Literaturseminar Neurobiologie WS 09/10 (JunProf A. Gottschalk):

(a.gottschalk@em.uni-frankfurt.de)

Ablauf des Seminars:

- Jeweils 1-2 Studenten (je nach Teilnehmerzahl) suchen sich einen Artikel aus (muss nicht aus der Liste sein, man kann auch selbst etwas Interessantes aus der aktuellen Literatur auswählen) und bereiten ein Seminar über dieses Paper vor. Es wird eine Liste mit den entsprechenden Terminen aushängen, so daß das Seminar für den Rest des Semesters koordiniert werden kann.

- Die Auswahl zum jeweiligen Termin auch nochmal per e-mail allen Teilnehmern bekanntgeben, bzw. PDF des Artikels mitschicken (aus dem Web, bzw. vom Seminarleiter erhältlich: a.gottschalk@em.uni-frankfurt.de), so daß jeder Teilnehmer das Paper lesen kann (und soll !).

- Die Seminarvorbereitung (mit Computerhilfe, Powerpoint o.Ä.) der jeweils Vortragenden soll umfassen:

o Kurzer Abriss über den wissenschaftlichen Hintergrund des Papers - wenn nötig, über das hinausgehend, was in der Einleitung des Papers steht
o Die Abbildungen aus dem Paper
o Ggf. kurze Erklärung der verwendeten Methoden, wenn sie nicht aus dem Paper selbst hervorgehen

Falls den Vortragenden etwas unverständlich ist, so kann dies gerne mit dem Seminarleiter 1-2 Tage vor dem Seminar nochmal geklärt werden

- Die jeweils Vortragenden stellen dann das Paper anhand der Daten/Abbildungen vor:

o Was wollten die Autoren untersuchen ?
o Wie haben sie das angestellt (welche Methoden etc.) ?
o Welche Ergebnisse wurden erzielt (Results) ?
o **ACHTUNG: Je ein/e andere/r Student/in stellt eine Abbildung oder ein Panel aus einer Abbildung vor**
o Was sind die Schlussfolgerungen der Autoren (Diskussion) ?

- Fragen dazu werden im Plenum besprochen, abschliessend erfolgt eine Bewertung des Papers (Diskussion angeleitet durch die Vortragenden bzw. den Seminarleiter):

o Belegen die Ergebnisse überzeugend die Schlussfolgerungen der Autoren ?
o Wurden die Ziele der Autoren erreicht ?
o Hätte es alternative (bessere ?) Möglichkeiten gegeben, diese Ziele zu erreichen ?
o Hat das Paper das entsprechende Arbeitsgebiet weitergebracht ?
o Welche Teilaspekte könnte man nun (wie ?) weiter untersuchen ?

Literaturseminar Neurobiologie WS 2009/10 (Gottschalk)

(a.gottschalk@em.uni-frankfurt.de)

Vorschlag: Jeweils Freitags, 11.00-13.00 Uhr, Seminarraum 1.01, N220

#	Datum	
1	**23.10.09**	
2	**30.10.09**	
3	**06.11.09**	
4	**13.11.09**	
5	**20.11.09**	
6	**27.11.09**	
7	**04.12.09**	
8	**11.12.09**	
9	**18.12.09**	
10	**15.01.10**	
11	**22.01.10**	
12	**29.01.10**	
13	**05.02.10**	
14	**12.02.10**	

LEHRBUCHEMPFEHLUNGEN:

Als noch eher leicht-verdauliche Einführung:

- **Reichert: Neurobiologie**
  Georg Thieme Verlag, 2000
- **Levitan und Kaczmarek: The Neuron, Cell and Molecular Biology**
  Oxford University Press, 2002

Etwas umfangreicher:

- **Bear, Connors, Paradiso: Neuroscience**
  Spektrum Verlag, 2009, 3. Auflage
- **Kandel, Schwarz, Jessell: Neurowissenschaften**
  Spektrum Akademischer Verlag, 1995

Fortgeschritten:

- **Squire, Berg, Bloom, du Lac, Ghosh, Spitzer: Fundamental Neuroscience**
  3rd edition, Academic Press, 2008
- **Kandel, Schwarz, Jessel: Principles of Neural Science**
  McGraw-Hill, 2000 → angeblich neue Auflage in 2010 geplant !
- **Dudel, Menzel, Schmitt: Neurowissenschaften**
  Springer, 2001

Literatur für das Neuro-Seminar WS 2009-2010 (A. Gottschalk)

1. Tierverhalten

1/1 Gordon, M. D. and K. Scott (2009). "Motor control in a Drosophila taste circuit." Neuron **61**(3): 373-84.

Tastes elicit innate behaviors critical for directing animals to ingest nutritious substances and reject toxic compounds, but the neural basis of these behaviors is not understood. Here, we use a neural silencing screen to identify neurons required for a simple Drosophila taste behavior and characterize a neural population that controls a specific subprogram of this behavior. By silencing and activating subsets of the defined cell population, we identify the neurons involved in the taste behavior as a pair of motor neurons located in the subesophageal ganglion (SOG). The motor neurons are activated by sugar stimulation of gustatory neurons and inhibited by bitter compounds; however, experiments utilizing split-GFP detect no direct connections between the motor neurons and primary sensory neurons, indicating that further study will be necessary to elucidate the circuitry bridging these populations. Combined, these results provide a general strategy and a valuable starting point for future taste circuit analysis.

1/2 Pirri, J. K., A. D. McPherson, et al. (2009). "A tyramine-gated chloride channel coordinates distinct motor programs of a Caenorhabditis elegans escape response." Neuron **62**(4): 526-38.

A key feature of escape responses is the fast translation of sensory information into a coordinated motor output. In C. elegans, anterior touch initiates a backward escape response in which lateral head movements are suppressed. Here, we show that tyramine inhibits head movements and forward locomotion through the activation of a tyramine-gated chloride channel, LGC-55. lgc-55 mutant animals have defects in reversal behavior and fail to suppress head oscillations in response to anterior touch. lgc-55 is expressed in neurons and muscle cells that receive direct synaptic inputs from tyraminergic motor neurons. Therefore, tyramine can act as a classical inhibitory neurotransmitter. Activation of LGC-55 by tyramine coordinates the output of two distinct motor programs, locomotion and head movements that are critical for a C. elegans escape response.

2. Axon Guidance und Dendriten Entwicklung

2/1 Hayashi, Y., T. Hirotsu, et al. (2009). "A trophic role for Wnt-Ror kinase signaling during developmental pruning in Caenorhabditis elegans." Nat Neurosci **12**(8): 981-7.

The molecular mechanism by which neurites are selected for elimination or incorporation into the mature circuit during developmental pruning remains unknown. The trophic theory postulates that local cues provided by target or surrounding cells act to inhibit neurite elimination. However, no widely conserved factor mediating this trophic function has been identified. We found that the developmental survival of specific neurites in Caenorhabditis elegans largely depends on detection of the morphogen Wnt by the Ror kinase CAM-1, which is a transmembrane tyrosine kinase with a Frizzled domain. Mutations in Wnt genes or in cam-1 enhanced neurite elimination, whereas overexpression of cam-1 inhibited neurite elimination in a Wnt-dependent manner. Moreover, mutations in these genes counteracted the effect of a mutation in mbr-1, which encodes a transcription factor that promotes neurite elimination. These results reveal the trophic role of an atypical Wnt pathway and reinforce the classical model of developmental pruning.

2/2 Parrish, J. Z., P. Xu, et al. (2009). "The microRNA bantam functions in epithelial cells to regulate scaling growth of dendrite arbors in drosophila sensory neurons." Neuron **63**(6): 788-802.

In addition to establishing dendritic coverage of the receptive field, neurons need to adjust their dendritic arbors to match changes of the receptive field. Here, we show that dendrite arborization (da) sensory neurons establish dendritic coverage of the body wall early in Drosophila larval development and then grow in precise proportion to their substrate, the underlying body wall epithelium, as the larva more than triples in length. This phenomenon, referred to as scaling growth of dendrites, requires the function of the microRNA (miRNA) bantam (ban) in the epithelial cells rather than the da neurons themselves. We further show that ban in epithelial cells dampens Akt kinase activity in adjacent neurons to influence dendrite growth. This signaling between epithelial cells and neurons receiving sensory input from the body wall synchronizes their growth to ensure proper dendritic coverage of the receptive field.

2/3 Yam, P. T., S. D. Langlois, et al. (2009). "Sonic hedgehog guides axons through a noncanonical, Src-family-kinase-dependent signaling pathway." Neuron **62**(3): 349-62.

Sonic hedgehog (Shh) plays essential roles in developmental events such as cell fate specification and axon guidance. Shh induces cell fate specification through canonical Shh signaling, mediated by transcription. However, the mechanism by which Shh guides axons is unknown. To study this, we developed an in vitro assay for axon guidance, in which neurons can be imaged while responding to a defined gradient of a chemical cue. Axons of dissociated commissural neurons placed in a Shh gradient turned rapidly toward increasing concentrations of Shh. Consistent with this rapid response, we showed that attraction by Shh does not require transcription. Instead, Shh stimulates the activity of Src family kinase (SFK) members in a Smoothened-dependent manner. Moreover, SFK activity is required for Shh-mediated guidance of commissural axons, but not for induction of Gli transcriptional reporter activity. Together, these results indicate that Shh acts via a rapidly acting, noncanonical signaling pathway to guide axons.

3. Ionenkanal Struktur

3/1 Kawate, T., J. C. Michel, et al. (2009). "Crystal structure of the ATP-gated P2X(4) ion channel in the closed state." Nature **460**(7255): 592-8.
P2X receptors are cation-selective ion channels gated by extracellular ATP, and are implicated in diverse physiological processes, from synaptic transmission to inflammation to the sensing of taste and pain. Because P2X receptors are not related to other ion channel proteins of known structure, there is at present no molecular foundation for mechanisms of ligand-gating, allosteric modulation and ion permeation. Here we present crystal structures of the zebrafish P2X(4) receptor in its closed, resting state. The chalice-shaped, trimeric receptor is knit together by subunit-subunit contacts implicated in ion channel gating and receptor assembly. Extracellular domains, rich in beta-strands, have large acidic patches that may attract cations, through fenestrations, to vestibules near the ion channel. In the transmembrane pore, the 'gate' is defined by an approximately 8 A slab of protein. We define the location of three non-canonical, intersubunit ATP-binding sites, and suggest that ATP binding promotes subunit rearrangement and ion channel opening.

4. Neuropathien / Degeneration / Mental Disorder / Sucht

4/1 Enard, W., S. Gehre, et al. (2009). "A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice." Cell **137**(5): 961-71.
It has been proposed that two amino acid substitutions in the transcription factor FOXP2 have been positively selected during human evolution due to effects on aspects of speech and language. Here, we introduce these substitutions into the endogenous Foxp2 gene of mice. Although these mice are generally healthy, they have qualitatively different ultrasonic vocalizations, decreased exploratory behavior and decreased dopamine concentrations in the brain suggesting that the humanized Foxp2 allele affects basal ganglia. In the striatum, a part of the basal ganglia affected in humans with a speech deficit due to a nonfunctional FOXP2 allele, we find that medium spiny neurons have increased dendrite lengths and increased synaptic plasticity. Since mice carrying one nonfunctional Foxp2 allele show opposite effects, this suggests that alterations in cortico-basal ganglia circuits might have been important for the evolution of speech and language in humans.

4/2 Nakatani, J., K. Tamada, et al. (2009). "Abnormal behavior in a chromosome-engineered mouse model for human 15q11-13 duplication seen in autism." Cell **137**(7): 1235-46.
Substantial evidence suggests that chromosomal abnormalities contribute to the risk of autism. The duplication of human chromosome 15q11-13 is known to be the most frequent cytogenetic abnormality in autism. We have modeled this genetic change in mice by using chromosome engineering to generate a 6.3 Mb duplication of the conserved linkage group on mouse chromosome 7. Mice with a paternal duplication display poor social interaction, behavioral inflexibility, abnormal ultrasonic vocalizations, and correlates of anxiety. An increased MBII52 snoRNA within the duplicated region, affecting the serotonin 2c receptor (5-HT2cR), correlates with altered intracellular Ca(2+) responses elicited by a 5-HT2cR agonist in neurons of mice with a paternal duplication. This chromosome-engineered mouse model for autism seems to replicate various aspects of human autistic phenotypes and validates the relevance of the human chromosome abnormality. This model will facilitate forward genetics of developmental brain disorders and serve as an invaluable tool for therapeutic development.

4/3 Tang, J. and J. A. Dani (2009). "Dopamine enables in vivo synaptic plasticity associated with the addictive drug nicotine." Neuron **63**(5): 673-82.
Addictive drugs induce a dopamine signal that contributes to the initiation of addiction, and the dopamine signal influences drug-associated memories that perpetuate drug use. The addiction process shares many commonalities with the synaptic plasticity mechanisms normally attributed to learning and memory. Environmental stimuli repeatedly linked to addictive drugs become learned associations, and those stimuli come to elicit memories or sensations that motivate continued drug use. Applying in vivo recording techniques to freely moving mice, we show that physiologically relevant concentrations of the addictive drug nicotine directly cause in vivo hippocampal synaptic potentiation of the kind that underlies learning and memory. The drug-induced long-term synaptic plasticity required a local hippocampal dopamine signal. Disrupting general dopamine signaling prevented the nicotine-induced synaptic plasticity and conditioned place preference. These results suggest that dopaminergic signaling serves as a functional label of salient events by enabling and scaling synaptic plasticity that underlies drug-induced associative memory.

4/4 Cai, S. Q. and F. Sesti (2009). "Oxidation of a potassium channel causes progressive sensory function loss during aging." Nat Neurosci **12**(5): 611-7.
Potassium channels are key regulators of neuronal excitability. Here we show that oxidation of the K(+) channel KVS-1 during aging causes sensory function loss in Caenorhabditis elegans and that protection of this channel from oxidation preserves neuronal function. Chemotaxis, a function controlled by KVS-1, was significantly impaired in worms exposed to oxidizing agents, but only moderately affected in worms harboring an oxidation-resistant KVS-1 mutant (C113S). In aging C113S transgenic worms, the effects of free radical accumulation were significantly attenuated compared to those in wild type. Electrophysiological analyses showed that both reactive oxygen species (ROS) accumulation during aging and acute exposure to oxidizing agents acted primarily to alter the excitability of the neurons that mediate chemotaxis. Together, these findings establish a pivotal role for ROS-mediated oxidation of voltage-gated K(+) channels in sensorial decline during aging in invertebrates.

4/5 Saxena, S., E. Cabuy, et al. (2009). "A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice." Nat Neurosci **12**(5): 627-36.
The mechanisms underlying disease manifestations in neurodegeneration remain unclear, but their understanding is critical to devising effective therapies. We carry out a longitudinal analysis in vivo of identified motoneurons selectively vulnerable (VUL) or resistant (RES) to motoneuron disease (amyotrophic lateral sclerosis, ALS) and show that subtype-selective endoplasmic reticulum (ER) stress responses influence disease manifestations. VUL motoneurons were selectively prone to ER stress and showed gradually upregulated ER stress markers from birth on in three mouse models of familial ALS (FALS). 25-30 days before the earliest denervations, ubiquitin signals increased in both VUL and RES motoneurons, but an unfolded protein response coupled with microglial activation was initiated selectively in VUL motoneurons. This transition was followed by selective axonal degeneration and spreading stress. The ER stress-protective agent salubrinal attenuated disease manifestations and delayed progression, whereas chronic enhancement of ER stress promoted disease. Thus, whereas all motoneurons are preferentially affected in ALS, ER stress responses in specific motoneuron subtypes influence the progressive manifestations of weakening and paralysis.

5. Neuronale Zellbiologie

5/1 Matsumoto-Miyai, K., E. Sokolowska, et al. (2009). "Coincident pre- and postsynaptic activation induces dendritic filopodia via neurotrypsin-dependent agrin cleavage." Cell **136**(6): 1161-71.
The synaptic serine protease neurotrypsin is essential for cognitive function, as its deficiency in humans results in severe mental retardation. Recently, we demonstrated the activity-dependent release of neurotrypsin from presynaptic terminals and proteolytical cleavage of agrin at the synapse. Here we show that the activity-dependent formation of dendritic filopodia is abolished in hippocampal neurons from neurotrypsin-deficient mice. Administration of the neurotrypsin-dependent 22 kDa fragment of agrin rescues the filopodial response. Detailed analyses indicated that presynaptic action potential firing is necessary for the release of neurotrypsin, whereas postsynaptic NMDA receptor activation is necessary for the neurotrypsin-dependent cleavage of agrin. This contingency characterizes the neurotrypsin-agrin system as a coincidence detector of pre- and postsynaptic activation. As the resulting dendritic filopodia are thought to represent precursors of synapses, the neurotrypsin-dependent cleavage of agrin at the synapse may be instrumental for a Hebbian organization and remodeling of synaptic circuits in the CNS.

5/2 Trimbuch, T., P. Beed, et al. (2009). "Synaptic PRG-1 modulates excitatory transmission via lipid phosphate-mediated signaling." Cell **138**(6): 1222-35.
Plasticity related gene-1 (PRG-1) is a brain-specific membrane protein related to lipid phosphate phosphatases, which acts in the hippocampus specifically at the excitatory synapse terminating on glutamatergic neurons. Deletion of prg-1 in mice leads to epileptic seizures and augmentation of EPSCs, but not IPSCs. In utero electroporation of PRG-1 into deficient animals revealed that PRG-1 modulates excitation at the synaptic junction. Mutation of the extracellular domain of PRG-1 crucial for its interaction with lysophosphatidic acid (LPA) abolished the ability to prevent hyperexcitability. As LPA application in vitro induced hyperexcitability in wild-type but not in LPA(2) receptor-deficient animals, and uptake of phospholipids is reduced in PRG-1-deficient neurons, we assessed PRG-1/LPA(2) receptor-deficient animals, and found that the pathophysiology observed in the PRG-1-deficient mice was fully reverted. Thus, we propose PRG-1 as an important player in the modulatory control of hippocampal excitability dependent on presynaptic LPA(2) receptor signaling.

6. Nervensystem Entwicklung

6/1 Betley, J. N., C. V. Wright, et al. (2009). "Stringent specificity in the construction of a GABAergic presynaptic inhibitory circuit." Cell **139**(1): 161-74.
GABAergic interneurons are key elements in neural coding, but the mechanisms that assemble inhibitory circuits remain unclear. In the spinal cord, the transfer of sensory signals to motor neurons is filtered by GABAergic interneurons that act presynaptically to inhibit sensory transmitter release and postsynaptically to inhibit motor neuron excitability. We show here that the connectivity and synaptic differentiation of GABAergic interneurons that mediate presynaptic inhibition is directed by their sensory targets. In the absence of sensory terminals these GABAergic neurons shun other available targets, fail to undergo presynaptic differentiation, and withdraw axons from the ventral spinal cord. A sensory-specific source of brain derived neurotrophic factor induces synaptic expression of the GABA synthetic enzyme GAD65--a defining biochemical feature of this set of interneurons. The organization of a GABAergic circuit that mediates presynaptic inhibition in the mammalian CNS is therefore controlled by a stringent program of sensory recognition and signaling.

6/2 Gauthier-Fisher, A., D. C. Lin, et al. (2009). "Lfc and Tctex-1 regulate the genesis of neurons from cortical precursor cells." Nat Neurosci **12**(6): 735-44.
The mechanisms that regulate symmetric, proliferative divisions versus asymmetric, neurogenic divisions of mammalian neural precursors are still not well understood. We found that Lfc (Arhgef2), a Rho-specific guanine nucleotide exchange factor that interacts with spindle microtubules, and its negative regulator Tctex-1 (Dynlt1) determine the genesis of neurons from precursors in the embryonic murine cortex. Specifically, genetic knockdown of Arhgef2 in cortical precursors either in culture or in vivo inhibited neurogenesis and maintained cells as cycling radial precursors. Conversely, genetic knockdown of Dynlt1 in radial precursors promoted neurogenesis and depleted cycling cortical precursors. Coincident silencing of these two genes indicated that Tctex-1 normally inhibits the genesis of neurons from radial precursors by antagonizing the proneurogenic actions of Lfc. Moreover, Lfc and Tctex-1 were required to determine the orientation of mitotic precursor cell divisions in vivo. Thus, Lfc and Tctex-1 interact to regulate cortical neurogenesis, potentially by regulating mitotic spindle orientation.

6/3 Linhoff, M. W., J. Lauren, et al. (2009). "An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic organizers." Neuron **61**(5): 734-49.

Delineating the molecular basis of synapse development is crucial for understanding brain function. Cocultures of neurons with transfected fibroblasts have demonstrated the synapse-promoting activity of candidate molecules. Here, we performed an unbiased expression screen for synaptogenic proteins in the coculture assay using custom-made cDNA libraries. Reisolation of NGL-3/LRRC4B and neuroligin-2 accounts for a minority of positive clones, indicating that current understanding of mammalian synaptogenic proteins is incomplete. We identify LRRTM1 as a transmembrane protein that induces presynaptic differentiation in contacting axons. All four LRRTM family members exhibit synaptogenic activity, LRRTMs localize to excitatory synapses, and artificially induced clustering of LRRTMs mediates postsynaptic differentiation. We generate LRRTM1(-/-) mice and reveal altered distribution of the vesicular glutamate transporter VGLUT1, confirming an in vivo synaptic function. These results suggest a prevalence of LRR domain proteins in trans-synaptic signaling and provide a cellular basis for the reported linkage of LRRTM1 to handedness and schizophrenia.

6/4 Woo, J., S. K. Kwon, et al. (2009). "Trans-synaptic adhesion between NGL-3 and LAR regulates the formation of excitatory synapses." Nat Neurosci **12**(4): 428-37.

Synaptic adhesion molecules regulate multiple steps of synapse formation and maturation. The great diversity of neuronal synapses predicts the presence of a large number of adhesion molecules that control synapse formation through trans-synaptic and heterophilic adhesion. We identified a previously unknown trans-synaptic interaction between netrin-G ligand-3 (NGL-3), a postsynaptic density (PSD) 95-interacting postsynaptic adhesion molecule, and leukocyte common antigen-related (LAR), a receptor protein tyrosine phosphatase. NGL-3 and LAR expressed in heterologous cells induced pre- and postsynaptic differentiation in contacting axons and dendrites of cocultured rat hippocampal neurons, respectively. Neuronal overexpression of NGL-3 increased presynaptic contacts on dendrites of transfected neurons. Direct aggregation of NGL-3 on dendrites induced coclustering of excitatory postsynaptic proteins. Knockdown of NGL-3 reduced the number and function of excitatory synapses. Competitive inhibition by soluble LAR reduced NGL-3-induced presynaptic differentiation. These results suggest that the trans-synaptic adhesion between NGL-3 and LAR regulates excitatory synapse formation in a bidirectional manner.

7. Neuronale Netzwerke / Gliazellen

7/1 Kerschensteiner, D., J. L. Morgan, et al. (2009). "Neurotransmission selectively regulates synapse formation in parallel circuits in vivo." Nature **460**(7258): 1016-20.

Activity is thought to guide the patterning of synaptic connections in the developing nervous system. Specifically, differences in the activity of converging inputs are thought to cause the elimination of synapses from less active inputs and increase connectivity with more active inputs. Here we present findings that challenge the generality of this notion and offer a new view of the role of activity in synapse development. To imbalance neurotransmission from different sets of inputs in vivo, we generated transgenic mice in which ON but not OFF types of bipolar cells in the retina express tetanus toxin (TeNT). During development, retinal ganglion cells (RGCs) select between ON and OFF bipolar cell inputs (ON or OFF RGCs) or establish a similar number of synapses with both on separate dendritic arborizations (ON-OFF RGCs). In TeNT retinas, ON RGCs correctly selected the silenced ON bipolar cell inputs over the transmitting OFF bipolar cells, but were connected with them through fewer synapses at maturity. Time-lapse imaging revealed that this was caused by a reduced rate of synapse formation rather than an increase in synapse elimination. Similarly, TeNT-expressing ON bipolar cell axons generated fewer presynaptic active zones. The remaining active zones often recruited multiple, instead of single, synaptic ribbons. ON-OFF RGCs in TeNT mice maintained convergence of ON and OFF bipolar cells inputs and had fewer synapses on their ON arbor without changes to OFF arbor synapses. Our results reveal an unexpected and remarkably selective role for activity in circuit development in vivo, regulating synapse formation but not elimination, affecting synapse number but not dendritic or axonal patterning, and mediating independently the refinement of connections from parallel (ON and OFF) processing streams even where they converge onto the same postsynaptic cell.

7/2 Nimmerjahn, A., E. A. Mukamel, et al. (2009). "Motor behavior activates Bergmann glial networks." Neuron **62**(3): 400-12.

Although it is firmly established that neuronal activity is a prime determinant of animal behavior, relationships between astrocytic excitation and animal behavior have remained opaque. Cerebellar Bergmann glia are radial astrocytes that are implicated in motor behavior and exhibit Ca(2+) excitation. However, Ca(2+) excitation in these cells has not previously been studied in behaving animals. Using two-photon microscopy we found that Bergmann glia exhibit three forms of Ca(2+) excitation in awake, behaving mice. Two of these are ongoing within the cerebellar vermis. During locomotor performance concerted Ca(2+) excitation arises in networks of at least hundreds of Bergmann glia extending across several hundred microns or more. Concerted Ca(2+) excitation was abolished by anesthesia or blockade of either neural activity or glutamatergic transmission. Thus, large networks of Bergmann glia can be activated by specific animal behaviors and undergo excitation of sufficient magnitude to potentially initiate macroscopic changes in brain dynamics or blood flow.

8. Neurotransmitter-Rezeptoren und post-synaptische Differenzierung

8/1 Gendrel, M., G. Rapti, et al. (2009). "A secreted complement-control-related protein ensures acetylcholine receptor clustering." Nature **461**(7266): 992-6.

Efficient neurotransmission at chemical synapses relies on spatial congruence between the presynaptic active zone, where synaptic vesicles fuse, and the postsynaptic differentiation, where neurotransmitter receptors concentrate. Diverse molecular systems have evolved to localize receptors at synapses, but in most cases, they rely on scaffolding proteins localized below the plasma membrane. A few systems have been suggested to control the synaptic localization of

neurotransmitter receptors through extracellular interactions, such as the pentraxins that bind AMPA receptors and trigger their aggregation. However, it is not yet clear whether these systems have a central role in the organization of postsynaptic domains in vivo or rather provide modulatory functions. Here we describe an extracellular scaffold that is necessary to cluster acetylcholine receptors at neuromuscular junctions in the nematode Caenorhabditis elegans. It involves the ectodomain of the previously identified transmembrane protein LEV-10 (ref. 6) and a novel extracellular protein, LEV-9. LEV-9 is secreted by the muscle cells and localizes at cholinergic neuromuscular junctions. Acetylcholine receptors, LEV-9 and LEV-10 are interdependent for proper synaptic localization and physically interact based on biochemical evidence. Notably, the function of LEV-9 relies on eight complement control protein (CCP) domains. These domains, also called 'sushi domains', are usually found in proteins regulating complement activity in the vertebrate immune system. Because the complement system does not exist in protostomes, our results suggest that some of the numerous uncharacterized CCP proteins expressed in the mammalian brain might be directly involved in the organization of the synapse, independently from immune functions.

8/2 Hayashi, M. K., C. Tang, et al. (2009). "The postsynaptic density proteins Homer and Shank form a polymeric network structure." Cell **137**(1): 159-71.
The postsynaptic density (PSD) is crucial for synaptic functions, but the molecular architecture retaining its structure and components remains elusive. Homer and Shank are among the most abundant scaffolding proteins in the PSD, working synergistically for maturation of dendritic spines. Here, we demonstrate that Homer and Shank, together, form a mesh-like matrix structure. Crystallographic analysis of this region revealed a pair of parallel dimeric coiled coils intercalated in a tail-to-tail fashion to form a tetramer, giving rise to the unique configuration of a pair of N-terminal EVH1 domains at each end of the coiled coil. In neurons, the tetramerization is required for structural integrity of the dendritic spines and recruitment of proteins to synapses. We propose that the Homer-Shank complex serves as a structural framework and as an assembly platform for other PSD proteins.

8/3 Lee, C. W., J. Han, et al. (2009). "Regulation of acetylcholine receptor clustering by ADF/cofilin-directed vesicular trafficking." Nat Neurosci **12**(7): 848-56.
Postsynaptic receptor localization is crucial for synapse development and function, but the underlying cytoskeletal mechanisms remain elusive. Using Xenopus neuromuscular junctions as a model, we found that actin depolymerizing factor (ADF)/cofilin regulated actin-dependent vesicular trafficking of acetylcholine receptors (AChRs) to the postsynaptic membrane. Active ADF/cofilin was concentrated in small puncta adjacent to AChR clusters and was spatiotemporally correlated with the formation and maintenance of surface AChR clusters. Notably, increased actin dynamics, vesicular markers and intracellular AChRs were all enriched at the sites of ADF/cofilin localization. Furthermore, a substantial amount of new AChRs was detected at these ADF/cofilin-enriched sites. Manipulation of either ADF/cofilin activity through its serine-3 phosphorylation or ADF/cofilin localization via 14-3-3 proteins markedly attenuated AChR insertion and clustering. These results suggest that spatiotemporally restricted ADF/cofilin-mediated actin dynamics regulate AChR trafficking during the development of neuromuscular synapses.

8/4 Lin, D. T., Y. Makino, et al. (2009). "Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation." Nat Neurosci **12**(7): 879-87.
The insertion of AMPA receptors (AMPARs) into the plasma membrane is an important step in the synaptic delivery of AMPARs during the expression of synaptic plasticity. However, the molecular mechanisms regulating AMPAR insertion remain elusive. By directly visualizing individual insertion events of the AMPAR subunit GluR1 in rodents, we found that the protein 4.1N was required for activity-dependent GluR1 insertion. Protein kinase C (PKC) phosphorylation of the serine 816 (S816) and S818 residues of GluR1 enhanced 4.1N binding to GluR1 and facilitated GluR1 insertion. In addition, palmitoylation of GluR1 C811 residue modulated PKC phosphorylation and GluR1 insertion. Finally, disrupting 4.1N-dependent GluR1 insertion decreased surface expression of GluR1 and the expression of long-term potentiation. Our study uncovers a previously unknown mechanism that governs activity-dependent GluR1 trafficking, reveals an interaction between AMPAR palmitoylation and phosphorylation, and underscores the functional importance of 4.1N in AMPAR trafficking and synaptic plasticity.

8/5 Petrini, E. M., J. Lu, et al. (2009). "Endocytic trafficking and recycling maintain a pool of mobile surface AMPA receptors required for synaptic potentiation." Neuron **63**(1): 92-105.
At excitatory glutamatergic synapses, postsynaptic endocytic zones (EZs), which are adjacent to the postsynaptic density (PSD), mediate clathrin-dependent endocytosis of surface AMPA receptors (AMPAR) as a first step to receptor recycling or degradation. However, it remains unknown whether receptor recycling influences AMPAR lateral diffusion and whether EZs are important for the expression of synaptic potentiation. Here, we demonstrate that the presence of both EZs and AMPAR recycling maintain a large pool of mobile AMPARs at synapses. In addition, we find that synaptic potentiation is accompanied by an accumulation and immobilization of AMPARs at synapses resulting from both their exocytosis and stabilization at the PSD. Displacement of EZs from the postsynaptic region impairs the expression of synaptic potentiation by blocking AMPAR recycling. Thus, receptor recycling is crucial for maintaining a mobile population of surface AMPARs that can be delivered to synapses for increases in synaptic strength.

8/6 Zhang, W., F. St-Gelais, et al. (2009). "A transmembrane accessory subunit that modulates kainate-type glutamate receptors." Neuron **61**(3): 385-96.
Glutamate receptors play major roles in excitatory transmission in the vertebrate brain. Among ionotropic glutamate receptors (AMPA, kainate, NMDA), AMPA receptors mediate fast synaptic transmission and require TARP auxiliary subunits. NMDA receptors and kainate receptors play roles in synaptic transmission, but it remains uncertain whether these ionotropic glutamate receptors also have essential subunits. Using a proteomic screen, we have identified NETO2, a brain-specific protein of unknown function, as an interactor with kainate-type glutamate receptors. NETO2 modulates the channel properties of recombinant and native kainate receptors without affecting trafficking of the receptors and also

modulates kainate-receptor-mediated mEPSCs. Furthermore, we found that kainate receptors regulate the surface expression of NETO2 and that NETO2 protein levels and surface expression are decreased in mice lacking the kainate receptor GluR6. The results show that NETO2 is a kainate receptor subunit with significant effects on glutamate signaling mechanisms in brain.

9. Synaptische Plastizität

9/1 Lee, S. J., Y. Escobedo-Lozoya, et al. (2009). "Activation of CaMKII in single dendritic spines during long-term potentiation." Nature **458**(7236): 299-304.

Calcium/calmodulin-dependent kinase II (CaMKII) plays a central part in long-term potentiation (LTP), which underlies some forms of learning and memory. Here we monitored the spatiotemporal dynamics of CaMKII activation in individual dendritic spines during LTP using two-photon fluorescence lifetime imaging microscopy, in combination with two-photon glutamate uncaging. Induction of LTP and associated spine enlargement in single spines triggered transient (approximately 1 min) CaMKII activation restricted to the stimulated spines. CaMKII in spines was specifically activated by NMDA receptors and L-type voltage-sensitive calcium channels, presumably by nanodomain Ca(2+) near the channels, in response to glutamate uncaging and depolarization, respectively. The high degree of compartmentalization and channel specificity of CaMKII signalling allow stimuli-specific spatiotemporal patterns of CaMKII signalling and may be important for synapse-specificity of synaptic plasticity.

10. Sensorische Neuronen und Systeme

10/1 Kamikouchi, A., H. K. Inagaki, et al. (2009). "The neural basis of Drosophila gravity-sensing and hearing." Nature **458**(7235): 165-71.

The neural substrates that the fruitfly Drosophila uses to sense smell, taste and light share marked structural and functional similarities with ours, providing attractive models to dissect sensory stimulus processing. Here we focus on two of the remaining and less understood prime sensory modalities: graviception and hearing. We show that the fly has implemented both sensory modalities into a single system, Johnston's organ, which houses specialized clusters of mechanosensory neurons, each of which monitors specific movements of the antenna. Gravity- and sound-sensitive neurons differ in their response characteristics, and only the latter express the candidate mechanotransducer channel NompC. The two neural subsets also differ in their central projections, feeding into neural pathways that are reminiscent of the vestibular and auditory pathways in our brain. By establishing the Drosophila counterparts of these sensory systems, our findings provide the basis for a systematic functional and molecular dissection of how different mechanosensory stimuli are detected and processed.

10/2 Lee, S. J., Y. Escobedo-Lozoya, et al. (2009). "Activation of CaMKII in single dendritic spines during long-term potentiation." Nature **458**(7236): 299-304.

Calcium/calmodulin-dependent kinase II (CaMKII) plays a central part in long-term potentiation (LTP), which underlies some forms of learning and memory. Here we monitored the spatiotemporal dynamics of CaMKII activation in individual dendritic spines during LTP using two-photon fluorescence lifetime imaging microscopy, in combination with two-photon glutamate uncaging. Induction of LTP and associated spine enlargement in single spines triggered transient (approximately 1 min) CaMKII activation restricted to the stimulated spines. CaMKII in spines was specifically activated by NMDA receptors and L-type voltage-sensitive calcium channels, presumably by nanodomain Ca(2+) near the channels, in response to glutamate uncaging and depolarization, respectively. The high degree of compartmentalization and channel specificity of CaMKII signalling allow stimuli-specific spatiotemporal patterns of CaMKII signalling and may be important for synapse-specificity of synaptic plasticity.

10/3 a multifunctional neural circuit." Nat Neurosci **12**(10): 1308-16.

The detection of approaching objects, such as looming predators, is necessary for survival. Which neurons and circuits mediate this function? We combined genetic labeling of cell types, two-photon microscopy, electrophysiology and theoretical modeling to address this question. We identify an approach-sensitive ganglion cell type in the mouse retina, resolve elements of its afferent neural circuit, and describe how these confer approach sensitivity on the ganglion cell. The circuit's essential building block is a rapid inhibitory pathway: it selectively suppresses responses to non-approaching objects. This rapid inhibitory pathway, which includes AII amacrine cells connected to bipolar cells through electrical synapses, was previously described in the context of night-time vision. In the daytime conditions of our experiments, the same pathway conveys signals in the reverse direction. The dual use of a neural pathway in different physiological conditions illustrates the efficiency with which several functions can be accommodated in a single circuit.

10/4 levels using distinct guanylate cyclases." Neuron **61**(6): 865-79.

Homeostatic sensory systems detect small deviations in temperature, water balance, pH, and energy needs to regulate adaptive behavior and physiology. In C. elegans, a homeostatic preference for intermediate oxygen (O2) levels requires cGMP signaling through soluble guanylate cyclases (sGCs), proteins that bind gases through an associated heme group. Here we use behavioral analysis, functional imaging, and genetics to show that reciprocal changes in O2 levels are encoded by sensory neurons that express alternative sets of sGCs. URX sensory neurons are activated by increases in O2 levels, and require the sGCs gcy-35 and gcy-36. BAG sensory neurons are activated by decreases in O2 levels, and require the sGCs gcy-31 and gcy-33. The sGCs are instructive O2 sensors, as forced expression of URX sGC genes causes BAG neurons to detect O2 increases. Both sGC expression and cell-intrinsic dynamics contribute to the differential roles of URX and BAG in O2-dependent behaviors.

11. Synaptische Vesikel Exozytose

11/1 Verstreken, P., T. Ohyama, et al. (2009). "Tweek, an evolutionarily conserved protein, is required for synaptic vesicle recycling." Neuron **63**(2): 203-15.

Synaptic vesicle endocytosis is critical for maintaining synaptic communication during intense stimulation. Here we describe Tweek, a conserved protein that is required for synaptic vesicle recycling. tweek mutants show reduced FM1-43 uptake, cannot maintain release during intense stimulation, and harbor larger than normal synaptic vesicles, implicating it in vesicle recycling at the synapse. Interestingly, the levels of a fluorescent PI(4,5)P(2) reporter are reduced at tweek mutant synapses, and the probe is aberrantly localized during stimulation. In addition, various endocytic adaptors known to bind PI(4,5)P(2) are mislocalized and the defects in FM1-43 dye uptake and adaptor localization are partially suppressed by removing one copy of the phosphoinositide phosphatase synaptojanin, suggesting a role for Tweek in maintaining proper phosphoinositide levels at synapses. Our data implicate Tweek in regulating synaptic vesicle recycling via an action mediated at least in part by the regulation of PI(4,5)P(2) levels or availability at the synapse.

11/2 Yao, C. K., Y. Q. Lin, et al. (2009). "A synaptic vesicle-associated Ca2+ channel promotes endocytosis and couples exocytosis to endocytosis." Cell **138**(5): 947-60.

Synaptic vesicle (SV) exo- and endocytosis are tightly coupled to sustain neurotransmission in presynaptic terminals, and both are regulated by Ca(2+). Ca(2+) influx triggered by voltage-gated Ca(2+) channels is necessary for SV fusion. However, extracellular Ca(2+) has also been shown to be required for endocytosis. The intracellular Ca(2+) levels (<1 microM) that trigger endocytosis are typically much lower than those (>10 microM) needed to induce exocytosis, and endocytosis is inhibited when the Ca(2+) level exceeds 1 microM. Here, we identify and characterize a transmembrane protein associated with SVs that, upon SV fusion, localizes at periactive zones. Loss of Flower results in impaired intracellular resting Ca(2+) levels and impaired endocytosis. Flower multimerizes and is able to form a channel to control Ca(2+) influx. We propose that Flower functions as a Ca(2+) channel to regulate synaptic endocytosis and hence couples exo- with endocytosis.

11/3 Zhu, Y., J. Xu, et al. (2009). "Two pathways of synaptic vesicle retrieval revealed by single-vesicle imaging." Neuron **61**(3): 397-411.

Synaptic vesicle recycling is essential for maintaining efficient synaptic transmission. Detailed dissection of single-vesicle recycling still remains a major challenge. We have developed a fluorescent pH reporter that permits us to follow the fate of individual vesicles at hippocampal synapses after exocytosis. Here we show that, during low-frequency stimulation, single-vesicle fusion leads to two distinct vesicle internalizations, instead of one, as in general perception: one by a fast endocytosis pathway (approximately 3 s), the other by a slow endocytosis pathway (after 10 s). The exocytosed vesicular proteins are preferentially recaptured in both pathways. RNAi knockdown of clathrin inhibits both pathways. As stimulation frequency increases, the number of endocytosed vesicles begins to match antecedent exocytosis. Meanwhile, the slow endocytosis is accelerated and becomes the predominant pathway. These results reveal that two pathways of endocytosis are orchestrated during neuronal activity, establishing a highly efficient endocytosis at central synapses.

12. Synaptische Vesikel Endozytose und Recycling

12/1 Darios, F., C. Wasser, et al. (2009). "Sphingosine facilitates SNARE complex assembly and activates synaptic vesicle exocytosis." Neuron **62**(5): 683-94.

Synaptic vesicles loaded with neurotransmitters fuse with the plasma membrane to release their content into the extracellular space, thereby allowing neuronal communication. The membrane fusion process is mediated by a conserved set of SNARE proteins: vesicular synaptobrevin and plasma membrane syntaxin and SNAP-25. Recent data suggest that the fusion process may be subject to regulation by local lipid metabolism. Here, we have performed a screen of lipid compounds to identify positive regulators of vesicular synaptobrevin. We show that sphingosine, a releasable backbone of sphingolipids, activates synaptobrevin in synaptic vesicles to form the SNARE complex implicated in membrane fusion. Consistent with the role of synaptobrevin in vesicle fusion, sphingosine upregulated exocytosis in isolated nerve terminals, neuromuscular junctions, neuroendocrine cells and hippocampal neurons, but not in neurons obtained from synaptobrevin-2 knockout mice. Further mechanistic insights suggest that sphingosine acts on the synaptobrevin/phospholipid interface, defining a novel function for this important lipid regulator.

12/2 de Wit, H., A. M. Walter, et al. (2009). "Synaptotagmin-1 docks secretory vesicles to syntaxin-1/SNAP-25 acceptor complexes." Cell **138**(5): 935-46.

Docking, the initial association of secretory vesicles with the plasma membrane, precedes formation of the SNARE complex, which drives membrane fusion. For many years, the molecular identity of the docked state, and especially the vesicular docking protein, has been unknown, as has the link to SNARE complex assembly. Here, using adrenal chromaffin cells, we identify the vesicular docking partner as synaptotagmin-1, the calcium sensor for exocytosis, and SNAP-25 as an essential plasma membrane docking factor, which, together with the previously known docking factors Munc18-1 and syntaxin, form the minimal docking machinery. Moreover, we show that the requirement for Munc18-1 in docking, but not fusion, can be overcome by stabilizing syntaxin/SNAP-25 acceptor complexes. These findings, together with cross-rescue, double-knockout, and electrophysiological data, lead us to propose that vesicles dock when synaptotagmin-1 binds to syntaxin/SNAP-25 acceptor complexes, whereas Munc18-1 is required for the downstream association of synaptobrevin to form fusogenic SNARE complexes.

12/3 Fredj, N. B. and J. Burrone (2009). "A resting pool of vesicles is responsible for spontaneous vesicle fusion at the synapse." Nat Neurosci **12**(6): 751-8.

Synapses relay information through the release of neurotransmitters stored in presynaptic vesicles. The identity, kinetics and location of the vesicle pools that are mobilized by neuronal activity have been studied using a variety of techniques. We created a genetically encoded probe, biosyn, which consists of a biotinylated VAMP2 expressed at presynaptic terminals. We exploited the high-affinity interaction between streptavidin and biotin to label biosyn with fluorescent streptavidin during vesicle fusion. This approach allowed us to tag vesicles sequentially to visualize and establish the identity of presynaptic pools. Using this technique, we were able to distinguish between two different pools of vesicles in rat hippocampal neurons: one that was released in response to presynaptic activity and another, distinct vesicle pool that spontaneously fused with the plasma membrane. We found that the spontaneous vesicles belonged to a 'resting pool' that is normally not mobilized by neuronal activity and whose function was previously unknown.

12/4 Hui, E., C. P. Johnson, et al. (2009). "Synaptotagmin-mediated bending of the target membrane is a critical step in Ca(2+)-regulated fusion." Cell **138**(4): 709-21.

Decades ago it was proposed that exocytosis involves invagination of the target membrane, resulting in a highly localized site of contact between the bilayers destined to fuse. The vesicle protein synaptotagmin-I (syt) bends membranes in response to Ca(2+), but whether this drives localized invagination of the target membrane to accelerate fusion has not been determined. Previous studies relied on reconstituted vesicles that were already highly curved and used mutations in syt that were not selective for membrane-bending activity. Here, we directly address this question by utilizing vesicles with different degrees of curvature. A tubulation-defective syt mutant was able to promote fusion between highly curved SNARE-bearing liposomes but exhibited a marked loss of activity when the membranes were relatively flat. Moreover, bending of flat membranes by adding an N-BAR domain rescued the function of the tubulation-deficient syt mutant. Hence, syt-mediated membrane bending is a critical step in membrane fusion.

13. Trafficking von Organellen und Proteinen

13/1 Jeyifous, O., C. L. Waites, et al. (2009). "SAP97 and CASK mediate sorting of NMDA receptors through a previously unknown secretory pathway." Nat Neurosci **12**(8): 1011-9.

Synaptic plasticity is dependent on the differential sorting, delivery and retention of neurotransmitter receptors, but the mechanisms underlying these processes are poorly understood. We found that differential sorting of glutamate receptor subtypes began in the endoplasmic reticulum of rat hippocampal neurons. As AMPA receptors (AMPARs) were trafficked to the plasma membrane via the conventional somatic Golgi network, NMDA receptors (NMDARs) were diverted from the somatic endoplasmic reticulum into a specialized endoplasmic reticulum subcompartment that bypasses somatic Golgi, merging instead with dendritic Golgi outposts. This endoplasmic reticulum subcompartment was composed of highly mobile vesicles containing the NMDAR subunits NR1 and NR2B, the microtubule-dependent motor protein KIF17, and the postsynaptic adaptor proteins CASK and SAP97. Our data demonstrate that the retention and trafficking of NMDARs in this endoplasmic reticulum subcompartment requires both CASK and SAP97. These findings indicate that NMDARs are sorted away from AMPARs via a non-conventional secretory pathway that utilizes dendritic Golgi outposts.

13/2 Macaskill, A. F., J. E. Rinholm, et al. (2009). "Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses." Neuron **61**(4): 541-55.

Energy use, mainly to reverse ion movements in neurons, is a fundamental constraint on brain information processing. Trafficking of mitochondria to locations in neurons where there are large ion fluxes is essential for powering neural function. Mitochondrial trafficking is regulated by Ca2+ entry through ionotropic glutamate receptors, but the underlying mechanism is unknown. We show that the protein Miro1 links mitochondria to KIF5 motor proteins, allowing mitochondria to move along microtubules. This linkage is inhibited by micromolar levels of Ca2+ binding to Miro1. With the EF hand domains of Miro1 mutated to prevent Ca2+ binding, Miro1 could still facilitate mitochondrial motility, but mitochondrial stopping induced by glutamate or neuronal activity was blocked. Activating neuronal NMDA receptors with exogenous or synaptically released glutamate led to Miro1 positioning mitochondria at the postsynaptic side of synapses. Thus, Miro1 is a key determinant of how energy supply is matched to energy usage in neurons.

13/3 Song, A. H., D. Wang, et al. (2009). "A selective filter for cytoplasmic transport at the axon initial segment." Cell **136**(6): 1148-60.

Distinct molecules are segregated into somatodendritic and axonal compartments of polarized neurons, but mechanisms underlying the development and maintenance of such segregation remain largely unclear. In cultured hippocampal neurons, we observed an ankyrin G- and F-actin-dependent structure that emerged in the cytoplasm of the axon initial segment (AIS) within 2 days after axon/dendrite differentiation, imposing a selective filter for diffusion of macromolecules and transport of vesicular carriers into the axon. Axonal entry was allowed for KIF5-driven carriers of synaptic vesicle protein VAMP2, but not for KIF17-driven carriers of dendrite-targeting NMDA receptor subunit NR2B. Comparisons of transport rates between chimeric forms of KIF17 and KIF5B, with the motor and cargo-binding domains switched, and between KIF5 loaded with VAMP2 versus GluR2 suggest that axonal entry of vesicular carriers depends on the transport efficacy of KIF-cargo complexes. This selective AIS filtering may contribute to preferential trafficking and segregation of cellular components in polarized neurons.