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Prof. Dr. Urs Emanuel Albrecht
«The circadian clock: orchestrating gene expression and physiology»

Prof. Dr. Dominique Muller
«Functional and structural plasticity of synaptic networks»

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VORWORT

Prof. Max M. Burger
Präsident des Stiftungsrates

Wann und wo immer Volkswirtschaften stagnieren, wird mit Nachdruck Innovation verlangt. Ein trendiges Thema, ein Thema, das deshalb der Forschung im Allgemeinen wieder einmal auch für Budget-Diskussionen einen gewissen Stellenwert gegeben hat. Zumindest solange die politische Realität dem erhöhten Stellenwert das Fundament nicht doch in aller Stille wieder entzieht.

Originalität und Innovation sind einerseits die Hauptpfeiler, auf denen Forschung basiert, und andererseits die wichtigsten Messparameter bei der Beurteilung von Forschungsprojekten.

Originalität bedeutet neue Ideen oder genauer: denken, was andere nicht gedacht haben. Innovation basiert auf der Neugierde, der Gier nach Neuem, auf einer Eigenschaft, die gesellschaftlich verpönt und für die Wissenschaftssoziologie akzeptabel, für die Forschung aber eine conditio sine qua non ist. Innovation und Originalität basieren auch auf einem gewissen Spieltrieb, was die Amerikaner als «Why not try» bezeichnen und was leider auch mit dem etwas negativ besetzten Begriff Risiko einhergeht. In Reformkreisen hat sich langsam der Begriff Risikobereitschaft eingebürgert, ein Begriff, der auch eine bessere Akzeptanz geniesst.

Wenn vom Forscher heute eine grössere Risikobereitschaft beim Abfassen und Einreichen seines Forschungsgesuches gefordert wird, so darf mit Fug und Recht auch von den zuständigen Evaluierungsgremien mehr Risikobereitschaft verlangt werden. Im medizinischen Ausschuss der Stiftung Prof. Dr. Max Cloëtta haben wir immer wieder Gesuche, die sehr innovativ – und damit eben auch recht risikoreich – waren, zur Bewilligung vorgeschlagen. Meist mit der Begründung, nicht nur der Idee, sondern auch dem jungen Wissenschafter eine Chance zu geben.

Aber Hand aufs Herz, wie viele Male sind wir im Nationalfonds, im Cloëtta-Ausschuss oder in Study Sections der National Institutes of Health (NIH) auf Nummer sicher gegangen, weil wir – wie auch die zugezogenen Experten – aus Erfahrung im Projekt ein zu grosses Risiko gesehen haben? Dieselben Probleme hat auch die privatwirtschaftliche Forschung, die drei Viertel der gesamtschweizerischen Forschungsausgaben abdeckt. Dort können die Folgekosten eines einzigen im Sand verlaufenen Projektes rasch über eine Milliarde Franken ausmachen.

Für die Grundlagenforschung ist Mut zum Risiko die Voraussetzung für den Erfolg, für den innovativen Durchbruch. Daran glauben Forscher und Evaluierungsgremien. Ob danach gehandelt wird, könnten nur Studien feststellen, die die Evaluierungsgremien oder deren Aufsichtsausschüsse selber durchführen. Solche Studien hängen vorerst einmal davon ab, ob die Beitragsempfänger einen aussagekräftigen Schlussbericht schreiben und die forschungsfördernden Organisationen diesen auch lesen und beurteilen. Beides sind leider keine Selbstverständlichkeiten.

Nur höchst selten ist die prognostische Validität der Einschätzung von Gesuchen – verglichen mit dem Erfolg des Forschungsprojektes (Zitationshäufigkeit + Qualität) – statistisch erfasst worden. Ein zaghafter Versuch mit allen NIH-Gesuchen kam vor 23 Jahren zum Schluss, dass höhere Beurteilungen durch Gutachter wohl zu mehr Zitationen führten, dass die Korrelation aber «very low» war (0.4). Und das war das Fazit des NIH, der wohl erfahrensten Organisation im Bereich Forschungsförderung, die den Goldstandard im Peer-Review-System darstellt. Könnte diese marginale Korrelation unter anderem auch auf eine beschränkte Risikofreudigkeit der Begutachtungsgremien zurückzuführen sein? D. h., wird vor allem «brave», nicht sehr risikofreudige Forschung gefördert, die dann nicht unbedingt meistzitierte Durchbrüche liefert?

Solange die Forscher selbst wie auch die Gutachtergremien Qualitätsbewusstsein, Zuverlässigkeit und Arbeitsethos bewahren, dürfen nicht nur die Forscher, sondern auch die Evaluierenden etwas mehr Mut zum Risiko aufbringen.

Nun zu unseren diesjährigen Preisträgern. Sie kommen beide aus dem Gebiet der Neurobiologie, dem wir auch unser Jubiläumssymposium vor zwei Jahren gewidmet haben. Die Tatsache, dass das Neuroscience Meeting in den USA von allen biomedizinischen Forschungskongressen am meisten Teilnehmer anzieht, zeigt, dass die Neurowissenschaften zu den derzeitigen Megatrends gehören. Dass wir aber bei der Auswahl unserer Preisträger nicht auf Trends, sondern ausschliesslich auf die Leistungen und das Potential achten, geht daraus hervor, dass nach über 15 Preisträgern erst dieses Jahr die Neurowissenschaften wieder zum Zug kommen – sogar zweifach!

Prof. Urs Albrecht vom Institut für Biochemie an der Universität Fribourg konnte überzeugend beweisen, dass zwei Gene in einem bestimmten Hirn-Kerngebiet die Voraussetzung dafür erfüllen, dass molekulare Uhren im Hirn für die Tag-Nacht-Zeitgebung funktionieren. Dieser so genannte zirkadische Rhythmus kann durch einen neuen Licht-Dunkel-Zyklus umgestellt werden. Beim Maulwurf funktioniert er lichtunabhängig. Dass solche neue Erkenntnisse unseres Preisträgers für das Verständnis von Schlafproblemen bei Nachtarbeitern oder bei Jetlag von Bedeutung sein werden, liegt auf der Hand.

Wir freuen uns, mehr darüber zu hören in der Laudatio von Prof. Ueli Schibler vom Département de Biologie Moléculaire der Universität Genf, der als erster in der Schweiz die genetisch-molekularbiologische Basis für die innere Uhr aufgebaut hat. Prof. Schibler wurde dafür 1986 mit dem Cloëtta-Preis ausgezeichnet.

Prof. Dominique Muller aus dem Département des Neurosciences Fondamentales am Centre Médical Universitaire in Genf hat international bestens anerkannte Beiträge zum Verständnis der synaptischen Plastizität geleistet. Er konnte als erster klar und eindeutig nachweisen, dass die Induktion der so genannten Langzeitpotenzierung mit histologisch nachweisbaren neuen Synapsen assoziiert ist. Bis dahin wurden in erster Linie funktionelle und nicht-anatomische Neubildungen für Lernprozesse und Gedächtnis verantwortlich gemacht.

Prof. Denis Monard vom Friedrich-Miescher-Institut in Basel, 1990 selber auch Cloëtta-Preisträger, wird uns einen ausführlicheren Überblick über das Forschungsgebiet von Prof. Dominique Muller und eine persönliche Würdigung geben.

Wie jedes Jahr möchte ich an dieser Stelle nicht nur meinen vier Kollegen vom medizinischen Ausschuss und den drei Mitgliedern des Stiftungsrates für ihren ehrenamtlichen Einsatz während des vergangenen Jahres herzlich danken, sondern auch Frau Brigitt Küttel und Frau Irène Thomann von unserer Geschäftsstelle für den perfekten Ablauf der Preisverleihung und der Verwaltung der Forschungsstellen.

DER CLOËTTA-PREIS 2005
WIRD VERLIEHEN

HERRN PROFESSOR DOKTOR

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FÜR SEINE INTERNATIONAL HERAUSRAGENDEN ARBEITEN
AUF DEM GEBIET DER CIRCADIANEN MOLEKULARBIOLOGIE.

ZÜRICH, 30. NOVEMBER 2005

FÜR DEN STIFTUNGSRAT:

DER PRÄSIDENT

DER VIZEPRÄSIDENT

EIN MITGLIED

Prof. Dr. Urs Emanuel Albrecht

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Marital status:	Married, 2 children

Education and Training

1982	Matura, Type C, Kantonsschule Limmattal, Urdorf, Switzerland
1988	Diploma in Biochemistry, Dipl. Phil. II, University of Zürich
1993	PhD in Zoology, Dr. Phil. Nat., University of Berne
1993–1997	Postdoctoral Fellow and Research Associate, Department of Biochemistry, Baylor College of Medicine, Houston, Texas, USA

Appointments

1998	Assistant Professor, Department of Biochemistry, Baylor College of Medicine, Houston, Texas, USA
1999	Assistant Professor, Max Planck Institute for Experimental Endocrinology, Hannover, Germany
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Awards and Prizes

1995–1996	Postdoctoral fellowship for advanced researchers from the Swiss National Science Foundation
1996+1998	V. C. Joshi Memorial Award, Dept. of Biochemistry, Baylor College of Medicine, Houston, USA
2001	AETAS foundation award
2005	Prize Prof. Dr. Max Cloëtta

Memberships and Committees

1989	USGEB/USSBE (Union of Swiss Societies for Experimental Biology)
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2001	Swiss Society for Neuroscience
2002–2005	Vice President of USGEB/USSBE (Union of Swiss Societies for Experimental Biology)
2003	Lifetime member Indian Society for Chronobiology

External reviewer for:
Deutsche Forschungsgemeinschaft (DFG)
INSERM, Paris, France
Israeli Science Foundation (ISF)
Netherlands Organisation for Scientific Research (NWO)
The Wellcome Trust, UK

KEY PUBLICATIONS

1. Sun Z. S., Albrecht U., Zhuchenko O., Bailey J., Eichele G and Lee C. C. «RIGUI, a putative mammalian ortholog of the *Drosophila period gene*». Cell 90:1003–1011, 1997.

2. Albrecht U., Sun Z. S., Eichele G. and Lee C.C. «A differential response of two putative mammalian circadian regulators, *mper1* and *mper2*, to light». Cell 91:1055–1064, 1997.

3. Zheng B., Larkin D.W., Albrecht U., Sun Z. S., Sage M., Eichele G., Lee C. C. and Bradley A. «The *mPer2* gene encodes a functional component of the mammalian circadian clock». Nature 400:169–173, 1999.

4. Motzkus D., Maronde E., Grunenberg U., Lee C. C., Forssmann W.-G. and Albrecht U. «The human *PER1* gene is transcriptionally regulated by multiple signalling pathways». *FEBS Lett.* 486:315–319, 2000.

5. Albrecht U., Zheng B., Larkin D., Sun Z. S. and Lee C. C. «*mPer1* and *mPer2* are essential components for normal resetting of the circadian clock». J. Biol. Rhythms 16:100–104, 2001.

6. Zheng B., Albrecht U., Kaasik K., Sage M., Lu W., Vaishnav S., Li Q., Sun Z. S., Eichele G., Bradley A., Lee C. C. «Non-redundant roles of the *mPer1* and *mPer2* genes in the mammalian circadian clock». Cell 105:683–694, 2001.

7. Abarca C., Albrecht U., Spanagel R. «Cocaine sensitization and reward are under the influence of circadian genes and rhythm». *Proc. Natl. Acad. Sci.* USA 99:9026–9030, 2002.

8. Oster H., Yasui A., van der Horst G. T. J., Albrecht U. «Disruption of *mCry2* restores circadian rhythmicity in *mPer2* mutant mice». *Genes Dev.* 16:2633–2638, 2002.

9. Oster H., Baeriswyl S., van der Horst G.T.J., Albrecht U. «Loss of circadian rhythmicity in aging *mPer1–mCry2*– mutant mice». *Genes Dev.* 17:1366–1379, 2003.

10. Spanagel R., Pendyala G., Abarca C., Zghoul T., Sanchis-Segura C., Magnone M. C., Lascorz J., Depner M., Holzberg D., Soyka M., Schreiber S., Matsuda F., Lathrop M., Schumann G. and Albrecht U. «The clock gene *Period2* influences the glutamatergic system and thereby modulates alcohol consumption». *Nat. Med.* 11:35–42, 2005.

THE CIRCADIAN CLOCK: ORCHESTRATING GENE EXPRESSION AND PHYSIOLOGY

Urs Albrecht

Timing is everything. How true is this proverb? Time has neither a beginning nor an end, and hence, it is difficult to define. What we experience as time is related to a reference point and, hence, relative. Living on earth has made us use the sun as reference and the 24-hour succession of light and darkness is probably the most pervasive epigenetic influence in the evolution from a single cell organism to man. This periodic succession of light and darkness provided the base for relative timing of biological processes over the 24 hours of a day. Because energy supply is the limiting parameter for survival, a system for optimal timing of energy expenditure and uptake developed. The mechanism of this system took the shape of a cycle reflecting the recurrence of sunrise and sunset, and is termed a «circadian clock» – a clock with a period of about one day (latin: *circa diem*). The internalization of environmental time within the organism not only allows organization of biological processes along the 24-hour time scale but also prediction of recurring events, such as availability of food and emergence of predators. The most compelling demonstration of the circadian clock's utility has been made by using cyanobacterial strains with different clock properties growing in competition with each other. Strains with a functioning circadian clock defeat clock-disrupted strains in rhythmic environments, however, this competitive advantage disappears in constant environments. The strains compete most effectively in a rhythmic environment when the frequency of their internal biological oscillator is similar to the environmental cycle (Ouyang et al., 1998; Woelfle et al., 2004). That this is also valid for multicellular eukaryotes has been demonstrated recently in studies using the plant *Arabidopsis thaliana*. Comparing wild type with long- and short-circadian period mutants indicates an advantage of matching the circadian period to the external light-dark cycle. Hence, wild type plants contain more chlorophyll, fix more carbon,

grow faster, and survive better than plants with circadian periods differing from their environment (Dodd et al., 2005).

The underlying principle of circadian clocks is successive gene activation in form of a cycle: the initial gene activation is regulated by the last one in the sequence, making up an auto-regulatory feedback loop for which one cycle takes about 24 hours. This principle is illustrated in figure 1. Positive elements activate the expression of negative elements, which in turn stop the activity of the positive elements. This system moves away from equilibrium before returning and hence, perpetual cycling is the consequence. Although the genes involved in this mechanism can differ in various organisms the principle illustrated in figure 1 is common to all of them (reviewed in Bell-Pedersen et al., 2005; Young and Kay, 2001).

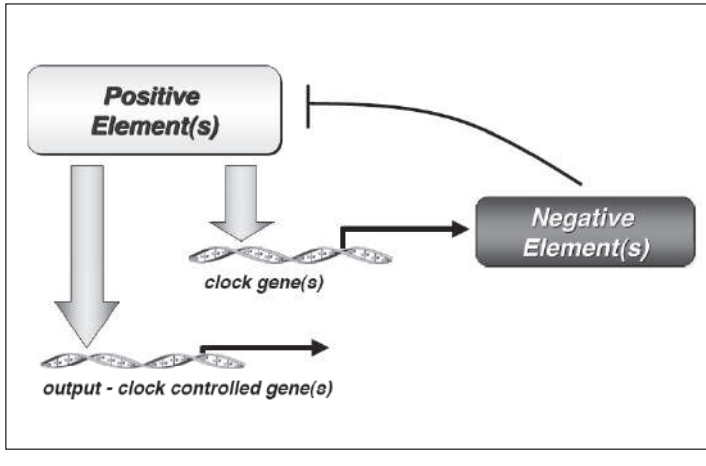

Figure 1:
General mechanism of the circadian clock. Positive elements activate expression of negative elements that inhibit the action of positive elements, thereby establishing an auto-regulatory feedback loop. The positive elements of the clock additionally activate clock-controlled genes transmitting time information to the whole organism.

Temporal information coded in this clock mechanism is only of if it is translated into a physiological meaning. This is achieved through coupling of the clock to biological pathways that are themselves composed of sequential gene activation. Connecting rate-limiting steps to the clock submits whole pathways to a circadian rhythm and hence, they become hands of the clock. This is also termed the clock's output (see figures 1 and 2).

The earth's orbit around the sun leads to seasons that manifest themselves, besides the temperature changes, in an altered length of a day's light period. To adapt to these changes the circadian clock is connected to mechanisms that allow it to stay in tune with nature. Sensory organs communicate environmental time information *via* signaling pathways to the clock, thereby synchronizing the internal circadian oscillators with the environment. The existence of such an input pathway in the circadian system (figure 2) is the reason why humans can adapt to different time zones and overcome jet lag.

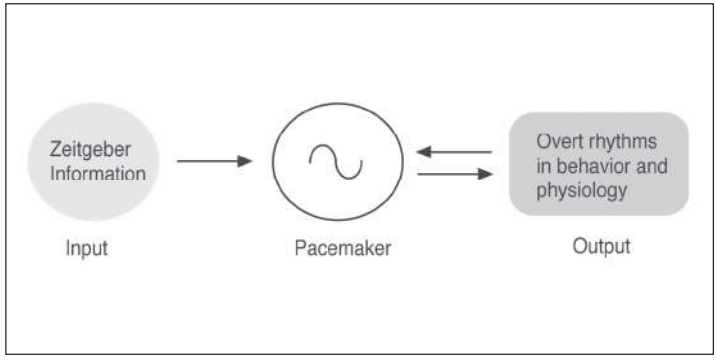

Figure 2:
Schematic diagram of the circadian system. It is composed of the input pathway, the pacemaker or clock and the output pathway. Note that the physiological state of the organism can influence the clock pacemaker to establish a crosstalk between clock pacemaker and output targets.

Figure 2 illustrates the exquisite position of the clock pacemaker in the circadian system. It synchronizes the rhythms in physiology with the environmental diurnal rhythm and hence is not only a metronome but also an integrator of both environmental and body signals. In unicellular and light permissive animals each cell has a circadian oscillator with its own photoreceptors that communicate with the clock to set its phase. This is different in opaque multi-cellular organisms such as mammals. Although each cell in the different organs contains a clock (Yamazaki et al., 2000; Yoo et al., 2004) not every cell of the body can be reached by light. Hence, a hierarchical organization of the individual clocks is necessary (reviewed in Hirota and Fukada, 2004). In mammals the retina and the master pacemaker, located in the *suprachiasmatic nuclei* (SCN) (see figure 3A), are the only known cells to be entrained by light. Consequently, the clocks in the peripheral organs, such as kidney and liver, must be synchronized by neuronal or humoral signals from the SCN. Diffusible factors from the SCN that have the potential to synchronize clocks in different structures of the brain are TGFα and prokineticin 2 (Cheng et al., 2002; Kramer et al., 2001). However, these factors seem not to be the main synchronizers of peripheral tissues because their receptors are lacking in most peripheral organs. Serum inducibility of clock genes in fibroblasts (Balsalobre et al., 1998) suggests that blood-born factors stimulate signal transduction pathways that influence the mammalian molecular oscillator in cells of peripheral tissues (figure 3A). Glucocorticoids might play an important role in this regard, because they can reset the circadian clock by changing *Per* gene expression (Balsalobre et al., 2000) and the hypophyseal-adrenal axis is regulating their expression (reviewed in Buijs and Kalsbeek, 2001). A number of other factors affect *Per* gene expression and clock phase, including interleukin-6 (Motzkus et al., 2002) and retinoic acid (McNamara et al., 2001).

To study the mammalian circadian transcriptional output, several laboratories have applied the systems-biology tool of transcriptional profiling (Panda et al., 2002; Ueda et al., 2002). Analysis of rhythmic genes in liver revealed their principal role in regulating metabolism, whereas genes cycling in the SCN are primarily involved in signaling and neurosecretion. However, the network topology of circadian trans-

criptional output remains elusive. It seems that three promoter elements are important for circadian regulation: E-boxes, targets of the CLOCK/BMAL1 complex, D-boxes, targets of DBP/E4BP4, and REV-ERBα/ROR-regulatory elements (RREs) (figure 3B). Combinations of these three elements in promoters allow generation of different phases and amplitudes of circadian transcription with the E-box element playing the critical role (Ueda et al., 2005).

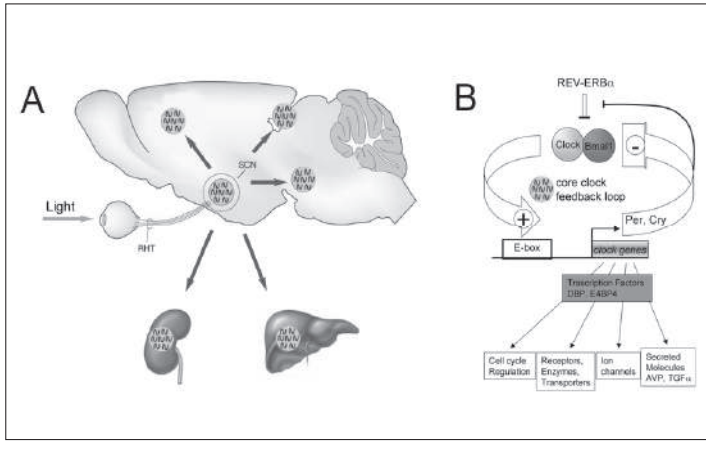

Figure 3:
The circadian system and clock mechanism in mammals. A) Hierarchical organization of the circadian system. Light activates specific photoreceptors in the retina from where the signal is transmitted via the retinohypothalamic tract (RHT) to the suprachiasmatic nuclei (SCN), the master pacemaker. The SCN emits signals to synchronize the clocks (circles with waved lines) in other brain structures and in peripheral organs such as the kidney and the liver. B) Clock mechanism in mammals. The transcriptional activators CLOCK and BMAL1 dimerize and promote transcription of Period (Per) and Cryptochrome (Cry) genes by binding to E-box elements present in their promoters, constituting the positive limb of the feedback mechanism. PER and CRY proteins are thought to inhibit the CLOCK/BMAL1 complex, thereby closing the loop (negative limb). REV-ERBα negatively regulates the Clock and Bmal1 genes and is influenced by PER and CRY. Additionally, protein kinases have important roles in the modulation of the activities of clock components. The positive limb of the loop can also activate output genes either directly (e.g. Avp) or indirectly via the regulation of other transcription factors such as DBP (reviewed in Reppert and Weaver, 2002).

The circadian timing system of mammals influences most physiological activities, including sleep and wakefulness, body temperature, intestinal peristaltics, hepatic activity, cardiovascular activity and precision of the sensory system (reviewed in Schibler et al., 2003). As illustrated in figure 3 these systems depend on the SCN, which receives photic information from classical rod and cone photoreceptors (Freedman et al., 1999; Ruby et al., 2002) as well as from melanopsin containing ganglion cells of the retina (Hattar et al., 2002; Ruby et al., 2002). This information is transmitted as electrical signals *via* the RHT (figure 3A). The neurotransmitters glutamate and pituitary adenylate cyclase-activating peptide released at the RHT synapses contacting the SCN, trigger the influx of calcium which results in an activation of several protein kinases (protein kinase A, PKA; protein kinase C, PKC; protein kinase G, PKG, cGK; mitogen-activated protein kinase, MAPK) (reviewed in Hirota and Fukada, 2004). This leads, besides the stimulation of immediate-early genes, to an activation of *Per1* and *Per2* genes, and the photic regulation of PER protein accumulation may play an essential role in tuning the circadian clock to daylight (Albrecht et al., 2001; Steinlechner et al., 2002). Support for this view comes from the finding that, in humans, a mutation in the casein kinase I binding domain of the *Per2* gene leads to hypophosphorylation and is associated with familial advanced sleep phase syndrome (FASPS) (Toh et al., 2001). This posttranslational regulation has a prominent effect on the clock and is further illustrated by the observation that a mutation in the casein kinase I delta can cause FASPS probably by affecting phosphorylation of clock components (Xu et al., 2005).

Single SCN neurons cultured *in vitro* display circadian rhythms in firing frequency and therefore, contain autonomous oscillators (Liu et al., 1997). Interestingly, the period length varies between the cells, causing desynchronization between them after prolonged time in culture. Therefore, in the intact organism the SCN neurons must be coupled. Coordinated activity of SCN neurons seems to be critically regulated by electrical synapses (Long et al., 2005) and vasoactive intestinal polypeptide (Aton et al., 2005). This coupling appears to be strong enough to maintain a circadian rhythm in rodents even when they are kept in constant darkness for months or even years. In contrast,

when exposed to constant light, the animals become behaviorally arrhythmic with time. This is not due to disruption of the circadian firing rhythm but is the result of desynchronization of clock neurons (Ohta et al., 2005).

The findings described above highlight the importance of synchronization. Internal body time needs to be aligned with external environmental time, and within the body, the clocks in different organs and within organs need proper orchestration if our body is to withstand predictable natural forces and work in an optimized fashion. However, in our 24-hour society shift work and transmeridian flights are a serious challenge for our circadian system, and through its coupling to physiological pathways also for our health. These challenges are relatively well handled in younger organisms, but with age, associated problems like sleep disturbances, digestive and cardiovascular problems, mental illness and alcohol abuse become more apparent. The reason for this is that circadian organization changes with age (Valentinuzzi et al., 1997; Yamazaki et al., 2002) and results in decrease of amplitude and fragmentation of the rest-activity cycle as well as in a reduced sensitivity to the phase-shifting effects of light (Valentinuzzi et al., 1997). These age-related phenotypes can be mimicked by alterations in the clock mechanism (Oster et al., 2003). A mutation in the Clock gene disrupts estrous cyclicity and maintenance of pregnancy (Miller et al., 2004) probably caused by an uncoupling of prolactin regulation from the clock (Leclerc and Boockfor, 2005). The findings described above illustrate a potential relation between the circadian system and the aging process.

A very prominent age-related process is the development of cancer. Because a defective clock seems to accelerate aging, it is not surprising to find that mice with a defective clock are more prone to develop cancer. The *Per2* gene plays a role in tumor suppression and in DNA damage response by regulating the temporal expression of genes involved in cell cycle regulation, such as *c-Myc, Cyclin D1, Cyclin A* and *Mdm-2* (Fu et al., 2002). In the regenerating liver of mice it has been shown that the circadian clock directly controls the expression of cell cycle-related genes, such as *wee1*, that in turn modulate the expres-

sion of active Cyclin B1-Cdc2 kinase, a key regulator of mitosis (Matsuo et al., 2003). Interestingly, circadian gene expression in fibroblasts continues during the cell division cycle and daughter cells have the same phase as their mother cell. It seems that the circadian oscillator gates cytokinesis to define time windows, and mitosis elicits phase shifts in circadian cycles (Nagoshi et al., 2004). However, the interaction between the circadian clock and the cell cycle appears to be reciprocal. In breast cancer the expression of *Per* genes is deregulated (Chen et al., 2005) and many tumor cells have lost circadian rhythmicity and daytime-dependent cell cycle progression (reviewed in Canaple et al., 2003). This difference between healthy and cancer cells might be exploited by delivering antiproliferative drugs at times when they are least toxic to normal cells. Encouraging results are now being obtained with the first attempts of using chronotherapy (reviewed in Mormont and Levi, 2003) and this might lead the way for more efficient and less harmful treatments of cancer patients. A prerequisite for the success of chronotherapy is the determination of an individual's body time. Reaching this goal is not an easy task, because of tissue sampling at multiple time points. However, a microarray-based method has been developed to determine individual body time in mice, from tissue harvested at a single time point (Ueda et al., 2004). How applicable this method is for humans, remains to be seen (reviewed in Albrecht, 2004) but it is a first step in the direction of individualized medicine, which could improve medical treatment.

Peripheral clocks are not exclusively influenced by the SCN in the brain. Feeding cycles can act directly on the clock in peripheral organs and uncouple their clock phase from the SCN (Damiola et al., 2000). This suggests that these clocks have an important role in the processing of nutrients and energy homeostasis. Several studies using transcriptome profiling support this view, showing that many cyclically expressed liver genes perform functions related to metabolism and detoxification (Panda et al., 2002; Storch et al., 2002). The connection between metabolism and the clock is particularly intriguing in view of the finding that DNA binding of Clock protein is regulated by the redox state of NAD cofactors (Rutter et al., 2001). This highlights the regulatory potential of metabolism on the clock, however, recent studies also

indicate that alterations in the circadian clock mechanism may change metabolism. Mice with mutations in the clock genes *Bmal1* and *Clock* show no diurnal variations in glucose and triglycerides, and gluconeogenesis is altered in these mutants, indicating that the circadian clock is involved in glucose homeostasis (Rudic et al., 2004). Additionally, *Clock* mutant mice become obese and develop metabolic syndrome of hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycemia and hypoinsulinemia (Turek et al., 2005). These findings lead to the speculation that in our society, due to irregular life style, our clock might become derailed, promoting abnormal eating habits and obesity. Indeed, studies with human volunteers indicate that short sleep duration is associated with reduced leptin levels, elevated ghrelin and an increased body mass index (Taheri et al., 2004).

Deregulation of metabolism has far reaching consequences. It not only leads to an alteration in body mass index but can also affect mental state. The amounts of glutamate, dopamine, serotonin and other neurotransmitters seem to be, at least in part, under the influence of the circadian clock via regulation of DBP or other unknown genes. Lack of three proline and acidic amino acid rich basic leucine zipper (PAR bZip) transcription factors, DBP (albumin D-site binding protein, Fig. 3B), HLF (hepatic leukemia factor) and TEF (thyrotroph embryonic factor) results in epilepsy. This is due to an alteration in pyridoxal kinase (Pdxk), which converts vitamin B6 derivatives into pyridoxal phosphate (PLP), the coenzyme of many enzymes involved in amino acid and neurotransmitter metabolism. In mice a lack of these PAR bZip factors results in decreased PLP, serotonin and dopamine levels in the brain leading to epilepsy (Gachon et al., 2004a). Another neurotransmitter, glutamate, is also affected in mice with an altered clock. Normally, the excitatory amino acid transporter 1 (EAAT1), which transports glutamate from the synaptic cleft into astrocytes, is expressed in a circadian fashion. A mutation in the *Per2* gene reduces the expression of EAAT1, and thus, glutamate levels in the synaptic cleft rise. However, the amount of glutamate does not reach toxic levels but they are sufficiently high to alter the behavior of these animals, e. g. adjustment of the clock to the day-night cycle (Albrecht et al., 2001) or alcohol consumption (Spanagel et al., 2005).

The first evidence for the involvement of the circadian clock in addictive processes has come from the observation that fruit flies mutant in clock genes do not sensitize to cocaine through regulation of tyrosine decarboxylase (Andretic et al., 1999). Similar observations have been made in mice (Abarca et al., 2002; McClung et al., 2005), suggesting that clock genes are involved in common modulator mechanisms of drug abuse-related behaviors (Yuferov et al., 2003). Interestingly, drugs modulate clock genes, as demonstrated by methamphetamine injection causing an increase of *Per* gene expression in the caudate putamen of the mouse (Nikaido et al., 2001). Metamphetamine treatment of rats alters the circadian expression rhythms of clock genes in the caudate putamen and the parietal cortex, and desynchronizes them from the SCN rhythms (Masubuchi et al., 2000). Drug action seems to involve common signal transduction pathways that are also part of the resetting mechanism of the circadian clock. For example, serotonin receptor agonists phase shift the circadian clock through an increase in cAMP production (Sprouse et al., 2004) and the drug Ecstasy (3,4 – methylenedioxymethamphetamine) alters this response (Biello and Dafters, 2001). Similarly, opioids affect the circadian system, suggesting a modulatory role for the clock in pain sensation (Vansteensel et al., 2005).

During episodes of depression the balance of a variety of neurotransmitters is disturbed. This balance might be tuned by the circadian clock as evidenced by the findings described above, and light probably plays an important role for orchestrating the temporal pattern of neurotransmitters for synchronization with the environment. An involvement of circadian clock related polymorphisms both in seasonal affective disorder (SAD) and in diurnal preference was found (Johansson et al., 2003), supporting the hypothesis of a link between circadian rhythms and seasonal depression. Whether a defective circadian clock plays a role in bipolar disorder (BD) is not clear, although there is evidence for a significant genetic etiology. However, gene-mapping efforts have been hampered by the complex mode of inheritance and the likelihood of multiple genes involved with small contribution. Because the circadian clock has a wide regulatory potential and extensive disruption in circadian function is known to occur among patients with BD during

relapse, it is plausible that circadian dysfunction underlies pathogenesis of BD (reviewed in Mansour et al., 2005).

The spatial and temporal distribution of electrical activity is a key modulator of the constructive and destructive processes that determine neuronal form and sculpt the pattern of neural circuitry. Therefore, one can speculate on the involvement of the circadian clock in the process of constant rearrangement. This may involve structural changes or modulation of the efficacy of synapses, both of which alter the functional properties of neural networks. This plasticity is crucial for numerous brain functions, most notably learning and memory, and may also explain addiction-related behaviors (see above). Circadian modulation of learning and memory has been investigated with varied results. In *Aplysia* long-term sensitization seems to be modulated in a circadian manner (Fernandez et al., 2003) and the clock gene *period* plays a key role in long-term memory formation in *Drosophila* (Sakai et al., 2004). Learning experiments in rats using the Morris water maze task demonstrated that circadian phase has an effect on learning performance (Valentinuzzi et al., 2004). However, mice mutant in the *Per1* or *Per2* gene do not differ in comparison to wild type animals in hippocampus-dependent learning (Zueger et al., 2005). Because these experiments have only used a limited set of tests under diurnal conditions more detailed studies are warranted in mammals.

The physiological and mental state of mammals alters throughout the day as manifested in the sleep-wake cycle. At the level of neurons this is paralleled by the steady depolarization of these cells during the day. Also, glucose metabolism in the brain is higher during wakefulness (Maquet et al., 2000) and the main astroglial glucose transporter *GLUT1* as well as the mitochondrial genes involved in oxidative phosphorylation are expressed during wakefulness. Similarly, genes of the glutamate-glutamine cycle (*glutamine synthase and glutaminase*) and regulatory genes for clustering glutamatergic receptors (*Homer/ Vesl, Narp*) are also expressed predominantly during wakefulness (Cirelli et al., 2004). While wakefulness has been associated with memory acquisition, sleep represents a favorable time for memory consolidation (Stickgold, 2001; Stickgold et al., 2001; Walker et al., 2002).

Many key components of the translational machinery are expressed at higher levels during sleep (Cirelli et al., 2004). Taken together these observations indicate that though sleep is a state of behavioral inactivity it is associated with the expression of many genes in the brain, and sleep and wakefulness favor different cellular processes. Wakefulness related transcripts may aid the brain in facing high energy demand, high synaptic excitatory transmission, high transcriptional activity and in the need for synaptic potentiation in the acquisition of new information as well as the cellular stress that may derive from one or more of these processes. Sleep, on the other hand, favors protein synthesis and complimentary aspects of neural plasticity such as synaptic depression. Interestingly, a mutation in a voltage-dependent potassium channel controlling membrane repolarization and transmitter release has been identified to be causal to short sleep in *Drosophila*. This mutation in the Shaker gene affects not only sleep duration but also leads to a reduced lifespan (Cirelli et al., 2005a).

Sleep is composed of homeostatic and circadian processes (Borbely, 1982; Borbely and Achermann, 1999) and hence the circadian clock is part of sleep. For example, mutations in *Per1* and *Per2* genes in the mouse influence allocation of sleep in the 24 hour day but these mutations do not affect the EEG slow-wave activity (Kopp et al., 2002). In contrast, *Cry1/2* double mutant mice exhibit high non-REM sleep (Wisor et al., 2002), highlighting a role of these clock components in homeostatic aspects of sleep. Microarray analysis of gene expression in *Drosophila* revealed that many wakefulness-related and sleep-related transcripts are modulated by time of day, suggesting an interaction at the molecular level between circadian and homeostatic mechanisms of sleep regulation (Cirelli et al., 2005b).

Perspectives

In the past few years many of the molecular components of the circadian clock have been identified in several organisms. This led to a general understanding of the clock mechanism and its potential to influence physiological processes. In the future the relationship

between metabolism and the circadian clock will receive more attention. This is because mitochondrial function depends partially on nuclear transcription of its enzyme complexes in the oxidative chain, which is directly related to generating the organism's energy currency ATP.

The clock mechanism itself will also be completed with satellite feedback loops, and inconsistencies in the current model will be addressed (see Gachon et al., 2004b). The regulation of clock components at the transcriptional and post-transcriptional levels will be major issues. Is regulation at the RNA level occurring in mammals as it is in other phyla (Kramer et al., 2003)? The determination of the three-dimensional structure of clock components (Yildiz et al., 2005) will boost our understanding in how these molecules interact with each other and with other molecules. This will help to decipher the precise function of the mammalian *Period* genes and answer the question whether they are co-activators or co-repressors of transcriptional activation.

At the cellular level future work will concentrate on the mechanisms by which cells couple and synchronize their circadian clock in a tissue. This will be greatly facilitated by the use of reporter constructs (Nagoshi et al., 2004), allowing direct monitoring of circadian clock activity in live cells. Such an *in vitro* system will be of great help to apply interfering RNA technology for evaluating the contribution of novel candidate clock genes.

At the physiological level one of the main challenges will be to unravel the role of the clock in mental capabilities. How does the clock influence the development of depression? Does it modulate pain sensation? What are the environmental contributions in altering the clock phase, causing aberrant mental states? Are there causative relationships between the clock, lifestyle and neurodegeneration causing Alzheimer's or Parkinson disease?

Individually tailored medicine is still a dream but with our knowledge on the circadian clock and clock-regulated processes improvement in efficiency of pharmacological agents will be possible. Individually

adapted treatment due to chronotyping of patients (Brown et al., 2005) will allow us in the future to improve timing of medical treatment, and thus, to reduce harmful side effects. Indeed, it appears that, for our health, timing is everything.

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REFERENCES

1. Abarca, C., Albrecht, U. and Spanagel, R. (2002) Cocaine sensitization and reward are under the influence of circadian genes and rhythm. *Proc. Natl. Acad. Sci.*, 99, 9026–9030.

2. Albrecht, U. (2004) Human molecular chronotyping in sight? *Genome Biol.*, 5, 246.

3. Albrecht, U., Zheng, B., Larkin, D., Sun, Z. S. and Lee, C. C. (2001) *mPer1* and *mPer2* are essential components for normal resetting of the circadian clock. *J. Biol. Rhythms*, 16, 100–104.

4. Andretic, R., Chaney, S. and Hirsh, J. (1999) Requirement of circadian genes for cocaine sensitization in *Drosophila. Science*, 285, 1066–1068.

5. Aton, S. J., Colwell, C. S., Harmar, A. J., Waschek, J. and Herzog, E. D. (2005) Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nat Neurosci*, 8, 476–483.

6. Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., Schütz, G. and Schibler, U. (2000) Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science*, 289, 2344–2347.

7. Balsalobre, A., Damiola, F. and Schibler, U. (1998) A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell*, 93, 929–937.

8. Bell-Pedersen, D., Cassone, V. M., Earnest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L. and Zoran, M. J. (2005) Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nat Rev Genet*, 6, 544–556.

9. Biello, S. M. and Dafters, R. I. (2001) MDMA and fenfluramine alter the response of the circadian clock to a serotonin agonist in vitro. *Brain Res*, 920, 202–209.

10. Borbely, A. A. (1982) A two process model of sleep regulation. *Hum Neurobiol*, 1, 195–204.

11. Borbely, A. A. and Achermann, P. (1999) Sleep homeostasis and models of sleep regulation. *J Biol Rhythms*, 14, 557–568.

12. Brown, S. A., Fleury-Olela, F., Nagoshi, E., Hauser, C., Juge, C., Meier, C. A., Chicheportiche, R., Dayer, J.-M., Albrecht, U. and Schibler, U. (2005) The period length of fibroblast circadian gene expression varies widely among human individuals. *PLoS Biol.*, in press.

13. Buijs, R. M. and Kalsbeek, A. (2001) Hypothalamic integration of central and peripheral clocks. *Nat Rev. Neurosci.*, 2, 521–526.

14. Canaple, L., Kakizawa, T. and Laudet, V. (2003) The days and nights of cancer cells. *Cancer Res*, 63, 7545–7552.

15. Chen, S. T., Choo, K. B., Hou, M. F., Yeh, K. T., Kuo, S. J. and Chang, J. G. (2005) Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. *Carcinogenesis*, 26, 1241–1246.

16. Cheng, M. Y., Bullock, C. M., Li, C., Lee, A. G., Bermak, J. C., Beluzzi, J., Weaver, D. R., Leslie, F. M. and Zhou, Q. (2002) Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature*, 417, 405–410.

17. Cirelli, C., Bushey, D., Hill, S., Huber, R., Kreber, R., Ganetzky, B. and Tononi, G. (2005a) Reduced sleep in Drosophila Shaker mutants. *Nature*, 434, 1087–1092.

18. Cirelli, C., Lavaute, T. M. and Tononi, G. (2005b) Sleep and wakefulness modulate gene expression in Drosophila. *J Neurochem.*, in press.

19. Cirelli, C., Gutierrez, C. M. and Tononi, G. (2004) Extensive and divergent effects of sleep and wakefulness on brain gene expression. *Neuron*, 41, 35–43.

20. Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F. and Schibler, U. (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev*, 14, 2950–2961.

21. Dodd, A. N., Salathia, N., Hall, A., Kévei, E., Toth, R., Nagy, F., Hibberd, J. M., Millar, A. J., Webb, A. A. R. (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science*, 309, 630–633.

22. Fernandez, R. I., Lyons, L. C., Levenson, J., Khabour, O. and Eskin, A. (2003) Circadian modulation of long-term sensitization in Aplysia. *Proc Natl Acad Sci USA*, 100, 14415–14420.

23. Freedman, M. S., Lucas, R. J., Soni, B., von Schantz, M., Munoz, M., David-Gray, Z. and Foster, R. (1999) Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science*, 284, 502–504.

24. Fu, L., Pelicano, H., Liu, J., Huang, P. and Lee, C. C. (2002) The circadian gene period 2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell*, 111, 41–50.

25. Gachon, F., Fonjallaz, P., Damiola, F., Gos, P., Kodama, T., Zakany, J., Duboule, D., Petit, B., Tafti, M. and Schibler, U. (2004a) The loss of circadian PAR bZip transcription factors results in epilepsy. *Genes Dev.*, 18, 1397–412.

26. Gachon, F., Nagoshi, E., Brown, S. A., Ripperger, J. and Schibler, U. (2004b) The mammalian circadian timing system: from gene expression to physiology. *Chromosoma*, 113, 103–112.

27. Hattar, S., Liao, H. W., Takao, M., Berson, D. M. and Yau, K. W. (2002) Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*, 295, 1065–1070.

28. Hirota, T. and Fukada, Y. (2004) Resetting mechanism of central and peripheral circadian clocks in mammals. *Zoolog Sci*, 21, 359–368.

29. Johansson, C., Willeit, M., Smedh, C., Ekholm, J., Paunio, T., Kieseppä, T., Lichtermann, D., Praschak-Rieder, N., Neumeister, A., Nilsson, L., Kasper, S., Peltonen, L., Adolfsson, R., Schalling, M. and Partonen, T. (2003) Circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. *Neuropsychopharmacol.*, 28, 734–739.

30. Kopp, C., Albrecht, U., Zheng, B. and Tobler, I. (2002) Homeostatic sleep regulation is preserved in mPer1 and mPer2 mutant mice. *Eur J Neurosci*, 16, 1099–1106.

31. Kramer, A., Yang, F. C., Snodgrass, P., Li, X., Scammell, T. E., Davis, F. C. and Weitz, C. J. (2001) Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. *Science*, 294, 2511–2515.

32. Kramer, C., Loros, J. J., Dunlap, J. C. and Crosthwaite, S. K. (2003) Role for antisense RNA in regulating circadian clock function in Neurospora crassa. *Nature*, 421, 948–952.

33. Leclerc, G. M., Boockfor, F. R. (2005) Pulses of prolactin promoter activity depend on a noncanonical E-box that can bind the circadian proteins CLOCK and BMAL1. *Endocrinology*, 146, 2782–2790.

34. Liu, C., Weaver, D. R., Strogatz, S. H. and Reppert, S. M. (1997) Cellular construction of a circadian clock: period determination in the suprachiasmatic nuclei. *Cell*, 91, 855–860.

35. Long, M. A., Jutras, M. J., Connors, B. W. and Burwell, R. D. (2005) Electrical synapses coordinate activity in the suprachiasmatic nucleus. *Nat Neurosci*, 8, 61–66.

36. Mansour, H. A., Monk, T. H. and Nimgaonkar, V. L. (2005) Circadian genes and bipolar disorder. *Ann Med*, 37, 196–205.

37. Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Meulemans, T., Luxen, A., Franck, G., Van Der Linden, M., Smith, C. and Cleeremans, A. (2000) Experience-dependent changes in cerebral activation during human REM sleep. *Nat Neurosci*, 3, 831–836.

38. Masubuchi, S., Honma, S., Abe, H., Ishizaki, K., Namihira, M., Ikeda, M. and Honma, K. (2000) Clock genes outside the suprachiasmatic nucleus involved in manifestation of locomotor activity rhythm in rats. *Eur. J. Neurosci.*, 12, 4206–4214.

39. Matsuo, T., Yamaguchi, S., Mitsui, S., Emi, A., Shimoda, F. and Okamura, H. (2003) Control mechanism of the circadian clock for timing of cell division in vivo. *Science*, 302, 255–259.

40. McClung, C. A., Sidiropoulou, K., Vitaterna, M., Takahashi, J. S., White, F. J., Cooper, D. C. and Nestler, E. J. (2005) Regulation of dopaminergic transmission and cocaine reward by the Clock gene. *Proc Natl Acad Sci USA*, 102, 9377–9381.

41. McNamara, P., Seo, S., Rudic, R. D., Sehgal, A., Chakravarti, D. and FitzGerald, G. A. (2001) Regulation of CLOCK and MOP4 by nuclear hormone receptors in the vasculature: a humoral mechanism to reset a peripheral clock. *Cell*, 105, 877–889.

42. Miller, B. H., Olson, S. L., Turek, F. W., Levine, J. E., Horton, T. H. and Takahashi, J.S. (2004) Circadian Clock mutation disrupts estrous cyclicity and maintenance of pregnancy. *Curr Biol*, 14, 1367–1373.

43. Mormont, M. C. and Levi, F. (2003) Cancer chronotherapy: principles, applications, and perspectives. *Cancer*, 97, 155–169.

44. Motzkus, D., Albrecht, U. and Maronde, E. (2002) The human PER1 gene is inducible by interleukin-6. *J Mol Neurosci*, 18, 105–109.

45. Nagoshi, E., Saini, C., Bauer, C., Laroche, T., Naef, F. and Schibler, U. (2004) Circadian gene expression in individual fibroblasts: cell-autononmous and self-sustained oscillators pass time to daughter cells. *Cell*, 119, 693–705.

46. Nikaido, T., Akiyama, M., Moriya, T. and Shibata, S. (2001) Sensitized increase of Period gene expression in the mouse caudate/putamen caused by repeated injection of methamphetamine. *Mol. Pharmacol.*, 59, 894–900.

47. Ohta, H., Yamazaki, S. and McMahon, D. G. (2005) Constant light desynchronizes mammalian clock neurons. *Nat Neurosci*, 8, 267–269.

48. Oster, H., Baeriswyl, S., van der Horst, G. T. J. and Albrecht, U. (2003) Loss of circadian rhythmicity in aging mPer1/mCry2 mutant mice. *Genes Dev*, 17, 1366–1379.

49. Ouyang, Y., Andersson, C. R., Kondo, T., Golden, S.S. and Johnson, C. H. (1998) Resonating circadian clocks enhance fitness in cyanobacteria. *Proc Natl Acad Sci USA*, 95, 8660–8664.

50. Panda, S., Antoch, M. P., Miller, B. H., Su, A. I., Schook, A. B., Straume, M., Schultz, P. G., Kay, S. A., Takahashi, J. S. and Hogenesch, J. B. (2002) Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell*, 109, 307–320.

51. Reppert, S. M. and Weaver, D. R. (2002) Coordination of circadian timing in mammals. *Nature*, 418, 935–941.

52. Ruby, N. F., Brennan, T. J., Xie, X., Cao, V., Franken, P., Heller, H. C. and O'Hara, B. F. (2002) Role of melanopsin in circadian responses to light. *Science*, 298, 2211–2213.

53. Rudic, R. D., McNamara, P., Curtis, A. M., Boston, R. C., Panda, S., Hogenesch, J. B. and FitzGerald, G. A. (2004) BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLOS Biol.*, 2, e377.

54. Rutter, J., Reick, M., Wu, L. C. and McKnight, S. L. (2001) Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science*, 293, 510–514.

55. Sakai, T., Tamura, T., Kitamoto, T. and Kidokoro, Y. (2004) A clock gene, period, plays a key role in long-term memory formation in *Drosophila. Proc. Natl. Acad. Sci.*, 101, 16058–16063.

56. Schibler, U., Ripperger, J. and Brown, S. A. (2003) Peripheral circadian oscillators in mammals: time and food. *J Biol Rhythms*, 18, 250–260.

57. Spanagel, R., Pendyala, G., Abarca, C., Zghoul, T., Sanchis-Segura, C., Magnone, M. C., Lascorz, J., Depner, M., Holzberg, D., Soyka, M., Schreiber, S., Matsuda, F., Lathrop, M., Schumann, G. and Albrecht, U. (2005) The clock gene Per2 influences the glutamatergic system and modulates alcohol consumption. *Nat Med*, 11, 35–42.

58. Sprouse, J., Reynolds, L., Braselton, J. and Schmidt, A. (2004) Serotonin-induced phase advances of SCN neuronal firing in vitro: a possible role for 5-HT5A receptors? *Synapse*, 54, 111–118.

59. Steinlechner, S., Jacobmeier, B., Scherbarth, F., Dernbach, H., Kruse, F. and Albrecht, U. (2002) Robust circadian rhythmicity of *Per1* and *Per2* mutant mice in constant light, and dynamics of *Per1* and *Per2* gene expression under long and short photoperiods. *J. Biol. Rhythms*, 17, 202–209.

60. Stickgold, R. (2001) Watching the sleeping brain watch us – sensory processing during sleep. *Trends Neurosci*, 24, 307–309.

61. Stickgold, R., Hobson, J. A., Fosse, R. and Fosse, M. (2001) Sleep, learning, and dreams: off-line memory reprocessing. *Science*, 294, 1052–1057.

62. Storch, K.-F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H. and Weitz, C. J. (2002) Extensive and divergent circadian gene expression in liver and heart. *Nature*, 417, 78–83.

63. Taheri, S., Lin, L., Austin, D., Young, T. and Mignot, E. (2004) Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLoS Med*, 1, e62.

64. Toh, K. L., Jones, C. R., He, Y., Eide, E. J., Hinz, W. A., Virshup, D. M., Ptacek, L. J. and Fu, Y.-H. (2001) An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science*, 291, 1040–1043.

65. Turek, F. W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., McDearmon, E., Laposky, A., Losee-Olson, S., Easton, A., Jensen, D. R., Eckel, R. H., Takahashi, J. S. and Bass, J. (2005) Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*, 308, 1043–1045.

66. Ueda, H. R., Chen, W., Adachi, A., Wakamatsu, H., Hayashi, S., Takasugi, T., Nagano, M., Nakahama, K., Suzuki, Y., Sugano, S., Iino, M., Shigeyoshi, Y. and Hashimoto, S. (2002) A transcription factor response element for gene expression during circadian night. *Nature*, 418, 534–539.

67. Ueda, H. R., Chen, W., Minami, Y., Honma, S., Honma, K., Iino, M. and Hashimoto, S. (2004) Molecular-timetable methods for detection of body time and rhythm disorders form single-time-point genome-wide expression profiles. *Proc. Natl. Acad. Sci.*, 101, 11227–11232.

68. Ueda, H. R., Hayashi, S., Chen, W., Sano, M., Machida, M., Shigeyoshi, Y., Iino, M. and Hashimoto, S. (2005) System-level identification of transcriptional circuits underlying mammalian circadian clocks. *Nat Genet*, 37, 187–192.

69. Valentinuzzi, V. S., Menna-Barreto, L. and Xavier, G. F. (2004) Effect of circadian phase on performance of rats in the Morris water maze task. *J Biol Rhythms*, 19, 312–324.

70. Valentinuzzi, V. S., Scarbrough, K., Takahashi, J. S. and Turek, F. W. (1997) Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. *Am J Physiol*, 273, R1957–1964.

71. Vansteensel, M. J., Magnone, M. C., van Oosterhout, F., Baeriswyl, S., Albrecht, U., Albus, H., Dahan, A. and Meijer, J. H. (2005) The opioid fentanyl affects light input, electrical activity and Per gene expression in the hamster suprachiasmatic nuclei. *Eur J Neurosci*, 21, 2958–2966.

72. Walker, M. P., Brakefield, T., Morgan, A., Hobson, J. A. and Stickgold, R. (2002) Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron*, 35, 205–211.

73. Wisor, J. P., O'Hara, B. F., Terao, A., Selby, C. P., Kilduff, T. S., Sancar, A., Edgar, D. M. and Franken, P. (2002) A role for cryptochromes in sleep regulation. *BMC Neurosci*, 3, 20.

74. Woelfle, M. A., Ouyang, Y., Phanvijhitsiri, K. and Johnson, C. H. (2004) The adaptive value of circadian clocks: an experimental assessment in cyanobacteria. *Curr Biol*, 14, 1481–1486.

75. Xu, Y., Padiath, Q. S., Shapiro, R. E., Jones, C. R., Wu, S. C., Saigoh, N., Saigoh, K., Ptacek, L. J. and Fu, Y. H. (2005) Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. *Nature*, 434, 640–644.

76. Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., Block, G. D., Sakaki, Y., Menaker, M. and Tei, H. (2000) Resetting central and peripheral circadian oscillators in transgenic rats. *Science*, 288, 682–685.

77. Yamazaki, S., Straume, M., Tei, H., Sakaki, Y., Menaker, M. and Block, G. D. (2002) Effects of aging on central and peripheral mammalian clocks. *Proc Natl Acad Sci USA*, 99, 10801–10806.

78. Yildiz, O., Doi, M., Yujnovsky, I., Cardone, L., Berndt, A., Hennig, S., Schulze, S., Urbanke, C., Sassone-Corsi, P. and Wolf, E. (2005) Crystal structure and interactions of the PAS repeat region of the Drosophila clock protein PERIOD. *Mol Cell*, 17, 69–82.

79. Yoo, S., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepka, S. M., Hong, H., Oh, W. J., Yoo, O. J., Menaker, M. and Takahashi, J. S. (2004) Period2: luciferase real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proc. Natl. Acad. Sci.*, 101, 5339–5346.

80. Young, M. W. and Kay, S. A. (2001) Time zones: a comparative genetics of circadian clocks. *Nat Rev Genet*, 2, 702–715.

81. Yuferov, V., Kroslak, T., Laforge, K. S., Zhou, Y., Ho, A. and Kreek, M. J. (2003) Differential gene expression in the rat caudate putamen after «binge» cocaine administration: advantage of triplicate microarray analysis. *Synapse*, 48, 157–169.

82. Zueger, M., Urani, A., Chourbaji, S., Zacher, C., Lipp, H. P., Albrecht, U., Spanagel, R., Wolfer, D. P. and Gass, P. (2005) mPer1 and mPer2 mutant mice show regular spatial and contextual learning in standardized tests for hippocampus-dependent learning. *J Neural Transm.*, in press.

LE PRIX CLOËTTA 2005
EST DÉCERNÉ À

MONSIEUR LE PROFESSEUR

DOMINIQUE MULLER

NÉ EN 1956, D'AESCHI (BE)
PROFESSEUR AU DÉPARTEMENT DES
NEUROSCIENCES FONDAMENTALES
CENTRE MÉDICAL UNIVERSITAIRE, GENÈVE,
POUR SES REMARQUABLES TRAVAUX DANS LE DOMAINE DE LA
PLASTICITÉ SYNAPTIQUE ET SON RÔLE DANS LES PROCESSUS
D'APPRENTISSAGE ET DE MÉMORISATION.

ZURICH, LE 30 NOVEMBRE 2005

AU NOM DU CONSEIL DE FONDATION:

LE PRÉSIDENT

LE VICE-PRÉSIDENT

UN MEMBRE

Prof. Dr. Dominique Muller

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Appointments

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KEY PUBLICATIONS

1. Muller, D., Joly, M. and Lynch, G. (1988) Differential contributions of quisqualate versus NMDA receptors to the induction and expression of long-term potentiation. *Science*, 242, 1694–1697.

2. Stoppini, L., Buchs, P.-A. and Muller, D. (1991) A simple method for organotypic cultures of nervous tissue. *J. of Neuroscience Methods* 37, 173–182.

3. Buchs, P.-A. and Muller D. (1996) LTP induction is associated with major ultrastructural changes of activated synapses. *Proc. Nat. Acad. Sci. U.S.A.* 93, 8040–8045.

4. Muller, D., Wang, C., Toni, N., Skibo, G., Cremer, H., Rougon, G. and Kiss, J. Z. (1996) PSA-NCAM is required for activity-induced synaptic plasticity. *Neuron* 17, 413–422.

5. Toni N, Buchs P.-A., Nikonenko I., Bron C. R. and Muller, D. (1999) LTP induces synaptogenesis by duplication of synaptic spines contacting a single axon terminal. *Nature* 402, 421–425.

6. Muller D., Djebbara-Hannas Z., Jourdain P., Vutskitz L., Durbec P., Rougon G. and Kiss J. Z. (2000) BDNF restores LTP in PSA-NCAM deficient hippocampus. *Proc. Nat. Acad. Sci. U.S.A.* 97:4315–4320.

7. Luscher, C., Malenka, R., Nicoll R. and Muller, D. (2000) Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nature Neurosci*. 3:545–550.

8. Jourdain, P., Nikonenko I., Alberi S. and Muller D. (2002) Anoxia/hypoglycemia induced remodeling of synaptic connections in hippocampal slice cultures *J. Neuroscience* 22:3108–3116.

9. Nikonenko, I., Jourdain P. and Muller, D. (2003) Presynaptic remodelling contributes to activity-induced synaptogenesis *J. Neuroscience* 23:8498–505.

10. Jourdain, P., Fukunaga, K. and Muller, D. (2003) Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation *J. Neuroscience* 23:10645–10649.

11. Boda B., Alberi S., Nikonenko I., Node-Langlois R., Jourdain P., Moosmayer M., Parisi-Jourdain L. and Muller D. (2004) The mental retardation protein PAK3 contributes to synapse formation and plasticity in hippocampus. *J. Neuroscience*. 24:10816–25.

FUNCTIONAL AND STRUCTURAL PLASTICITY OF SYNAPTIC NETWORKS

Dominique Muller

Introduction

When Ramon y Cajal described the morphological complexity of cortical neurones with their numerous dendritic spines, small protrusions that are the site of communication between neuronal cells, he proposed several ideas about their function that are still the subject of intense investigations. He proposed for example that these small biological connections could be reinforced by exercise, thus contributing to learning and that new connections could be established through an increased ramification and growth of dendritic arborisations (Ramon y Cajal, 1911). This was purely speculative at the time, but he established in this way the first conceptual bases for the notion of synaptic plasticity. This still holds true nowadays and synaptic plasticity is still considered as one of the fundamental properties of brain networks accounting for many aspects of higher brain functions, including memory and cognition. Despite this importance the molecular mechanisms underlying these properties are still far from being understood. Major progress has been realized within the last 5–10 years, essentially through the development and refinement of imaging and transfection technologies that now allow us to visualize and investigate the function of identified synaptic boutons while altering the expression of specific molecular constituent. It has become possible in this way to start understand at the molecular level the mechanisms that contribute to the various aspects of synaptic plasticity.

Modulation of synaptic efficacy

One of the main concepts about synaptic plasticity has been formulated by D. Hebb. (Hebb, 1949). He proposed a learning mechanism based

on lasting changes in the efficacy of synaptic communication between two neurons and triggered by a coordinated activity of these neurons. Later physiological studies by Bliss and colleagues revealed the existence of a synaptic property, referred to as long-term potentiation or LTP that closely matched the predictions of D. Hebb (Bliss and Lomo, 1973; Bliss and Collingridge, 1993). It was found in many cortical regions that activation of a synapse with high frequency trains mimicking the firing of neurons observed during learning, lead to an increase in the efficacy of neurotransmission. The change was very fast; it resulted in larger synaptic responses in the target cell (figure 1) and thus increased the probability that this cell would also be activated and fire. Overall, induction of LTP changes the population of neurons that respond to a stimulus, a phenomenon that may be considered as a learning mechanism for a neuronal network. A key feature of this property is its stability: the increase in transmission efficacy is generated within a few seconds, but the change remains effective for extended periods of time, up to several weeks for the longest experiments carried out in living animals (Abraham, 2003). It thus represents a perfect mechanism for the long-term storage of information. In addition to this, LTP has also been shown to be synapse-specific, to exhibit associative properties and to be required for many behavioral situations involving learning tasks (Malenka, 2003). This property is thus currently considered as the most important mechanism allowing neuronal networks to adapt their behavior and function in an activity-dependent manner. Its involvement in memory has been demonstrated in many examples of transgenic animals unable to perform in learning or memory tasks due to a selective blockade of LTP, or conversely that learned better due to enhanced LTP (Tsien et al., 1996; Tang et al., 1999).

Molecular mechanisms underlying synaptic potentiation

A fascinating and highly controversial issue about this property has been to understand the mechanisms responsible for the long-lasting increase in synaptic transmission (Malenka and Nicoll, 1999; Luscher et al., 2000). While the debate is still going on (Emptage et al., 2003), it is generally agreed that a major role is played by postsynaptic recept-

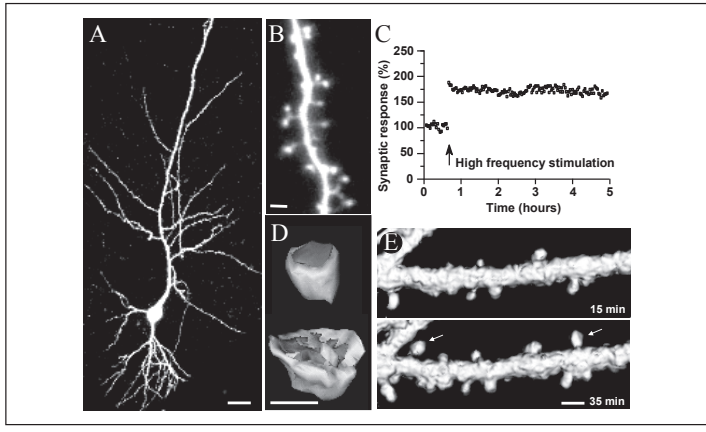

Figure 1:
Illustration of properties of synaptic plasticity. A. Hippocampal CA1 neuron in an organotypic slice cultures expressing EGFP following transfection using a biolistic approach (bar: 20 μm). B. Higher magnification of a dendritic segment illustrating the presence of numerous dendritic spines characterized by various shapes, length and morphologies (bar: 2 μm). C. Illustration of the increase in synaptic efficacy (LTP) induced by high frequency stimulation of a group of CA3 neurons in a hippocampal organotypic slice culture. D. Illustration of the heads of dendritic spines with their postsynaptic densities (gray zones) reconstructed from serial EM sections. The top image is characteristic of a thin spine while the bottom image represents a mushroom-type spine with a segmented postsynaptic density (perforated synapse). This type of spines was found to increase following induction of LTP in slice cultures. E. Formation of two new dendritic spines, as observed with 2-photon confocal imaging in organotypic slice cultures following application of a short hypoxia/hypoglycaemia protocol that triggers a lasting synaptic potentiation.

ors (Malinow and Malenka, 2002). There are two main types of excitatory glutamate receptors at these synapses referred to as NMDA and AMPA receptors according to their selective agonists. NMDA receptors have been shown to be responsible for triggering the synaptic changes. They are activated only under conditions where firing of the two connected neurons is synchronous and blocking these receptors prevents induction of LTP. Once activated, they allow calcium to enter the postsynaptic spine, which in turn activates various signaling messengers that lead to the lasting increase in synaptic efficacy. How this is pro-

duced remains controversial, but again, much evidence points to an involvement of AMPA type of receptors. We provided the first evidence that enhancement of AMPA transmission was involved in the increase in synaptic efficacy (Muller et al., 1988; Muller and Lynch, 1988). This has now been confirmed in many different ways and the current view of how LTP occurs proposes that the increase in AMPA receptor mediated transmission results from two main mechanisms: i) the phosphorylation by the enzyme calcium/calmodulin dependent protein kinase II (CaMKII) of the GluR1 subunit of AMPA receptors which increases the conductance of the ionic channel associated with the receptor (Barria et al., 1997; Benke et al., 1998; Lisman et al., 2002); and ii) an increased turnover rate of AMPA receptors that brings additional receptors to the synapse, producing in this way larger signals (Malinow and Malenka, 2002). An important finding brought into light by these studies has been the highly dynamic aspect of receptor mobility and insertion in neuronal membranes. It appears that receptors such as AMPA receptors continuously cycle through constitutive and activity-dependent mechanisms between synaptic or dendritic membranes and intracellular compartments. The number of receptors present at the synapse can be thus rapidly and tightly controlled, probably through the presence of scaffold proteins that determine the size of the postsynaptic density containing the receptors, and through the existence of a recycling machinery (Park et al., 2004). The possibility to transfect cells with vectors that express specific proteins or with antisense oligos or siRNAs that prevent expression of wild type proteins has made possible to start analyze the role of specific molecules in these mechanisms. In a recent collaborative work carried out with Dr. H. Hirling from EPFL in Lausanne, we contributed to the identification of one of the proteins that regulate the recycling of AMPA receptors. In a screen for proteins involved in vesicle trafficking, Dr. Hirling discovered the protein NEEP21, a protein specifically expressed in neurons and highly enriched in early endosomes (Steiner et al., 2002). He found that suppression of this protein using an antisense approach significantly altered the expression of AMPA receptors at the membrane. This prompted a more careful analysis of the participation of NEEP21 in the control of synaptic transmission and plasticity at excitatory synapses. By analyzing synaptic transmission, receptor recycling and synaptic plasticity in cells

transfected with a NEEP21 antisense, S. Alberi, in the laboratory, found that elimination of NEEP21 reduced synaptic transmission and prevented synaptic plasticity, probably by diminishing the number of AMPA receptors expressed at the synapse and altering the recycling of the receptor (Alberi et al., 2005). He could also show that this effect specifically concerned the GluR2 subunit of the AMPA receptor, as the conductance properties of the receptor were altered by interference with NEEP21 function (Steiner et al., 2005). Together these studies provided further evidence that the trafficking of receptors tightly controls the efficacy of synaptic transmission and allowed to identify one of the first molecules participating in this control.

Structural plasticity

In addition to changes in receptor numbers or properties, another mechanism often considered to account for the stability of synaptic plasticity involved a structural remodeling of the synapse and eventually also the creation of new synaptic contacts. To address this issue, we developed several years ago an electron microscopic analysis of activated synapses that revealed the occurrence of major morphological changes at activated synapses (Buchs and Muller, 1996; Toni et al., 1999; Toni et al., 2001; Nikonenko et al., 2002). These concerned two major aspects: changes of existing synapses and appearance of new types of synapses.

Induction of synaptic potentiation was found to be associated with an enlargement of the size of the dendritic spines, an increase in size of the postsynaptic density, modifications of its distribution and formation of perforated synapses characterized by multiple postsynaptic densities (Buchs and Muller, 1996). These morphological changes could represent the structural correlate of the increased trafficking of receptors. The increase in size of dendritic spines was recently confirmed using sophisticated imaging techniques (Matsuzaki et al., 2004) and might reflect several interesting mechanisms. It could result from membrane insertion associated with receptor recycling, or from a reorganization of the actin cytoskeleton in order to adapt the spine to the new synaptic structure, or it might also reflect the activation of local mechanisms of

protein synthesis together with the migration of polyribosomes within the spine (Ostroff et al., 2002). A current view of synaptic plasticity mechanisms is that the long-lasting stability would depend upon the synthesis of new proteins, including receptors, constituents of the post-synaptic density or signaling molecules that would be responsible for maintaining and stabilizing the contact with the presynaptic structure. Several recent studies have indeed directly demonstrated the capacity of neurons to locally regulate the synthesis of several important synaptic proteins (Kang and Schuman, 1996; Ostroff et al., 2002; Ju et al., 2004; Sutton and Schuman, 2005). According to this view, synchronous activity would stimulate the growth of dendritic spines and the formation of large, mushroom-type spines that would be more effective, due

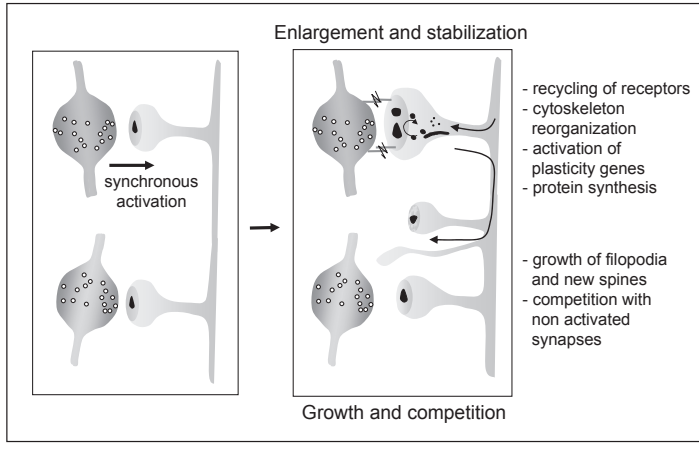

Figure 2
Schematic diagram summarizing the different mechanisms contributing to activity-induced synaptic plasticity. The diagram represents the possible changes that take place on a small dendritic segment with two spine synapses following synchronous activation of the top synapse. The activated synapse becomes strengthened through expression of new receptors associated with an enlargement of the spine head and transformation in a more stable mushroom-type spine. Activity further triggers the growth of filopodia or new spines that will enter in competition with the non-activated synapse for further stabilization.

to a larger number of receptors and, at the same time, become stabilized through the synthesis of specific proteins (Kasai et al., 2003). In contrast, synapses that would not be used regularly or activated in synchrony would rather remain immature, with a small number of receptors and probably also a shorter life time. An interesting idea that emerges from this proposal is the notion of competition between synapses: those synapses that are used in a coordinated, synchronous manner, because they would refer to coherent information, would become strengthened and stabilized at the expense of those that would not participate in neuronal communication.

Synaptogenesis

Implicit with this notion of competition between synapses is the idea that there must be a continuous process that eliminates and forms new synapses throughout life, even if this process concerns only a small fraction of synaptic contacts. This mechanism would allow synaptic networks to adapt to new experiences and new situations and would thus form the basis for learning processes. In contrast the more stable and larger synapses would constitute the backbone of the network architecture and account for properties such as long-term memory.

The idea of synapse competition and turnover has been very difficult to test experimentally essentially for technical reasons. However, recent improvements in confocal imaging techniques and particularly the introduction of 2-photon confocal approaches has open new possibilities to observe over prolonged periods of time the behavior and function of identified synapses, even under in vivo conditions (Trachtenberg et al., 2002). These approaches revealed important new information about synaptic properties. First, they demonstrated the highly dynamic nature of dendritic spines. These are highly enriched in actin and they appear to continuously change shape and organization in an activity-dependent manner (Matus, 2000; Yuste and Bonhoeffer, 2001). The function of this intrinsic motility remains unclear, but it certainly highlights the important capacity for plasticity of dendritic spines. Second, they allowed to demonstrate that the activity patterns that induced LTP

not only resulted in strengthening of synapses, but also promoted the growth of new protrusions, filopodia and spines (Maletic-Savatic et al., 1999; Engert and Bonhoeffer, 1999; Toni et al., 1999; Jourdain et al., 2002). The phenomenon occurred quite quickly following stimulation leading within 10–30 minutes to the appearance of new synaptic structures. We studied these mechanisms particularly under conditions of brief ischemia, which through an important release of glutamate, results in a synchronous activation of many synapses and thus mimics synaptic potentiation. Under this condition as following induction of LTP, we could identify growth mechanisms that affected both the pre- and postsynaptic structures (Jourdain et al., 2002; Jourdain et al., 2003; Nikonenko et al., 2003).

At the postsynaptic level, activity triggers the growth of filopodia that extend over the course of tens of minutes and then usually retract and stabilize as new dendritic spines. Alternatively, in many instances, activity directly stimulates the appearance of new dendritic spines that appear after about 15–30 minutes, but form very quickly within 1–2 minutes. Whether these new spines are functional and how long they persist remains unclear, but several examples, including observations from 3D-EM reconstructions suggested that they could represent the formation of new synaptic contacts (Toni et al., 1999). At the EM level, we even found a marked increase in images in which a single nerve terminal contacted two distinct, adjacent spines on the same dendrite, a phenomenon that clearly suggested a process of synapse duplication as contributing to the potentiation effect (Toni et al., 1999).

At the presynaptic level, we could also detect growth mechanisms induced by activity: they were seen as filopodia-like protrusions arising from stimulated varicosities or sometimes also directly from the axon (Nikonenko et al., 2003; Muller and Nikonenko, 2003). These protrusions appeared rapidly, within 10–20 minutes after stimulation, and could be studied at the EM level using 3D reconstruction. In this way we could analyze the characteristics of the contacts that were established by these protrusions. We found that within 20 minutes, 80% of them were engaged in a synaptic contact detectable by the presence of

a postsynaptic density. More interesting even, we found that the vast majority of these protrusions contacted first a dendritic shaft, while after 30 minutes almost 90% of them were in fact involved in a synaptic contact with a dendritic spine. These observations thus clearly supported the idea that activity can also trigger presynaptic growth mechanisms that may be involved in the formation of new dendritic spines, following establishment of an initial synaptic contact on the dendritic shaft (Muller and Nikonenko, 2003). Together these various studies provided clear evidence that neuronal and synaptic activity are able to promote synaptogenesis and that remodeling of synaptic networks is likely to be part of the mechanisms that contribute to information processing. The possibility to image even under in vivo conditions the behavior of identified cortical neurons and their dendritic spines further showed that this continuous process of synapse formation and elimination occurs in different cortical regions; it varies as a function of age and can be modulated by sensory stimulation (Trachtenberg et al., 2002; Shepherd et al., 2003; Holtmaat et al., 2005). It is thus only now really that this whole idea of synapse competition will start to be amenable to experimental tests.

Molecular control of cognition

It follows from the different concepts described above, consistent with the original ideas formulated by Ramon y Cajal, that higher brain functions critically depend upon a tight control and balance between mechanisms that, on the one hand, allow a plasticity of the function and organization of synaptic networks, in order to ensure continuous learning and adaptation, and, on the other hand, mechanisms that stabilize and maintain the connections critically involved in behaviors that must be conserved or memorized. Failure to operate correctly one or the other mechanism is likely to be detrimental to cognitive function. By studying the role of specific molecules involved in these aspects of plasticity, one should therefore gain information on important mechanisms for cognitive processing and, conversely, studies of selective deficits in cognition should highlight signaling pathways that might be critical for synaptic plasticity. We recently started this kind of approaches by anal-

yzing the mechanisms underlying specific, genetic forms of mental retardation in which the mental handicap is the only deficit and due to a mutation of a specific gene. Many different genes have now been identified as responsible for X-linked forms of mental retardation (Ramakers, 2002; Ropers et al., 2003). We focused in our work on the gene PAK3, the expression of which is modulated by induction of synaptic plasticity (Boda et al., 2002). Using a transfection approach through which we could express various mutants of PAK3 gene or suppress the expression of PAK3 gene in neurons, we found that this kinase does indeed contribute to synaptic plasticity mechanisms (Boda et al., 2004). Suppression of PAK3 resulted in the formation of aberrant, elongated spines and a decrease in the number of stable, mushroom-type spines; it also interfered with the formation of synaptic contacts, since many of these aberrant spines were de-afferented and since, in the remaining spines, the size of the postsynaptic densities was also markedly reduced. Furthermore, these morphological alterations were associated with defects of synaptic transmission and an impairment of LTP. As PAK3 belong to a family of molecules involved in Rho-GTPase signaling (Luo, 2000), it is likely that PAK3 produces these effects by interfering with cytoskeleton regulations that underlie spine and synapse dynamics (Edwards et al., 1999; Bokoch, 2003). Several other recent studies of other mental retardation genes or even of autism have started to unravel the importance of numerous molecular constituents of synapses as well as of unsuspected mechanisms for the function and plasticity of synaptic networks (Kaufmann and Moser, 2000; Zhang et al., 2003; Govek et al., 2004). Together the molecular complexity of the machinery that regulates synaptic mechanisms is thus probably directly related to its functional importance for the brain.

Conclusion and perspectives

While some of the general ideas about synaptic network plasticity have been proposed about a century ago, the fine details and importance of these concepts for brain function start only now to be better understood and their molecular mechanisms to be accessible to experimental analysis. The results of recent studies lead to the conclusion that both

modifications of synaptic function and structure and mechanisms of synapse formation and elimination contribute to the processing of information by synaptic networks. These two aspects are probably tightly inter-related to allow at the same time adaptation of brain networks to new situations and stabilization through competition of effective connections. These mechanisms are controlled by a complex machinery that starts only to be unravelled. It involves the numerous proteins localized in the postsynaptic density, the various receptors and molecules that regulate their trafficking, cell-cell signalling molecules that allow communication between pre- and postsynaptic structures, all second messenger pathways that regulate cytoskeleton organization and dynamics and the mechanisms that control gene transcription and local protein synthesis. Understanding the specific role of each of these molecules in the various aspects of plasticity will represent a major challenge for the coming years in view of the critical importance that these mechanisms play in functions as diverse as learning and memory, rehabilitation, mental retardation or even psychiatric diseases such as schizophrenia.

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REFERENCES

1. Abraham, W. C. (2003) How long will long-term potentiation last? *Philos. Trans. R. Soc. Lond B Biol. Sci*., **358**, 735–744.

2. Alberi, S., Boda, B., Steiner, P., Nikonenko, I., Hirling, H. & Muller, D. (2005) The endosomal protein NEEP21 regulates AMPA receptor-mediated synaptic transmission and plasticity in the hippocampus. *Mol. Cell Neurosci*., **29**, 313–319.

3. Barria, A., Muller, D., Derkach, V., Griffith, L. C. & Soderling, T. R. (1997) Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science*, **276**, 2042–2045.

4. Benke, T. A., Luthi, A., Isaac, J. T. & Collingridge, G. L. (1998) Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature*, **393**, 793–797.

5. Bliss, T. V. & Collingridge, G. L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, **361**, 31–39.

6. Bliss, T.V. P. & Lomo, T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of anaesthetized rabbit following stimulation of the perforant path. *J. Physiol. (Lond.)*, **232**, 331–356.

7. Boda, B., Alberi, S., Nikonenko, I., Node-Langlois, R., Jourdain, P., Moosmayer, M., Parisi-Jourdain, L. & Muller, D. (2004) The mental retardation protein PAK3 contributes to synapse formation and plasticity in hippocampus. *J. Neurosci*., **24**, 10816–10825.

8. Boda, B., Mas, C. & Muller, D. (2002) Activity-dependent regulation of genes implicated in X-linked non-specific mental retardation. *Neuroscience*, **114**, 13–17.

9. Bokoch, G. M. (2003) Biology of the p21-activated kinases. Annu. Rev. Biochem., **72**, 743–781.

10. Buchs, P. A. & Muller, D. (1996) Induction of long-term potentiation is associated with major ultrastructural changes of activated synapses. *Proc. Natl. Acad. Sci. USA*, **93**, 8040–8045.

11. Edwards, D. C., Sanders, L. C., Bokoch, G. M. & Gill, G. N. (1999) Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat.Cell Biol.*, **1**, 253–259.

12. Emptage, N. J., Reid, C. A., Fine, A. & Bliss, T.V. (2003) Optical quantal analysis reveals a presynaptic component of LTP at hippocampal Schaffer-associational synapses. *Neuron*, **38**, 797–804.

13. Engert, F. & Bonhoeffer, T. (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature*, **399**, 66–70.

14. Govek, E. E., Newey, S. E., Akerman, C. J., Cross, J. R., Van, d., V & Van Aelst, L. (2004) The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. *Nat.Neurosci.*, **7**, 364–372.

15. Hebb, D. O. (1949) *The Organization of Behavior Wiley*, New York.

16. Holtmaat, A. J., Trachtenberg, J. T., Wilbrecht, L., Shepherd, G. M., Zhang, X., Knott, G.W. & Svoboda, K. (2005) Transient and persistent dendritic spines in the neocortex in vivo. *Neuron*, **45**, 279–291.

17. Jourdain, P., Fukunaga, K. & Muller, D. (2003) Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. *J. Neurosci.*, **23**, 10645–10649.

18. Jourdain, P., Nikonenko, I., Alberi, S. & Muller, D. (2002) Remodeling of hippocampal synaptic networks by a brief anoxia-hypoglycemia. *J. Neurosci.*, **22**, 3108–16.

19. Ju, W., Morishita, W., Tsui, J., Gaietta, G., Deerinck, T. J., Adams, S. R., Garner, C. C., Tsien, R.Y., Ellisman, M. H. & Malenka, R. C. (2004) Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat. Neurosci.*, **7**, 244–253.

20. Kang, H. & Schuman, E. M. (1996) A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science*, **273**, 1402–1406.

21. Kasai, H., Matsuzaki, M., Noguchi, J., Yasumatsu, N. & Nakahara, H. (2003) Structure-stability-function relationships of dendritic spines. *Trends Neurosci.*, **26**, 360–368.

22. Kaufmann, W. E. & Moser, H.W. (2000) Dendritic anomalies in disorders associated with mental retardation. *Cereb. Cortex*, **10**, 981–991.

23. Lisman, J., Schulman, H. & Cline, H. (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat. Rev. Neurosci.*, **3**, 175–90.

24. Luo, L. (2000) Rho GTPases in neuronal morphogenesis. *Nat. Rev. Neurosci.*, **1**, 173–180.

25. Luscher, C., Nicoll, R. A., Malenka, R. C. & Muller, D. (2000) Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nat. Neurosci.*, **3**, 545–550.

26. Malenka, R. C. (2003) The long-term potential of LTP. *Nat. Rev. Neurosci.*, **4**, 923–926.

27. Malenka, R. C. & Nicoll, R. A. (1999) Long-term potentiation – a decade of progress? *Science*, **285**, 1870–1874.

28. Maletic-Savatic, M., Malinow, R. & Svoboda, K. (1999) Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science*, **283**, 1923–1927.

29. Malinow, R. & Malenka, R. C. (2002) AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.*, **25**, 103–26.

30. Matsuzaki, M., Honkura, N., Ellis-Davies, G. C. & Kasai, H. (2004) Structural basis of long-term potentiation in single dendritic spines. *Nature*, **429**, 761–766.

31. Matus, A. (2000) Actin-based plasticity in dendritic spines. *Science*, **290**, 754–758.

32. Muller, D., Joly, M. & Lynch, G. (1988) Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. *Science*, **242**, 1694–1697.

33. Muller, D. & Lynch, G. (1988) Long-term potentiation differentially affects two components of synaptic responses in hippocampus. *Proc. Natl. Acad. Sci. USA*, **85**, 9346–9350.

34. Muller, D. & Nikonenko, I. (2003) Dynamic presynaptic varicosities: a role in activity-dependent synaptogenesis. *Trends Neurosci.*, **26**, 573–575.

35. Nikonenko, I., Jourdain, P., Alberi, S., Toni, N. & Muller, D. (2002) Activity-induced changes of spine morphology. *Hippocampus*, **12**, 585–591.

36. Nikonenko, I., Jourdain, P. & Muller, D. (2003) Presynaptic remodeling contributes to activity-dependent synaptogenesis. *J. Neurosci.*, **23**, 8498–8505.

37. Ostroff, L. E., Fiala, J. C., Allwardt, B. & Harris, K. M. (2002) Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron*, **35**, 535–545.

38. Park, M., Penick, E. C., Edwards, J. G., Kauer, J. A. & Ehlers, M. D. (2004) Recycling endosomes supply AMPA receptors for LTP. *Science*, **305**, 1972–1975.

39. Ramakers, G. J. (2002) Rho proteins, mental retardation and the cellular basis of cognition. *Trends Neurosci.*, **25**, 191–199.

40. Ramon y Cajal, S. (1911) *Histologie du Système Nerveux de l'Homme et des Vertébrés* Maloine, Paris.

41. Ropers, H. H., Hoeltzenbein, M., Kalscheuer, V., Yntema, H., Hamel, B., Fryns, J. P., Chelly, J., Partington, M., Gecz, J. & Moraine, C. (2003) Nonsyndromic X-linked mental retardation: where are the missing mutations?. *Trends Genet*., **19**, 316–320.

42. Shepherd, G. M., Pologruto, T. A. & Svoboda, K. (2003) Circuit analysis of experience-dependent plasticity in the developing rat barrel cortex. *Neuron*, **38**, 277–289.

43. Steiner, P., Alberi, S., Kulangara, K., Yersin, A., Sarria, J. C., Regulier, E., Kasas, S., Dietler, G., Muller, D., Catsicas, S. & Hirling, H. (2005) Interactions between NEEP21, GRIP1 and GluR2 regulate sorting and recycling of the glutamate receptor subunit GluR2. *EMBO J.*.

44. Steiner, P., Sarria, J. C., Glauser, L., Magnin, S., Catsicas, S. & Hirling, H. (2002) Modulation of receptor cycling by neuron-enriched endosomal protein of 21 kD. *J. Cell Biol.*, **157**, 1197–1209.

45. Sutton, M. A. & Schuman, E. M. (2005) Local translational control in dendrites and its role in long-term synaptic plasticity. *J. Neurobiol.*, **64**, 116–131.

46. Tang, Y. P., Shimizu, E., Dube, G. R., Rampon, C., Kerchner, G. A., Zhuo, M., Liu, G. & Tsien, J. Z. (1999) Genetic enhancement of learning and memory in mice. *Nature*, 4**01**, 63–9.

47. Toni, N., Buchs, P. A., Nikonenko, I., Bron, C. R. & Muller, D. (1999) LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature*, **402**, 421–425.

48. Toni, N., Buchs, P. A., Nikonenko, I., Povilaitite, P., Parisi, L. & Muller, D. (2001) Remodeling of synaptic membranes after induction of long-term potentiation. *J. Neurosci.*, **21**, 6245–51.

49. Trachtenberg, J. T., Chen, B. E., Knott, G. W., Feng, G., Sanes, J. R., Welker, E. & Svoboda,K. (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature*, **420**, 788–94.

50. Tsien, J. Z., Huerta, P. T. & Tonegawa, S. (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell*, **87**, 1327–38.

51. Yuste, R. & Bonhoeffer, T. (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu. Rev. Neurosci.*, **24**, 1071–89.

52. Zhang, H., Webb, D. J., Asmussen, H. & Horwitz, A. F. (2003) Synapse formation is regulated by the signaling adaptor GIT1. *J. Cell Biol.*, **161**, 131–142.

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