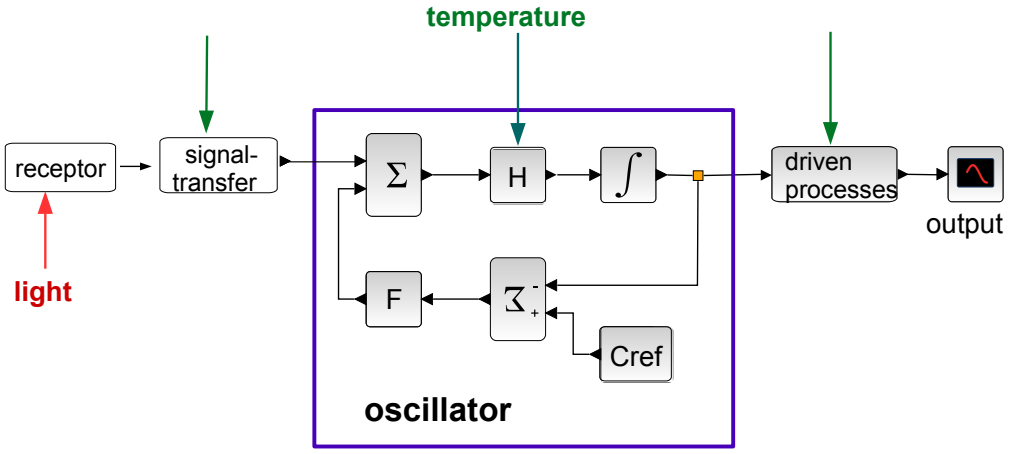


Figure 1.14.: Light shifts the rhythm of the *Kalanchoe* petal movement. The processes (turgor) controlled by the oscillator (blue box) are also phase-shifted and the petal movement serves as a hand of this shift. The scheme shows, that temperature (green arrows) enters the system at different parts of the model: The signal transfer is sped up by higher temperatures and slowed down by lower temperatures, the oscillator is phase-shifted (advanced by higher temperatures), and the driven processes have a larger lag at lower temperatures than at higher temperatures. After Engelmann and Heilemann 1981.

If we want to know how an oscillator functions and how it is build, we can use different strategies. One is, to treat the oscillating system with a certain substance. If its rhythm is sped up or slowed down by it, a *state variable* could

have been affected, that is, an essential part of the oscillator. But not necessarily. It might have changed a *parameter* of the oscillator only. How can we distinguish between both? Here the singular state is of help.

Kalanchoe flowers are well suited to find out, whether certain substances, which change the speed of a rhythm, affect the clock-mechanism. This is done in the following way: We induce in flowers by the special illumination an arrhythmic state. If we add a few days later a substance, which is believed to affect the clockwork (that is to change a state variable), the oscillator should start oscillating again. If the substance does not affect the clockwork, the arrhythmic condition should last. In spite of this, a substance can still change period length (by influencing a parameter). For both cases examples exist.

In figure 1.15 some curves of *Kalanchoe* flowers are shown, which illustrate what was said. First some examples for inducing arrhythmia by red light, white light and UV-light are shown (figure 1.15, a-c). A second light pulse can initiate the oscillation of the petals, if it is strong enough.

Interestingly, a temperature pulse (6 °C, figure 1.15d and e) reestablishes the rhythm, if long enough applied (1 h does not suffice, 4 h are enough). Likewise, a 4 h treatment of the *Kalanchoe* flowers with nitrogen re-induces the petal rhythm (figure 1.15, f).

Lithium salts slow down the circadian clock in different organisms (Engelmann, 1987). It was therefore tempting to test whether the clock is directly affected by the substance. The experiments were done with *Kalanchoe* flowers and were successful: By adding Lithium chloride to the water arrhythmic flowers could be induced to oscillate again (see figure 1.16). Vanadate given as sodium orthovanadate, however, is not able to bring back oscillations to the arrhythmic flowers (figure 1.16). This substance inhibits the ATPases in the plasmalemma. They therefore do not seem to be involved in the mechanism of the oscillator directly, although vanadate shifts the petal rhythm, depending on the phase in the cycle of the petal movements at which it was presented (Eckhardt and Engelmann, 1984). Polyethylen glycol (PEG) is also able to induce rhythmic petal movements in arrhythmic flowers (figure 1.16. PEG withdraws water from the flowers and changes in this way the turgor of the motor cells. Since turgor is responsible for the movement of the motor cells, the result is not surprising.

Continuously offered methyl jasmonate (60ppm) shortened the period length of the *Kalanchoe* flower clock by about 1.5 h (Engelmann et al, 1997). We tried therefore, whether this substance makes arrhythmic flowers to oscillate again. This was *not* the case (figure 1.17).

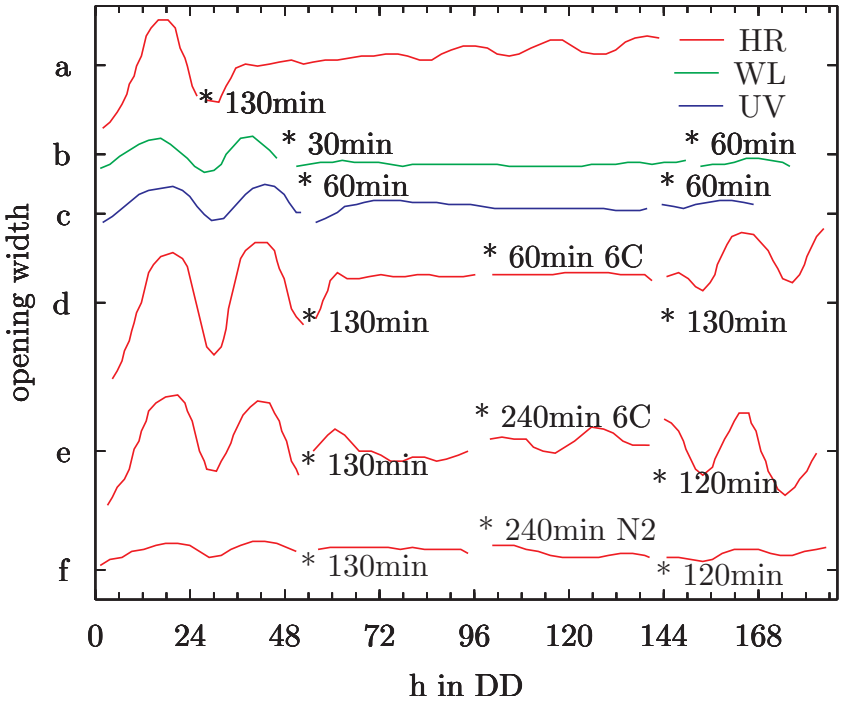


Figure 1.15.: Examples for arrhythmia and re-initiation: a: Red light pulse (HR, 130 min duration 30 h after onset of constant conditions strongly damped, but started to oscillate again after some time. b: Rhythm annihilated (instead of HR, 30 min of white light WL). Second light pulse (60 min WL) petals oscillate again. c: 60 min UV induces arrhythmia. A second UV-pulse of same strength and duration: petals oscillate again. d: During arrhythmia temperature is lowered for 60 min to 6 °C: Petals stop moving. A light pulse of 130 min HR starts the rhythm again. e: A 240 min lowering of temperature to 6 °C starts the oscillator again. A second light pulse (120 min HR) increases amplitude. f: Induction of movement in arrhythmic petals by 4 h nitrogen pulse. From Engelmann et al 1978.

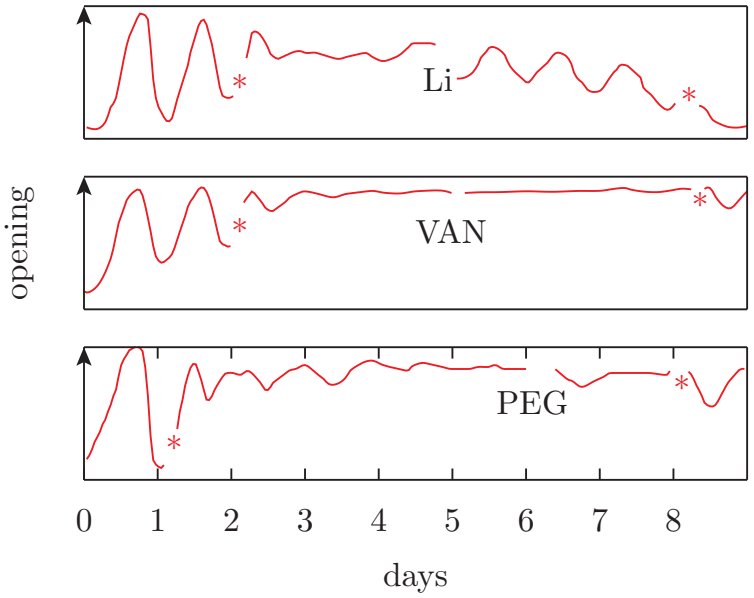


Figure 1.16.: Arrhythmic *Kalanchoe* flowers were induced to oscillate again by adding a lithium chloride- and polyethylen glycol solution (PEG), but not by a vanadate (VAN) solution. The petal movement was damped heavily by a critical red light pulse (*). The flowers were treated at the marked time (Li, VAN, PEG) for 4 h with LiCl (2 mM), vanadat (10mM) or polyethylenglycol solutions, washing it off and continuing the recording. A second light pulse (*) was used to test, whether the flowers are still able to oscillate (the cut flowers -and also flowers on the plant- loose after some time the ability to oscillate). After (Lude, 1995).

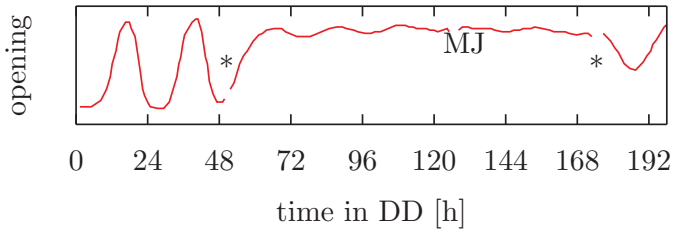


Figure 1.17.: A *Kalanchoe*-flower was made arrhythmic by a critical red light pulse (*) and than treated for 3 h with gaseous methyljasmonate. It was not able to initiate the oscillations. A second light pulse (*) shows, that the flower was still able to oscillate. After (Engelmann et al, 1997).

1.7. Arrhythmic appearance is not always arrhythmia

If one finds an apparent arrhythmic situation, it has carefully to be checked whether the clocks responsible for it are indeed not running any more. Scattered clocks could mess up a rhythm to a degree where a rhythm would disappear. Here comes another example from the *Kalanchoe* studies, which shows that an apparent arrhythmic petal movement can in fact be composed of running clocks. It was found in experiments designed to test a phase shifted rhythm with a second light pulse. The arrhythmic appearance of the sixteen measured flowers is in fact brought about by two effects, namely an initial, but transient damping and phase scattering. Only the common recording of all flowers has led to a seemingly arrhythmic output. Figure 1.18 shows the results and testing by simulations.

Another example might be the report of (Steinlechner et al, 2002). He finds arrhythmia in the locomotor activity rhythm of Siberian hamsters after applying two light pulses. Of course, he recorded the locomotor activity of single hamsters. But the circadian center in the suprachiasmatic nucleus of the hypothalamus in the brain is composed of many oscillators which seem to be grouped in at least two different populations. If they have reacted in a comparable way to the two light pulses as was found in the case of the two pulse experiment in *Kalanchoe* flowers, we would deal with running oscillators, which became, however, desynchronized. More details on page 83.

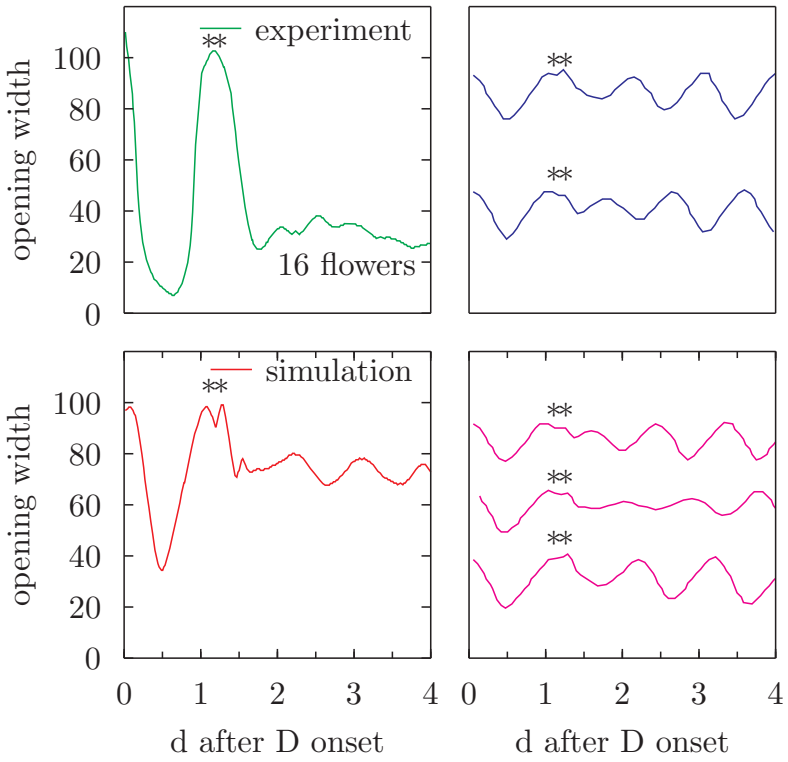


Figure 1.18.: A cuvette containing sixteen flowers of *Kalanchoe* plants were illuminated with two light pulses a few h apart from each other (**). The recorded output (all flowers were recorded with one electrical photocell; therefore the behavior of the individual flowers is not known) looks pretty much arrhythmic (green curve top left). It can be shown, however, that the apparent arrhythmia is the result of two effects of the light pulses: Initial, but transient damping and phase scattering of the petal movement. The first effect is shown in the right upper curves (blue), the second effect in the right lower curves (magenta). If these effects are added together, we obtain the left lower simulated curve (red) which is similar to the green one on the top. After (Johnsson et al, 1973).

2. Models for rhythms

Why are models used? They help us to understand complex processes better such as the weather, the economy (book of Dewey and Dakin (2011) and figure 2.1) and the production (e.g. planning of production under uncertainties, see Mula et al (2006)), technical developments, numerous issues in science. To one of those belong also biological rhythms, topic of this book.

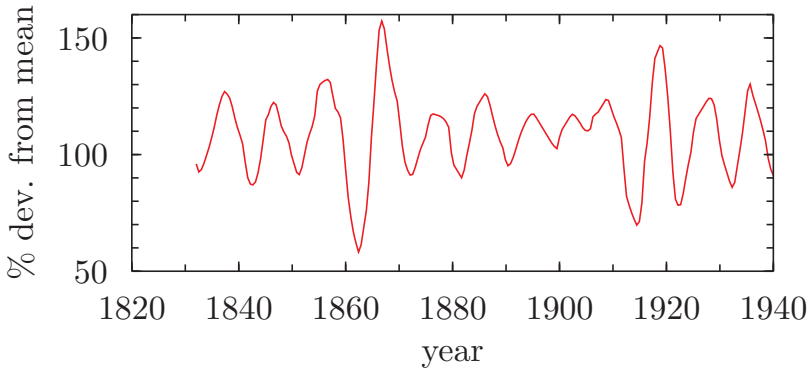


Abbildung 2.1.: Nine-year rhythm of wholesale prices between 1830 and 1940, after Dewey and Dakin (2011).

There are numerous models, which try to describe rhythms in organisms. Some of them are pure mathematical and describe the variables as differential equations, others use functional diagrams of the control theory, again others describe rhythms in word and picture. Goal of these models is, to generalize the results of observations and experiments and to make predictions, which can be tested by new experiments (Johnsson et al, 2015). For quite some time the *molecular basis* of circadian rhythms was studied experimentally, but in the meantime *modeling* has become important again (Bordyugov et al, 2013; Richards and Gumz, 2013; Gebicke-Haerter et al, 2013; Rué and Garcia-Ojalvo, 2013; Dalchau, 2012; Hogenesch and Ueda, 2011; Kaplan and Bechtel, 2011; Liu et al, 2010; Zhang and Kay, 2010; Yamada and Forger, 2010; Hubbard et al, 2009; Roenneberg et al, 2008; Leloup and Goldbeter, 2008; Beersma, 2005).

In modeling biological rhythms, besides the basic circadian system the inputs

and outputs have to be characterized. Light is the most important input. It is absorbed in photoreceptor molecules and leads to a signal, which is transferred to the circadian clock. Models have to take into account also the outputs of the clock. Whereas amplitude and phase of the rhythmic *outputs* differ from the amplitude and phase of the *oscillator*, the period is the same. In addition, these outputs can also feed back to the inputs as well as to the oscillator. In this way the photoreceptor system as an input to the clock can be influenced by feedback from the clock. For example the iris muscle of the eyes in mammals is under circadian control, and circadian leaf movements in plants influence the light flux falling onto the leaf blade.

Furthermore, during longer illumination the systems can adapt in such a way, that the same light pulse can act differently in longer dark periods. All this has to be considered in modeling.

Especially interesting are situations, which are unexpected and special. To this belongs the singular state, which has been talked about already in this book. These situations allow especially well, to test a model critically. If a model can not simulate arrhythmia after a special light pulse, it has to be changed or replaced by another one.

For biological clocks models with feed back are often used (see Witte and Engelmann (2016)). The long periods of circadian clocks originate by time delay during the feedback[1]. As an example we mention the transcription-translation feedback model, in which the time delay is brought about by the transcription, translation, transport and synthesis/degradation of clock components.

One has to take into account also, that organisms possess many cellular oscillators. If they are strongly coupled mutually, the whole system behaves like *one* oscillator. However, organisms might also have different oscillators, which behave differently. Multioscillator models have to take care of this.

The circadian system of man is an example for it. According to older publications it consists of two mutually interacting oscillators (Wever, 1979; Kronauer et al, 1982). One of it controls among others the activity rhythm, the other controls among others the temperature rhythm. Normally both oscillators are mutually coupled and oscillate in phase, but under special circumstances such as in an environment without time cues different periods can show up. More recent publications indicate, that the human circadian system can be better described by a model with more than two oscillators (Nakao et al, 2002; Kalsbeek et al, 2012).

Even in unicellulars a multioscillator system can be found (Roenneberg and Mittag, 1996; Daan et al, 2001). In many animals such as e.g. *Drosophila* several oscillators are used to model the circadian system.

[1] see also figure 2.11 and page 34

In the following we will firstly mention examples for models such as the predator-prey model and a feedback model of Johnsson and Karlsson (1972) and Karlsson and Johnsson (1972) (which is later -from page 36 onward- explained in more detail). Afterward we will describe methods to build models and how to handle the freely available program Scilab (from page 26 onward). In further sections we get to know properties of linear (from page 27 onward) and nonlinear networks (from page 29 onward, details in Witte and Engelmann (2016)). General feedback networks will be described from page 32 onward. The feedback model of Johnsson and Karlsson (1972) and Karlsson and Johnsson (1972) (page 36) was used by Lewis (1999) to derive a similar model which is found on page 41. For coupled oscillator networks see also Witte and Engelmann (2016).

In several of these sections we will try to reach the point of singularity also in the models by using an external pertubation such as a light pulse to induce arrhythmia.

2.1. Examples for models

A good example for oscillations in populations is that between predator and prey. In figure 2.8 it is shown, how the size of the populations varies with time (e.g. years). The prey propagates, and with a certain time delay the population of the predator, which lives on the prey, increases too. As a result more and more prey is taken, and the population of prey decreases. The predator get thus less food and its number declines. This leads to oscillations, which show a characteristic period length (in the case shown about 18 years).

There are numerous examples for such oscillations between populations of carnivores and herbivores in small food chains such as the ecosystem of the Kaibab plateau in Arizona, where the deer are hunted by coyotes and wolfs (see: Kaibab Plateau).

The population variations can be described by the Lotka-Volterra model:

$$\frac{dB}{dt} = f \cdot B - s \cdot BR \text{ and } \frac{dR}{dt} = g \cdot BR - d \cdot R \tag{2.1}$$

B and R are the corresponding population sizes, f is the propagation rate of the prey, s its mortality rate. It depends on R. The propagation rate of predator is g, d its mortality rate. BR indicates the meeting probability of R and B. The rhythms of predator- and prey populations, as shown in figure 2.8, can also be displayed as a phase diagram, whereby the number of predators is plotted against the number of prey (figure 2.8, below).

Further examples for modeling are, among many others, nerve systems, the

coordinated action of enzymes in systems such as the control of glycolysis in yeast, the control of transpiration on the surface of leaves (see Witte and Engelmann (2016))

Important is the following feedback model, which will be described by an example. Although it is a short period oscillator, it is easily observable and can be used for illustration.

2.2. Oscillator model of the balance system

Two Swedish physicists from the University in Lund had studied the gravity-induced pendulum movement of sunflower seedlings. They proposed a model which describes these oscillations in the plants successfully. Figure 2.2 shows, how a seedling of about 6 cm height bends to the side after having laid the pot horizontally for 20 min. After a certain time the tip of the seedling bends,

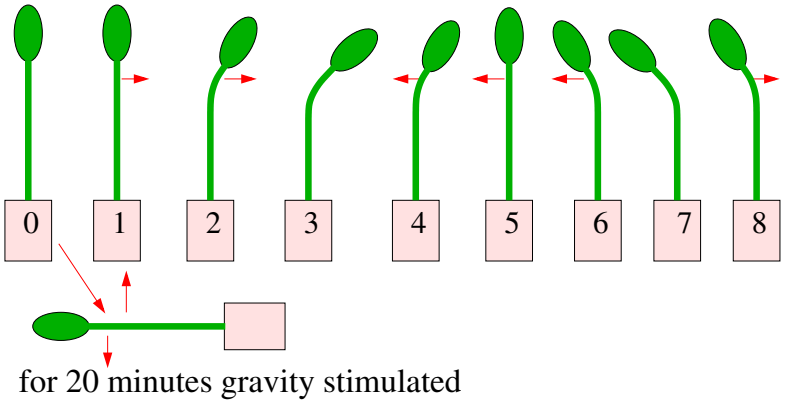


Figure 2.2.: A sunflower seedling is put horizontally for 20 min (0) and afterward back to vertical (1). The gravity stimulates the seedling which begins to bend laterally after putting it back in a vertical position (2). It bends still further (3), until in the state of maximal bending the difference of the hormone concentrations at the two sides has disappeared. A new gravitropic stimulus stimulates the hypocotyl anew. It bends now to the other side (4), overshoots the plumb line (5) and bends back again (6), until a new gravitropic stimulation occurs (7) leading to a counter-reaction (8). A pendulum like movement results. The red horizontal arrows indicate the direction of bending. According to Engelmann and Johnsson 1998.

although the pot has been put upward again. It reaches a state where a counter-reaction is induced by the influence of gravity. The tip bends back, overshoots the plumb line, is again gravistimulated and bends into the other direction

(figure 2.3). In this way a pendulum like movement occurs. Depending on the

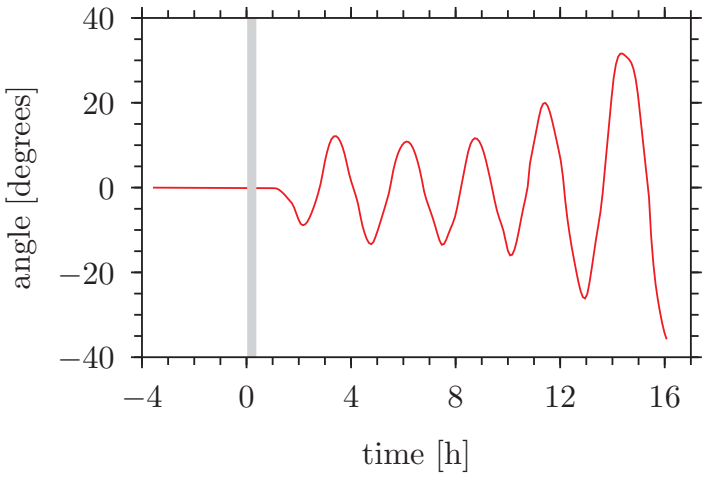


Figure 2.3.: Periodic pendulum movement of a sunflower seedling after a 20 min stimulation by gravity (marked gray).

temperature, the period lengths of the oscillations are in the range of 125 min (at 25 °C) to 265 min (at 15 °C). Responsible for the bending are gravity stimulations by small particles in the cells of the plants, which lead to shifts in growth hormones (see Engelmann: Growth).

The model is shown in figure 2.4. It is a *feedback* loop, in which a reference

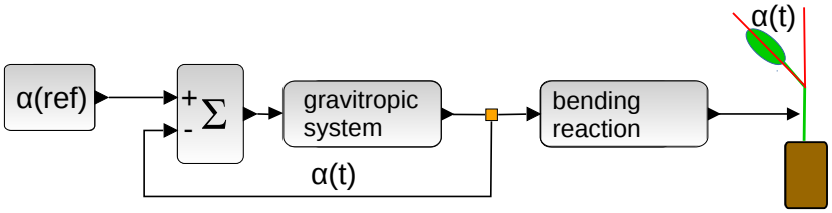


Figure 2.4.: Feedback model of pendulum movement. A reference value $\alpha(ref)$ (vertical growth) is compared with the actual value $\alpha(t)$ (bending of the plant). If the position of the tip of the plant deviates from the plumb line, an error signal in the balance system is amplified, weighted and compared again with the reference value after a time delay (feedback loop). Choosing the parameters in a suitable way, the system oscillates and simulates fairly well the to- and fro-movements of the sunflower seedlings. See Johnsson (1971).

value (vertical growth) is compared with the actual value (bending of the plant). If it deviates from the plumb line, an error signal is amplified, weighted and the

signal compared again with the reference value after a time delay. Choosing the parameters in a suitable way, the system oscillates and simulates fairly well the to- and fro-movements of the sunflower seedlings across the plumb line. With this model one can study the results of a second gravitational stimulation presented at different times after the first one.

When Anders Johnsson and Hage Karlsson got to know the studies on the *Kalanchoe* flowers, they tried to simulate also these oscillations with their feedback model and were quite successful in doing so (figure 2.5). They were also able

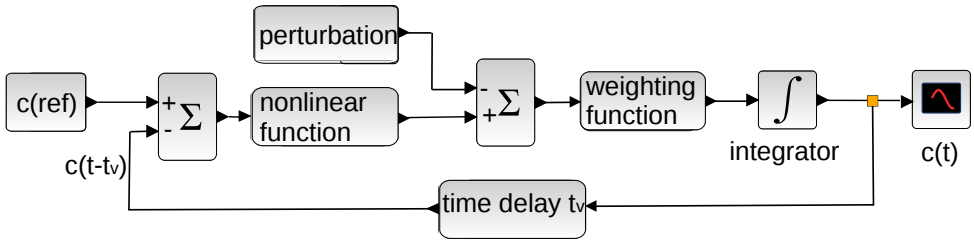


Figure 2.5.: Feedback model of the *Kalanchoe* petal movement. A reference value $c(ref)$ is compared with the actual value $c(t)$, which could be the concentration of a substance. In the first subtractor the difference is formed, amplified by a nonlinear function and weighted in a weighting function (see figure 2.17); after integration and a time delay t_v the signal $c(t - t_v)$ is again via a feedback loop compared with the actual value $c(ref)$ in . Perturbations by e.g. a light pulse are perceived at the second subtracter. The oscillation $c(t)$ is shown in . After Johnsson and Karlsson (1972); Karlsson and Johnsson (1972). See also figure 2.16.

to simulate the effects of temperature- and light pulses. Choosing a suitable entrance point into the feedback loop for the disturbances, the phase response curves (see section 1.2) of the *Kalanchoe*-flowers could also be well modeled.

2.3. Working with models

A number of useful programs are available for setting up models. They can be used to build a network out of functional units which describe the connection between systems for special tasks (e.g. synthesis of a substance or comparing concentrations) and the functional sequence between them. Subsequently, based on these models, simulations can be performed. Then it is checked whether they agree with the experimental results and whether they can be used for predictions.

One of these programs is *Scilab*. It is an 'open source' program and can be downloaded for free from the Internet for various operating systems (e.g.

Linux, Windows or Mac) under www.scilab.org. To become familiar with it, we recommend the book by Campbell et al (2006). In chapter 3 (modeling and simulation in Scilab) different types of models and the tools for the simulations are explained. Before, the properties of the program, the data structure and representation, import and export of data, external routines are treated. For us the more important second part of Scilab is Xcos[2] (called before Scicos). It offers a graphic block diagram editor, which allows to construct dynamical systems. They in turn can be used to perform simulations. Numerous modules (blocks) exist already in the xcos palettes[3]. In chapter 10 of the book, examples are presented, among them the predator-prey model.

To get to know Xcos, one should begin with the demonstration examples (click the Scilab Demo button **?** in the menu).

2.4. Properties of linear networks

To explain general properties of model building it is reasonable to differentiate between *linear* and *nonlinear* networks. For linear networks the superposition principle is valid: The overall signal can be ascertained by subdividing the stimulating signal and adding each of the corresponding individual signals at the nodes (superposition principle). The same would happen, if the stimulating signal would not have been subdivided and the overall signal would have been directly produced.

If for instance the 6 molar concentration of a substance is doubled, it does not matter, whether it is subdivided in 1 molar and 5 molar, each part doubled and afterward added (2 times 1 molar and 2 times 5 molar gives 12 molar) or whether it is directly doubled (6 molar times 2 results also in 12 molar). This does, however, not work, if the network is saturated at a 5 molar concentration. The result would be 2 times 5 molar, namely 10 molar. Here the superposition principle is not valid and the network would be *nonlinear*.

Linear networks are easier to analyze, but most of the networks occurring in nature are complicated and nonlinear. They can be described, however, by „linearizing around a working point“ as simple linear networks, which can be calculated more easily; by using them one can at least produce approximations.

For linear networks the following relation is valid

$$y(x_a + x_b) = y(x_a) + y(x_b) \tag{2.2}$$

and it can be described as a linear differential equation system of first order, as

[2] the files with the functional diagrams end with xcos. The compressed files end with zcos

[3] Therefore the user does not need to construct new modules from scratch, although it can be done, if necessary. These modules can be reused.

differential equations in form of a matrix, or as *one* differential equation of higher order (see in Witte and Engelmann (2016)):

A linear net with two variables x_1 and x_2 can be exemplified in the following way (where second derivatives are introduced):

$$\frac{dx_1}{dt} = a \cdot x_1 + b \cdot x_2, \quad \frac{dx_2}{dt} = c \cdot x_1 + d \cdot x_2 \tag{2.3}$$

Dissolving the right equation for x_1 and introducing it in the left equation, we receive

$$\frac{d^2x_2}{dt^2} - (a+d) \cdot \frac{dx_2}{dt} + (a \cdot d - b \cdot c) \cdot x_2 = 0 \tag{2.4}$$

In figure 2.6 this function is presented (how to derive the function network from the equation is described in Witte and Engelmann (2016)).

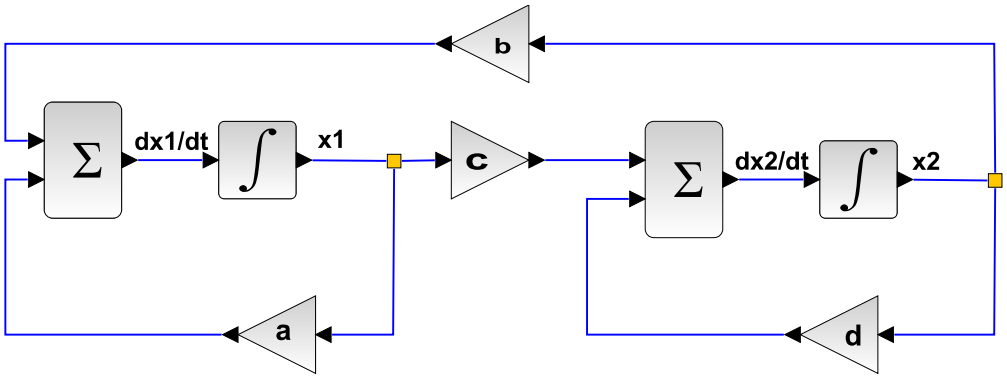


Figure 2.6.: Functional diagram of a linear network, whereby the left part corresponds to the left term in equation 2.3 and the right part to the right term in equation 2.3. a, b, c, and d are factors, see page 29.

In a linear net a *singular point around the zero point* can occur: For the stationary case the derivative should be zero at the singularity

$$X' = 0 = A \cdot X \tag{2.5}$$

in which X contains the state variables $[x_1, x_2, x_3 ... x_n]$ for a n-degree system. For these X-values an oscillation is not possible, i.e.

$$\frac{dx_1}{dt} = 0 = ax_1 + bx_2, \frac{dx_2}{dt} = 0 = c \cdot x_1 + d \cdot x_2 \tag{2.6}$$

Possible solutions are either

1. $x_1 = 0$ and $x_2 = 0$ (trivial case) or
2. $a \cdot d - b \cdot c = 0$, that is

$$x_2 = -\frac{a}{b}x_1 = -\frac{c}{d}x_1 \tag{2.7}$$

That is, no special values are required for x_1 or x_2. Instead x_1 and x_2 have to stay in a certain ratio, which is determined by the system parameter a, b, c, d. It follows, that a to d (independent of x_1 and x_2) have also to stay in a certain ratio.

In this way -at least extreme- oscillations in a predator-prey system can be prevented. This could hold also for oscillations in the economy, thereby perhaps avoiding crises due to strong deviations of the amplitudes.

2.5. Nonlinear nets, singularity point, predator-prey model

The general description of a nonlinear net occurs by a nonlinear differential equation with a nonlinear function f:

$$X' = f(X) \tag{2.8}$$

where X contains state variables, e.g. x_1 and x_2 in figure 2.7. The stationary case (singularity point) would be:

$$X' = 0 \tag{2.9}$$

An example for the nonlinear net is the predator-prey model (see also page 23), which can be described by the equation

$$\frac{dx_1}{dt} = a \cdot x_1 - b \cdot x_1 \cdot x_2, \quad \frac{dx_2}{dt} = c \cdot x_1 \cdot x_2 - d \cdot x_2 \tag{2.10}$$

whereby x_1 represents the number of prey and x_2 the number of predators. The propagation rate of the prey is a, the killing rate of the prey is c, the birth rate of the predator is b, the death rate of the predator is d.

The functional diagram is shown in figure 2.7 and the changes in population size of prey and predator in figure 2.8 , upper curves. The phase diagram, which plots size of the prey population against the size of the predator population, is shown in the curve below.

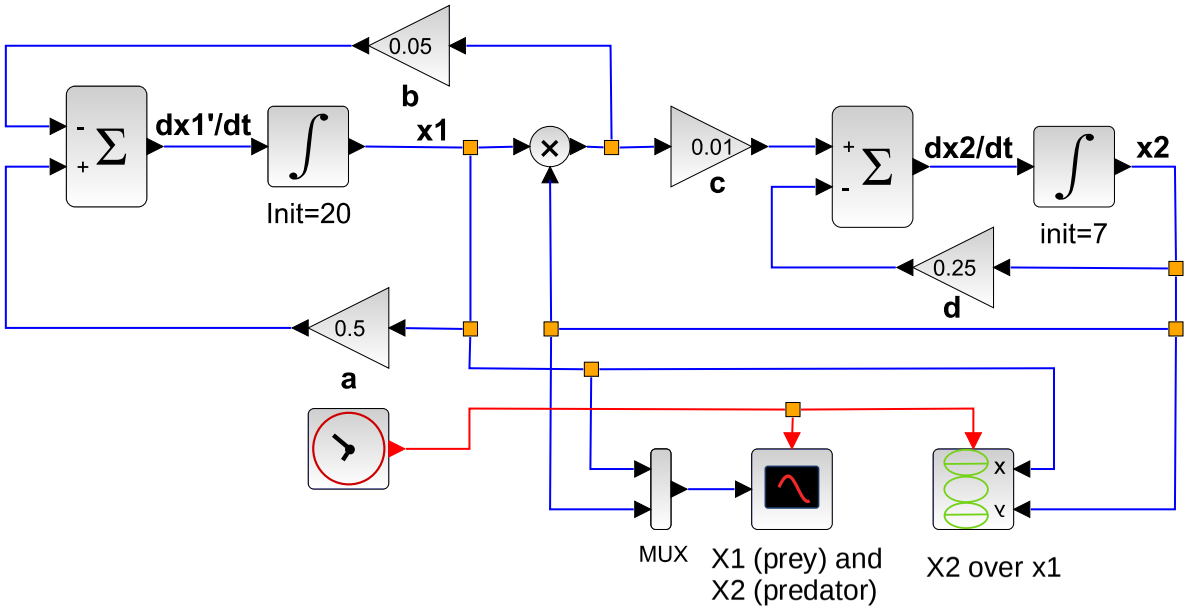


Figure 2.7.: Functional diagram of the predator-prey model of a nonlinear network, whereby the left part corresponds to the left term in equation 2.11 and the right part the right term in equation 2.12. Propagation rate of the prey is a, the one of the predator is b, the death rate of the prey is c, the one of the predator d. Simulation examples for $a = 0.5$, $b = 0.05$, $c = 0.01$, $d = 0.25$, $int = 20$ for the start valued of the prey and $int = 7$ for the start values of the predator (as indicated in the integrator). CLOCK_c is a pulse generator for a uniform presentation of the time course for prey and predator; the multiplexer MUX allows two entrances into the CSCOPE further to the right, and the CSCOPXY even further to the right enables the output of two signals, one for the prey (x1), the other for the predator (x2), as shown. The curves produced by the simulation and the phase diagram are shown in figure 2.8

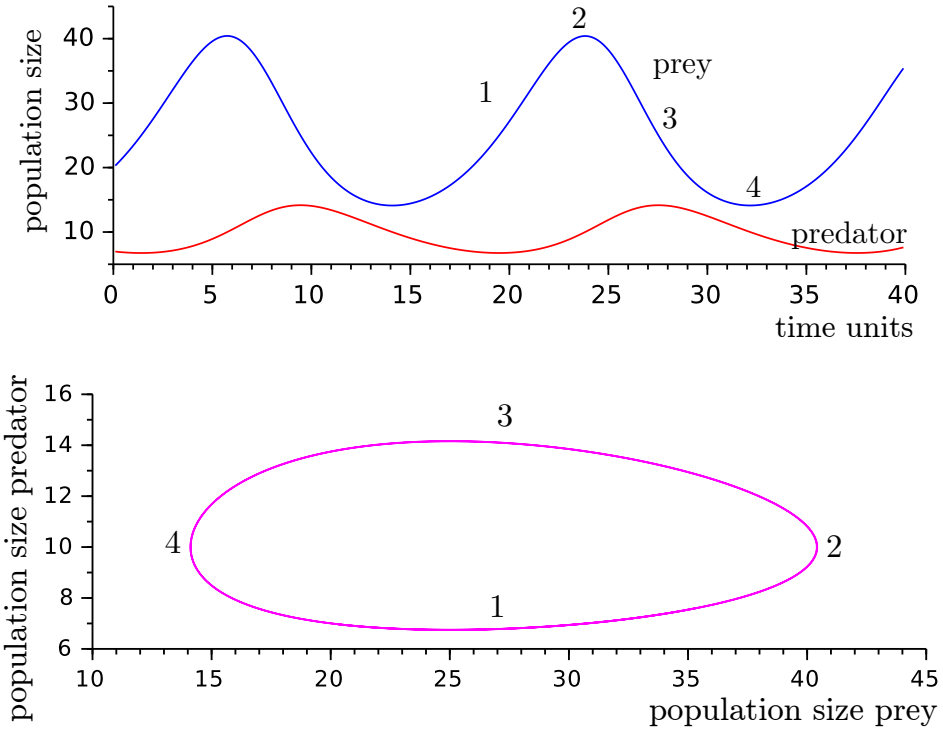


Figure 2.8.: Alterations in the population size of prey (blue) and predator (red) after simulation with the model in figure 2.7 and (bottom) phase diagram for rhythmic variations between the number of predators and prey. The population size of the prey first increases (1), and -time delayed- the population size of the predator (2). Afterward the size of the prey population decreases (3) and finally also the predator population (4).

For singularity points holds:

$$\frac{dx_1}{dt} = 0 = a \cdot x_1 - b \cdot x_1 \cdot x_2 = x_1(a - b \cdot x_2) \tag{2.11}$$

$$\frac{dx_2}{dt} = 0 = c \cdot x_1 \cdot x_2 - d \cdot x_2) = x_2(c \cdot x_1 - d) \tag{2.12}$$

The solution is

1. x_1=0 and x_2=0 (trivial case, both populations = 0, no dynamics) or
2. $x_1 = \frac{d}{c}$ and x_2=$\frac{a}{b}$

To reach the point of singularity, the ratios of the coefficients a to b have to equal the value of x_2 and the ratios of the coefficients d to c have to equal the value of x_1, i.e. the ratio of propagation rate of the prey a and the propagation rate of the predator b must be the same as the death rate of the predator d and the death rate of the prey c.

If the initial values according to equation 2.11 and 2.12 are changed in such a way, that the point of singularity is reached, i.e. $a/b = x_2 = int = 10$ and $d/c = x_1 = int = 25$ (initial values for predator and prey), the number of predators and prey stays constant (see figure 2.9).

2.6. General feedback networks

In general feedback networks a reference value (e.g. the concentration $c(t)_{Ref}$) is compared with the actual value (e.g. the actual concentration $c(t)$), a correcting quantity derived from the difference (e.g. further synthesis of a desired substance) and then compared again with the reference value. Two situations have to be distinguished:

1. the forward branch F_V, in which the difference between actual and desired value is processed (e.g. to change a concentration), and
2. the backward branch F_R, in which the actual value is fed back to be compared with the reference value (e.g. to delay and reduce a concentration).

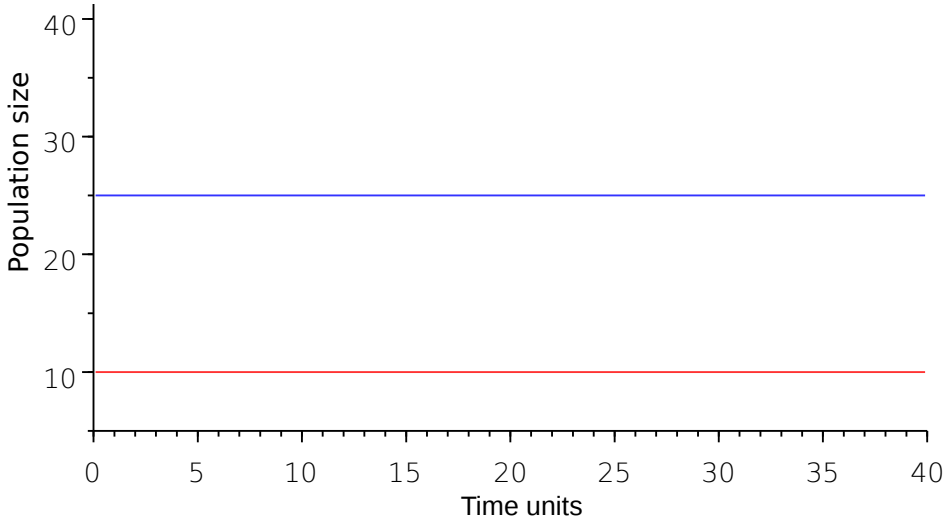


Figure 2.9.: To reach the singularity point, the ratios of the coefficients a to b (see page 29) have to equal the value of x_2 and the ratios of the coefficients d to c have to equal the value of x_1. In this case *no* oscillations occur in the predator-prey population, the size of the population of the prey (blue) and of the predator (red) in the simulation in figure 2.8 *do not change.* In nature this would correspond to an equilibrium.

A graphic illustration of this model shows figure 2.10). To show oscillations, the following conditions must be fulfilled:

If a signal z is given to the input (e.g. a pulse like change of the reference concentration), the back fed signal f_{Ra} (e.g. the concentration at the input to be compared with) has to be at least as large as the input signal z, to avoid damping out of the system.

If the model is not described in the time domain (e.g. by differential equations), but in the frequency domain (e.g. by a Fourier transformation), two conditions follow from what was said before :

1. The value of the product of forward- and backward-amplification $|F_V \cdot F_R|$ has to be larger than 1.

2. The phase of the back feeding signal (e.g. a sinus oscillation) has to equal the input signal or a multiple of its period length.

Further necessary conditions for *all* networks are:

- The oscillation occurs always around a singular point.

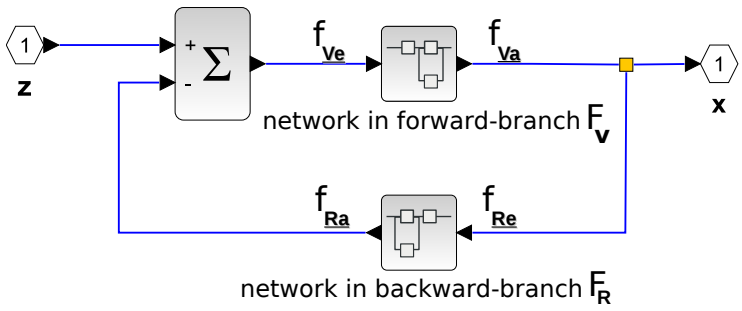


Figure 2.10.: General feedback model for the generation of an oscillation. In the forward branch F_V the signal is after subtraction in Σ influenced in a superblock (which can consist of several parts) and afterward changed again in a superblock in the backward branch F_R, until it finally reaches the subtractor again to pass the loop anew.

- In the case of *linear* networks the singular point is located at X = 0, i.e. oscillations take always place around the zero point, if the average value of z with respect to time is zero; otherwise the oscillations will occur around the mean value (e.g. c_{ref})

In the case of a simple *Feedback model with one delayer only* a delay is used only in the feedback branch, and an impulse is put on the input (Witte and Engelmann, 2016). Form and size of the impulses are not changed, but at the comparison at the input its polarity is altered. This leads to a negative impulse. in the next turn it becomes positive, because its polarity changes again. Altogether the period length becomes twice as large as the delay time (upper curve in figure 2.11).

If in this simple feedback model an *Integrator* is added to the forward branch (figure 2.12), the signal is delayed by a fourth of the period length. Altogether the period is *four times* as large (bottom curve in figure 2.11, see also Witte and Engelmann (2016)).

2.7. Network synthesis-example with point of singularity

A modified van der Pol Oscillator (after Wever) with the differential equation

$$x'' + 0.5 \cdot (x^2 + x^{-2} - 3)x' + (1 + 0.6x)x = z \tag{2.13}$$

and the perturbing signal z is shown in the functional diagram in figure 2.13.

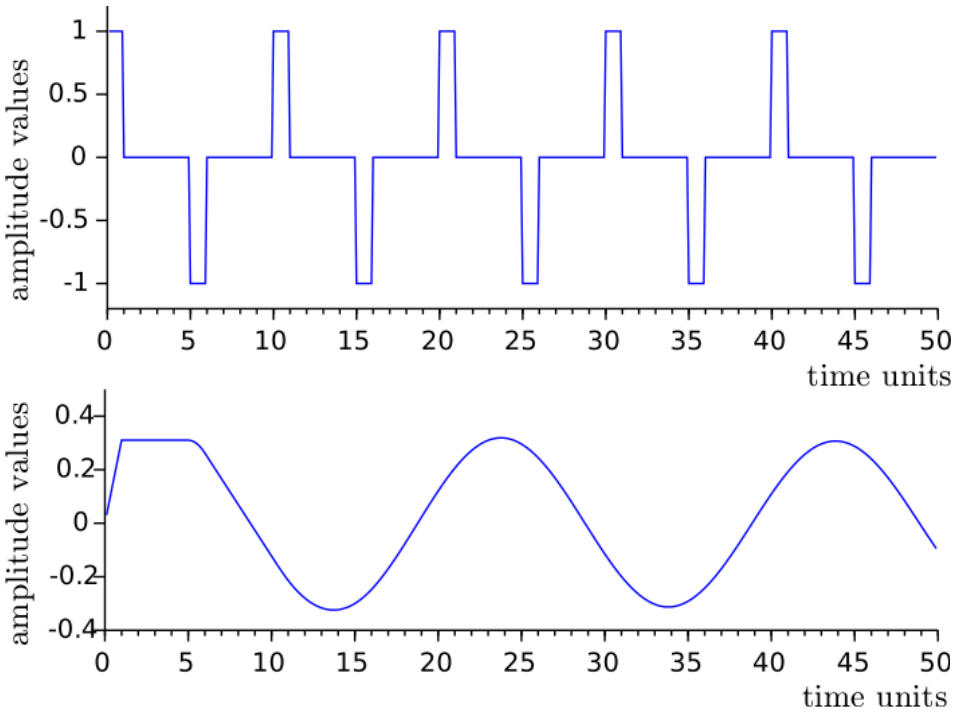


Figure 2.11.: Top: Output signal of a simple feedback model with only one time delay similar to figure 2.12. The period length is twice as large as the delay time of the time delay element.
Bottom: If an integrator is added to the forward branch of this simple feedback model, the signal is filtered and further delayed by a quarter of the period length. By and large a sinus oscillation results and the periods are four times as large as the delay times.

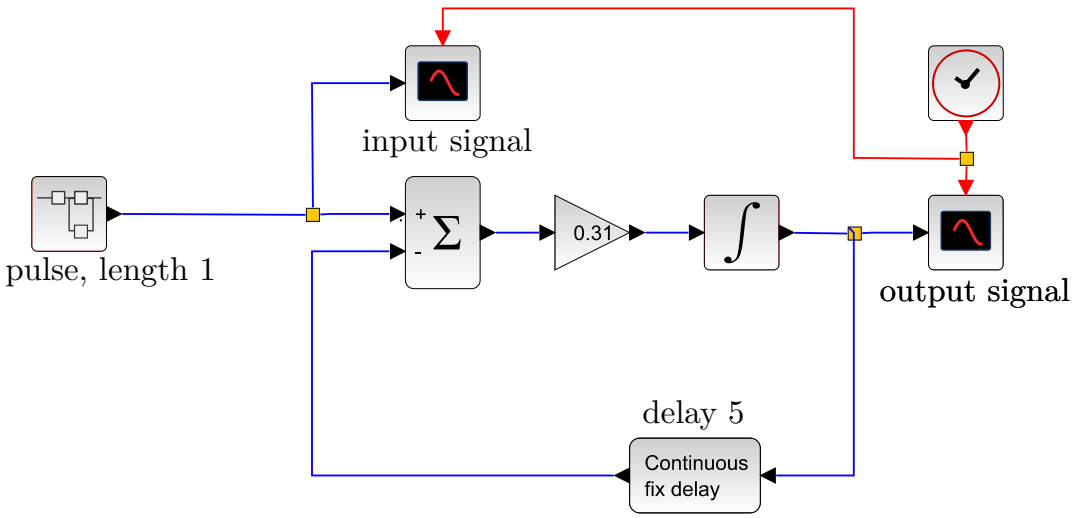


Abbildung 2.12.: Example for a feedback model with integrator and delayer (Continuous fix delay), which delays by 5 time units (e.g. hours, days). Time courses of input- and output signals shown in the CSCOPE elements. The timing of the output occurs via the CLOCK_c element.

How this diagram is derived from the equation is explained in Witte and Engelmann (2016).

If a perturbing pulse is given to the left integrator, an oscillation occurs as pictured in figure 2.14, where x is plotted against time. If x' is plotted against x, the phase diagram in the lower part of figure 2.15 results.

2.8. Feedback model of Johnsson and Karlsson

The feedback model of Johnsson and Karlsson was mentioned already in section 2.2. It is shown in figure 2.16 as a functional diagram, and in figure 2.17 at the left the functional block F and at the right the functional block HeV.

The usefulness of the model was tested by applying a single light pulse perturbation at various phases of the rhythm of the *Kalanchoe* petal movement. The induced phase shifts of the rhythm could be simulated by the model. Likewise the phase shifting effects of temperature pulses were successfully simulated with the model.

A critical test of the model is the experimentally induced arrhythmia by a very special light pulse at a certain phase, that is, to send the oscillator in the point of singularity. The following parameters were used for this purpose:

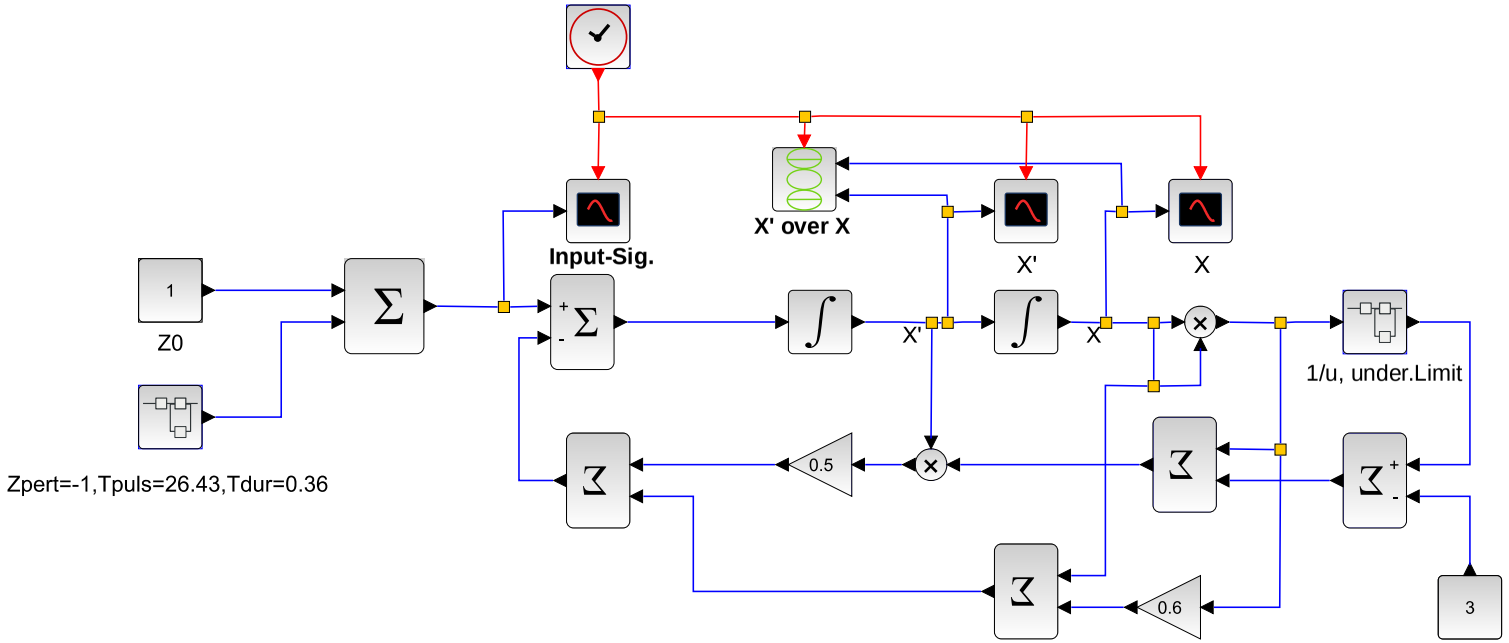


Figure 2.13.: Van der Pol oscillator, which was brought by a perturbing pulse Zpert in a non oscillating condition (point of singularity, see figures 2.14 and 2.15). This functional model is derived in Witte and Engelmann (2016) from the Van der Pol equation and explained there in more detail. It has been modified in such a way, that (in Zpert) a dark pulse with the strength -1 at the time Tpuls = 26.43 was given for a certain duration (here 0.36 time units) during the continuous light (Z0=1). The signal after the first adder can be viewed as the input signal (EinSig) in the CSCOPE element . The time courses of the parameter x (figure 2.14) and its derivative x' (not shown) can be followed in two further CSCOPE elements (top right) and displayed as a phase diagram x' as a function of x in the element CSCOPXY (see figure 2.15). After Pedersen and Johnsson (1994) and see Witte and Engelmann (2016).

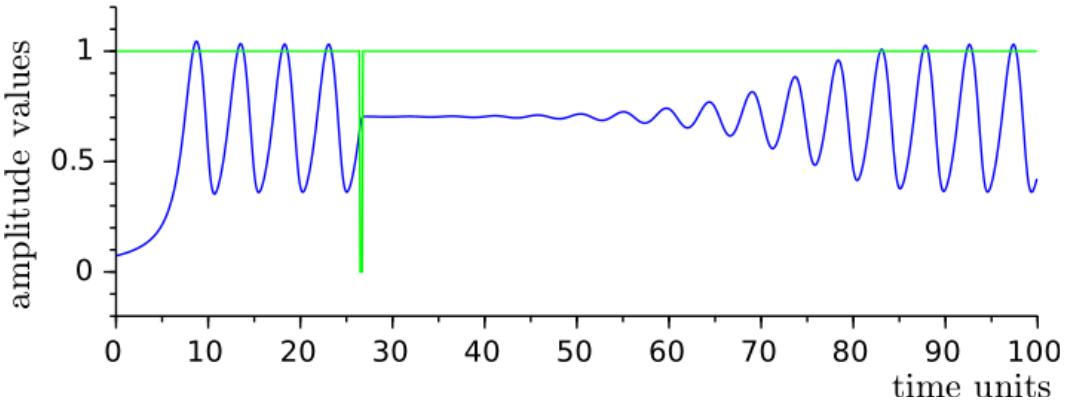


Figure 2.14.: Output signal x in the modified van der Pol oscillator after Wever (see figure 2.13). After a perturbing pulse the system has been brought into a singular state for about 10 time units. Afterward the oscillator begins to oscillate again.

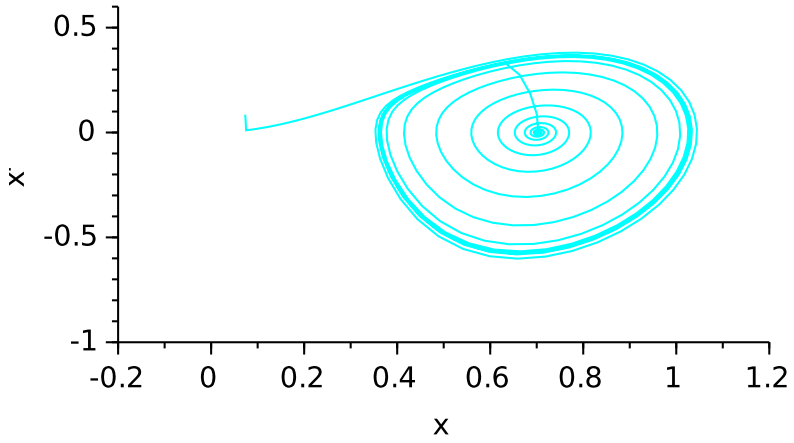


Figure 2.15.: If x', the first derivative of x, is plotted against x, a phase diagram is obtained. The fast move into the point of singularity and the slow return to the limit cycle is seen. The onset of the oscillation is marked by a stroke at the left of the limit cycle, but is irrelevant here.

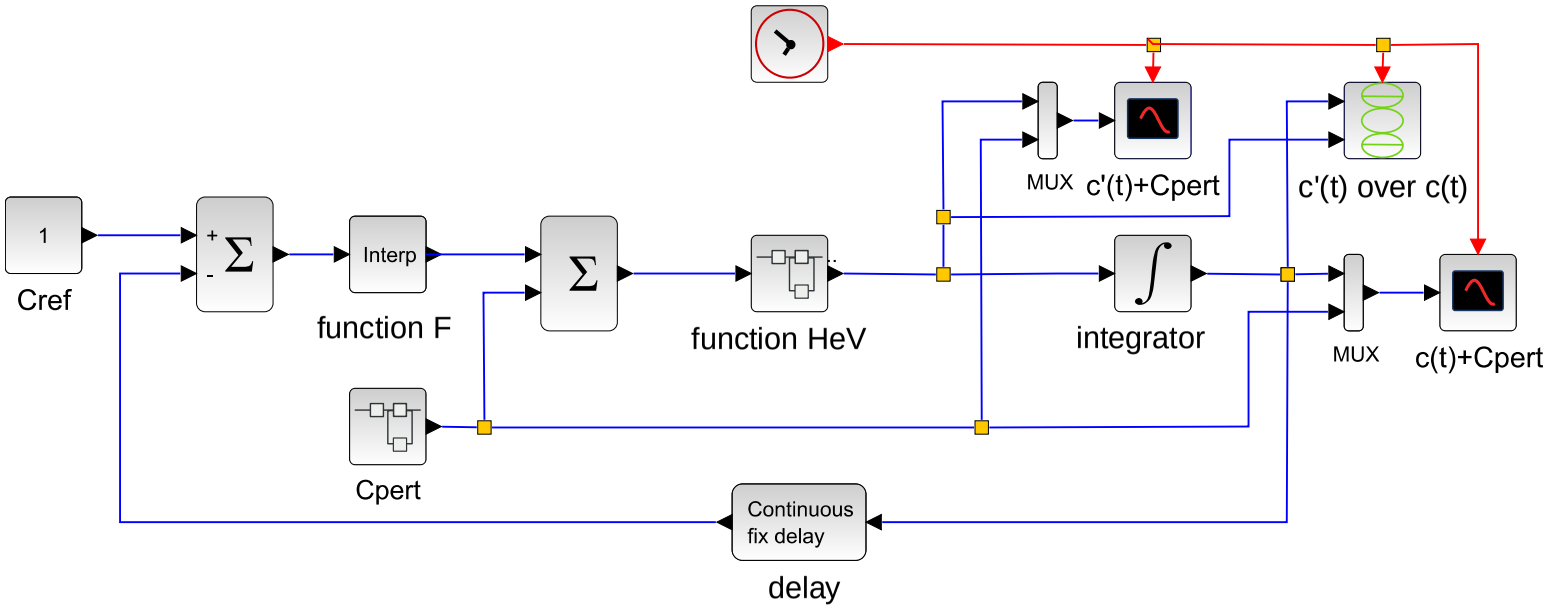


Figure 2.16.: The feedback model of Johnsson and Karlsson (see also figure 2.6) is displayed with Scilab in form of a functional diagram. In figure 2.17 the functions F (left) and HeV (=H-function with external delay, in superblock) are shown. Via Cpert perturbations k (e.g. a light pulse) can be administered between the function F and the function HeV (the signal will be transferred via the multiplexer MUX (top) together with the $c'(t)$ signal to the output (top) and displayed as a curve/pulse. Via a further MUX (right) it is, together with the $c(t)$ signal, brought to an additional output (right). The $c(t)$ signal is in the continuous-fix-delay element delayed and fed back to the subtractor (top left), where it is compared with the Cref value. Then it traverses again the loop. $c'(t)$ over $c(t)$ is shown in CSCOPXY (top right) as a phase diagram (see lower part of figure 2.18) and also brought to the right output. After Johnsson and Karlsson (1972); Karlsson and Johnsson (1972).

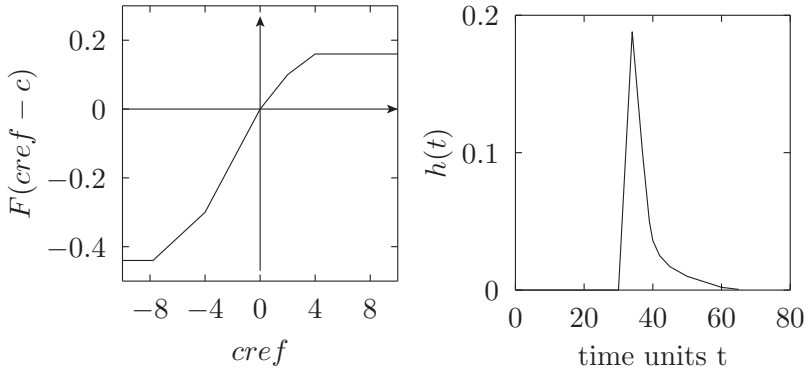


Figure 2.17.: Functional blocks of the feedback model of Karlsson and Johnsson. To the left the function F and to the right the function H is shown (in figure 2.16 these functions are represented by and). The function F shows, how the output of cref $-$ c depends on the feedback signal cref. It has an upper and a lower limiter, which prevent too high amplitudes of the oscillation. The function H (in Fig 2.16 HeV with delay) presents a kind of memory: What happened until the 30th time unit is not remembered, what happened around the 32nd time unit is optimally remembered, and afterward the memory is declining until it is completely lost from the 60th time unit onward. After Johnsson and Karlsson (1972).

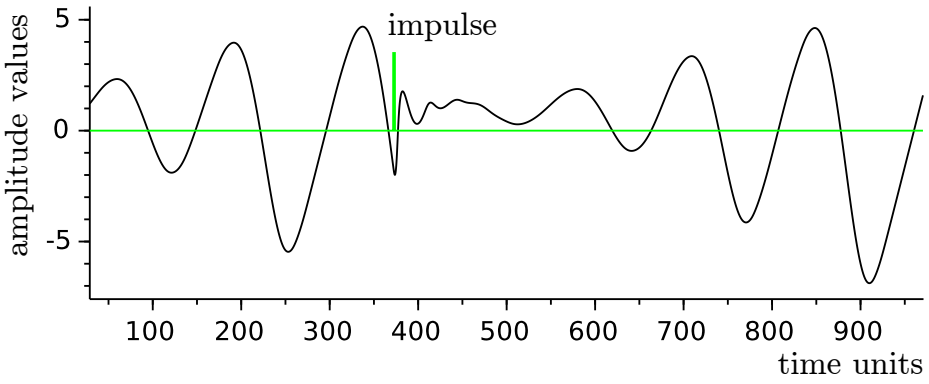


Figure 2.18.: Examples for (only briefly existing) arrhythmia by an impulse (green); results of simulations of the feedback model of Johnsson and Karlsson in figure 2.16.

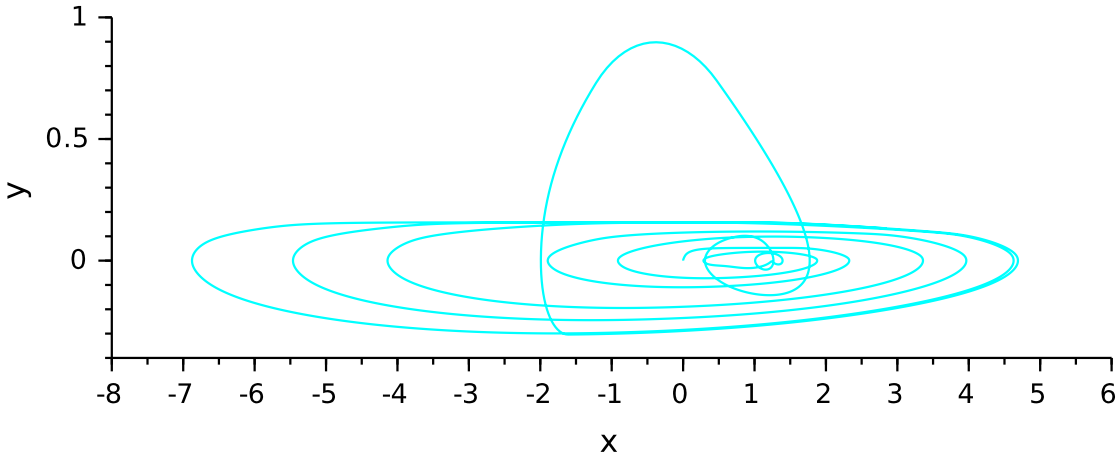


Figure 2.19.: Phase diagram ($y = x'$ as a function of x) of simulations of the feedback model of Johnsson and Karlsson in figure 2.18.

- perturbing pulse after 372 time units with 3.5 amplitude units
- delay of 30 time units
- period length about 140 time units.

The period amounts to a bit more than four times the delay time as a consequence of feedback and time delay (see page 34 and Witte and Engelmann (2016). The curve for $c(t)$ is shown in figure 2.18 . Compare it with the experimentally obtained curve in figure 1.7. If $c'(t)$ is plotted against $c(t)$, the phase diagram in the lower part of figure 2.19 is obtained.

2.9. Feedback model of Lewis

Die New Zealand Weta *Hemideina thoracica* is a night active insect and belongs to the family of the Orthoptera. The locomotor activity is controlled in a circadian way even under constant conditions for months. Lewis at the university of Auckland has studied with his team intensively the circadian rhythm of these animals and has developed a model (see figure 2.20 and Lewis (1999); Gander and Lewis (1979)), which is based on the feedback model of Johnsson and Karlsson (see figure 2.16). A time-delayed signal (current value) of the output (substance X) is fed back and compared with a reference value and the difference between both values determines the synthesis of a substance X. X is partly lost by diffusion via membranes (loss block). The output signal (substance X) is

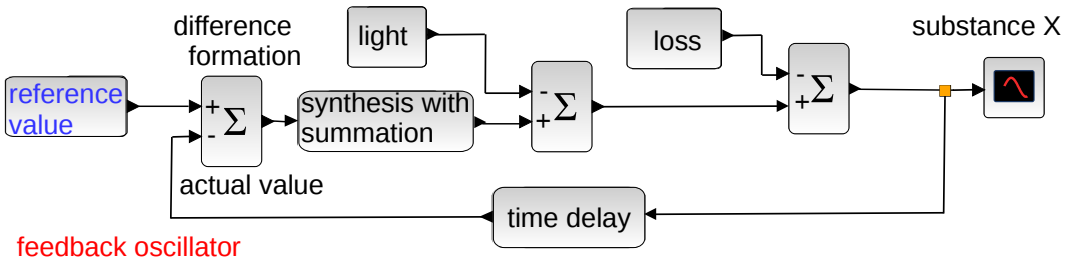


Figure 2.20.: In the feedback model of Lewis a reference value is compared with the actual value and the difference determines synthesis. Disturbances by light influence the oscillation. Loss of the substance X via membranes is a further element of the system. The time course of the concentration of X can be followed in CFSCOPE. The time delayed feedback of the current value is compared with the reference value by forming the difference, before the loop is passed again. See Lewis (1976); Gander and Lewis (1979).

time delayed and fed back as the actual value. X varies in a circadian way and controls daily processes such as the locomotor activity.

The model is thus similar to a refrigerator: It cools, if the reference temperature (e.g. 6 °C) is exceeded and the temperature sensor switches on. Normally the temperature drops below the reference value. Heat enters the refrigerator even with closed doors and the temperature increases slowly, until the compressor starts working. Perturbations (opening of the refrigerator) influence the oscillations.

The model of Lewis simulates successfully the effect of light- (Lewis, 1976) and temperature pulses (Gander, 1976, 1979) on the rhythm. It can simulate further experimental observations such as the *splitting* of the rhythm in several components, spontaneous changes in period length under free run (figure 2.21) and so called aftereffects, if it is assumed, that the circadian system consists of two rhythm generators, which are mutually coupled (Lewis et al, 1991) or that we are dealing with a population of weakly coupled oscillators (Christensen and Lewis, 1983, 1982; King, 1988). Such coupled oscillator networks can be displayed with Scilab/Xcos in such a way (functional diagram in figure 2.23), that the temperature- and light effects are simulated and the amplitude of the oscillation heavily affected (see figure 2.22). More details in Witte and Engelmann (2016).

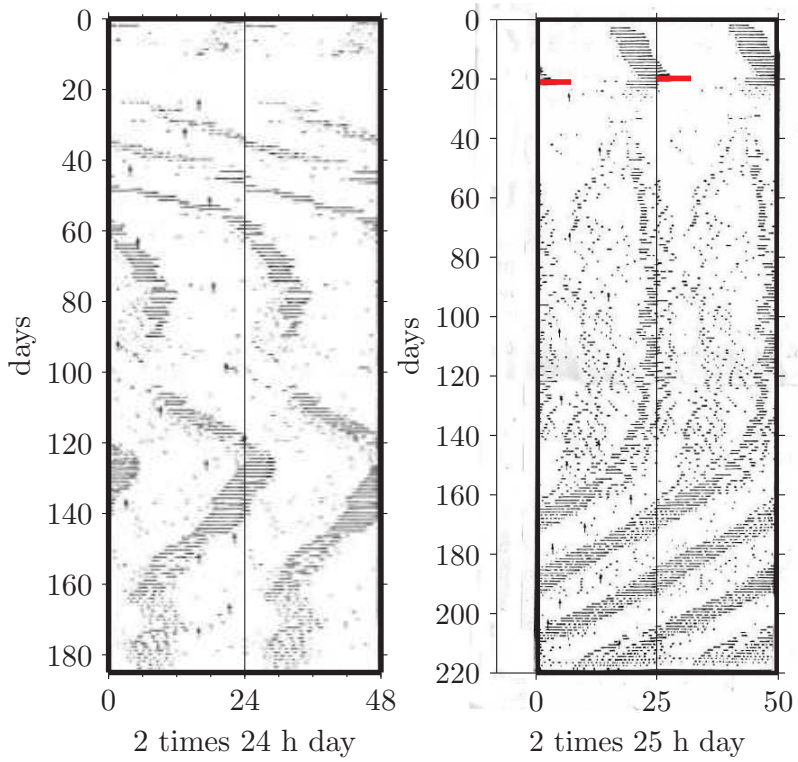


Figure 2.21.: The actogram of a Weta shows spontaneous changes in period length (left) and splitting (right, splitting induced by an 8 h light pulse, marked red; activity plotted here in a 25 h frame!). Double plots: Activity of the first and second day are displayed next to each other, below the activity of the second and third day and so on. Arrows indicate feeding times. From Christensen and Lewis (1982).

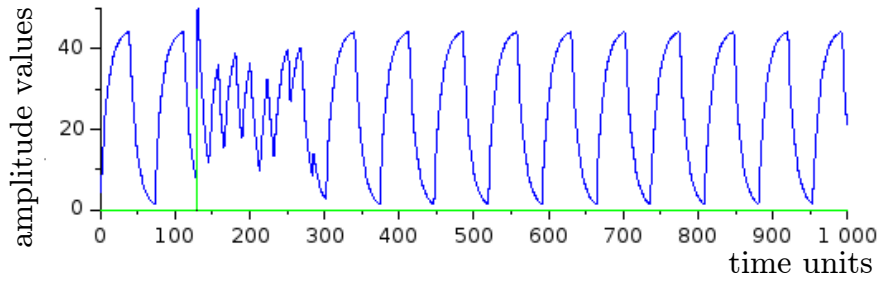


Figure 2.22.: The Lewis feedback model was used to simulate the disturbance of the activity rhythm of the Weta (blue curve) by a light pulse (green vertical line) with Scilab. After Lewis and Saunders (1987) and Christensen and Lewis (1983).

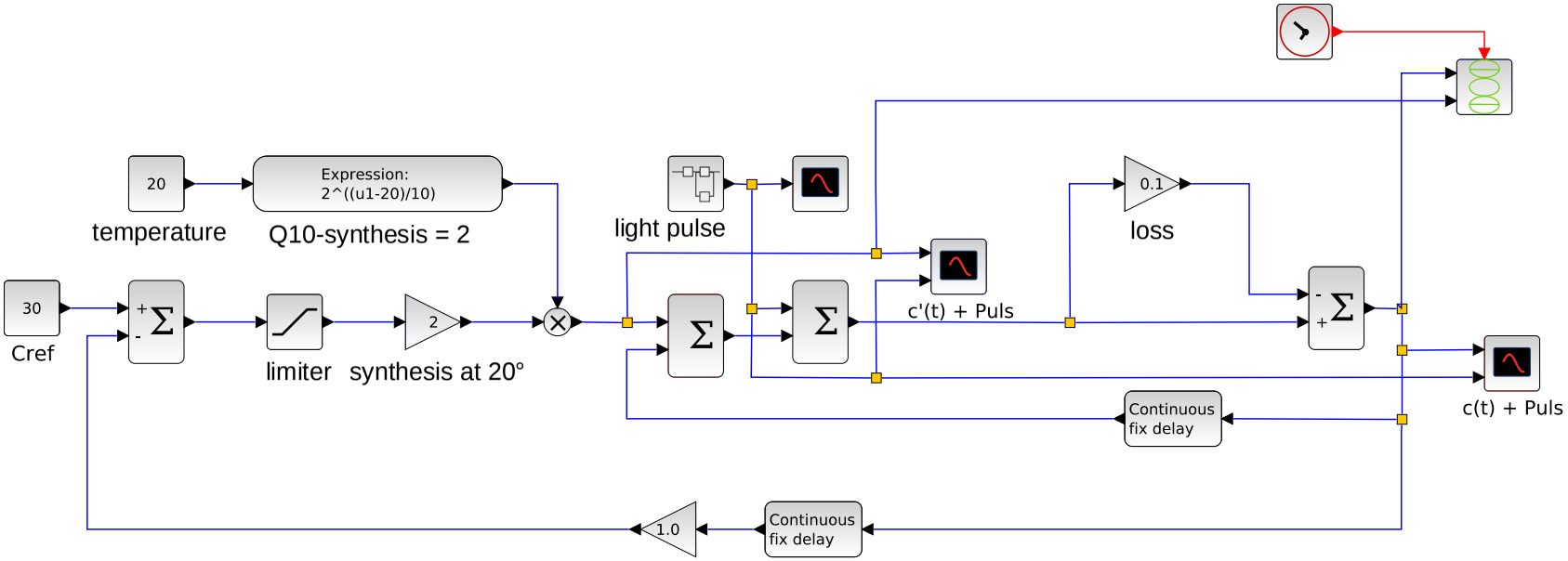


Figure 2.23.: The Lewis feedback model (figure 2.20) for the activity rhythm of the Weta was modified in order to show besides the light effects (top center) also the temperature effects (top left) on the synthesis of the substance X with a $Q_{10} = 2$. The outputs represent the position of the light pulses and the time course of the curves $c(t)$ and $c(t')$. A limiter in the functional diagram prevents a too large synthesis of the substance X. $c'(t)$ is shown in CSCOPXY as a function of $c(t)$ and could be used to view the phase diagram (figure in Witte and Engelmann (2016)). After information in Gander (1976); Lewis (1976).

3. Eclosion rhythm of *Drosophila* and arrhythmia

In this chapter we will treat the eclosion rhythm of *Drosophila*, which Arthur Winfree used to describe the point of singularity.

The fruit-fly *Drosophila* is one of the preferred animals of biologists. They are easily and rapidly reared and propagated, there are many mutants and the genome is completely known since several years. As most other organisms, the fruit-fly expresses daily rhythms, two of which are described in a book (Fliegende Uhren, Engelmann). One of the responsible oscillators controls *eclosion* of the flies out of the pupal case, after the maggot of the last larval stage has pupated and turned into a fly (figure 3.1 and 3.2).

Figure 3.1.: After fruit-flies have passed several larval stages and grown in size, they pupate (left, see also video Drosophila). In the pupal case the maggot is rebuild to a fly (metamorphosis). With a balloon at the top of the head the fly opens the flap (operculum) of the pupal case and emerges (right, see also video Schlüpfrhythmus Drosophila).

This *metamorphosis* of a larva into a fly in the puparium takes a few days. Finally the new fly is ready to eclose from the pupal case. But the fly waits until an internal daily clock tells that it is now time to emerge. A balloon protrudes at the forehead with which the pupal case is opened at a pre-formed place (*operculum*), and the fly can escape from the puparium. Other flies, which have developed far enough, emerge also around this time, namely in the early morning. Later

Abbildung 3.2: Fruitfly *Drosophila melanogaster* deposits eggs (photography by Dennis Pauls and Christian Wegener, Würzburg). About 2.5 mm long.

and in the following night no flies emerge. On the next morning green light is given again for those flies, which are now developed far enough. This continues for several days, until all flies have emerged. Figure 3.3 shows eclosion in time windows during a week.

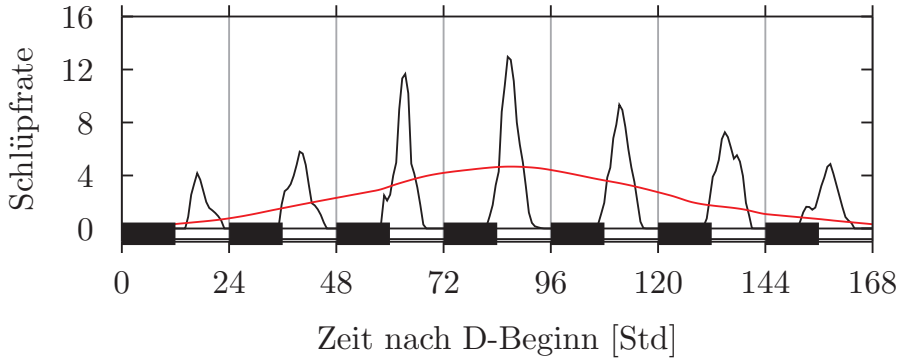


Figure 3.3.: Fruit-flies emerge preferentially in time windows in the morning only (black curves). Later and in the following night no flies eclose. On the next morning green light is given again for the flies, which are now developed far enough. This continues for several days, until all maggots have turned into flies and these are ready to emerge. If the maggots are reared under constant conditions (temperature, continuous light), the flies would eclose as indicated by the red curve: At the begin none of the flies is yet ready to eclose, after a few days many flies eclose, and afterward eclosion rate declines, until all animals have emerged. But under these conditions there is no rhythmic eclosion. Modified after Maier (1973); Winfree (1988).

We are dealing with a population rhythm, since it can be seen only by recording the eclosion of a large number of flies. Mind you that the individual fly ecloses just ones during its life. But since eclosion of each fly is restricted by the internal clock to a certain time window of the day, the rhythm can be recognized.

That eclosion is controlled by an *internal clock* and not just by the light-dark-cycle, can be seen by the eclosion of animals kept in the dark: It continues to

occur in a circadian fashion (see figure 3.4). This clock can be synchronized by light. It takes care that the flies eclose at the right time of the day from their pupal cases.

3.1. Phase response curves

The rhythm can be shifted with light, as we have seen already in the *Kalanchoe*-flowers (section 1.2). A single, short pulse is already sufficient. The eclosion rhythm of the illuminated animals still follows the same measure, namely ca. 24 h. Depending on the phase at which the pulse hits the pupae, the rhythm is advanced or delayed. Animals which were illuminated *before* their 'midnight-point' show a delayed rhythm. Light *after* the midnight-point advances the rhythm. In figure 3.4 a light pulse was applied, which delayed the eclosion rhythm.

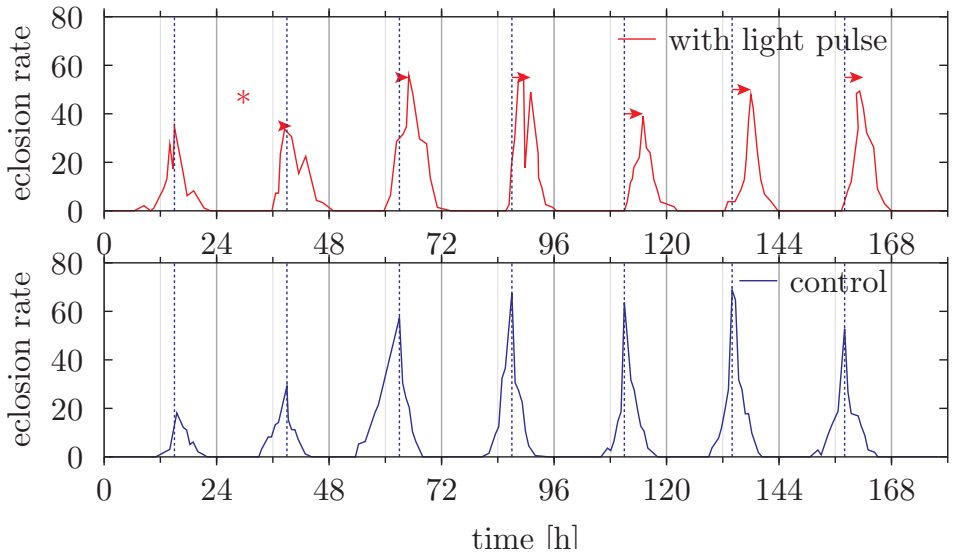


Figure 3.4.: A light pulse shifts the circadian eclosion rhythm of *Drosophila pseudoobscura*. Lower curve: Control without light pulse; the rhythm was induced by transfer of the pupae from LL to DD (red safe light). Upper curve: a light pulse given at a certain time (*) shifts the eclosion maxima in respect to the corresponding maxima of the controls (broken blue lines), as shown by the red arrows (by about 1,2,4,4,4,4 h).

In these experiments each group of pupae is illuminated just ones, but e.g. 3 h later as the previous group. The shifts are quite strong with illuminations of 10 min duration. If the groups of pupae are, however, illuminated for 1 sec

only, the shifts of the rhythm are much smaller. Here too the rhythm is delayed by a light pulse given before the midnight-point, and advanced by light after the midnight-point. But around the midnight-point we do hardly see any shift, whereas longer light pulses at this time had shifted the rhythm quite strongly.

The phase response curves which can thus be constructed are -as in the rhythm of the *Kalanchoe* petals (section 1.2)- of the strong (10 min-light pulse) and of the weak (1 sec light pulse) type (see figure 3.5). If the results are plotted in such a

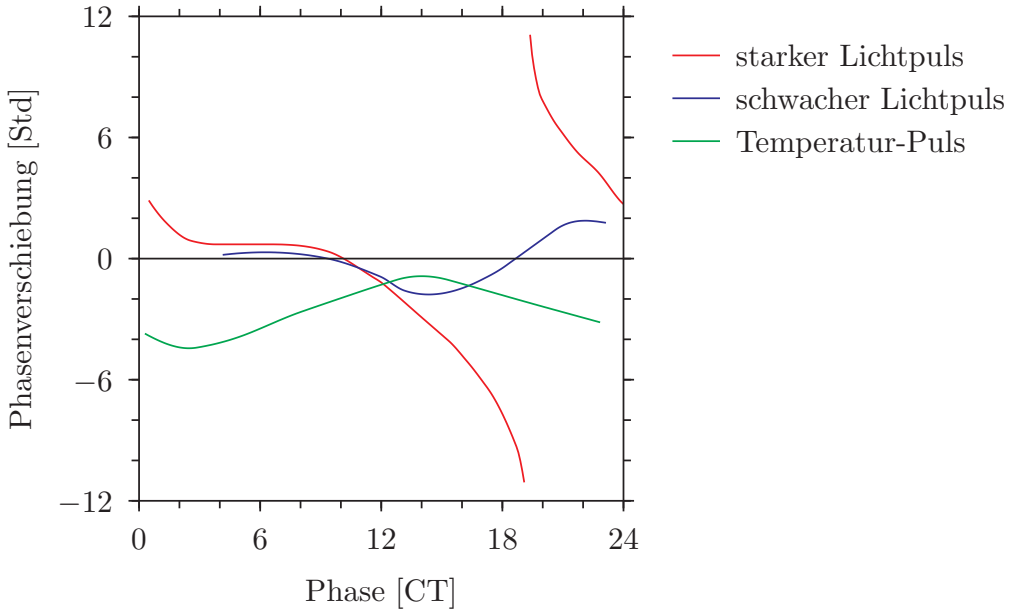


Figure 3.5.: How the eclosion rhythm of *Drosophila pseudoobscura* reacts on light pulses and temperature pulses: Many experiments like the one shown in figure 3.4, were performed in order to obtain the red and blue phase response curve. The light pulses were given at various times ('phases') during the circadian cycle (x-axis, given in circadian time). The phase shifts of the light pulses were plotted as a function of phase on the y-axis. Is the rhythm advanced by the light pulse, the values lie above the zero line, if they delay, they lie below the zero line. Strong light pulses lead to a strong phase response curve (red curve) and weak light pulses a weak phase response curve (blue curve). Note, that between CT 5 and 10 the shifts are small. The green curve reflects the phase shifting effect of temperature pulses given at various times. They delay the rhythm in all phases. After Pittendrigh and Minis (1964); Zimmerman et al (1968).

way that the new phase after the light pulse lie on the y axis and the old phase, at which the light pulse was given, lie on on the x axis, we get curves as shown in

figure 3.6. The eclosion peaks after a strong reaction to light pulses lie generally horizontally and after a weak reaction generally diagonally.

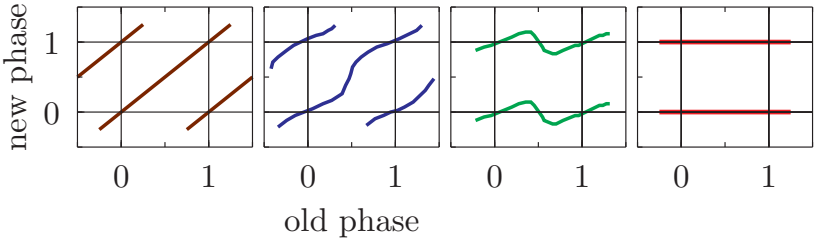


Figure 3.6.: Strong and weak phase response curves to light were plotted in such a way, that the time of illumination during the cycle is plotted horizontally (from 0 to 1), and the new phase after the light pulse vertically. Without light pulse the eclosion peaks occurred, as expected, during times represented by the diagonal (left diagram). New and old phase are identical. If the light pulse is very strong, the maxima after the stimulus are all found at the same new phase, independent of the time of illumination (right diagram). The blue diagonally running curves in the second diagram correspond to a weak phase response curve, the green one running horizontally corresponds to a strong one (third diagram from the left). After (Winfree, 1983).

In the laboratory of Pittendrigh at the Princeton-University (Princeton, New Jersey) the eclosion rhythm of *Drosophila pseudoobscura* was studied intensively. The phase shifting by light pulses was also an important tool to understand the properties of the circadian oscillator better. Arthur Winfree had studied engineering sciences and was therefore familiar with mathematical methods to look into rhythms. One of these methods is topology, which will be dealt with in section 4.3. He noticed that the phase response curves were either of the weak or the strong type and knew from this, that singularities must exist. If the oscillating system was put into a singular state by a special disturbance, the rhythm should disappear. But what is the best way of finding such a singular point?

3.2. How to find a singularity

Prerequisite for finding a singularity is, that the disturbance leads to both, weak and strong phase response curves, if offered at different phases of the daily cycle. This is the case, as we have seen in section 3.1. To search for such a point of singularity is like hunting for a needle in the hay stack. Fortunately there is a trick which helps to find it relatively fast (Winfree, 1987a). Here again topology

is of much help. In principle we have to find the time at which a strong light pulse has its largest phase shifting effect and to find a light strength, which is just not able anymore to lead to a weak reaction, but can not evoke a strong reaction. In figure 3.7 it is shown, how to proceed. It is the fastest way to find the point of singularity. Details are given in the figure and the legend.

3.3. How to measure the eclosion rhythm with a time machine

Although the method used to find the white hole as just described simplifies the search for the white hole considerably, we still need quite a number of experiments to pinpoint it. Therefore Arthur Winfree has constructed a time machine, which allows automatic recording of the eclosion rhythm of flies, after the various groups have been illuminated with different intensities of light pulses (figure 3.8). With this machine and the strategy just explained (section 3.2) Winfree found indeed a combination of the duration of the light pulse and a phase, after which the eclosion rhythm disappeared. Figure 3.9 shows two examples, where after a critical illumination the animals do not eclose rhythmically anymore.

3.4. Singular eclosion

The time machine has shown that the eclosion rhythm can be extinguished, if a special stimulus is given at the right time. But are we really dealing with arrhythmia? It might well be that this special pulse has shifted the rhythms of the various individual flies in completely different phases. The individual clocks would still be running, but not in synchrony anymore. How could we test this?

A specialty of the phase response curve of *Drosophila* might be of help. During a long time of the day phase it does not show any reaction to a light pulse (figure 4.3, dead zone). This makes sense, since during the day phase light is prevailing.

What would a light pulse do, if it hits a population of flies in their pupal cases, all of which possess a running circadian clock, but which are scattered in all possible phases of the cycle? As an alternative we would deal with a population of flies in the pupae, which have lost their rhythms by the special light pulse. If they experience a new light pulse, the rhythm would be restarted in all pupae and a clear uni-modal eclosion peak would result. If, however, the clocks are running in all kinds of phases, the light pulse would hit a large part of the pupae in phases, which lie in the dead zone. They do not experience any phase shift. More pupae will also be found, the clocks of which are in a phase where the

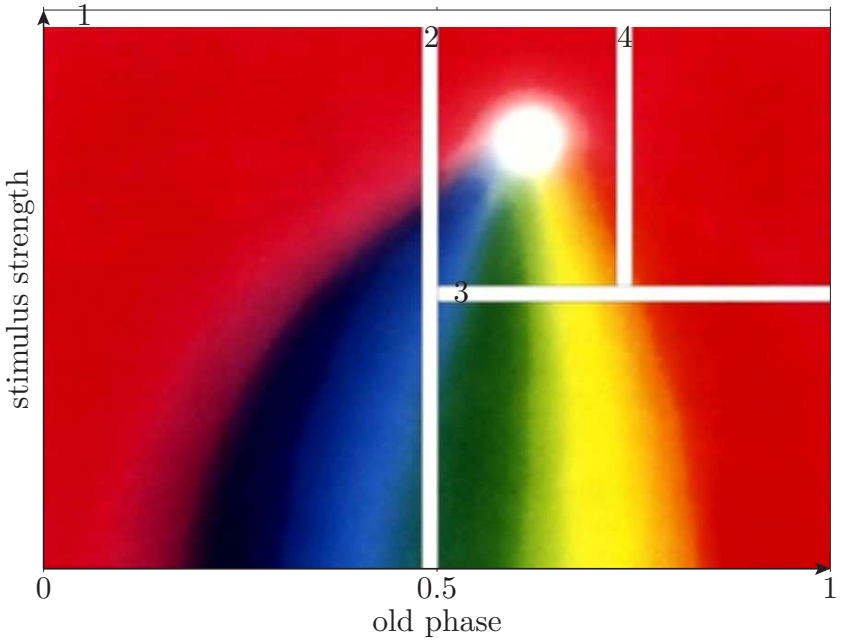


Figure 3.7.: The fastest way to find a white hole experimentally is to plot stimulus size against phase, at which the stimulus is offered. First we administer a very strong stimulus at the different phases (strip 1). If in all cases the same new phase (here red) is found, we are dealing with a strong reaction of the rhythm to the light. Next we offer a stimulus at a phase in the middle between two eclosion peaks, and vary its strength in the different groups from weak to strong (strip 2). The new phases are color coded. Since the rims of the left rectangular do not show a complete spectrum of colors (but changes only from blue to red), the white hole must be located in the right rectangular. We now apply the stimulus with half the strength at phases to the right of the middle of the old phase (strip 3). The lower part of it does not have at its rim a complete scale of color spectrum, the white hole must therefore lie in the upper part. In the next experiment at old phase 0.75 (3/4 of the x-axis) the stimulus strength is varied from 1/2 to 1 (full strength, strip 4). The right part of the rectangular has around its rims the same phase (red), and does therefore not contain the white hole. It must lie in the left part of the rectangular and can now be determined by some further experiments. After Winfree (1987a).

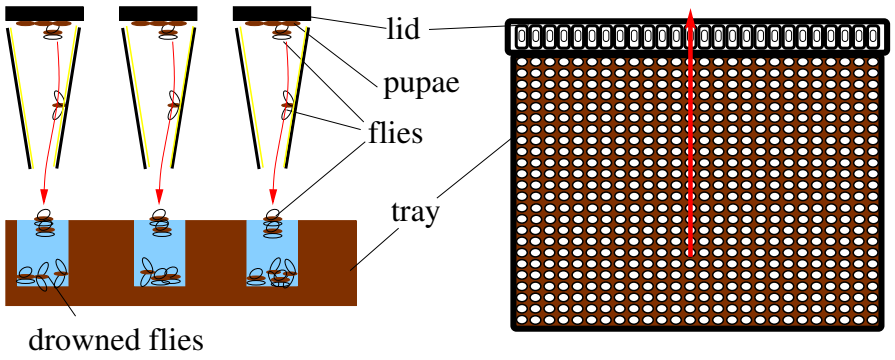


Figure 3.8.: Time machine for recording eclosion of fruit-flies. A row of 26 Teflon (slippery, yellow) funnels with lids, at the under side of which pupae are glued. Emerging flies slip (red arrow) in holes of a plastic plate and drown in water (blue, with some detergent). Right: The plate has 26 (width) times 24 (length) holes and is shifted automatically every hour by one stripe of holes (red arrow). The flies emerging at the different hours of the day are from 26 treated groups (e.g. each one illuminated 1 h later, thus 24, with two controls at the border) fall down, drown and can be counted later. After a day the plate has to be replaced by a new one. The illumination can also be performed automatically. See (Winfree, 1987a).

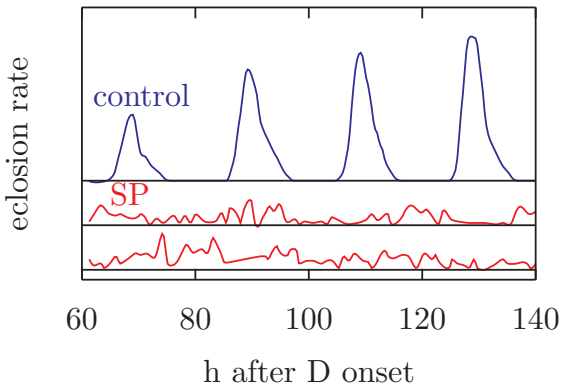


Figure 3.9.: A blue light pulse of distinct duration given at a critical time point during the daily cycle makes the eclosion rhythm disappear (SP, middle and bottom diagram). The flies emerge randomly distributed. The upper curve shows a control, which was not illuminated.

light pulse shifts the rhythm maximally. Between these two ranges are those phases, where the phase response curve shows its steepest part. They occur less frequently as compared to the two ranges just mentioned. The result of a second light pulse, which hits the running, but desynchronized oscillators, is a bi-modal curve (figure 3.10).

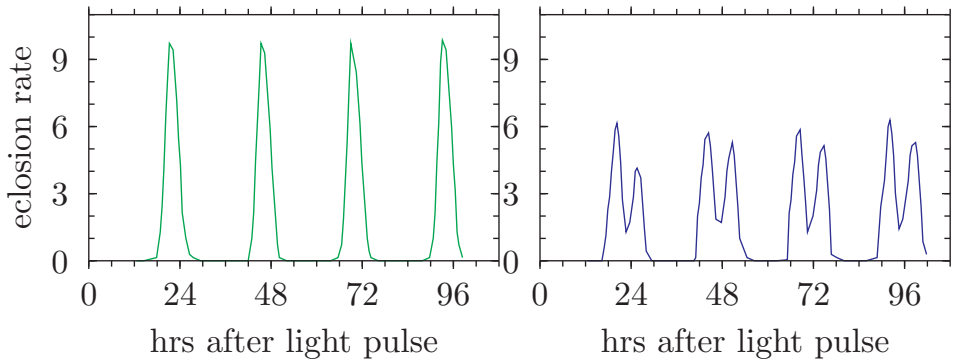


Figure 3.10.: With a light pulse in a population of *Drosophila* pupae made 'arrhythmic' after a singular pulse it can be checked, whether the flies have been made indeed arrhythmic by the first pulse or whether they were only desynchronized, so that each fly still has its rhythm, but in the different flies in different phases. In the first case (arrhythmia) the second pulse induces a new rhythm of eclosion with only one peak per day (left figure). In the second case it will result in a daily bi-modal eclosion (right). The experiment gives results as in the left curve. It speaks in favor of stopped clocks in the individual flies. After (Winfree, 1987a).

This can be tested experimentally. Arrhythmic eclosion occurs for instance, if the animals are kept for several days in continuous light. If they are transferred into darkness and eclosion is recorded, one peak is found per day.

Desynchronous cultures can be obtained by transferring groups of animals from continuous light at different times into darkness and mix them. All animals have now running clocks, but they are in different phases. If a light pulse is given to this mixed population, we obtain a bi-modal eclosion curve for the day. This is what we would expect according to the thoughts on page 50.

Now comes the *experimentum crucis*: We test the population, which was treated with a singular light pulse, with a second light pulse. It results in a uni-modal eclosion curve. The special treatment with a singular light pulse did indeed extinguish the rhythm and did not send the different clocks into various phases.

3.5. The singular conditions differ in sequential cycles

If the treatment, which results in the first cycle after transfer to continuous darkness in arrhythmia, is applied in the second or third cycle, a lower intensity is sufficient, to extinguish the rhythm, as we have seen already in *Kalanchoe* (section 1.4). The same light intensity as in the first cycle will shift the rhythm, but will not produce arrhythmia. How can this be explained?

Most likely it is not a difference in the clockwork. Instead, the light receptors might become more sensitive during the longer dark periods. Less light is now sufficient, to produce a signal which stops the clock. In figure 3.11 it is shown, how long the illumination has to be in the first, second and third cycle, in order to induce arrhythmia.

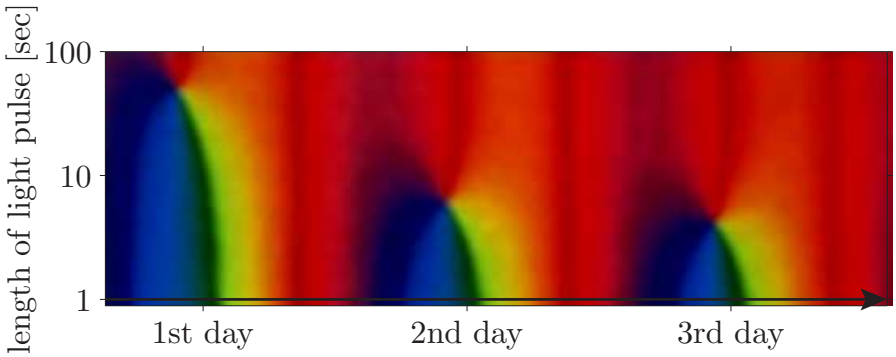


Figure 3.11.: If the white hole is determined not only on the first, but also on the second and third cycle after onset of darkness, different conditions result: On the following days shorter light pulses are needed to induce arrhythmia. It is likely that the light receptors are more sensitive to light after a long dark period as compared to the first day. Nach (Winfree, 1987a).

3.6. Does a long weak stimulus act like a short strong one?

In light reactions the product of light intensity and duration is often responsible for the effect. A weak long light pulse or a short strong one can thus be used to lead to the same result. Is this true also for the special light pulse, which made the eclosion rhythm of *Drosophila* arrhythmic?

To stop the eclosion clock, a pulse of blue light of 50 sec duration and a strength of 10 μWcm^{-2} is normally used. The dose is thus 500 $\mu Wcm^{-2}sec$. Experiments were performed, in which at a constant dose the duration and intensity was varied (figure 3.12). Surprisingly, arrhythmia can indeed be induced over a

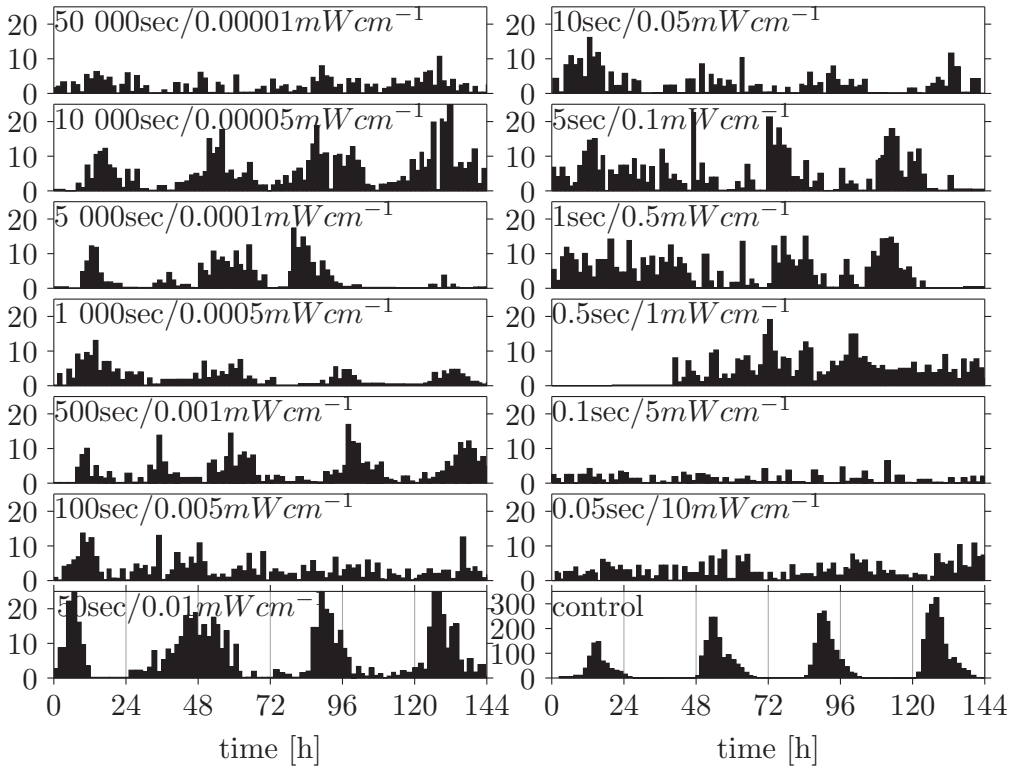


Figure 3.12.: To extinguish the eclosion rhythm, the product of light duration and -intensity must have a certain value. The combinations tried are given at the curves. The amount of arrhythmia is shown in figure 3.13. Note the different scale of the y axis in the control! After (Chandrashekaran and Engelmann, 1976).

considerably large range. Thus, even a pulse of 50 000 sec duration (about 13.9 h) and 0.01 μWcm^{-2} shows this special rhythm-extinguishing effect (its dose corresponds to that of the standard dose, Chandrashekaran and Engelmann 1976). At the other extreme a pulse of 0.04 sec only with a correspondingly high intensity (12 500 μWcm^{-2}) leads to arrhythmia (figure 3.13). In all cases the pulse had to *begin* at the critical phase.

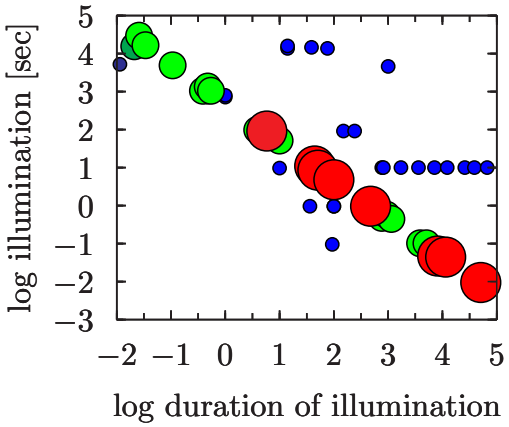


Figure 3.13.: To extinguish the eclosion rhythm, the product of duration of the light pulse (x-axis) and its strength (y-axis) has to have a constant value. The amount of arrhythmia is shown by the different diameters and colors of the circles. Red circled and large: Strong arrhythmia (R-values above 100), green circled and medium sized: quite arrhythmic (R-value between 50 and 100). Blue and small: (R-values under 50). Examples are shown in figure 3.12. After (Chandrashekaran and Engelmann, 1976).

3.7. Testing temperature effects on the clock with a singular pulse

With a singular pulse the effect of temperature on the eclosion clock of *Drosophila* can be tested (Hamm et al, 1975). The singular stimulus has to be given at 20 °C at the circadian time 18.5 (6.5 h after the end of the last light period) and causes strong damping of the eclosion rhythm (red curve in figure 3.14). If, however, the temperature during the illumination is reduced to 6 °C (symmetric to the 10 sec-illumination), the animals eclose rhythmically. In order to obtain strong damping, the critical light pulse at 6 °C has to be given 1.5 to 2 h earlier (blue curve in figure 3.14).

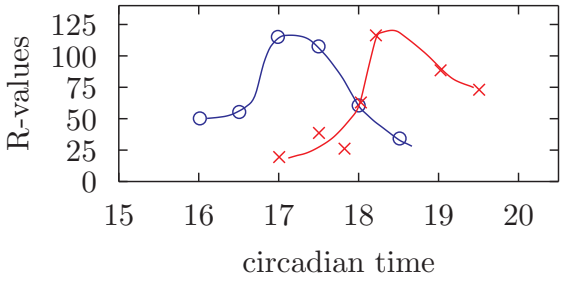


Figure 3.14.: At a lower temperature (6 °C) a singular light pulse has to be given 1.5 to 2 h earlier (blue curve) as compared to the normal 20 °C temperature (red) in order to obtain arrhythmia (high R-values): On the path to the clock must be an element which slows the signal at lower temperatures (Hamm et al, 1975).

There must be an element on the path to the clock, which slows the signal at a lower temperature. The same was deduced from experiments, in which light pulses at various phases shifted the rhythm. If during the illumination time the low temperature was given, the phase response curve to light was shifted in comparision to the one at 20 °C (see figure 2 and 3 in Hamm et al (1975)).

So far we have dealt with circadian rhythms in stopping the oscillations. In the following we will present an example for an ultradian rhythm.

4. Heart rhythm and point of singularity

On November 7, 1914 a janitor at the McGill University in Montreal finds a 28 year old physiologist in his laboratory. He lies in a tangle of electrical cables under the laboratory desk. A recorder is fixed on his chest near the heart, which still records the faltering heart beats. Georg Mines dies without coming back to consciousness.

What happened? Each day about thousand people die alone in the USA from sudden cardiac death. In most cases it is caused by fibrillation of the heart. The heart does not beat coordinated any more as is the case in the normal heart. Fibrillation might occur without warning even in healthy persons.

In this case the sudden cardiac death was caused by a self-experiment. Mines wanted to find out, whether weak electrical currents are also able to induce fibrillation. He had experimented with animals and wanted now to know whether it can be done also in humans (Winfree, 1983). In the following it is explained, what happened.

4.1. Normal heart activity

The heart of mammals is a complex organ. It consists of the left and right atrium which function as 'injection chambers' and the two ventricles, the 'pumps' (see figure 4.1). The contractions of the heart start in the left and right atrium and affect afterward the ventricle. A wave of electrical impulses passes thereby the muscle cells of the heart tissue and coordinates the four chambers. The muscle fibers contract, if the internal potential of the cells is triggered by an action potential. The negative potential breaks down briefly (depolarization) and is rebuild again after a refractory phase (repolarisation). During the refractory phase a normal stimulus can not trigger an action potential.

Special cells in the sinus node at the upper rim of the left and right atrium are pacemaker for these stimulations. They make the left and right atrium to contract. In addition the atrioventricular node between the left and right atrium

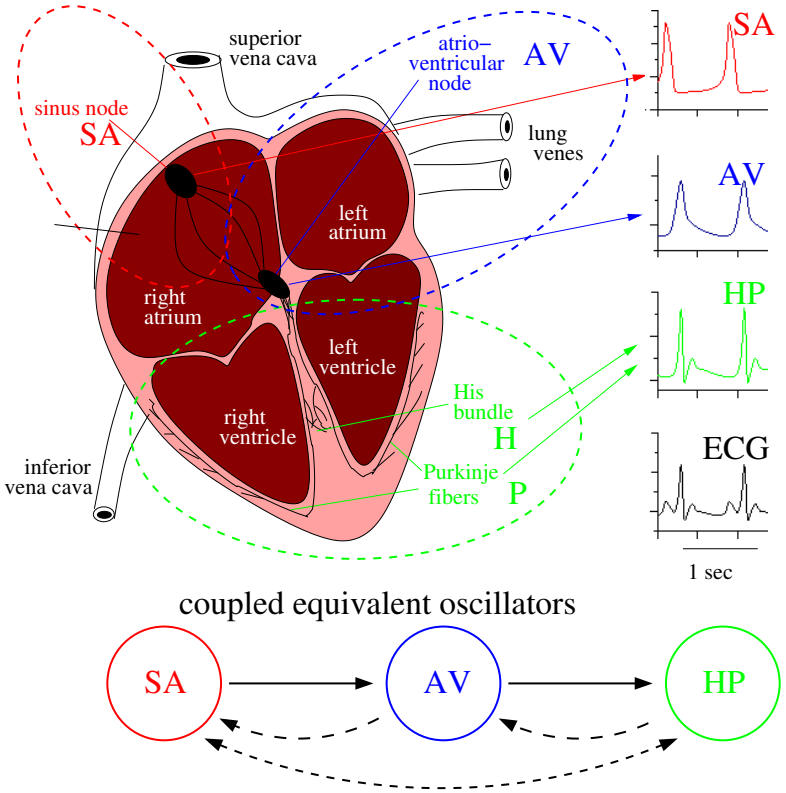


Figure 4.1.: The heart of mammals consists of the left and right atrium which function as 'injection chambers' and the left and right ventricles, the 'pumps'. Oxygen deficient blood from the body reaches the heart via the superior and inferior *vena cava* and is pumped from the heart to the lung via the lung veins. There the blood is enriched again with oxygen and distributed to the body (see Herzfunktion). Electrical impulses from the sinus node (SA) are primary pacemaker and synchronize the contraction of the left and right atrium. Impulses are furthermore conducted to the atrioventricular node (AV). From here the impulses are conducted via specialized structures (stem of the His bundle (H), bundle branches) and the Purkinje fibers (P) to the muscles of the main chamber of the heart and take care that (normally) they contract as a whole uniformly and periodically in the right beat rhythm (see Herzerregung). To the right the time course of the excitation, at the bottom the sequence, with possible backlashs (broken arrows). After Gois and Savi 2009; Zebrowski et al 2007.

are excited. It is a second pacemaker and induces via the Purkinje fibers[1] the ventricle muscles to contract (see figure 4.1).

The internal frequency of the sinus node determines the heartbeat. It is about 1 sec during rest. Nerve impulses from the brain, from different ganglia and from internal organs are able to speed up or slow down the heartbeat. This occurs normally synchronously in the whole heart. The synchrony might, however, fail in the case of infarct, unusually high hormone- or ion concentrations, chemical stress, physical damage or by a strong electric stroke.

4.2. Fibrillation

During fibrillation the coordination of the heartbeat is disturbed. Mines had proposed in a publication which he had submitted to a journal briefly before his dead, that fibrillation could be brought about by circulating waves in the heart. He had stimulated the heart of animals electrically between two contractions and thereby varied the time of the stimulus systematically. Most of the stimuli did not show any permanent effect. However, under certain conditions fibrillation was induced. For this to happen it was important that the time of stimulation was a critical one ('vulnerable phase of heart action').

4.3. Topology and fibrillation

Topology is a special area of mathematics. It is concerned with properties, which stay constant in spite of quantitative changes. Topological properties do not change even if a figure or a physical system is steadily deformed. If one looks at an image by using a distorting lens, it is topologically still equivalent to the original image. If one knows the topological properties of a system, predictions can be made even if the mechanism and the quantitative aspects of the system are not known.

Topology is useful also for describing the fibrillation of the heart chambers. The time between heartbeat and onset of stimulus is called *coupling interval*, the time between stimulus and the next heartbeat *latency* (see figure 4.2). Depending on the time and the strength of the stimulus the next heart beats will occur either earlier or later as compared to the expected time point (see figure 4.3). In the case of weak stimuli a *weak rescheduling* is found: the beats lie *after the stimulus* close to the time points at which the beats are expected if the heart would not have been stimulated (on the diagonal lines declining to the right, left part of figure

[1] a widely arborized nerve network with specially rapid conduction of electrical impulses

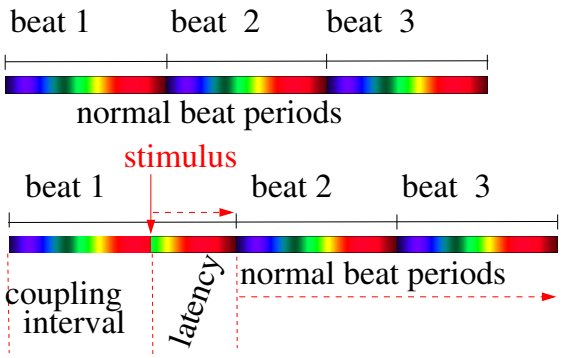


Figure 4.2.: Coupling interval (beat 1 to stimulus) and latency (time between stimulus and beat 2, beat 3, beat 4) and disturbances of the heartbeat by a stimulus. The natural beat period (time between two sequential beats) is about 1 sec. The stimulus shifts the beat by a certain amount, but the beat period stays constant. After Winfree (1987b).

4.3). In the case of stronger stimuli a *strong rescheduling* is found: The beats after the stimulus are generally further away from the time points at which the beats would occur without stimulating the heart and they lie more horizontally (right part of figure 4.3). In the case of a very strong stimulus all the following beats would occur at the same time, independent on the coupling interval at which the stimulus was offered. Strong and weak rescheduling was indeed found, when the sinus node of a rabbit heart was stimulated and the time points of the next beats were determined.

In order to simplify the results, the areas 1-2, 2-3 and 3-4 were superimposed (area 0-1 was discarded, because the phase shifts due to the stimuli were not yet consolidated). If the stimuli had shifted the next beats of the heart only without affecting the following periods, the beats (shown as points) lie on top of each other. In this way we have converted the different latencies to one and we get thus a representative curve (bottom part of figure 4.3). If the phases are coded as colors of the spectra and plotted in a diagram with coupling interval as the horizontal axis and stimulus strength as the vertical axis, figure 4.4 results.

The points at the lower rim represent almost no reaction to a very weak stimulus and the colors run therefore from red through the whole spectrum to red again. At the upper rim the reaction of a very strong pulse is shown. Under these conditions of a strong rescheduling only a *part* of the spectrum is shown, from (left) green to yellow, orange, yellow, green and blue back to green (right). At the two sides of the figure the color sequence is identical, since the coupling interval at zero and at the time point one period later are the same.

Topology predicts, and this can also be demonstrated, that on a surface it is not

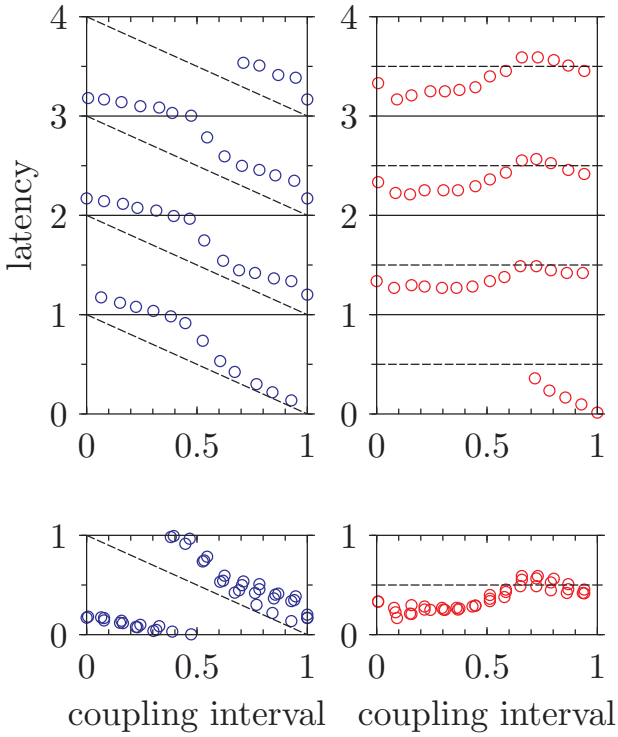


Figure 4.3.: Top curves: At certain times (*coupling interval* = interval between stimulus and preceeding heart beat, x-axis) the heart of a rabbit was weakly (left, blue) or strongly (right, red) stimulated. Latency (interval between stimulus and the following beats) plotted vertically. In the case of *weak* rescheduling (left) the beats (blue circles) follow *after the stimulus* by and large the diagonal broken lines declining to the right. In the case of *strong* rescheduling the beats (red circles) follow by and large the horizontal broken lines.
Bottom left (blue): The values of the three top curves (blue) were superimposed and the new phase position of the heart beats (latency minus n*period) again shown as a function of the phase, at which the stimulus was given. This *weak* rescheduling shows, that after a weak stimulus the heart beats are still found in all phases of the spectrum.
Bottom right (red): Again the values of the three top curves (red) were superimposed for the *strong* rescheduling. It shows, that after a strong stimulus the heart beats are not any longer found in all phases of the spectrum, but only in a part of it. After Winfree (1983).

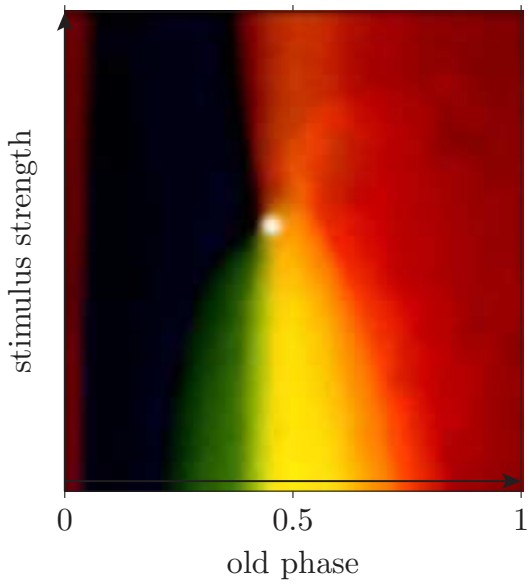


Figure 4.4: Coupling interval and stimulus strength: The phases of the heart rhythm were coded as colors of the spectrum (below the horizontal arrow). Coupling interval (old phase) is the horizontal axis and stimulus strength the vertical axis. In a critical range (around the middle of a beat interval at 0.5) the stimulation of a heart with a certain strength hits a singular point. It is surrounded by all phases (spectral colors), itself however phase-less. After (Winfree, 1987a).

possible to find for each point a weak transition. On a flat circle for instance not all points can be retracted to the periphery while at the same time all neighboring points of the circular area are still neighbors on the periphery. A soap film in a soap film loop for instance can only retract to the rim of the loop if punched somewhere.

There, where all spectral colors meet, exists a singular point (*while whole*) [2]. This state is reached at a certain combination of coupling interval and stimulus strength. Such a singular stimulus lies between weak and strong rescheduling, that is, not at the rim of the rectangular.

These expectations were confirmed by computer-simulations of nerve cell activities. Singularities (*white holes*) were found with a spectral ring around them. They have been found in the meantime also experimentally in giant axons of cuttlefish, in the Purkinje fibers of dogs (figure 4.5) and in the sinus node of cats. In the heart these singularities are larger than expected and responsible for heart fibrillation.

4.4. Circulating waves

The heart has, however, a spatial pattern. Arrhythmias are expressed therefore more often as fast circulating waves and not so much as uncontrolled or

[2] Arthur Winfree calls it 'black hole'. Since this expression is used already for certain conditions in the universe and the holes in the figures of Winfree are almost always shown as white, we prefer the name *white hole*

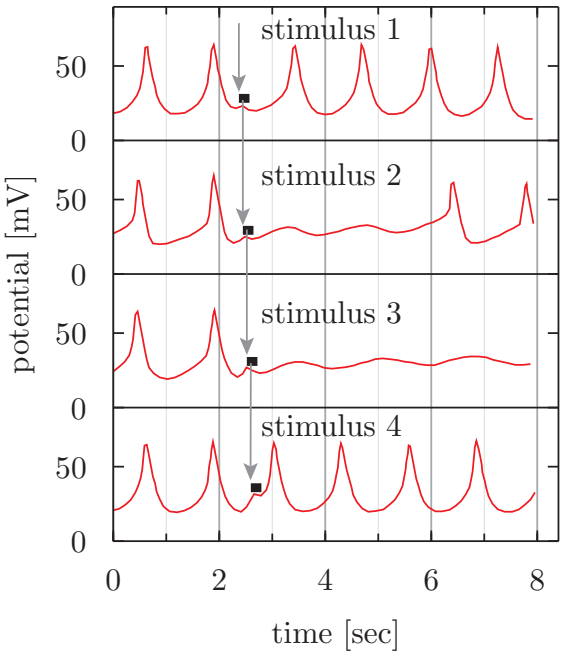


Figure 4.5: Singular stimulus in Purkinje fibers of a dogs heart: The curves show the spiking of Purkinje fibers, brought about by a continuously applied constant electrical current. However, at the marked time points an additional stimulus of 200 msec duration was applied. Given shortly after a beat (stimulus 1, top curve) it delays the next beat. Added still later (stimulus 2, second curve) it stops the spiking for a few cycles. A pulse given still later at the *vulnerable phase* (stimulus 3, third curve) stops the spiking completely. This corresponds to the white point in figure 4.4. A pulse after the vulnerable phase advances the next beat (stimulus 4, last curve). After (Winfree, 1983).

suspended beats. Fibrillation occurs, if the normal contraction of the heart is spatially disorganized.

The circulating waves are connected with singularities. The impulses in the heart are transmitted from each cell in full strength to the neighboring cells. If a continuous circling in the heart should come about, the time for one turn has to be longer than the refractory time, which follows the action potentials. Otherwise the wave would disappear after a turn, since it hits fibers, which are still refractory. Certain kinds of arrhythmia are based on waves, which circulate around hindrances such as orifices of blood vessels or a dead area of tissue. They might even circulate in a compact and healthy tissue of the ventricular muscles.

How heart fibrillation could come about is shown in figure 4.6 and explained in the legend.

Thereby it is of importance that the nerves are not uniformly distributed over the heart tissue. If the nerve endings lie close together, the nerve impulse is stronger, whereas it is weaker if the endings are less dense. In this way a gradient of the stimulus strength is build perpendicular to the pacemaker wave. In this area a singular point must be found, in which a stimulus does not lead to a definite latency. This point is surrounded by a rainbow of a complete sequence of latencies. They are connected with *isochrons* (lines of identical phases), which

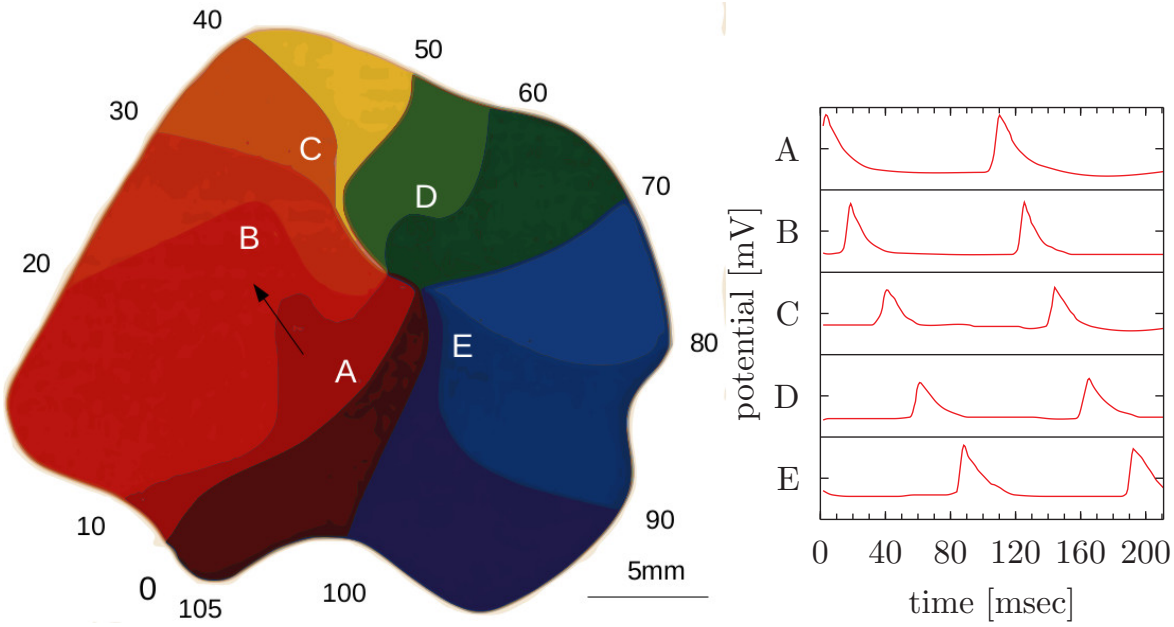


Figure 4.6.: Fibrillation could occur if an action potential in the heart muscle tissue circulates from A to B (arrow), C, D, E and back to A as shown here in a rabbit heart. A turn takes 105 msec, and the numbers around the colored figure represent the time in 10 sec steps from 0 to 105 sec. At the locations A to E the circulating potentials were recorded for two cycles and are shown in the curves A to E at the right. After (Winfree, 1983).

surround the singularity star-like and react to a stimulus with the same phase shift. As a consequence discharges occur which circle around the singularity. The area has been calculated to be in the order of about 1 cm, and this has also been observed in individual circling waves of the heart. If the heart contains many inhomogeneous locations, the individual waves would split into many small ones which could lead to fibrillation.

All stimuli which might lead to fibrillation can be plotted in a diagram with stimulus size against coupling interval (figure 4.7). The result is a disk, which

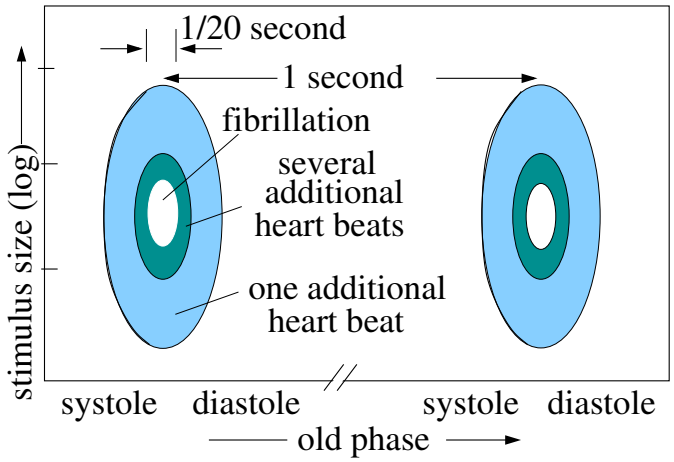


Figure 4.7.: Areas in which a stimulus of a given strength (vertical axis) leads to one additional heartbeat (blue) or several additional heartbeats (cyan). Given in a critical range (singular point) a stimulus can induce fibrillation. The singularity is not larger than 1/20 of the cycle (from one heartbeat to the next). The stimulus which induces fibrillation, covers about one order of magnitude. After (Winfree, 1987a).

consists of an outer range, where a stimulus induces one extra heart beat, of an inner range, where several additional beats are brought about, and a central range, where fibrillation is induced. This disk is rather large. Knowing about these areas, one can in the case of complicated surgery on the open heart induce an artificial, but reversible stop of the heart beat.

4.5. Singular events in the practice

The conditions under which a rescheduling singularity becomes a circulating wave, and why it might either fall apart into several smaller ones or just fade away is unknown. If one could foresee when heart fibrillation occurs, it might

be prevented by medications and an important step would have been done to prevent sudden cardiac death.

The sudden heart-circulation-collaps *(Commotio cordis)* is one of the most frequent reasons for the sudden heart standstill during sport of young people. It is well known in the USA (Aliyev et al, 2009), but has rarely be described in Europe (Maron and Estes, 2010). One report of such a case by (Solberg et al, 2011) should serve to illustrate the sudden collapse:

During a Norwegian football game of the highest league in June 2009 two players ran towards each other and collided at high speed while jumping up to catch the ball. The left side of the thorax of one of the players was hit by the knee of the opponent and he fell down loosing consciousness. He showed cramps and the team doctor diagnosed 32 sec after the collision, that his pulse was gone and he was not breathing anymore. He was strongly sweating and his face was ash gray. The doctor interpreted the situation as a sudden heart standstill. He put the player in a stable lateral position and started immediately a heart-lung reanimation by giving a blunt beat to the sternum, which was, however, unsuccessful. After a further beat to the sternum the player regained consciousness. He was brought to a hospital and checked by electrocardiogram, echo cardiographs and for biochemical markers and other data, which were, however, all normal, except a slight increase of liver enzymes, which normalized during the next 24 h. The player could leave the hospital on the next day.

In the last 50 years only one further incidence was mentioned in Europe. The difference could be due to the rougher sports such as baseball and ice hockey in America. But ice hockey is also common in Europe. After (Solberg et al, 2011) it is therefore more likely, that *Commotio cordis* is under-diagnosed in Europe. Team doctors should be aware of this and perform a fast and competent heart-lung-reanimation. Automatic defibrillators used externally should be available at open places and sports arenas. Due to the increasing intensity and the high speeds in modern sports the probability of *Commotio cordis* increases and one should reckon with it also in Europe. The risk of fatal events is high and the survival rate is only around 15 % (Maron et al, 2002).

Commotio cordis is known for a long time. It is defined as a blow against the chest leading to a vicious heart arrhythmia. The risk of death depends on the location where the blow hits the chest, on the speed and the physical structure of the object (higher risk with compact objects (Link et al, 1998)), and especially on the time in respect to the phase of the heart rhythm, at which the blow occurs. An unfavorable combination of these factors can lead to a vicious arrhythmia in the affected person. A hit 10–30 msec before a T-wave (see figure 4.15) has the highest probability to lead to ventricular fibrillation. It is interesting, that the probability is highest, if the beat causes a mean pressure of 350 mm Hg in

the left ventricle, whereas a higher pressure reduces the risk. Furthermore the probability was highest as a speed of the projectile of 60 km/h, whereas here too a *higher* speed reduced the probability.

4.6. Heart models with coupled oscillators

To find models for the heart rhythm one can either orient one self on the physiology or on the signal production. In the first case one starts from cells, which would lead in plants as well as in animals to very extensive and complicated models and needs high computing power. Therefore it is tried to find macro models, which describe the most important properties.

In the case of the heart models can for instance be used, which consist of one to three oscillators. They produces the rhythm of the sinus node and additionally the rhythm of the AV node and His bundle with the Purkinje fibers. The oscillators are mutually coupled, whereby the sinus node has the highest eigenfrequency which triggers the other two oscillators (AV-node and Purkinje fibers) from outside (see figure 4.1). Coupling is especially strong between sinus- and AV-node and between AV-node and His-bundle with the Purkinje fibers. Other couplings (see broken arrows in the lower part of figure 4.1) are indeed present, but much weaker. Therefore, they do not need to be considered in model building.

4.6.1. Pacemaker cells in the heart

Neurons can produce autonomously electrical impulses after an external trigger, and most of them relay them for producing certain effects at the target location (e.g. contraction of muscles). The first studies and quantitative descriptions of ion channels of a cell, which produce an impulse, were undertaken by Hodgkin and Huxley (1952) on the giant axon of squids, for which they earned the Nobel prize. Their equations described the produced impulses correctly, but were quite complicated. FitzHugh (1961) was able to simplify them substantially without loosing the correct presentation of the impulses. Independently Nagumo et al (1962) achieved the same and this lead to the collective FitzHugh-Nagumo-model:

$$\begin{aligned} \dot{v} &= v - \tfrac{1}{3}v^3 - w + I_{ext} \\ \tau\dot{w} &= v - a - bw \end{aligned} \tag{4.1}$$

where v is the membrane potential, w and τ auxiliary variables and I_{ext} an external current; $\dot{v}$ and $\dot{w}$ are, as usual, the first derivatives of v and w.

FitzHugh called this model also Bonhoeffer-van-der-Pol-oscillator, since the equations for $a = b = 0$ as a special case describe the van-der-Pol-oscillator. Even without external stimulation oscillations occur, which reproduce the potential of a neuron producing oscillations. In the general case instead of the parameter a and b of the original Van der Pol oscillator the model can be extended in an other way, to describe important properties of the action potentials and to influence in a simpler way the frequency and stability of the oscillation without changing the form of the signal significantly (see Grudzinski and Zebrowski (2004)). This lead to the modified equation:

$$\ddot{x} + \alpha(x - v_1)(x - v_2)\dot{x} + x(x + d)(x + e)/ed = 0, \quad d, e, \alpha > 0 \tag{4.2}$$

The corresponding Scilab-model is shown in figure 4.8. It allows not only to describe the action potential of a single neuron, but also the action potentials of numerous and uniform neurons (e.g. in the sinus-node).

This modified Van-der-Pol-oscillator is, however, not stable against external influences. If an impulse is administered to the input (model for it in Witte and Engelmann (2016)), it might -depending on the time of application and amplitude- lead to a skipping of oscillations (figure 4.9, upper curve) or a complete standstill (figure 4.9, lower curve).

4.6.2. Coupling of the sinus- with the atrioventricular node

The sinus node of the heart consists of several uniform heart cells, which can produce autonomously an oscillation. Since they correspond to the oscillation of a single heart cell, the model of Grudzinski and Zebrowski (2004) (figure 4.8) can be used also for the whole sinus node. This node influences the AV node, which consists also of uniform oscillation-producing heart cells, which, however, possess a lower eigenfrequency. The sinus node acts thus due to its coupling with the AV node as a pacemaker node: The heart cells of the AV node produce with the same frequency as the heart cells of the sinus node an action potential. Both oscillations - the one of the sinus node and the delayed one of the AV node - lead due to a weighted superposition an altered signal, which can describe also pathological properties of a real EKG signal. An equivalent model in Scilab/xcos is shown in figure 4.11.

Depending on the coupling factors from the sinus node to the AV node both oscillators can oscillate with their eigenfrequencies and react like a single oscillator to external influences, e.g. with a partial or complete interrupt of the oscillation or -in the case of stronger coupling- with the same frequency. If the eigenfrequency of the AV node is too low and can not follow the signal of the sinus node, an interruption occurs (see Witte and Engelmann (2016)). In the

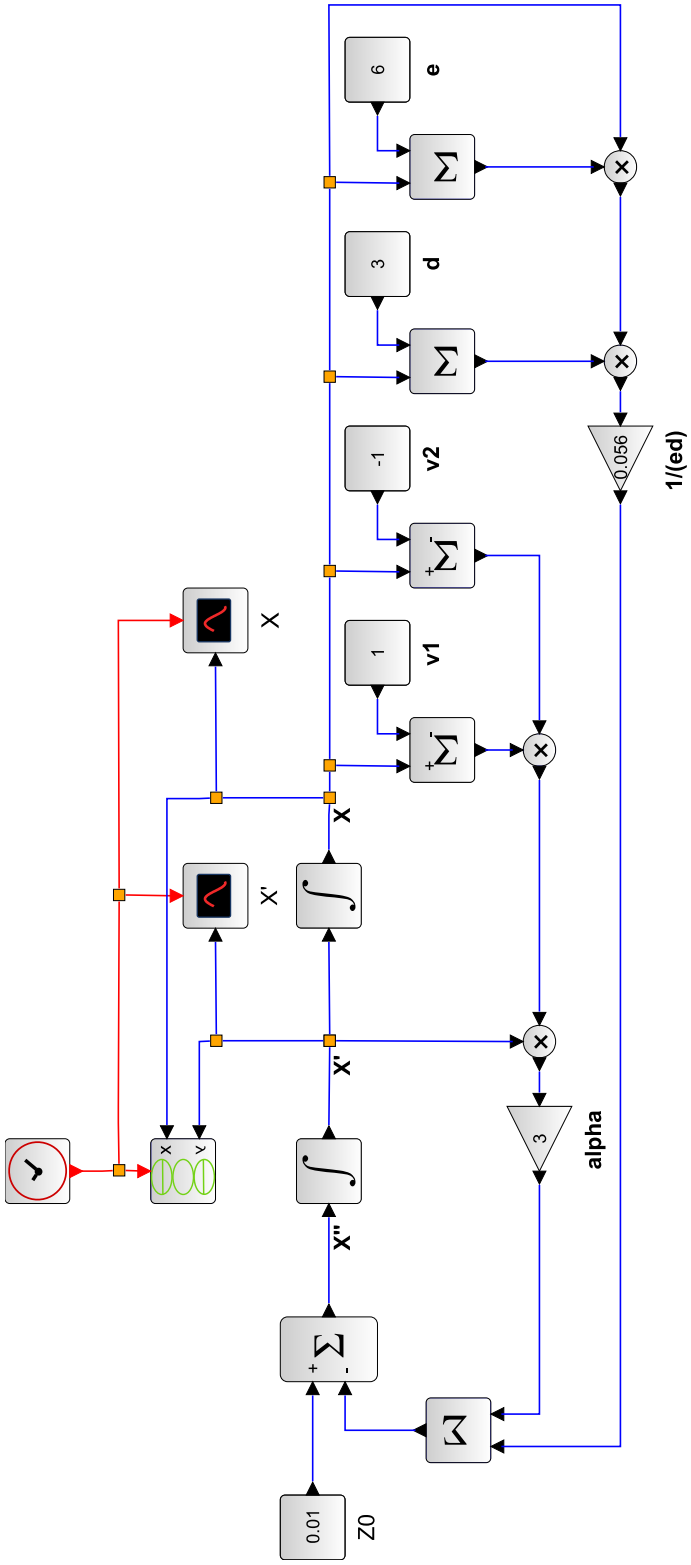


Figure 4.8.: A Van-der-Pol-Oscillator after Grudzinski and Zebrowski (2004) according to equation 4.2 simulates the action potential of a neuron which produces oscillations or of a whole neuron bundle of equal cells (e.g. of the SA- or AV-nodes in the heart). The parameter α changes the diastolic as well as the refractory time of a heart beat, ν_1 and ν_2 change the value of the resting potential, and the parameters e and d serve to vary the period length of the heart beats, but nothing else -the form of the beat stays unchanged. Thus all parameters can change the heart rate, but in different ways.

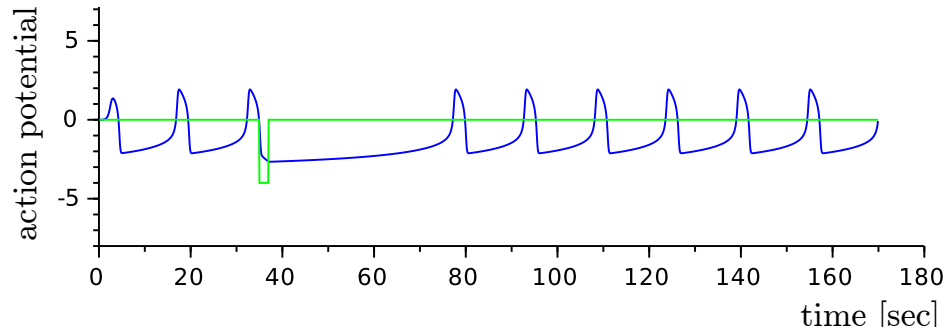


Figure 4.9.: Action potential x(t) of a Van-der-Pol-oscillator (in figure 4.8) stops briefly (2 time units, amplitude -4), whereby the timing of the impulse is decisive for the result. See also Witte and Engelmann (2016).

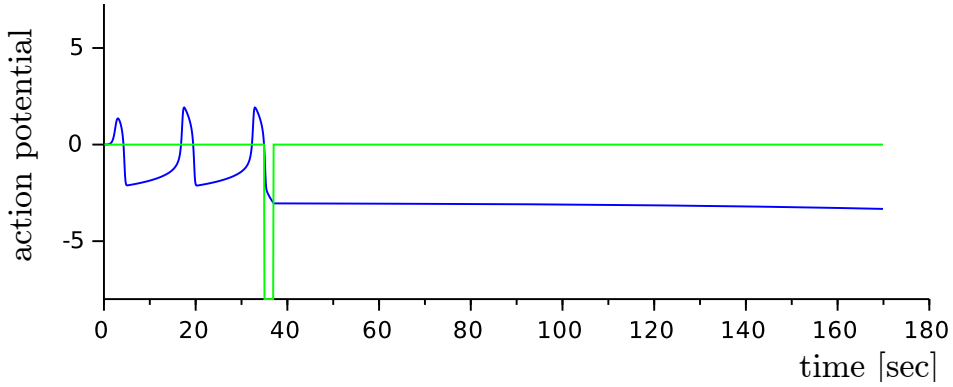


Figure 4.10.: Action potential x(t) of a Van-der-Pol-oscillator (in figure 4.8) stops completely (amplitude -7); again the timing of the impulse is decisive. See also Witte and Engelmann (2016).

worst case under too differing eigenfrequencies of the sinus- and AV nodes the oscillations of the AV node (which is responsible for the blood pumping of the main chamber of the heart, see figure 4.1) cease and the heart stops beating (see figure 4.12).

4.6.3. Coupling of all three pacemaker centers

With the two oscillators, which describe the coupling of the sinus- with the AV-node, many properties of the heart beat can be explained already. The various signals are, however, not yet similar to those which are recorded as EKG signals $x_{EKG}(t)$ at the surface of the body (see figure 4.14 for an original signal). The reason is, that the His bundle and the Purkinje fibers also represent

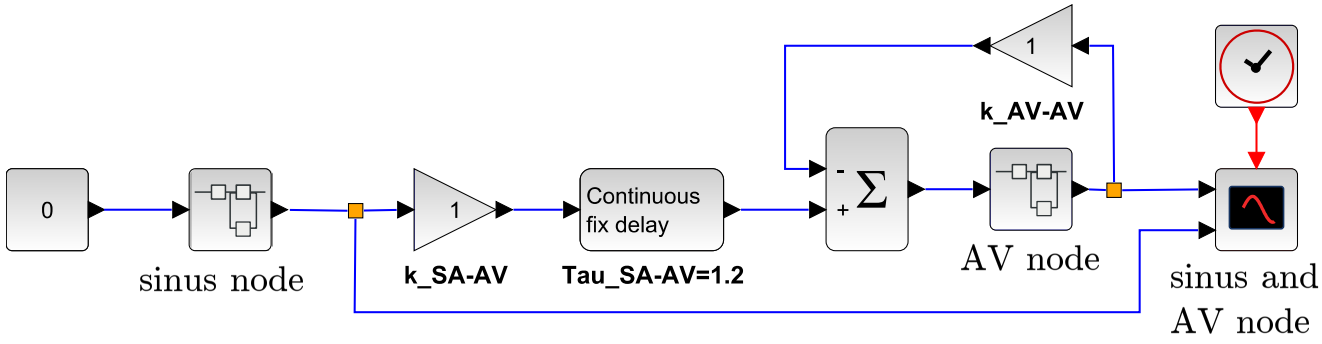


Abbildung 4.11.: Oscillator model with SA- and AV node of the heart after Zebrowski et al (2007), whereby here an additional delay $\tau_{SA-AV} = 1,2$ of the sinus node signal was added, before this reaches the control input of the AV node. k_{SA-AV} and k_{AV-AV} are damping factors between sinus- and AV nodes and feedback of the AV node from its output to its input. Both have the value 1. The sinus node as well as the AV node are represented in Scilab/Xcos by a small separate network (superblock, see also Witte and Engelmann (2016)).

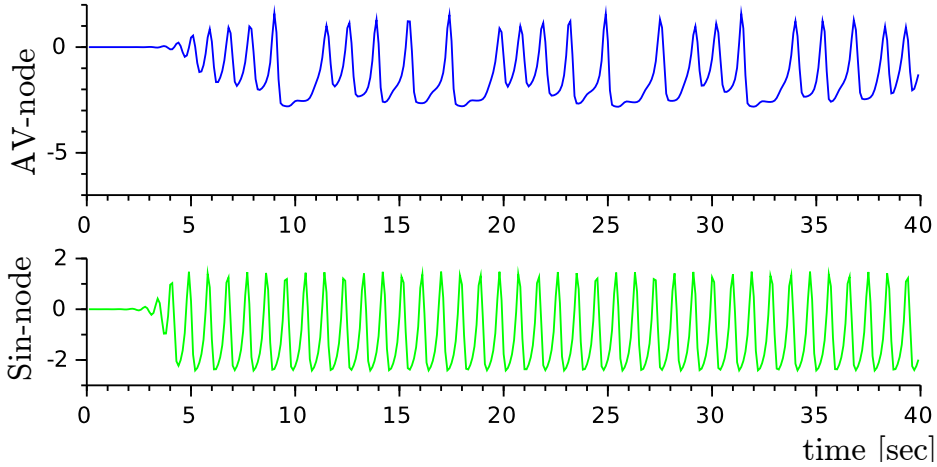


Figure 4.12.: Simulation of the signals of the sinus node (bottom, green) and of the non-synchronizable oscillation of the AV node (top, blue) in the heart using the Grudzinski-Zebrowski model in figure 4.11. With stronger coupling of the sinus node and the AV node the oscillations of the AV node would stop completely after irregular miss-firing, i.e. would reach the point of singularity ($k_{SA-AV} = 12$, $k_{AV-AV} = 1$, $e_{SA} = 12$ und $e_{AV} = 7$). See also Witte and Engelmann (2016).

an oscillator of its own; both are sharing parts of the EKG signal (see figure 4.1), and their output signals superimpose with the one of the sinus nodes, thus acting additionally on the surface of the body. The EKG signal can therefore be linearly approximated by a weighted addition of these signals and a damping factor (see Gois and Savi (2009) and Witte and Engelmann (2016)). The stimulation of the heart pump by the sinus node via the atrium and afterward via the AV node affecting the main chamber results from the coupling of the three oscillators in one direction, namely from the SA- to the AV- and further to the HP-oscillator, as shown in the lower part of figure 4.1.

A corresponding model in Scilab/xcos (Witte and Engelmann, 2016) shows additionally perturbations by alterations of the EKG basis line (e.g. at changed skin contact) and noise (e.g. at 50 Hz electromagnetic interference, influences of fluorescent lamps and radio waves). The produced signals of the three pacemaker systems and the resulting EKG signal without perturbation is shown in figure 4.13. It nicely illustrates the single parts which constitute the EKG signal. The P-waves are due to the signals of the sinus nodes and the QRS complex arises from the interplay of AV-node and His bundle with Purkinje fibers. The T wave (see figure 4.15) is mainly due to the His bundle and the Purkinje fibers.

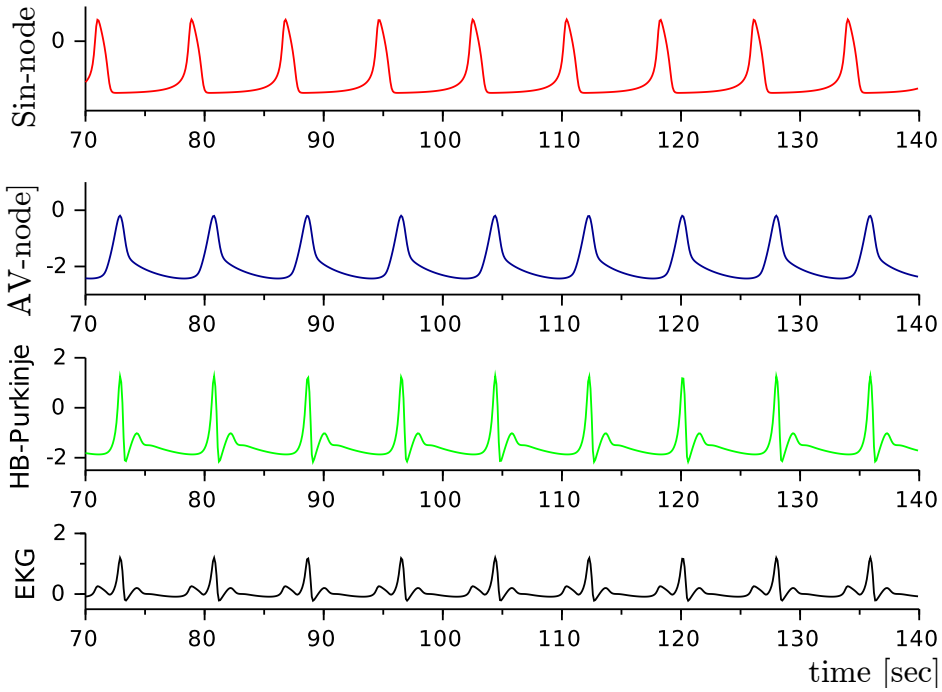


Figure 4.13.: Simulation of EKG signals by the three pacemakers sinus node (top, red), AV node (below, blue) and HP-Purkinje-fibers (green) in the heart. The EKG (black) is composed of the three curves. Three-oscillator-heart model by Gois and Savi (2009), see also Witte and Engelmann (2016).

The three-oscillator-heart model by Gois and Savi (2009) also allows to simulate EKG's in various heart diseases, e.g.

- AV-rhythm
- His bundle rhythm
- Atrium- and main chamber fibrillation and flutter
- AV-block
- missing heart beats
- extra systoles
- Sinus-brady- and Tachycardia

An especially tragic case is the sudden heart death. It can be induced by e.g. an external impulse, and can also be simulated with this model. Figure 4.16

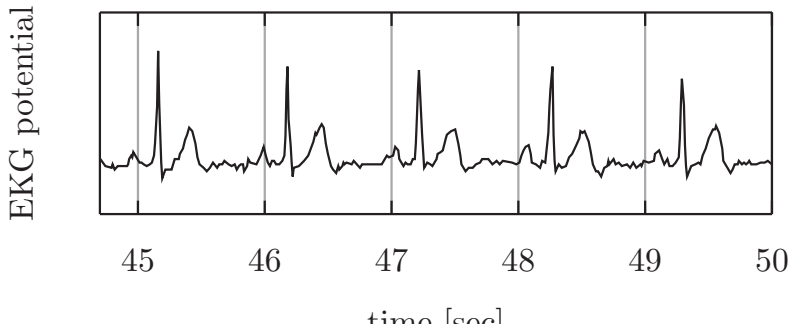
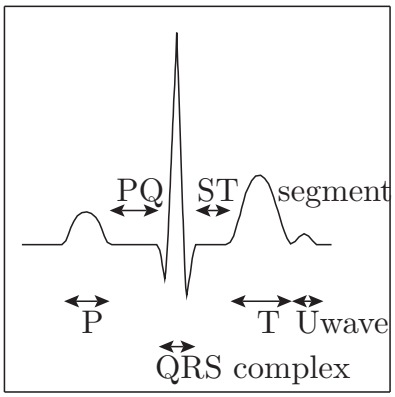


Figure 4.14.: EKG signal from Karl-Heinz Witte, own recording. Compare with figure 4.13 lowermost curve and see figure 4.15.

Figure 4.15: Scheme of EKG signal (see figure 4.14) and the designations: P, T and Q waves, PQ and ST segments and QRS complex.



illustrates the effect of a short negative impulse at the sinus node with a duration of 0.216 and an amplitude of 1 at the 80th time unit: Two heart beats are dropped, before the normal sinus rhythm appears again.

If this impulse lasts only 0.157 % longer, namely 0.21634 instead of 0.216 time units, an asystole occurs and the heart stops beating (sudden heart death, see figure 4.17).

Heart rhythms -as recorded in the EKG, thus show under certain conditions and specific perturbations arrhythmic passages or complete failures of the heart beat. In the next chapter we will deal with another example for arrhythmia, which was observed in the photoperiodic reaction of a shortday plant.

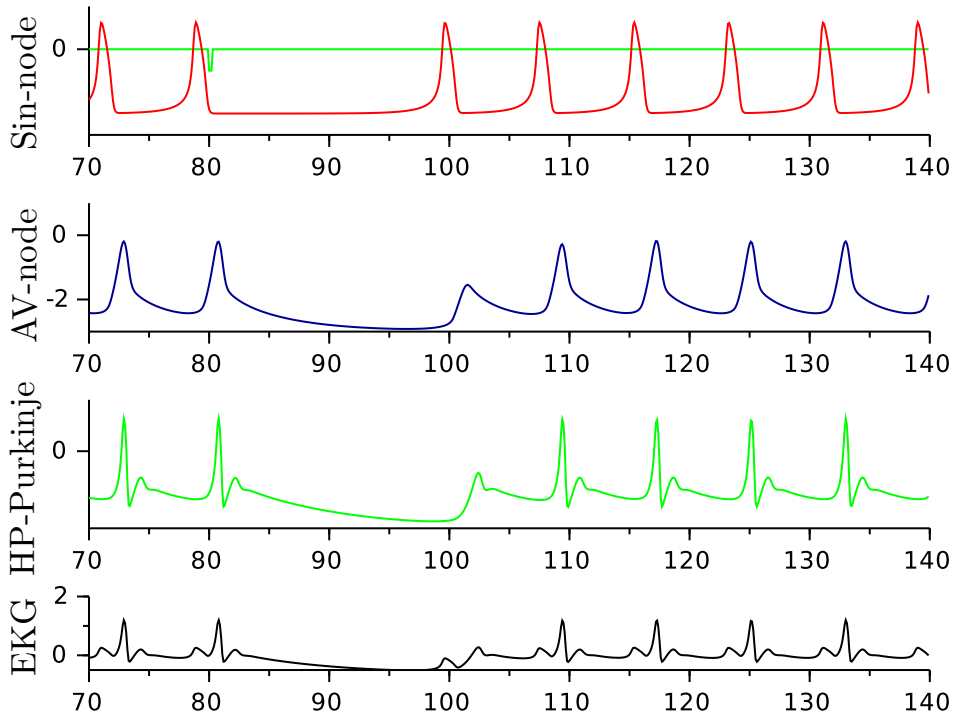


Figure 4.16.: Simulation of signals of the EKG with the three-oscillator heart model of Gois and Savi (2009), see also Witte and Engelmann (2016). Similar to figure 4.13, but with an external negative impulse at the sinus node (green) at time 80 with an amplitude of 1 and a duration of 0.216 sec. At least two heart beats cease, before the normal sinus rhythm continues. Time in sec.

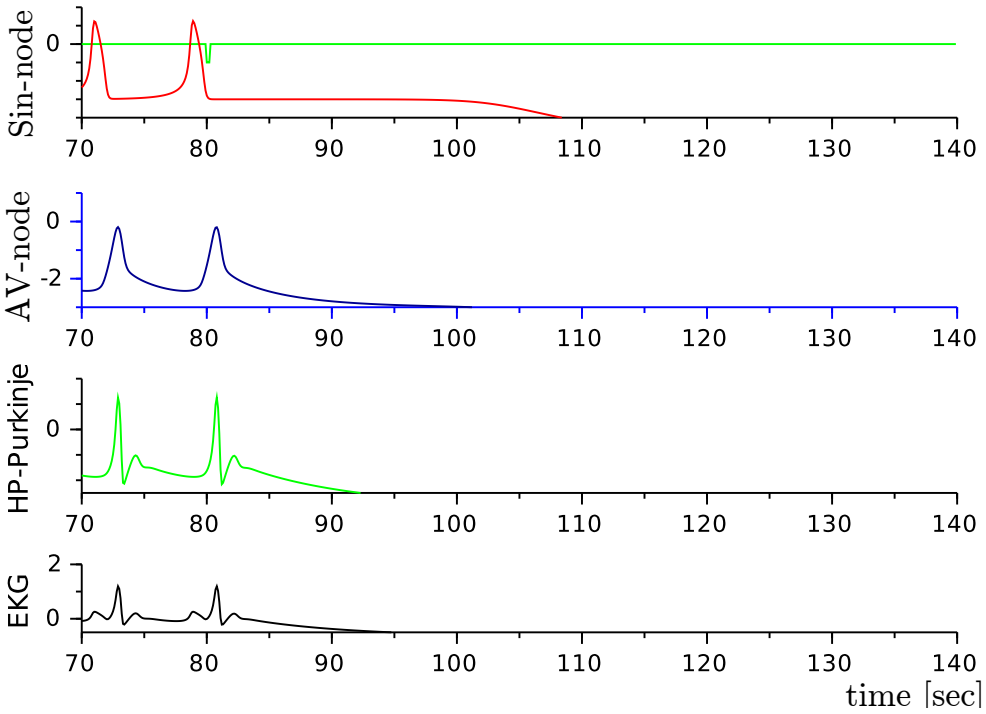


Figure 4.17.: Similar to figure 4.13, but with an external negative impulse at the sinus node (green) at time 80 with an amplitude of 1 and a length of 0.21634 sec (only 0.157 % longer as compared to the example in figure 4.13. An asystole occurs: The heart stops beating, the point of singularity is reached.

5. Arrhythmia in the photoperiodic reaction of the Morning Glory

An interesting case of arrhythmia was found in the morning glory *Ipomoea nil* (before *Pharbitis nil*). First a few remarks to the plant which belongs to the bindweed family (Convolvulaceae). Seeds of the variety Violet can be purchased from the Marutane Trading Co. in Kyoto, Japan. They are seeded and kept at 27-28 °C in continuous light. Two days after seeding (details see Rhythmen-in-Organismen) they germinate and can already be induced to flower, when the cotyledons have unfolded. A single dark period of at least 9 to 10 h is sufficient for inducing flower formation. Since the changes in the apex can be seen already a few days after induction, the plants qualify outstandingly for photoperiodic experiments.

Thus, (Takimoto and Hamner, 1964) had performed experiments, in which a longer dark period of 48 hours was preceded by an 8 h dark period. In between light periods of 2, 4 and 6 h were administered before the onset of the long dark period. In the long dark period the photoperiodic sensitivity was tested by applying single pulses of 5 min red light, which was given to different groups at various times. It turned out, that after a 4 and a 6 h light period a rhythm was detectable, but not after a 2 h light period.

These experiments were repeated by (Bollig, 1970), but the results interpreted differently. The two hour light period would accordingly be a pulse, which hit the circadian oscillator in its singular point and induced arrhythmia. She varied the experiments by offering a dark period of 58 h in the control group. Experimental groups obtained after 8 h darkness a light period of 15 min up to 4 h. In the following dark period various groups were irradiated every 4 h (each group ones) with 5 min of red test light (2330 $erg/cm^2/sec$) until the 58th h and the photoperiodic reaction (number of flowers) determined after 2 to 3 weeks. The results are shown in curves in figure 5.1.

The results can be interpreted in the following way: A circadian rhythm is induced with the onset of the dark period, which reaches after 8 h a critical phase, at which an appropriate light pulse annihilates the rhythm. The pulse should not be too short and not too long. The short red light pulses do not influence

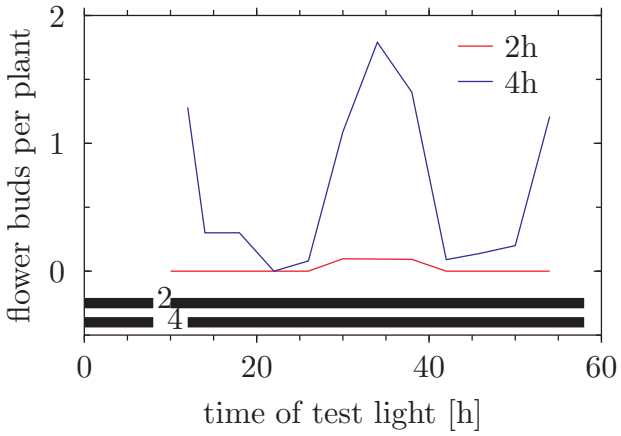


Figure 5.1.: Photoperiodic reaction of *Pharbitis nil* in a 58 h dark period, in which after 8 h a white light period of 0.5 (blue) or 2 h was given. The following long dark period was scanned every 4 h with a 5 minute red test light and the average number of flowers determined after two to three weeks. For interpretation see text. After Bollig (1970).

the rhythm, but induce a photoperiodic reaction which varies in its strength depending on the phase at which they are given.

To confine more accurately the conditions, which lead to arrhythmia, the time point of the inserted light period (instead of white light red light was used) and its duration was varied. It turned out, that a 45 minute (red!) light period between the 7th and 10th h induce arrhythmia; thus they are acting relative broad. The duration can also be varied, since 45 as well as 60 minutes red light induce arrhythmia.

It can be concluded, that an oscillator controls the photoperiodic sensitivity of the flower induction and that the oscillator can be sent by a light pulse into a singular state. This state prevents flower induction. The result is a strong indication, that Bünnings hypothesis, a circadian oscillator is responsible for the timing of the photoperiodic reaction, is correct.

The question remains, whether this critical pulse has indeed stopped the oscillator. If so, a second light pulse (of 4 h duration) given after the critical one restarts the oscillation again independent of its time point; flowers should be induced afresh. That was indeed found: In all cases the second light pulse induced maximal flower formation. If, however, the second light pulse hits an oscillator, which was not arrhythmic (because it met a non-critical first light pulse), the amount of flower induction depends on the phase at which the test light occurred.

It would have been, however, much more convincing, if another hand of the

circadian oscillator had been used which is not connected with flower induction. It was therefore checked, whether leaf movement or transpiration varied in a circadian manner. But that was under the chosen conditions not the case. One should test further processes in order to find an oscillating hand of the circadian clock. Suitable would for instance be the expression of clock-controlled genes such as *cab*. This would allow to find out, whether a light sensitive main oscillator controls this hand *and* the timing of the photoperiodic flower induction: If this is true, an arrhythmic oscillator should put all peripheral oscillators into arrhythmia. Alternatively several oscillators could exist, which are mutually coupled and synchronized with each other by external time cues. If the oscillator, which is responsible for the photoperiodic timing, is stopped by a critical light pulse, the other physiological oscillators must not necessarily be stopped. It was indeed shown, that in *Ipomoea nil* the photoperiodic flower induction is controlled by another oscillator than the one which drives the leaf movement (Bollig, 1975).

6. Further examples for the point of singularity

6.1. Circadian examples

After the studies of Winfree in *Drosophila* it was tried to induce arrhythmia in other organisms too. Since eclosion of *Drosophila* is a population rhythm, which shows up in groups of numerous flies only, it was tempting to look for an example, in which already in an individual animal a rhythm can be observed for a longer time. Eric Peterson in England found in mosquitoes and their activity a suitable object. They are active during twilight in the evening and morning. If the activity of the animals is recorded individually, a critical light pulse can indeed be found which affects the rhythm massively (Peterson, 1980, 1981b,a).

In another insect it was tried in vain. Gottfried Wiedenmann in Tübingen tried in cockroaches to stop them from showing their activity rhythmically by using a critical illumination (Wiedenmann, 1977). The same was found in *Drosophila*-flies: It was not possible to extinguish the activity rhythm.

In unicellular algae arrhythmia could be induced in several cases. In *Euglena* cell division, which occurs under certain conditions in a circadian manner, was made arrhythmic by a critical light pulse. The cells divided afterward at random times, and not anymore in certain time windows, which are opened by the circadian clock. In *Chlamydomonas*, a 10 to 20 µm sized unicellular green alga of fresh water and wet soil, Johnson and Kondo (1992) observed, that a light pulse of a particular strength administered at a critical phase lets the phototaxis rhythm disappear. In *Lingulodinium polyedra*[1], a dinoflagellate, a critical dose of anisomycin (300 nM) stopped the glow rhythm (Taylor et al, 1982).

In the Siberian hamster *Phodopus sungorus* arrhythmia was induced in the locomotor activity, the body temperature, the sleep-wake rhythm and the melatonin level. In this case a light pulse was applied during the night, which shifted the rhythm, and in the next night a second light pulse was given, which delayed the rhythm (Steinlechner et al, 2002; Ruby et al, 2004; Barakat et al, 2005). Arrhythmia occurred in the next 2 to 5 days and did not disappear, although the animals stayed all the time in a LD.

[1] old name *Gonyaulax*

Grone et al (2011) studied in these animals the expression of clock genes in the suprachiasmatic nucleus (SCN), the central pacemaker for circadian rhythms. The mRNA did not vary any more in a daily pattern. That could mean, that the individual oscillators in the SCN cells were not synchronized any more mutually (were desynchronized) or, that the amplitudes of the oscillations were reduced to such an amount, that the singular point was reached. The latter was predicted already by Leloup and Goldbeter (2008) for the light inducible clock genes per1 and per2. Here it was found, that bmal1 did not oscillate either. The mRNA of the clock genes were expressed poorly, indicating, that the circadian system was in a singular state.

In humans were also indications for arrhythmia. Since the circadian system can be brought into a singular state only, if it reacts strongly to a stimulus (a phase response of type 0, see figure 3.5), this had to be shown first. Czeisler et al (1989) succeeded in doing it by recording the body temperature[2]. Jewett et al (1991) showed further, that unconventional light stimuli applied on three days at a phase, at which the circadian system reacts maximally to light, the amplitude was reduced considerably and in some cases the rhythm dissappeared completely. An earlier model of the circadian systems of humans (Kronauer, 1990) was therefore improved and tested successfully (Jewett and Kronauer, 1998; Jewett et al, 1999, 1994; Kronauer et al, 1982).

Ukai and Ueda (2010); Ukai et al (2007) checked experimentally, whether desynchronous or arrhythmic oscillators were responsible for the disappearance of the rhythm. They introduced melanopsin in mammalian cells (Rat-1 cultures), thus making them light sensitive. The rhythm was measured continuously. They interpreted the results as showing, that the arrhythmia was due to desynchronous oscillators.

6.2. Red bread mould *Neurospora crassa*

The red bread mold *Neurospora crassa* is a filamentous fungus which belongs to the class of the Sordariomycetes. It forms under darkness (or in red safelight) daily conidia, a special form of spores. In race tubes they are seen as orange bands (see figure 6.1 and Ruoff-Neurospora) and temperature steps (warmer or colder) shift this circadian (about 24 h) rhythm, as shown by phase response curves. In the case of strong pulses or steps they are of the type 0, in the case of weak onces of the type 1 (figure 6.2). If the perturbation is administered at the right time (CT 16 with temperature steps, CT 19 with a 15 sec light pulse) and in

[2]This can be done also by replacing a long light pulse of 6.5 h by six 15 min light every hour, although the illumination time amounts to 23 % only (Gronfier et al, 2004; Jewett et al, 1994).

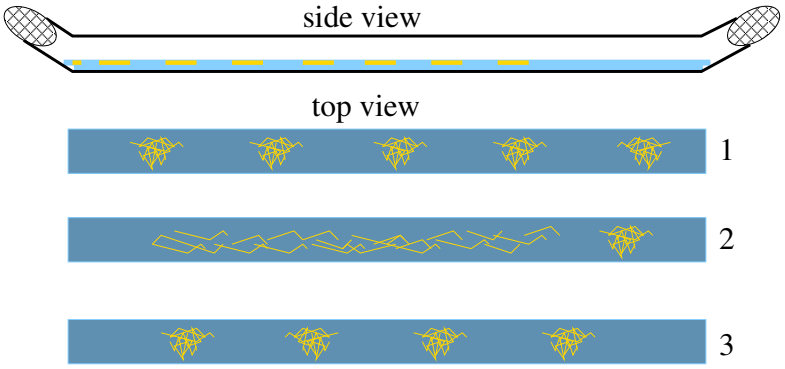


Figure 6.1.: Race tube (top, side view) half filled with a growth medium (cyan) is inoculated at one end (very left, pink) with *Neurospora* spores and kept at a temperature of 21^0 C in red safelight. The conidia germinate, grow over the medium and form in a circadian pattern bands of conidia (yellow). Transfer to 25^0 C leads to advance (top view 1) respectively delay phase shifts (top view 3), depending on the time of transfer. At a special phase at CT 15 arrhythmia is induced for a few days (top view 2); the bands disappear and conidia are formed throughout the growth uniformly. After a few days conidia formation becomes rhythmic again. Scheme after figure 1D in Huang et al (2006).

an appropriate strength, the rhythm disappears and the conidia formation occurs for a few days uniformly (Huang et al, 2006). Afterward the conidia are again produced in bands. Thus the point of singularity is unstable and the system returns to the limit cycle.

The molecular basis of the *Neurospora* clock is well known (see figure 6.3 and its legend). FRQ and WCC are important components. It was shown, that FRQ does not oscillate anymore in a circadian pattern after the singular treatment. It could be shown, that a weak perturbation is sufficient to re-initiated the rhythm. This speaks in favor of true arrhythmia and against a desynchronization, at which the individual oscillators are still oscillating, but in different phases; therefore taken as a whole, no rhythm can be recognized. Would the oscillators respond in this way, a stronger pulse had to be given in order to synchronize them again.

That FRQ is a state variable could be shown by using a wt,qaFRQ construct, in which the expression of the frq gene is under the control of a promotor, which is inducible by quinic acid. A 2 h treatment at the right time with this substance (which is subsequently washed out) brings the rhythm of conidiation for 3 to 4 days to a halt.

These experimental results speak in favour of an amplitude model for the *Neurospora* clock (Lakin-Thomas et al, 1991), which does not oscillate anymore in the singular state.

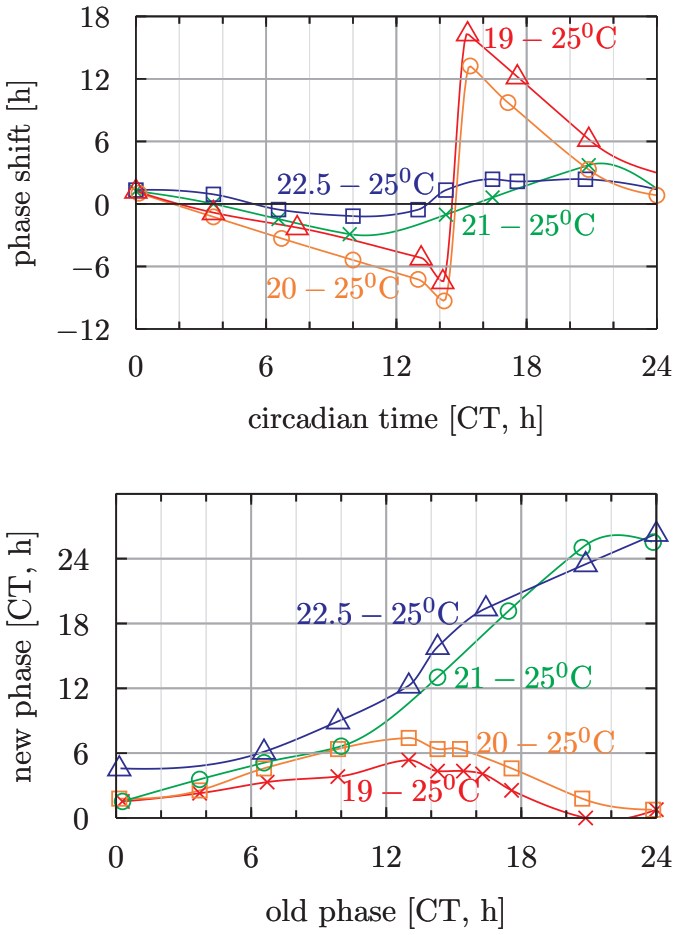


Figure 6.2.: Phase response curves of the *Neurospora* clock. Top: Phase shifting effect of a temperature step up at different phases (x-axis) and various values ($19-25^0$C red, $20-25^0$C orange, $21-25^0$C green, $22.5-25^0$C blue). Phase delays plotted downward, advances upward. Weak responses (green and blue curves) show slight shifts, strong responses (red and orange) strong onces. Bottom: New phase (after step-up of temperature, y-axis) plotted against old phase, at which step-up occured (x-axis). Weak responses are curves close to the diagonal (the diagonal curve would represent no shift at all), strong responses are more horizontal. After Huang et al (2006).

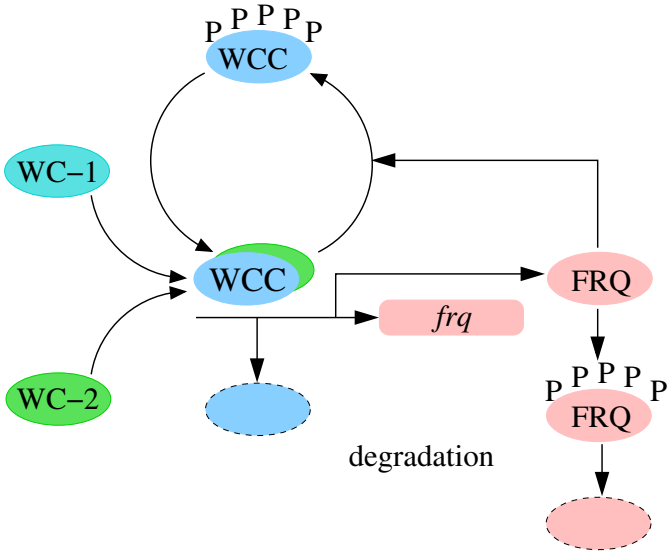


Figure 6.3.: Molecular model of the *Neurospora* clock, simplified. The transcription factors WHITE COLLAR-1 (WC-1) and WHITE COLLAR-2 (WC-2) form a heterodimer WHITE COLLAR COMPLEX (WCC). The non-phosphorylated form activates at the begin of the dark period the transcription of the frequency gene *frq* and is degraded (dashed bright blue oval). The FREQUENCY protein (FRQ) accumulates, reaches at noon a maximum and is slowly phosphorylated. Hyperphosphorylated FRQ is degraded (dashed pink oval) by proteosomes. FRQ promotes the phosphorylation of the WCC by kinases. Hyperphosphorylated WCC will become inactive, leading to decreased transcription of *frq* and a negative regulation of FRQ. The phosphorylated WCC is more stable as compared to the *hypo*phosphorylated. Increase of FRQ thus increases the amount of WCC. After Tseng et al (2012).

6.3. Examples for annual and ultradian rhythms

The examples given so far have concentrated circadian rhythms. However, arrhythmia was found also in the *annual* rhythm of an insects, the varied carpet beetle *Anthrenus verbasci* (Miyazaki et al, 2007). It exhibits an annual rhythm in pupation Blake (1959) even under constant conditions. The rhythm is temperature compensated and can be shifted by a four week longday treatment, whereby the rhythmus is delayed in the early (subjective) winter and advanced in the late winter (Miyazaki et al, 2005). The phase response curve is of the strong type and resembles circadian rhythms free running in continuous darkness. Arrhythmia resulted, if the animals were treated with four weeks of longdays between these delaying and advancing phases. A treatment of two weeks of longday only resulted in a weak phase response curve.

It is, however, also possible to induce arrhythmia in oscillations, which are shorter than circadian (daily) rhythms, the so called *ultradian* rhythms. In the following some details are given:

Arthur Winfree found in the glycolysis of yeast cells, which occurs under certain conditions rhythmically, arrhythmia, if a critical stimulus (oxygen-pulse) was administered (Winfree, 1987a).

In the case of the transpiration rhythm (water vapor is given off via the stomata) of oat leaves a stable singular point was demonstrated, which could be induced by a specific light pulse (Johnsson et al, 1979).

In the plant *Codariocalyx motorium* the rhythmic up- and down-movement of the lateral leaflets was stopped by a direct electric current pulse, if administered at a certain phase (see figure 6.4 and Johnsson et al (2012)).

It is possible, that fibrillation of the heart is not the only instance of arrhythmia in humans. Respiration is also controlled by an ultradian oscillator. (Paydarfar et al, 1986) stimulated the upper larynx nerve of cats. This nerve transmits normally impulses, which shorten inspiration and lengthen expiration. The scientists varied onset and duration of the stimulus and recorded the activity of the phrenic nerves between brain stem and diaphragm. Depending on the stimulus duration they obtained strong and weak phase shifts (see left and right upper diagrams in figure 6.5). A contour map shows singularities at a stimulus of 0.75 sec shortly before the (expected) inspiration. In the experiment the inspiration of the adult cat was not stopped[3], but the observed phases after the treatment were unpredictable. It might be possible that in some young cats such a critical pulse stops respiration. A similar situation could be responsible for the sudden infant death in children.

[3] the singular state was thus not stable

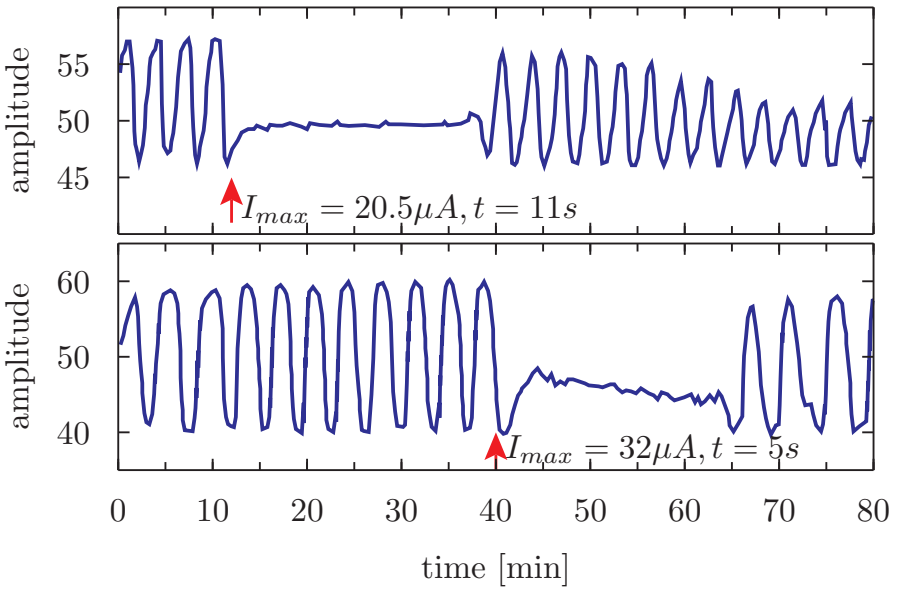


Figure 6.4.: Two examples for the induction of arrhythmia by a direct current pulse during the downward position (red arrow, strength and length next to the arrows) of the lateral leaflet of *Codariocalyx motorium*. After some time the leaflets start oscillating again spontaneously. After (Johnsson et al, 1993)

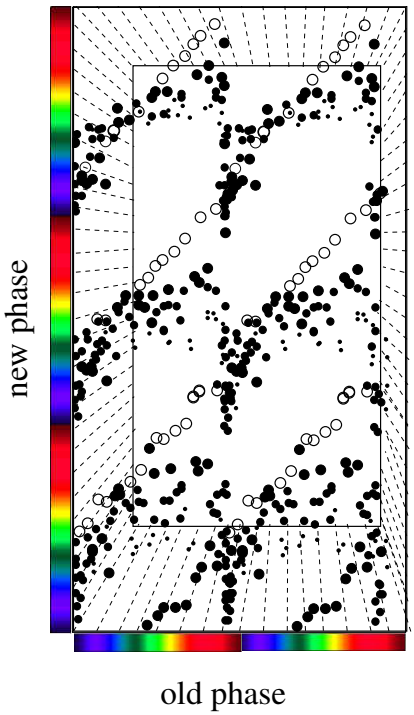


Figure 6.5: Stimulation of the upper larynx-nerve of a cat shortened inspiration and lengthened expiration. Onset and duration of stimulus were varied and the activity of the phrenic nerve between brain stem and diaphragm recorded. Strong (back, small point, for 2 sec) and weak phase shifts (front, large points) resulted. Each point stands for the onset of inspiration. A respiration cycle takes 5 sec (two cycles shown). If looking upon the image from above and coding phases with colors, the contour map in figure 6.6 is obtained. From (Winfree, 1987a).

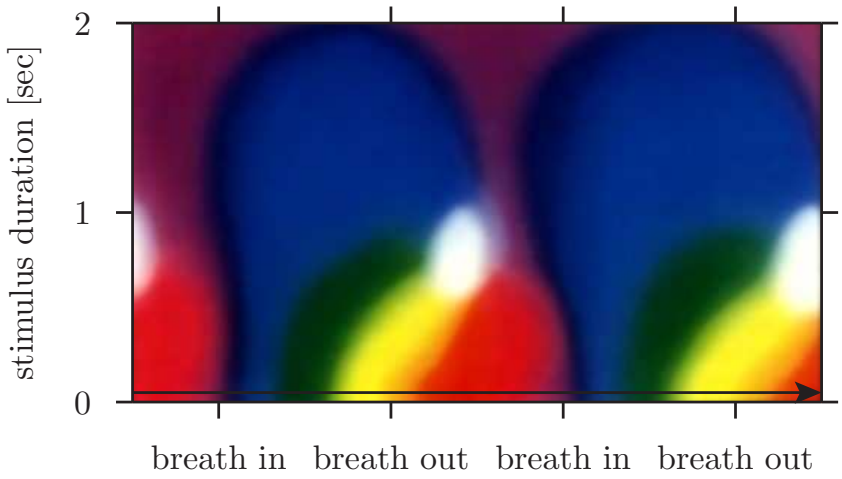


Figure 6.6.: A contour map of the respiration rhythm of the cat: If looking upon figure 6.5 from above and coding phases with colors, this contour map is obtained with three singularities. After Winfree (2004).

A. Appendix

A.1. Basic terms in chronobiology

Here some terms are explained, which are mentioned in the book partly at various locations and which can not be explained in a short way in the glossary.

A.1.1. Oscillations and their properties

Using the *Kalanchoe* petal movement as an example we explain here some properties of oscillations. If a flower is kept in a 12:12 h LD switch over and transferred at the end of the last light period into DD (weak green light), we observe the following (see figure A.1): In an LD the flowers begin to open in the morning, are open during noon and begin to close again in the evening, being closed maximally during the night. The period length is 24 h. If the movement is recorded during DD, the period shortens to 22 h and the amplitude of the oscillation declines (damping).

If a light pulse is given during DD, the rhythm is shifted. Amount and direction of this shift depends on the strength of the light pulse and from the time, at which the light pulse is given. Light during the subjective day time shifts only slightly (see figure A.2 lower curve), but given during the night shortly before the minimum of the petal opening a light pulse delays the opening of the flower and the maxima of the curve appear later as compared to the undisturbed controls (see figure A.2 central curve compared to lower curve, the control). Given shortly after the minimum a light pulse advances the opening of the flowers and the maxima of the curve occur earlier as expected from the controls (see figure A.2 upper curve).

If other flowers are now illuminated by a light pulse at various phases and the resulting shifts of the respective rhythms are compared to the controls, a phase response curve can be constructed. The kind of plotting it differs and is explained in the following.

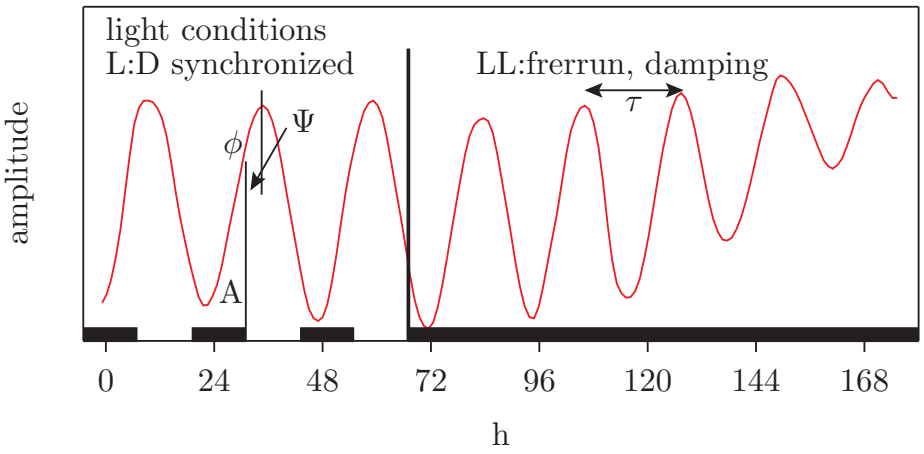


Figure A.1.: Description of an oscillation: An organism with an endogenous (internal) oscillator is synchronized by the LD of the environment (in the example 12:12 h LD). The period length amounts to 24 h. Afterward LL is offered. Now the organism shows 'freerun´ with a period length shorter than 24 h. Furthermore (in this case) the rhythm damps out in LL. Phase ϕ is a time point on the curve (first vertical line), period length τ is the interval between corresponding phases such as two following maxima of the oscillation (double arrow), amplitude A (height of the first vertical line) is used generally to indicate the y value of a point on the curve with phase ρ, but also, to characterize the y value of the maximum (actually this value should be called 'maximal amplitude´). Phase relationshipΨ is the interval between maximum (second vertical line) and an external event such as the change from darkness and light (end of second bar above the x axis).

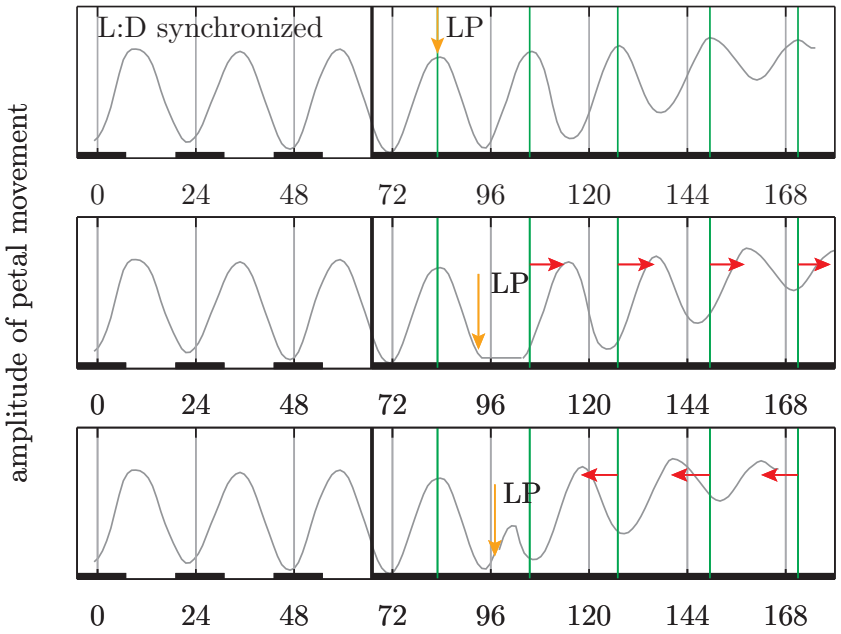


Figure A.2.: If a light pulse is offered during DD to an oscillating system (here *Kalanchoe* flowers), the rhythms is shifted. Amount and direction of this shift depends on the time, at which the light pulse is given. Light during the subjective day time shifts only slightly (lower curve), light in the subjective night shortly before the minimum of the flower opening delays the rhythmus (middle curve). A light pulse given briefly after the minimum advances the rhythm (bottom curve).

A.1.2. Phase response curve

The best way to understand the phase shift of rhythms is to display the results as shown in figure A.3 for *Kalanchoe* petal movements. Plotted are only the times of maxima and minima of the petal movement and the scheme of the illumination. Furthermore many flowers were recorded simultaneously, since the values vary from flower to flower somewhat. Would the flowers not react to a light pulse (e.g. at very weak intensities or ineffective wavelengths of the light), the maxima and minima would occur at the same time as the one of the controls.

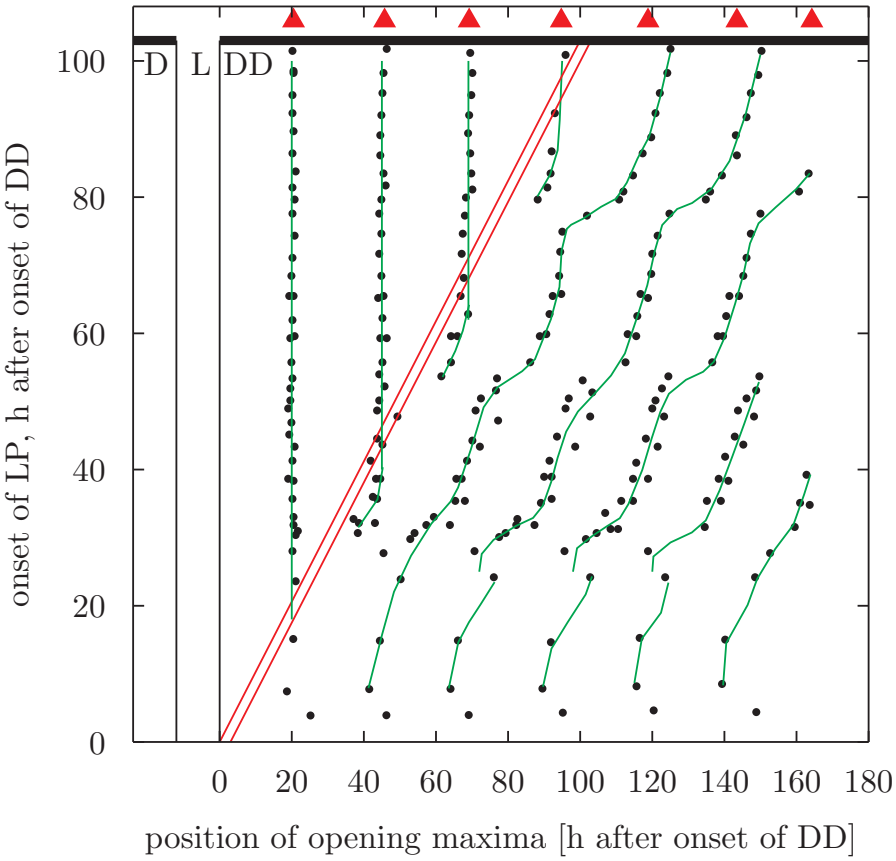


Figure A.3.: *Kalanchoe* flowers (16 in a cuvette) from a LD 12:12h into weak green light ('DD') were recorded and received group-wise 3 h light (red double line, time: y axis). Position of maximal opening (dots) plotted (triangles: control). The green curves try to give the time points of the maxima. More details in the text. Data from (Engelmann et al, 1974).

Would, however, the light pulses exert a strong effect, the maxima and minima would lie parallel to the onset of the dark period. At intermediate light intensities

two different types are obtained; the weak phase response type has no strong shifts as compared to the control and at the time of the opening minimum no shift at all is found. In the case of the strong phase response type the shifts especially shortly before and after the opening minimum are substantial and might amount to half a period, that is 11 h (remind you: in DD the petal movement *of Kalanchoe* has a free run period of 22 h instead of 24 h). During the minimum of the opening the shifts are here the strongest (in the weak response type no shifts were here observed).

To recognize the shifts of the rhythms better, we use a method of Winfree, in which the period of an oscillation is coded with the color spectrum of the visible light. We assume, that the shift of the oscillator, which underlies the petal movement rhythm, occurs immediately, although the petals need some time before reflecting directly the state of the oscillator (so called transient cycles). This kind of display helps, if we vary the strength of the light pulse and obtain a whole cohort of PRCs. This display is especially useful, if we use it in the way of Winfree (see figure 1.8 and 6.5).

In chronobiological publications often a slightly different kind of display is used. Hereby only the deviations from the control value are plotted, which leads to a PRC as shown in figure 3.5. If the corresponding values of the time axis x (the corresponding phases) are added to the y values, we obtain the kind of display used by Winfree, in which new phase is plotted against old phase. More informations in Johnson (1992).

A.2. The players

For the non-biologists a short presentation of the 'player' in this book, as far as they have not yet been shown in the preceeding text.

Yeasts see Hefe, *Euglena gracilis* see Euglena, *Lingulodinium polyedrum* (old name *Gonyaulax polyedra*) see figure A.4.

The plants Morning Glory *Ipomoea nil* (old name *Pharbitis nil*), oat *Avena sativa*, sunflower *Helianthus annuus* , *Kalanchoe blossfeldiana* see figure A.4.

Furthermore shown are the fruitfly *Drosophila melanogaster*. It belongs like the mosquito *Culex* and the cockroach *Periplaneta americana*, the varied Carpet beetle *Anthrenus verbasci* (see Anthrenus), the New Zealand Weta *Hemideina thoracica* (see Weta) to the insects. In the same figure the Siberian hamster *Phodopus sungorus*.

Figure A.5 shows portraits of Irene Bollig-Buchanan, Erwin Bünning, Anders Johnsson, Hage Karlsson, Colin Pittendrigh, Maroli Chandrashekaran, Gottfried Wiedenmann and Arthur Winfree.

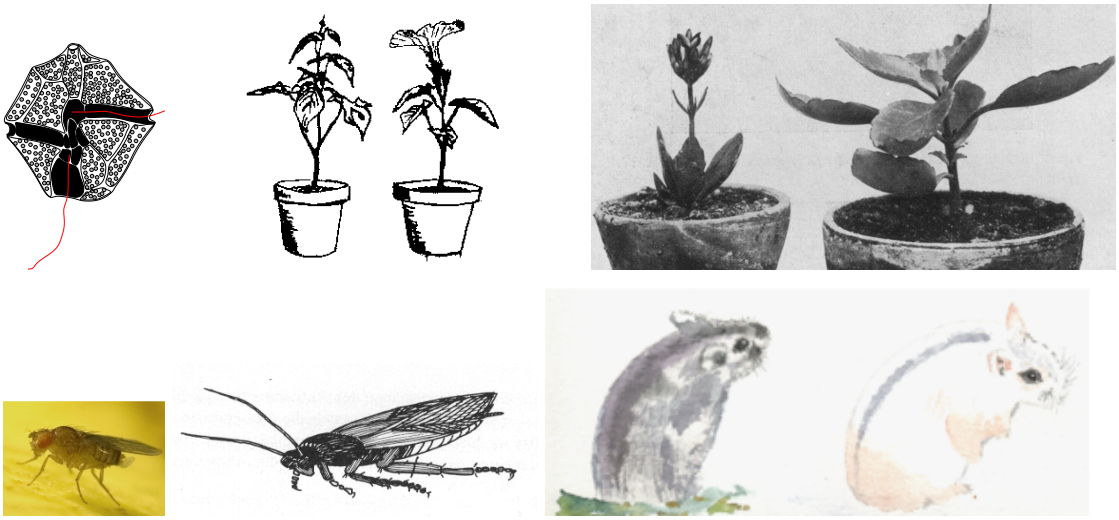


Figure A.4.: Various algae, plants and animals which have been mentioned in this book. Top left *Lingulodinium polyedrum*, 50 μm diameter, next to it *Ipomoea nil* (ca 20 cm height), to the right *Kalanchoe blossfeldiana* (ca 25 cm height).
Bottom left *Drosophila melanogaster* (about 2.5 mm long), next to it *Periplaneta americana* (ca 4 cm long), to the right *Phodopus sungorus* (about 8 cm long).

Figure A.5.: Top row from left: Irene Bollig-Buchanan, Erwin Bünning, Anders Johnsson, Hage Karlsson,
bottom row Colin Pittendrigh, Maroli Chandrashekaran, Gottfried Wiedenmann (with doctoral cup after examination), and Arthur Winfree (with his son).

A.3. Further books

Rhythmic processes are wide spread in nature. For those, who want to inform themselves on rhythms in organisms, The author WE was written the following books, in which further literature can be found: *Rhythms of Life; Hoe plants grow and move*; *Flower clocks, time memory and time forgetting*; *Flying clocks- The clocks of Drosophila*; *Bio-calendar - The year in the life of plants and animals; Clocks, which run according to the moon - Influence of the moon on the earth and its life*; *Lithium ions against depression: Is the internal clock involved in endogenous depressions? Experiments in Spitsbergen; Rhythms in structures of organisms*; *Our internal clocks - Biological time measurement in humans and other mammals*.

These books are available under http://tobias-lib.uni-tuebingen.de/ - see there under 'search' and author Engelmann.

A.4. Thanks, requests, addresses

We are thankful to Anders Johnsson, Trondheim (Norway) for pointing out errors, shortcomings, unintelligible or unclear parts in the book, and to Carl.H.Johnson, Nashville (TN, USA) for pointing out references.

This is a preprint version for Research Gate and we would appreciate your comments. The German version is available upon request.

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Index

Name Index

Glossary, Abbreviations

action potential	or electrical stimulation is a temporary deviation of the membrane potential of a cell from the resting potential.
amplitude	or oscillation amplitude is the maximal deviation of an oscillation from the arithmetic mean. In biology and technique peak amplitude is often used.
Anthrenus verbasci	varied Carpet beetle belongs to the family of the Dermestidae. Like the museum beetle *Anthrenus museorum* and the carpet beetle *Anthrenus scrophulariae* it is a common pest of materials.
arrhythmia	stopping an oscillation by a special treatment such as a light pulse at a certain phase. Used also for an irregular or lacking heartbeat.
ATP	adenosine triphosphate, an universal energy carrier of organisms. ATPases are enzymes, which split ATP in ADP and phosphate. This sets energy free, which can drive other reactions.
circadian	from (lat.) circa -about and dies (lat.) day - about 24 hours.
circadian time CT	is the time of a circadian day. Thus, if the free run period is 26 h, a circadian h would be 1/26, that is 65 min instead of 60 min. A subjective day lasts from CT 0 to CT 12, the subjective night from CT 12 to CT 24 (= CT 0). CT 0 is arbitrarily chosen to be the time, at which in a free run after a 12:12 h LD (e.g. in DD) light would occur again.
clock genes	are important for the functioning of the circadian clock.

coefficient	from Latin coefficere -co-operate, is a number or variable, which is added to another mathematical expression as a factor. The coefficient can be a parameter or an index
control theory	is a section of applied mathematics. It deals with dynamic systems in science, technique, medicine, economy, biology, ecology, and treats the influence of their inputs.
Culex	is a mosquito genus containing many species. It belongs to the family Culicidae.
damping	of an oscillation: The amplitude of the oscillation decreases.
depolarization	change of the membrane potential in the positive or negative direction.
diagram	ancient greek diagramma -figure, contour; a graphic display of data.
diastole	is the is the period during which the heart refills with blood following the systole.
differential equation	is an essential tool of mathematical modeling.
Drosophila	belongs to the family Drosophilidae.
EKG	or electrocardiogram from greek. kardía -heart, and gramma -written is the recording of the sum of electric activities of all heart muscle fibers.
endemic	plants and animals restricted to a certain, well defined area.
Euglena gracilis	belongs to the genus *Euglena*, a flagella bearing eukaryotic unicellular in the class of Euglenoida.
extrasystole	is a heart beat outside the normal heart rhythm; a heart rhythm disturbance. Extrasystoles occur frequently in young people, are, however, usually without significance

feed back	is a mechanism in signal amplifying or information processing systems, in which a part of the output feeds directly or indirectly back to the input.
frequency domain	The analysis or display is done after a transformation, e.g. by Fourier- or Laplace-transformation.
functional diagram	displays functional relations (functions) by graphical symbols. Complex connections can thus be expressed more precisely as compared to using words and more ostensive as in formulars.
giant axon	in squids, are 100 to 1000 times as thick as in mammals (up to 1 mm diameter). Allow a fast conduction of action potentials.
glycolysis	from greek glykys, sweet, and lysis, decomposition. Stepwise decomposition of monosaccharides (simple sugars) in animals, plants, fungi, and bacteria. Central process of metabolism.
growth hormones	or auxines (greek auxano - I grow) are a group of natural (phytohormones) and synthetic growth regulators.
harmonic oscillation	sinusoidal oscillation.
Helianthus annuus	belongs to the genus *Helianthus* in the family Asteraceae.
Hemideina thoracica	or New Zealand Weta belongs to the family of Anostostomatidae. Distributed mainly on the Southern hemisphere.
His bundle	or bundle of His are special heart muscle cells that transmit the electrical impulses from the atrioventricular node to the Purkinje fibers
Hodgkin and Huxley	Hodgkin and Huxley described experiments about the movement of ions in a nerve cell during an action potential and put together all of the information into a mathematical model. Sir Andrew Fielding Huxley, born 22 November 1917, English physiologist and biophysicist. Fellow of the Royal Society of London. 1963 Nobel Prize with Hodgkin and Eccles.

hypothalamus	from greek hypo - under and thalamós - chamber, an area between the interbrain near the crossing of the optical nerves.
image recognition	or image analysis, to recognize special features.
irradiance	is the radiant flux (power) received by a surface per unit area. SI unit is watt per square metre (W/m2). Irradiance is often called intensity but leads to confusion with radiant intensity.
larynx nerve	the superior laryngeal nerve is a branch of the vagus nerve, descends, by the side of the pharynx, behind the internal carotid artery, and divides into two branches, the external and the internal laryngeal nerve.
light pulse	or light impulse.
limit cycle	is a closed curve (cycle), if stable; unstable: the system approaches a singular point.
Lingulodinium polyedrum	is a dinoflagellate with about 50 µm diameter.
matrix	is a row of elements, e.g. number, in a tabular. They can be used in certain ways for calculations.
methyl jasmonate	methyl ester of the jasmonic acid. A phytohormone: Inhibits growth, participates in senescence, forms jasmonate-induced proteins and protease inhibitors, component of the defense mechanism of plants against herbivores, induces the formation of secondary substances of plants.
mRNA	messenger RNA, transfers information of a gene and is synthesized during transcription by the enzyme RNA-polymerase.
parameter	from greek para - besides and metron -measure; a characterizing property, size or number.
period	or period length: Duration of a single oscillation, time intervall, after which a process is repeated again.

Periplaneta americana	American cockroach belonging to the family of Blattidae, anthropophilic species and pest of stored products.
phase	or phase position or phase angle presents the actual position during the course of a periodic event; phase shift presents the shift of an oscillation by an external or internal event. Phase difference is the phase relation between two oscillators or between the input- and output signal of a system.
phase diagram	plots the derivative x' of a quantity x.
phase response curve	reaction of an oscillation to a perturbation: the resulting shifts are plotted as a function of the phase, at which the perturbation occurred.
Phodopus sungorus	Djungarian hamster. Seven to nine cm long, weight 20 to 45 g. Fur of the dorsal side gray to dark brown and white at the ventral side during the sommer, during the winter both sides white.
photocell	measures the intensity of light.
photoperiodism	is the dependency of growth, development and behaviour in organisms on daylength.
photoreceptor	light sensitive receptor cells of an eye or -on the molecular level- of certain light sensitive pigments (photopigments). Plants and fungi as well as unicellular algae and bacteria do also possess light receptors such as phototropines, phytochromes and cryptochromes.
plasmalemma	or cell membrane or plasma membrane is a biomembrane surrounding the living cell. Consists of a lipid double layer. About six to ten nm thick. Confines the cell from the surrounding.
polyethylen glycol	or PEG, a polymere. Fluid or solid, depending on the length of the chain; chemically inert, water soluble and non toxic.
puparium	from Latin pupa, is the hardened skin of the last larval state of an insect. Often used (as here) for the pupal casing.

Q_{10}	indicates the temperature dependence of a process. At a Q_{10} of 2 the process would procede at twice the speed at a 10^0 higher temperature
reciprocity	or antiproportionality between two values, the product of which is constant, e.g. between light intensity and -duration (Bunsen-Roscoe-law).
refractory time	is the time after the triggering of an action potential, during which the nerve cell can not react to a new stimulus.
repolarization	or electrical stimulation is a temporary deviation of the membran potential of a cell from the resting potential.
resting potential	is the negative potential of an unexcited nerve cell. It is brought about by the unequal ion concentration between the extracellular space and the cytoplasm.
singular light pulse	induces under proper conditions (phase, at which administered, and strength) arrhythmia.
singularity	an isolated point with extraordinary behavior.
sinus-brady	from greek bradykardía, slow heart beating. Describes a heart beat below 60 beats per minute in grownups
state variables	describe the energy content of the storage elements in a technical dynamic system. They are, e.g. the potential of a condensor, the current in an inductivity, the potential and kinetic energies of a spring-mass-damping system. The number of state variables is the dimension of the state space.
suprachiasmatic nucleus	or SCN is an area in the ventral hypothalamus of the mammalian brain. About 0.8 mm sized, located below the 3rd ventricle above the crossing of the optic nerves. Center of the internal clock of mammals.
synchronization	from ancient greek syn - together and chrónos - time, the timing of sequencial events, clocks and time cues. The events occur thus synchroneously or in a certain sequence.

system parameter	change with time. Example: The mass of a rocket.
systole	is the contraction period of the heart (especially the ventricles), during which blood is pumped into the aorta by the left ventricle and into the pulmonary trunk by the right ventricle.
tachykardia	from greek tachykardía-, fast heart beating. Describes a heart beat beyond 100 beats per minute in rest.
time cue	or Zeitgeber affect externally the internal clock of an organism in such a way, that they are synchronized with the environment. For plants and animals light is usually the most effective one.
time domain	the analysis or display is done as a function of time.
time lapse	accelerated filming of movements; the frame frequency at the recording is lower as compared to the one at the play back. At the replaying with normal speed the recorded scene seems to run faster; thus the slow movement is better recognizable.
topology	from greek tópos -place and logos -teaching, doctrine; is an area of mathematics. It is concerned with the properties of mathematical structurs which are conserved under steady deformation.
transcription	of a gene from DNA to RNA.
translation	is the synthesis of proteins via mRNA molecules.
turgor	or turgor pressure: Pressure of the cell sap on the cell wall.
ultradian rhythm	is shorter than a daily rhythm, typically in the range of minutes to several hours.
Van der Pol	Balthasar van der Pol, born 27. Januar 1889 in Utrecht, died 6. Oktober 1959. Dutch electro-engineer and physisist. Studied in Utrecht mathematics and physics; 1916 to 1917 university college London; Cavendish-laboratory in Cambridge. Research institute of Philips company in Eindhoven until 1949. Published 1920 his work on nonlinear oscillations.

Van der Pol Oscillator	with nonlinear damping and self-excitation. For small amplitudes the damping is negative, the amplitude increases. From a certain threshold onward the damping becomes positive. The system passes on to a limit cycle.
Varied carpet beetle	*Anthrenus verbasci* belongs to the Dermestidae, also called museum beetle. As its relative, the cabinet beetle *Anthrenus museorum* and the carpet beetle *Anthrenus scrophulariae* is a material pest, feared in insect collections.
Watt-second	1 watt-second is 3600 Joule. 1 Joule, symbol J, is a derived unit of energy in the International System of Units. It is equal to the energy transferred to an object when a force of one newton acts on that object in the direction of its motion through a distance of one metre. It is also the energy dissipated as heat when an electric current of one ampere passes through a resistance of one ohm for one second.
yeasts	are unicellular fungi. Propagate by budding or dividing, belong to the Ascomycetae. Example: Bakers yeast *Saccharomyces cerevisiae*
Zeitgeber	affect the internal clock of an organism from outside thus synchronizing it with the environment. For plants and animals light is usually the most effective one.