Drosophila as a model to study mechanisms underlying alcohol induced behaviors

Henrike Scholz

Alcohol is a widely abused drug and alcohol dependence is a very severe health hazard worldwide. The relapse of treated alcoholics is 90%. This is in part due to the fact that the molecular and neuronal bases of addictive behaviors e.g. how ethanol changes neural circuits on the cellular and subcellular level are not fully understood yet. Ethanol tolerance is a behavior strongly associated with alcoholism. This mechanism is thought to promote higher ethanol intake and the development of an addictive brain. We have previously established *Drosophila* as a genetic models system to study the neuronal bases of ethanol induced behaviors like ethanol tolerance[1] and olfactory ethanol preference[2]. We have shown that despite changes of network function cellular stress caused by ethanol also alters ethanol induced behaviors[3] and therefore locomotor out.

Here, we will explain and demonstrate experiments that allow us to identify mechanisms involved in mediating behaviors that are associated with alcoholism. Studying the effect of ethanol on the nervous system will also provide new insights into mechanism how decisions to move are normally generated in the brain. We will discuss the advantages and limitations of this model system to study the mechanistic bases of behaviors.

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Web site: http://www.neuro.uni-koeln.de/neuro-scholz.html?&L=0

How to find a new chemosensory receptor gene in a day

Sigrun Korsching

The sense of smell has the peculiarity that activation of individual olfactory receptors may be sufficient to generate particular innate behaviors (Hussain et al., 2013). It is known that individual olfactory sensory neurons choose only a single receptor gene out of a large gene family, and that all neurons expressing that receptor gene converge their axons onto a common glomerulus. However, very little is known about the properties of neuronal circuits linking a particular glomerulus, i.e. a particular olfactory receptor, with an innate behavior. Furthermore, to understand the relevance of individual receptors to neuronal representation of odors the complete olfactory receptor gene family should serve as reference frame.

The recent progress in genome and transcriptome sequencing is outpacing the progress in gene prediction, even though publicly available data bases nowadays usually come with quite a bit of automated gene prediction. In particular, large gene families such as those encoding olfactory receptor genes are usually not predicted completely, and predicted sequences sometimes need manual corrections. Thus a person knowing her bioinformatics can rather quickly identify new olfactory receptor genes, *cf.* (Saraiva and Korsching 2007).

Here we propose to identify novel chemosensory receptor genes in vertebrate genomes. We will begin with a lesson introducing methods for gene identification, gene search and phylogenetic analysis, which is necessary for gene validation, *cf.* (Baldauf 2003). Then, each student will be assigned their own genome and gene family to study. The student will search for genes, collect a handful of candidate genes, and run a phylogenetic analysis with reference gene sets, which we will provide. Each student will report their candidate genes and discuss them using the phylogenetic tree generated by the student.

Hussain A, Saraiva LR, Ferrero D, Ahuja G, Krishna VS, Liberles SD, Korsching SI (2013) High-affinity olfactory receptor for the death-associated odor cadaverine. *Proc Natl Acad Sci U S A*, 110(48):19579-84.

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Task-Specificity of Processing of Sensory Feedback

Ansgar Büschges

The neural control of posture and movement in all legged animals utilizes the same sensory feedback signals from the extremities. These signals are related to the generated movements and forces that act on the legs. The way these sensory feedback signals are processed in the central nervous system, however, depends strongly on the actual behavioral task at hand. These tasks encompass, for instance, posture control during standing or movement control during different forms of locomotion, such as curve walking, forward or backward walking (1,2,3). Today, detailed knowledge is available with regard to sensory signals that are processed in such a plastic and state-dependent manner and the neural pathways that process this sensory information. Current research focuses on the neural mechanisms in charge of priming neural network operation in a task-specific fashion. The introductory lecture will present the present state of knowledge on this topic in the field of motor control (e.g.1, 2).

In the summer school, experimental approaches to studying task-specific processing of sensory signals in the invertebrate locomotor system will be presented.

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Role of Sensory Feedback in Interleg Coordination in *Drosophila melanogaster*

Ansgar Büschges

Legged locomotion is shared by almost all terrestrial animals and is assumed to have become highly optimized during evolution with respect to its functional needs, like being robust for unpredictable environment or stereotypical for high speeds. In insects inter-leg coordination varies with locomotion speed, from metachronal wave gait at slow speeds through tetrapod coordination at intermediate speeds to tripod coordination during fast locomotion. To date, the underlying neural control is still poorly understood; especially the question to which extent sensory feedback from each leg contributes to inter-leg coordination across the range of locomotor speeds deserves closer attention. The introductory lecture will present the present state of knowledge in the field of motor control (e.g. 1).

Two recent studies on drosophila suggest that sensory feedback does play a role for inter-leg coordination and the resulting movement patterns in fruit flies (2, 3).

In the summer school experimental approaches to studying the differential role of load and movement feedback for inter-leg coordination over a large range of walking speeds in the fruit fly *Drosophila melanogaster* will be presented.

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Website:

http://www.neuro.uni-koeln.de/neuro-bueschges.html

The cellular mechanisms of coordination – How does the system encode relevant information?

Carmen Wellmann

Coordination of neuronal networks is a crucial task in many behaviors of vertebrates and invertebrates. We find synchronized neuronal networks in the brain, were the resulting patterns are measured in form of EEGs as alpha, beta, gamma and delta – waves (oscillations). These are widely regarded as functionally relevant signals of the brain. Synchronized neuronal networks are also necessary for the locomotor output, independent of its form (swimming, crawling, walking or flying).

In all these systems a big effort is made to understand the cellular mechanisms of the synchronization. In the swimmeret system of crayfish (1), we found a perfect example, were not only the components of the pattern generating kernel were described (2), but were also the coordinating network is known and can be analyzed (3, 4).

Recent studies in the crayfish swimmeret system show that the coordinating information is encoded in two neurons from each segment – one is projecting anteriorly and one posteriorly to all other neuronal networks in the system (4). We recently demonstrated that the anterior projecting coordinating neuron activity is shaped through a direct chemical synapse from one pattern generating nonspiking neuron in the network. Information in these coordinating neurons can be encoded in two ways: in the duration of the burst and in the frequency of the spikes per burst. But how does the network change these two parameters? Is one only driven through the information from the network and the other from the surrounding excitation level?

During this summer school course the students will learn electrophysiological techniques of extracellular recordings with pin electrodes of the systems' motor output and recordings with suction electrodes of the coordinating neurons. With different transmitter (cholinergic agonists) the excitation of the system will be changed and the parameters of the coordinating neurons and motor neurons will be recorded. The topic will be if one of the transmitters in the system is able to change only one parameter (duration of burst or/and interspike frequency) in the encoded coordinating information.

References:

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Transcranial magnetic stimulation of the human motor cortex

Silvia Gruhn, Gereon Fink

Transcranial magnetic stimulation (TMS) is a well established tool in human neuroscience to interfere with cortical activity. A very short (< 50 µs) magnetic pulse delivered through the scalp induces an electrical field in the underlying brain tissue that stimulates cortical neurons and/or its axons. When applied in a train of several hundred to thousand pulses (repetitive TMS, rTMS), excitability of cortical neurons can be altered from minutes up to an hour depending on stimulation frequency or its pattern. The neurobiological mechanisms underlying such lasting effects are not yet clear but are often suggested to resemble those of long-term potentiation (LTP) and long-term depression (LTD). We will discuss the technical principles of TMS in its various application forms. Then, a practical session will be offered where TMS to the motor cortex will be demonstrated. We will show how TMS can be used to measure physiological properties of the motor cortex in-vivo, and how this tool may selectively disturb activity of certain areas, thereby providing evidence for the causal role of a given brain region for a particular behaviour.

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Disruption of motor network connectivity post-stroke and its noninvasive neuromodulation.
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http://neurologie-psychiatrie.uk-koeln.de/neurologie/team

Neuroendocrine control of energy homeostasis in the zebrafish
(Hammerschmidt lab)

During recent years, the zebrafish (Danio rerio) has become a powerful alternative animal model to study various aspects of physiology, including the neuroendocrine control of energy homeostasis (1). As in mammals, Agrp and Pomc neurons are present in the hypothalamus of the zebrafish forebrain, regulating food intake, energy expenditure and somatic growth (2). Some of these effects are mediated via the hypothalamic-hypophyseal axis, which is set up during late embryogenesis (3), while others most likely involve other neurocircuits. We have generated transgenic lines in order to visualize and manipulate various hypothalamic circuits involved in control of energy homeostasis.

During the course, you will be briefly introduced to the zebrafish model and have the chance to examine zebrafish embryos at various stages of development (1 cell stage – 5 days post fertilization). Using confocal laser scanning microscopy, you will examine the neuronal circuits of selected hypothalamic cell populations in zebrafish transgenic larvae (agrp:EGFP and pomc:EGFP transgenic lines, among others). Furthermore, two physiological experimental setups will be introduced to you: First of all you will record swimming behaviour in a zebrafish mutant which lacks Agrp neurons (among other neuronal cell populations) and compare the physical activity to a control group. Finally, a method to quantify metabolism in zebrafish utilizing a custom-made oxygen swimming chamber will be presented in a short demonstration.

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http://www.uni-koeln.de/math-nat-fak/ebio/en/Research/Hammerschmidt/hammerschmidt.html

Control of Energy Homeostasis
Kloppenburg Lab
http://cecad.uni-koeln.de/Prof-Peter-Kloppenburg.82.0.html

Energy homeostasis is tightly controlled by neuronal circuits in the arcuate nucleus of the hypothalamus to adjust food intake and energy expenditure according to the availability of fuel sources in the periphery of the body. Aging, extreme diets, and diseases can deregulate these neuronal systems. Thus, defining age and diet-associated changes in this network is critical to the understanding of what makes organisms increasingly susceptible to metabolic disorders over their life-span. To better understand and counteract obesity, type 2 diabetes, and associated metabolic disorders, increasing efforts are being made to define the homeostatic control mechanisms that regulate body weight and energy homeostasis.

The long-term goal of our laboratory is to analyze and define the biophysical parameters and cellular mechanisms that cause age- and diet-dependent changes in intrinsic firing characteristics and synaptic properties in the neuronal circuits that control energy homeostasis.

During the course we will a) discuss current models regarding the regulation of feeding behavior and b) introduce electrophysiological and optophysiological approaches that help to reveal the cellular mechanisms of energy homeostasis.

References:

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From gene identification to functional analysis and therapy of motor neuron diseases

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As a group based at the Institute of Human Genetics, the Wirth lab is involved in gene identification particularly in finding genes implicated in motor neuron diseases, such as spinal muscular atrophy but also of other non-neuronal diseases, for example osteoporosis [1, 2]. We are part of a large European consortium called "NEUROMICS" aiming to identify novel genes for neurogenetic disorders. You will get insight into how a novel disease-causing gene can be identified by whole exome sequencing using next generation sequencing and various bioinformatics-based filter techniques to find the causative pathogenic variant.

Candidate pathogenic variants need to be further validated by functional analyses using cellular and animal models. We are using zebrafish or mice in our lab [2-4]. You will learn how primary motor neurons can be analysed under the microscope and see which phenotype morphant zebrafish with defects in motor neurons present. We will also show you how some of these defects can be rescued.

Finally, a very elegant way to correct aberrant splicing of genes or to downregulate certain genes involved in the pathology of a disease is the use of antisense oligos (ASOs). You will get some impression on how we are using antisense oligos to treat spinal muscular atrophy.

Literature:
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Analysis of mitochondrial morphology and function in situ in the mouse brain

Shuaiyu Wang, Sara Murru, Elena Rugarli

Mitochondria are essential organelles involved in ATP production, Fe-S cluster biogenesis, calcium buffering, and several metabolic biosynthetic pathways. Mitochondrial homeostasis depends on regulated biogenesis and efficient quality control mechanisms, and is key to cell survival. Dysfunctional mitochondria can trigger apoptotic cell death, and play an important role in the pathogenesis of several diseases, especially neurodegenerative diseases (1). In this experimental station, we will explain and demonstrate experiments in mouse models of neurodegeneration linked to mitochondrial dysfunction. Students will learn how to perform histological staining of the brain, how to evaluate mitochondrial respiratory activity in situ by using histochemical techniques, and how to visualize mitochondrial morphology in vivo using appropriate reporter lines. Students will have the opportunity to perform the experiments themselves, to critically assess the results, and to learn how mouse genetics can be employed to study neurodegenerative processes.

References

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Subthalamic deep brain stimulation in a hemiparkinsonian rat model

Alexander Drzezga, Heike Endepols

Deep brain stimulation in the subthalamic nucleus (DBS-STN) is a successful therapeutic approach to treat Parkinson's motor symptoms, but functional network changes underlying DBS-STN are not well understood. However, there is evidence that DBS-STN increases glutamatergic neurotransmission in the substantia nigra pars reticulata (SNr) and in the striatum [1]. To investigate metabolic effects associated with changes in synaptic activity, we combine DBS-STN with [^{18}F]fluorodeoxyglucose (FDG) positron-emission-tomography (PET) in a rat hemiparkinson model [2].

Parkinson-like symptoms are induced by injecting 6-hydroxydopamine (6-OHDA) unilaterally into the medial forebrain bundle. Automated gait analysis and [^{18}F]fluoro-dihydroxy-phenylalanin (FDOPA) PET are used to confirm motor impact and dopaminergic lesion, respectively. For DBS-STN an electrode is inserted through a chronically implanted guide cannula aiming at the ipsilesional STN. The exact position of the cannula and required electrode depth are determined using magnetic resonance imaging (MRI). During stimulation using a chronically implanted neurostimulator (Medtronic), we investigate DBS-STN effects on motor and cognitive performance. Simultaneous FDG-PET shows functional neural networks activated by DBS-STN.

Station: University Hospital of Cologne, Clinic of Nuclear Medicine / Inst. of Radiochemistry and Experimental Molecular Imaging

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