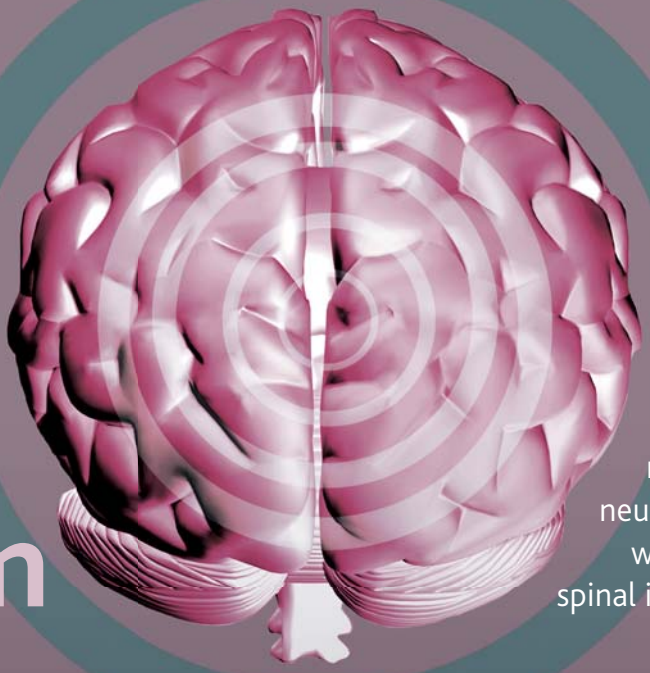
Brain rhythm



Neurophysiologist **Associate Professor Jürg Streit** is investigating the role of rhythm generation in neural networks; work he hopes will enhance testing for novel spinal injury treatment approaches

To begin, what is the main focus of your research project?

The overarching goal of all of our research projects to date has been to learn how neurons organise and interact in circuits to perform emergent network functions. Practically all functions of the central nervous system emerge on the level of neuronal networks. On the level of whole brains, such networks are interacting in a complex and distributed way, translating into behaviour. On the level of individual cells that form the networks, genetic and epigenetic mechanisms provide the neurons with a rich repertoire of biochemical and electrical responses. Our main research interest lies directly between these two levels, to understand which functions emerge when neurons form a small network together that is not embedded into a whole brain.

How are you investigating these research topics?

The generation of population responses in defined small networks can be ideally investigated in simplified systems like cultures of neuronal explants, in which both the network level and the level of individual neurons are available for recording. A key function of such networks is the generation of rhythmic activity. In the spinal cord, such rhythms are used to drive repetitive movements that are, for example, used for locomotion. In the cortex, rhythms are involved in motor control, perception and memory processes. Therefore, we study the mechanisms of rhythm generation in neural networks in organotypic explant cultures.

What strategies are currently in place to increase functional recovery after spinal cord lesions? Can we expect to see stem cell treatment for spinal cord injuries in the near future?

In the last few years the search for strategies to improve functional recovery after spinal cord injury has been a very active field. Several strategies were intensively investigated in animal models and some have led to clinical trials. Many of these strategies are related to the promotion of axonal growth factors or to the blocking of molecules that inhibit axonal growth. The most promising among these is the antibody treatment blocking the Nogo-A growth inhibitor that was discovered by Professor Martin Schwab's group in Zürich. Additonally, clinical trials are being conducted or planned for stem cell treatments and for electrostimulation of spinal cord circuits. Nevertheless, a real breakthrough in the sense that significant functional recovery could be evoked in completely paralysed patients has not been achieved yet. To reach this goal, it will probably require a combination of several strategies. As a tool for testing such combinatorial strategies *in vitro*, we are on the way to developing a model based on spinal cord slice cultures, in which functional recovery after lesions can be tested and quantified.

How much work have you been able to conduct to forge partnerships with industry and complete the circle from bench to bedside?

We have been pleased at our progress in this regard. We have developed the multielectrode array system in close collaboration with the EPFL (the Swiss Federal Institute of Technology) in Lausanne. Out of this collaboration, an EPFL spin-off has been developed that is producing customised multielectrode array devices. This is a great advantage for others and for us because it allows adaptation of the design of the multielectrode arrays to suit individual experimental purposes.

What are the next steps in your research that you have planned?

We are now focused on three goals. Firstly, to proceed with our studies into the mechanisms of rhythm generation in spinal cord and cortical networks. In particular, we want to know whether another candidate for an inward current, the calcium-dependent cation current (I_{CAN}) also contributes to rhythm generation. Secondly, we want to discover whether motoneurons can directly activate rhythms in spinal cord circuits. Finally, we are keen to investigate functional recovery after lesions in our model of two adjacent spinal cord slices in culture. We have found that such slices are functionally coupled in the sense that the spontaneous rhythms in both are synchronised. Following a lesion that separates the slices after three weeks in culture, synchronisation of the rhythms in the two slices is lost. We take these cultures as a model for the limited regeneration potential of spinal cord circuits. We want to test whether the insertion of stem or neuronal precursor cells into the lesion site can restore the functional coupling of the two slices following the lesions.

Challenging traditional views **on rhythm mechanisms**

Researchers at the **University of Bern** are investigating rhythm generation mechanisms of simple neural networks in culture in order to help build a deeper understanding of more complex networks in the central nervous system

COMPLEX NETWORKS OF neurons control the way the human central nervous system functions, which makes studying these networks and their role in generating, for example, locomotion, fairly challenging. By understanding how these responses are defined in smaller networks, such as neuronal cultures, hypotheses relating to the more complex systems can potentially be extrapolated. A team of scientists based at the University of Bern's Department of Physiology in Switzerland has taken this approach to help improve knowledge surrounding the inner workings of the human central nervous system.

MECHANISMS OF ACTIVATION AND DEACTIVATION

Specifically, the researchers are looking at the neural networks of organotypic explant cultures to learn more about the mechanisms of rhythm generation in these systems. Their work covers a number of key themes, including understanding pattern generation in spinal cord slices, working on an *in vitro* model of propriospinal regeneration, discovering more about activities within the cortical networks and exploring the effects of grafted stem cells on infected hippocampal slice cultures on multielectrode arrays. Professor Jürg Streit, the project's team leader, explains that neuronal network rhythm generation always includes mechanisms for network activation and deactivation: "In a network that consists of excitatory and inhibitory synaptic connections, feedback synaptic inhibition is a possible mechanism of network deactivation". They have found, especially in the cortex cultures they have used, that the deactivation mechanisms have some control over the rhythm frequency. Thus, understanding more about the deactivation mechanisms ultimately helps to improve knowledge about the speed of the rhythms.

STUDYING CORTICAL SLICE CULTURES

The first time rhythms were identified in the cerebral cortex was back in the 1920s when electroencephalogram equipment supported the discovery that the frequency of these rhythms strongly correlates with levels of consciousness. The findings suggested that these rhythms are not necessarily an important factor in cortical processing, because when people are unconscious these rhythms are the deepest and slowest. More recent breakthroughs in this field, however, are pointing towards barely detectable localised rhythms as playing a contributing role in important cortical functions, such as memory, sensory perception or motor control. According to Streit, this is why they are interested in looking at, and comparing how, the mechanisms that drive rhythm generation in cortical networks are related to those previously described in spinal cord networks.

To enable these investigations to be undertaken, the laboratory has developed a culture of cortical slices that they have taken from neonatal rats on multielectrode arrays. Through their work, the team has been able to confirm that these cultures generate bursts of oscillating spontaneous activity, which Streit seperates into fast and slow oscillations with different mechanisms. They are now working on answering several outstanding questions that relate to mechanisms underlying rhythm generation. Through this, they hope to be able to detect whether or not the same mechanisms of rhythm generation as they have described in spinal cord networks are also operational in cortical circuits.

THE ROLE OF PERSISTENT SODIUM CURRENT

Several studies into how locomotion is controlled within spinal cord networks have proposed a number of mechanisms that may be responsible; for example, the natural pacemaker current mechanism. Although this has yet to be clearly identified in mammalin spinal cords, there are potential candidates for initiating this process. One of these is the persistent sodium current (I_{NaP}); a current that is thought to flow during a special state of the classical voltage-dependent sodium channels. Streit and his team were among the first researchers to propose the role of such currents in the generation of rhythmic activity in spinal cord networks. However, he believes that there are still many unanswered questions about the function of these currents and how they are initiated: "Complex rhythms, like fictive locomotion, are certainly not produced

EMERGENT PROPERTIES AND RHYTHM GENERATION IN CULTURED NEURAL NETWORKS

OBJECTIVES

Using whole cell patch clamp and extracellular recordings by multielectrode arrays, the objective is to record rhythmic activity in slice cultures of embryonic spinal cord and of neonatal cerebral cortex to illuminate the cellular and network mechanisms underlying rhythm generation. Based on these cultures, the group also studies functional recovery following lesions *in vitro*.

KEY COLLABORATORS

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ASSOCIATE PROFESSOR JÜRG STREIT studied medicine at the University of Bern from 1975. Following an internship at the District Hospital Litembo, Tanzania in 1980, he completed his MD at the Faculty of Medicine in 1984. Postdoc research at the Max Planck Institute for Psychiatry in Munich and the Institute of Physiology in Bern ensued until, in 1994, Streit completed his Habilitation at the Faculty of Medicine in Bern. From 2002 he has been an Associate Professor in the Department of Physiology. His main interests are functions that emerge on the level of isolated neuronal networks. For this, his team has developed a multielectrode recording system for organotypic cultures.

There is every possibility that, by improving understanding of the role of activation of rhythm generation, therapeutic interventions after spinal cord injury may be able to 'switch on' these rhythms

by a pacemaker mechanism that is based on a single ionic current but more likely on a combination of intrinsic neuronal properties and network interactions".

What they have learnt is that I_{NaP} plays a major role in the generation of spontaneous rhythmic activity. This is a significant finding because a critical issue for deciphering the mechanisms that are involved in rhythm generation in neuronal networks is network activation, which involves spontaneous intrinsic activation of neurons. The researchers consider I_{NaP} to be a good candidate for this activation and believe that additional candidates may well be found in the future. There is every possibility that, by improving understanding of the role of activation of rhythm generation, therapeutic interventions after spinal cord injury may be able to 'switch on' these rhythms.

MULTISITE EXTRACELLULAR AND INTRACELLULAR RECORDINGS

To investigate electrical activity within the network, as well as within individual neurons, the team combines multielectrode arrays and whole cell patch clamps. To record at the network level they employ an array of flat metal microelectrodes, upon which the neuronal explants are then cultured. For these investigations they have used 64 platinum-covered electrodes covering around 2 mm^2. The patch clamp method for single cell recordings allows the investigators to measure both the membrane potential of individual neurons as well as the ionic current flow through the ion channels of the membrane at given voltages: "Combining these two methods means we can span the range of levels from population activity to the activation of specific ion channels," notes Streit.

OBSERVING EARLY STAGE MOTONEURON CONTRIBUTION

The University of Bern researchers have observed that motoneurons contribute to rhythm generation at a very early stage of activation. This surprising finding means the team is in a position to challenge the classical view that rhythms are generated in networks of spinal interneurons and then synaptically transmitted to motoneurons as an output stage. From Streit's perspective, one theory to help explain this contribution is that axonal collaterals help to form a positive feedback on spinal circuits: "A positive feedback would be a novel mechanism that may be relevant for activity-dependent degeneration or to form and refine circuits in the spinal cord during development". At this stage they only have indirect evidence for this mechanism within their experiments. They are now planning the next stage of experiments in the hope of confirming whether motoneurons can be directly responsible for initiating network activity. A discovery of this importance would have a significant impact on the way spinal cord injury recovery and rehabilitation is undertaken.

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