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Living between the Clocks – the Inner Clock in Biology and Medicine

Chair: Prof. Dr. Till Roenneberg

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Life between Clocks



*Till Roenneberg studied zoology, genetics and biochemistry. In his graduate work, he investigated the mammalian visual system and received his Ph.D. in 1983 at the University of Munich. From 1985 to 1988, he worked in Woody Hastings' lab at Harvard University on the circadian system of the unicellular alga *Gonyaulax polyedra*. Since 1988, he is head of the centre of Chronobiology at the Institute for Medical Psychology at the Medical Faculty of the University of Munich. In 1993, he habilitated in medical psychology and neurobiology. His research concentrates on the circadian system ranging from the molecular level to behaviour using both micro-organisms and humans as model systems.*

The circadian clock impacts practically all functions in our body, ranging from activating genes to modifying behaviour and cognitive functions. Although circadian clocks continue to cycle in constant conditions without information about the daily changes of the environment (e.g., light and darkness, warm and cold), it has evolved (in organisms of all phyla) not to ensure temporal organisation in a constant environment but to optimise the daily sequence of events within a predictably changing world. Thus, understanding how the circadian clock “entrains” to the daily environmental changes is a prerequisite to understand the function of this ubiquitous and important biological mechanism.

Entrainment of the circadian clock on an individual level manifests itself in how the clock embeds itself into the 24-hour-day. In humans, this can be studied in different “chronotypes” – some people go to sleep and wake up early, others late. The distribution of chronotypes in a population forms a bell shape with the extreme early types (“larks”) at one end and the extreme late types (“owls”) at the other. Like in the distribution of body height, where very short and tall people are a minority, most humans within a given population deviate more or less from the average.

Chronotype is partly influenced by genetics, but also by other factors (e.g., light and age). Modern society greatly affects the circadian clock: by predominantly working indoors, we are exposed to much less light than in former times. The 24-hour society, shift work, and frequent travel over many time zones all challenge the daily programme of our bodies. While entrainment evolved to be a harmonic balance between a cyclic

environment and the circadian clock, most of us now live *between* rather than *with* the external and the internal clocks with consequences for our health and quality of life. A detailed understanding of the circadian clock and its entrainment is a prerequisite to counteract these difficulties.

*Professor Dr. Serge Daan FRSC
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Optimal Timing in Nature



Zoologist Serge Daan holds the prestigious Niko Tinbergen chair of Behavioral Biology in Groningen. He received his Ph.D. from the University of Amsterdam and then became postdoc and longtime collaborator of the two founders of modern chronobiology: Jürgen Aschoff at the Max Planck Institut für Verhaltensphysiologie in Andechs and Colin Pittendrigh at Stanford University. Daan is recipient of the Alexander von Humboldt Research Prize. His research focuses on the functional analysis of behavioural timing in animals and humans.

Biologists today accept Darwin's principle of evolution by natural selection as the sole general basis for the diversity and complexity of life. Genetic variation is generated by random mutation and by sexual reproduction. Nature weeds out the weakest genetic configurations. The present genotypes reflect the success of those that - in past generations - left most offspring to survive. In a periodic world due to cosmic rotation, timing is all-important. Internal programs controlling the timing of behaviour are thus honed and tuned by natural selection, generation by generation.

Natural selection acts on the physiological and behavioural properties of the individual. These are determined partly by a central oscillator, partly by processes downstream from the oscillator. Internal oscillator properties are reflected for instance in cycle length and sensitivity to the external synchronizing stimuli, notably light for 24 h and annual rhythms. Together they determine the phase relationship between internal and external time. Experimental studies demonstrate how the environment selects for genotypes where the internal closely matches the external period. But tuning goes way beyond producing this match. The sensitivity towards light is tuned to optimize stable entrainment, the variation in daylength has induced flexible adjustment of internal behavioural programs. Many of these processes probably affect evolutionary fitness via the survival of the individual, by the gradual accumulation of small physiological benefits each day. Some have a sudden effect on the risks involved in single life time events, such as birth or metamorphosis. Others yet affect the timing and success of reproduction of the individual. Together they have shaped internal circadian timing into one of the fundamental properties of life as evolved on and adapted to a planet in eternal rotation.

*Professor Johanna H. Meijer, PhD
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The clock in the brain



Johanna H. Meijer studied Neurosciences at the University of Leiden, Netherlands. She performed her PhD at Dalhousie University (Canada) and at the Universities of Groningen and Leiden (Netherlands). She received a fellowship of the Dutch Royal Academy of Sciences, and is now working at the Department of Neurophysiology in Leiden. Main interests are circadian clock properties, light effects on circadian clocks, seasonal rhythms and sleep.

The rotation of the earth results in large but predictable changes in environmental conditions. The environmental fluctuations have affected life from the beginning of evolution onwards. Circadian clocks have evolved as an adaptation to these changes. The presence of innate endogenous clocks allows animals to anticipate to changes in the environment rather than to follow them passively. In mammals, the circadian clock resides in the suprachiasmatic nucleus (SCN) which is located at the base of the hypothalamus. Neurons of the SCN have a genetic basis for rhythm generation and several so called 'clock genes' have been identified. The genes become functionally important in the central nervous system as their protein products affect the membrane potential of the neurons. The circadian changes in membrane potential result in rhythmic generation of electrical impulses which are transmitted to other areas of the brain. In our lab we record electrical activity from SCN neurons by non-invasive stationary electrodes, which allow for long-term recordings of the clock. When we expose animals to a shift in the light dark cycle, we find that a dissociation can occur between clock gene expression in SCN neurons on the one hand and electrical output of the clock cells on the other hand. We also show that animals adjust easier to delays than to advances of the environmental cycle. Moreover we obtain evidence that readjustment problems are caused not only by the clock itself, but more by the areas outside the clock, which appear to attenuate the phase shifting responses. The results are important for understanding the organization of circadian clocks, all the way from the genetic level to attributes arising at the tissue level. The results also shed light on phenomena associated with shift work or jet-lag.

*Professor Russell G. Foster
Division of Neuroscience and Mental Health
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Shedding Light on the Clock



Russell Foster received his B.Sc. and Ph.D. from the University of Bristol. Subsequently working at the Universities of Giessen and Nijmegen before joining the biology faculty at the University of Virginia in 1988. In 1995 he returned to the UK and joined the Chairman of the Department of Integrative & Molecular Neuroscience, Imperial College School of Medicine. His interests span circadian and visual neurobiology, but are mainly focused upon the molecular and physiological mechanisms that regulate and generate circadian rhythms.

A clock is not a clock unless it can be set to local time, and the near 24 hour molecular rhythm in the SCN is normally adjusted by daily exposure to darkness and light. Studying these light detecting mechanisms led to the discovery of a previously unrecognized light sensing mechanism within the eye. These sensory cells are independent of the rod and cone receptors that we use to see. Indeed, some people can lack any sense of conscious vision, due to genetic disease of the rod and cone photoreceptors, but are still able to use their eyes to regulate their circadian clock using novel receptors. Until recently discussion that the eyes of humans and other mammals might contain a novel photoreceptor mechanism generated either bewilderment or hostile rebuttal by most eye researchers. It seemed impossible that something as important as another group of light-sensing cells could have been missed. The rationale was that the eye has been the subject of serious study from some 150 years, and in broad terms we understand how the eye functions. Photosensory rods and cones of the outer retina transduce light, and the cells of the inner retina provide the initial stages of signal processing before topographically mapped signals travel down the optic nerve to specific sites in the brain for advanced visual processing. All responses to light were ascribed to this process.

Studies in mice and humans with hereditary retinal disorders during the 1990's produced some very puzzling results. Despite that fact that most of the rods and cones had been lost, and no conscious light perception was present, circadian entrainment to the light/dark cycle could still occur. It seemed extraordinary that the sensitivity of the circadian system to light did not parallel the loss of either rod or cone photoreceptors, or the loss of visual function. This work paved the way for the development of a

transgenic mouse model (*rd/rd cl*) which was engineered to lack all functional rods and cones. Despite the ablation of the classical photoreceptors, both circadian entrainment and the regulation of pineal melatonin remained intact in these animals. There had to be another light sensing mechanism within the eye. The cellular localisation of the non-rod, non-cone ocular photoreceptors has been based upon a number of different lines of evidence. The most convincing approach employed the isolated rodless + coneless *rd/rd cl* mouse retina in combination with calcium (Ca^{2+}) imaging techniques. Approximately 1% of the neurons in the retinal ganglion cell layer responded to light directly and they employ an opsin/vitamin A photopigment with a maximum sensitivity in the "blue" part of the spectrum, at a wavelength of 479 nm (λ_{max} 479nm). The photopigment was originally termed opsin photopigment (OP)⁴⁷⁹. Although the biochemistry of the photopigment had been deduced, the molecular identity of OP⁴⁷⁹ remained a mystery. We now know that melanopsin forms this photopigment. Melanopsin is not only expressed in the directly photosensitive ganglion cells, but its genetic ablation in mice lacking all functional rods and cones abolished circadian responses completely. These studies demonstrate that melanopsin is critical for the normal function of photosensitive ganglion cells, but on their own could not explain how melanopsin worked. The function of melanopsin has been assessed very recently by combining the expression of melanopsin protein with physiological assays of cellular photosensitivity. Remarkably, melanopsin can confer photosensitivity to a variety of non-photosensitive cell types.

It is perhaps significant that much of the original work on the discovery of this new photoreceptor system came from circadian biologists who were not themselves vision scientists. Ralph Hodgson said: "Some things have to be believed to be seen" and the struggle to convince vision scientists that the eye contained another light sensor is a wonderful example of Hodgson's point.

*Professor Anna Wirz-Justice
Centre for Chronobiology
University Psychiatric Hospitals Basel, Switzerland*

Healthy and Happy Clocks



Anna Wirz-Justice was born in New Zealand, studied chemistry at the University of Otago, received her Ph.D. from University College London and began working in neuropsychiatry during a postdoc in Paris. She is head of the Centre for Chronobiology at the University Psychiatric Clinics in Basel, Switzerland. Main interests are the effects of light and melatonin on mood, performance, thermoregulation, sleep, circadian and seasonal rhythms (leading to chronotherapeutic applications for affective and sleep disorders).

The main premise of human chronobiology is that temporal order is essential for health. Psychological, behavioural, physiological and hormonal rhythms are specifically and functionally synchronised (entrained) with respect to sleep and the day-night cycle. The converse premise implies that temporal disorder must have clinical correlates. Rhythm abnormalities are particularly characteristic of mood disorders. Many rhythms are phase advanced with respect to the sleep-wake cycle, diminished in amplitude and/or with day-to-day variability in entrainment. These altered rhythms could be either a cause or an effect of altered mood state. Both could independently reflect abnormalities in a third system, such as psychomotor activity. In addition, sleep disturbances are inextricably linked with depressive illness. Both sets of observations have led to tests of the therapeutic potential of circadian or sleep manipulations.

Winter depression was first modelled on regulation of animal behaviour by seasonal changes in daylength, and led to application of the major zeitgeber light as the first successful chronobiological treatment in psychiatry. Light therapy has great promise for many other disorders, e.g. bulimia, premenstrual disorder, depression during pregnancy, and, importantly, as adjuvant to antidepressant medication in major non-seasonal depression, as well as for sleep-wake cycle disturbances in Alzheimer's Dementia. The pineal hormone melatonin is both a zeitgeber for the human circadian system as well as possessing direct sleep promoting effects: it does not modify mood. A night of total sleep deprivation, paradoxical as it sounds, is the most rapid antidepressant known. Even late (after 2am) partial sleep deprivation, or a phase advance

of sleep can induce mood improvement, indicating that a circadian component is important in the therapeutic response (i.e. being awake in the second half of the night). Happy clocks therefore, require correct internal and external synchronisation to maintain stable mood state.

*Professor Debra J. Skene
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Circadian Rhythm Disorders and Treatment Strategies



Debra Skene received her B.Pharm, MSc and PhD in Pharmacology at Rhodes University and the Medical University of Southern Africa (MEDUNSA) in South Africa. Following post doctoral research in the UK and France, she was appointed a Lecturer at the University of Surrey in 1992. She is currently Professor of Neuroendocrinology and Head of the Neuroendocrinology Research Group in the School of Biomedical and Molecular Sciences, University of Surrey, UK.

Professor Skene's research focuses on the human circadian system looking at the causes, consequences and treatment of circadian rhythm disorders. Photic and nonphotic regulation of the circadian system as well as optimisation of treatment of circadian rhythm disorders by light and melatonin is a major research objective. Her research is funded by the BBSRC, MRC, Wellcome Trust and the EU (Framework 5 and FP6 Marie Curie programmes). She is currently Secretary-Treasurer of the European Pineal and Biological Rhythms Society. She also serves as a Senior Member at Appointment and Promotion Boards of the University of Surrey.

Circadian rhythm disorders can result from an abrupt shift in time (as experienced following travel across time zones or by rotating shift workers), be a result of circadian phase misalignment (e.g. delayed and advanced sleep phase insomnia) or desynchronisation from the 24 h day (e.g. non-24 h sleep/wake disorder seen most often in totally blind subjects). Most physiological and behavioural symptoms can be attributed to circadian desynchrony, for example, poor sleep, daytime napping, tiredness and reduced performance during waking hours. The acute and phase shifting effects of light and exogenous melatonin have justified their use in the treatment of circadian rhythm disorders. Current research is directed towards optimising these therapies (e.g. intensity and spectral composition of light; time of administration, dose and formulation of melatonin).

*Professor Rainer Dietrich
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Separate circadian rhythm of the ‘language organ’?



Rainer Dietrich, born 1944, graduated in German Language and Literature and Philosophy at the University of Saarbrücken; Dr. phil. 1972 with a dissertation in Computational Linguistics. As a professor of Applied Linguistics of the University of Heidelberg, he specialized in language acquisition and cognitive linguistics in general. He is currently Professor of Psycholinguistics at the Humboldt-University of Berlin. His main research interests are in the field of language processing and second language acquisition. He heads the psycholinguistic experimental lab of the faculty of Arts II.

The major issues of the lab’s research are the logic of natural, non-tutored second language acquisition and the time course of language processing; over the last five years, Prof. Dietrich headed an interdisciplinary research project on group interaction in high risk environments.

The sciences form a big family, and we frequently differentiate between two branches: Natural Sciences and Humanities. My discipline, psycholinguistics, occupies a fascinating dual position that straddles this distinction. In terms of its subject matter and the questions it raises, it is undoubtedly one of the humanities. As far as methods are concerned, however, it could be labelled as a natural science.

The ability to master a language is one of the few skills exclusive to man amongst living creatures. We all acquire language as a matter of course – ‘on the job’, so to speak, simply by using it. One of the most fascinating questions in psycholinguistics deals with how man accomplishes the quasi-miraculous task of learning a language – especially in an age in which we are unable to connect with far less demanding systems as chess, for example. One of the most plausible explanations for this is the assumption of an innate language faculty. Language is learned automatically beginning with the first items of linguistic input from the child’s surrounding. Unaffected by fragmentary and ungrammatical input sentences, self interruptions, speech errors and inarticulate utterances, we build up a perfect and complete cognitive grammar for the processing of well formed utterances in the target language.

If language is part of our biological equipment the question arises as to whether its mechanism is subject to the tic-toc of the circadian rhythm as is organ and every cell of our bodily system.

The question was hardly put until now and on a theoretical basis, there is more than just the alternative of 'yes' or 'no'. Forming an integrated component of man's cognitive system, language performance and its circadian variation might well turn out to be completely synchronized with the variability of attention and vigilance. On the other side, there are also, strong theoretical reasons suggesting the existence of a separate daily rhythm of the language organ, even of separate rhythms of the various linguistic functions like utterance production, comprehension, and articulation. I will introduce the major components of the linguistic system and present recent findings pro and contra the assumption of sensitivity for circadian rhythm of the language organ.

Dr. Martha Merrow

Reiksuniversiteit Groningen, Behavioural Biology

The clock in the genes



Martha Merrow, born 1957 in the USA, Rosalind Franklin Research Fellow at the Reiksuniversiteit Groningen, the Netherlands. Studied Biology at Middlebury College in Vermont, received her Ph.D. at Tufts University School of Medicine in Immunology in 1991 and her Habilitation at Ludwig-Maximilians-Universität, Munich, in Medical Psychology and Chronobiology. Main research interests include molecular mechanisms of circadian systems, especially in microorganisms, the challenges of describing the genetics behind complex traits, the adaptive function of biological clocks, and modeling a circadian oscillator.

One of the surprising characteristics of circadian clocks is their self-sustained rhythm in constant conditions. Furthermore, these biological clocks are remarkably precise and robust (resistant to irrelevant external perturbations). Because they regulate behaviour and physiology in organisms of all phyla, understanding their molecular mechanism has been pursued with great enthusiasm, using many strategies.

The circadian clock is essentially a quantitative trait (e.g., When active? When inactive?) and thus genetics has been highly successful. A forward genetics approach of mutagenesis and screening has been used to uncover clock genes in mice, *Drosophila*, *Neurospora*, *Arabidopsis* and cyanobacteria, among other genetic model systems. The results show that a transcription/translation feedback loop is a key regulator of circadian clock function. The general theme involves a transcriptional activator complex driving expression of a downstream clock gene (complex), which then feeds back negatively on its own transcription. More recently, in most systems, auxiliary feedback loops have been described so that a network of interacting feedbacks is recognized as part of this growing 'core'. In addition, the critical role of phosphorylation has also become apparent.

Is clock genetics 'solved'? Although clock research could serve as a model for functional genomics, some of the most important insights are still to come. Genetics experiments show that many additional clock genes remain to be discovered even in the primary model systems. How big will the clock gene network become? The clock in the genes of humans, one of the most exciting stories on the horizon, is still in the early stages of description. Given the rich variety of human chronotypes, and the challenges in genotyping complex traits, this will be an endeavor full of surprises and new insights.

*Professor Andrew Millar
Institute of Molecular Plant Science
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Clocks in plants



Andrew Millar was born in London, grew up in Luxembourg and studied genetics at Cambridge University. He began working on plant circadian rhythms during his Ph.D. at The Rockefeller University, New York, and continued in the NSF Centre for Biological Timing at the University of Virginia, Charlottesville, USA. He has recently moved from the University of Warwick to the Chair of Systems Biology at Edinburgh University. His laboratory uses both experimental and theoretical approaches to understand the design principles of biological networks, the mechanisms of the plant clock and the physiological importance of clocks for plant growth.

The circadian clock is an intricate, even delicate regulator of plant physiology, yet at least one of the selective pressures that drove its evolution is brutally simple. Plants must be exposed to sunlight for photosynthesis and sunlight is not available continuously. Each day's solar energy propels plant metabolism into a spate of photosynthetic carbon fixation, which must end at nightfall. Locomotion would not alleviate the problem. Plants, like other organisms, have adapted to the day/night cycle by evolving the circadian system, which drives matching rhythms in very many aspects of metabolism and physiology. About 10% of genes in the model plant species, *Arabidopsis thaliana*, are controlled by the clock. The oscillating gene activities underlie the visible rhythms of petal opening, leaf angle and tissue growth rate, as well as less obvious rhythms such as the tolerance to freezing temperatures, which is highest at night.

Plant clocks share the main properties of circadian regulation in other organisms. Circadian rhythms in plants persist under constant environmental conditions and entrain to the day/night cycle, ensuring that rhythmic processes occur at an appropriate time of day. If entrainment is altered even quite subtly, plant growth and photosynthesis can be drastically reduced (by up to 50% over a few weeks). We are seeking to understand which of the clock-controlled molecules causes this effect, and whether it could be harnessed for benefits to agriculture or horticulture. The gene regulators that generate plant rhythms also share the complex interactions characteristic of other clockworks, though they

probably evolved independently. Mathematical analysis suggests that the complexity of biological clockworks makes their timing more flexible, so clockworks from all organisms might be expected to share similar designs even if they are composed of different molecules. This general principle may apply more generally to control networks in biology.

*Professor Joseph S. Takahashi
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The Body Clock – from Clock Genes to Cell Clocks



Joseph S. Takahashi was born in Japan, studied biology at Swarthmore College, received his Ph.D. from the University of Oregon at Eugene and was a Research Associate in pharmacology at the National Institutes of Health in Maryland. He is the Walter and Mary E. Glass Professor in the Life Sciences, an Investigator in the Howard Hughes Medical Institute, and Director of the Center for Functional Genomics at Northwestern University. His main interests are the molecular mechanism of circadian clocks in mammals, mouse genetics, genomics, and the genetic basis of complex behaviors.

My colleagues and I study the mechanism of circadian rhythms in vertebrates. Circadian rhythms are of interest because they represent an evolutionarily conserved adaptation to the environment that can be traced back to the earliest life forms and because in animals circadian behavior can be analyzed as an integrated system - beginning with genes leading ultimately to behavioral outputs. Initially, our work focused on the localization of circadian oscillators in nervous systems of birds and mammals. Then, led to a more mechanistic study of how these oscillators functioned by isolating them *in vitro*. The key breakthroughs in the field however came from genetics and the identification of 'clock genes' first in *Drosophila* and *Neurospora*.

In the last decade, we have used the mouse as a tool for the discovery of genes that control circadian behavior. In work with William F. Dove, my colleagues and I used a phenotype-driven mutagenesis strategy to isolate the first circadian rhythm mutant in the mouse (named *Clock*). This led to the identification of the *Clock* gene by a combination of transgenic "rescue" and positional cloning. These experiments revealed that *Clock* encoded a novel member of the basic-helix-loop-helix - PAS family of transcription factors. In subsequent work, we found that CLOCK and its partner BMAL1 act as the positive elements of a transcriptional feedback loop that generates circadian oscillations. The primary targets of CLOCK are the *Period* and *Cryptochrome* genes which comprise the negative feedback elements of the oscillator. Recently, we have continued to use genetics to dissect circadian behavior and have extended this approach to other types of complex behaviors.