
# EUSynapse

From molecules to networks: Understanding synaptic physiology and pathology in the brain through mouse models

Integrated Project
Life Sciences, Genomics and Biotechnology for Health / Studying the Brain and Combating Diseases of the Nervous System

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# Final Activity Report

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EUSynapse Kick-Off Meeting: Palacio de Jabalquintoin (Baeza/Spain) December 14, 2005

# Project objectives

To prepare the ground for the analysis and therapy of neurological and psychiatric disorders, EUSynapse was established as a European research consortium whose focus was on the comprehensive analysis of the molecular mechanisms underlying synaptic information processing. Taking full advantage of the availability of mammalian genome sequences and of the power of mouse genetics it was our goal to develop the mouse as the prime model organism for studying synaptic function. The consortium has brought together scientists who are leading in developing mouse models, resulting in one of the largest collections of synaptic mouse mutants worldwide including strong candidates for disease models. The consortium also united world-leading cell biologists and physiologists in their aim to concentrate on synaptic preparations derived from mice, thus allowing for a combination of molecular perturbation of synaptic function in a single species that was unprecedented so far.

Considering the complexity of synaptic function, research was to be conducted at hierarchically organized levels of complexity: the level of individual molecules and multimolecular assemblies, the level of individual synapses, the level of synaptic integration in individual cells and small networks in vitro, and finally the level of complex networks that are closest to the in-vivo situation. The use of genetic mouse models that are available to all partners was thought to provide a unique opportunity to overcome the boundaries between the different levels of analysis. It allowed for forming links between basic molecular mechanisms governing individual steps in synaptic transmission and complex functions of neuronal networks and the intact brain. The experimental work was to be complemented by developing theoretical models and by implementing a database that will allow for easy data access and retrieval. It was anticipated that the combined efforts of the consortium will lead to novel insights into causes of synaptopathies and hence to new avenues for their treatment.

Using an integrated approach at hierarchically increasing levels of complexity, the consortium planned to significantly further our understanding of synaptic function using the mouse as the prime model organism. Based on the ability to manipulate expression levels of synaptic proteins, the role of these proteins in synaptic transmission and in more complex brain functions was expected to be better understood, providing links to human diseases and identifying candidate drug targets for therapeutic intervention in synapse-related brain diseases (synaptopathies). Major milestones included

- Refinement of techniques for the analysis of presynaptic function including the development of novel optical methods, the generation of reporter mouse models expressing fluorescently tagged proteins, and the adaptation of pre-existing high resolution techniques such as capacitance patch clamping and multiple electrode recordings to mouse preparations.
- Establishment of genetic procedures to manipulate expression levels in mouse model synapses, complemented with proteomic and DNA-array analyses to monitor the changes induced by altered expression. Focus was to be on the calyx of Held as model synapse, but the procedures were planned to be standardized and adaptable to other brain regions.
- Determination of the mechanisms by which active zones and postsynaptic densities are assembled, which comprise the foundation of synaptogenesis in the nervous system, taking advantage of mouse models containing key proteins of active zones and PSDs tagged to fluorescent proteins.
- Identification of candidate genes involved in autism, bipolar disease, schizophrenia, and language disorders using material derived from human patient collectives with documented diagnostic and treatment records. Initial characterization of the role of the

candidate genes in synaptic function by changing expression levels and/or introducing mutations.

- Determination of the calcium dynamics and the dynamics of the molecular steps influenced by calcium in the presynaptic terminal, thus developing standardized paradigms to be applied to studies of synaptic plasticity in mice. Identification of presynaptic protein complexes and development of methods for their quantification, leading to the development of standardized screening assays.
- Refinement of techniques to measure short term plasticity, and application of these techniques to mouse models with synaptic phenotypes, focussing on hippocampal neurons and the calyx of Held as prime model systems.
- Determination of the rules of assembly, polarized trafficking and signal transduction of both ionotropic and metabotropic glutamate receptors during physiologically relevant network activities.
- Development of new approaches to study the function of small neural networks in mice, using one sensory and two cortical regions as models. Transgenic reporter lines were to be used to identify neuronal subpopulations. Standardization of network recordings for the analysis of mouse disease models, and the development of computer simulations, with an initial focus on autism models.

# Project execution

## *Introduction*

The research activities of EUSynapse were divided into six subprojects. The goal of the first subproject was to advance existing and develop new tools for the study of presynaptic function and, furthermore, to adapt and improve genetic techniques for the manipulation of the synapse in complex preparations such as brain slices. The other five subprojects were dealing with various aspects of synaptic functions at different levels of complexity. These included synapse assembly and dynamics (SP2), the cell biology of the presynapse with special emphasis on calcium dynamics and the synaptic vesicle cycle (SP3), short-term plasticity to be studied in selected model preparations (SP4), the assembly, trafficking and signalling mechanisms of glutamate receptors (SP5), and finally the role of synaptic transmission in the function of neural networks in the healthy and diseased brain (SP6).

With a few exceptions the objectives the consortium set out to tackle have been largely achieved. During the course of the project, collaboration between different members of the consortium has been intensified, leading to synergies that could not have been achieved in any of the individual laboratories alone.

The results of the work carried out by the consortium have been published in 75 original papers in international scientific journals. In addition, 4 international meetings have been organized. In particular, the consortium has launched a new meeting series, the “European Synapse Meeting” (the first held in Bordeaux in the year 2008, the second in Göttingen in November 2009. A third meeting will take place in July 2010 in Amsterdam as a satellite meeting to the FENS conference jointly representing three EU-funded consortia (EUSynapse, EuroSPIN and SynSys). In addition, summer schools and student exchange visits were organized and open not only to members of the consortium but also from other European research institutions. The spectrum of participants ranged from early PhD-students to experienced Post-Docs. The courses covered introductions to neuroscience techniques (e.g. Bordeaux Summer School), behavioural rodent phenotyping, single-cell recording-, imaging-, viral transfection techniques and many more. Please refer to subproject 7 (Dissemination and training) and the section on *Dissemination and use* at pages **Fehler! Textmarke nicht definiert.**ff for a comprehensive collection of our results.

Work within the consortium was mainly coordinated in Göttingen and during the annual meetings of all partners and their deputies, with regular telephone conferences held in between the annual meetings for further coordination. With the exception of Faust Pharmaceuticals, a SME that left the consortium in the year 2008 because of a change in their companies research focus, all partners have been fully involved until the end of the project in November 2009.

## *Subproject 1: Molecular and optical tools to study synaptic terminals*

### Overall goals

To develop treatment of diseases related to the synapse (synaptopathies) a detailed knowledge about the molecules involved at individual steps of synaptic transmission is required. It is not sufficient to understand the processes of neurotransmitter release and transmitter action *per se*, but also the mechanisms which underlie the various forms of synaptic plasticity, both long- and short-term, need to be identified at the molecular level. In particular, it is necessary to know precisely at which particular step in the long chain of events during synaptic transmission (action potential generation and modulation, $Ca^{2+}$ influx,

$Ca^{2+}$ -buffering and homeostasis, vesicle priming, vesicle fusion etc.) a given molecule serves its function. Electrophysiological and molecular techniques regarding the postsynaptic events are relatively well established. Nerve terminals, however, where neurotransmitter release and its modulation reside, are much less accessible. Therefore, the goal of the first subproject was to sharpen the tools regarding presynaptic processes. Work was carried out along two major lines of research. The first was directed towards developing new imaging assays for presynaptic function. The second goal was to manipulate protein expression levels in functionally intact preparations such as brain slices using molecular tools such as viral gene transfer.

## Major results

### Development of novel optical tools

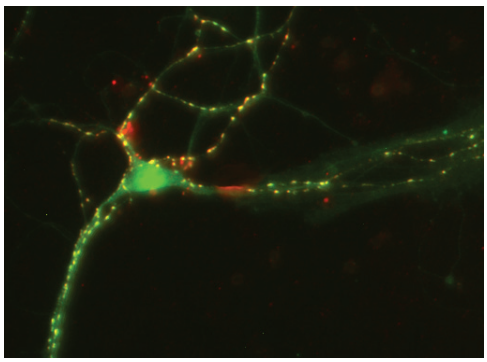
In the course of this part, transgenic mice expressing GFP-tagged synaptic vesicle proteins were used to monitor synaptic vesicle recycling, using the neuromuscular junction as prime model synapse. In the initial phase of the work, mice expressing synaptophluorin (GFP-tagged synaptobrevin) were used. This work led to the identification of hotspots for exocytosis but the low expression levels of the protein limited temporal and spatial resolution. For this reason, we tested a variety of alternative fusion proteins, including synaptotagmin-phluorin and synaptophysin-phluorins. The latter yielded better signals for both dissociated neuronal cultures and nerve terminals in slice preparations. Still, however, signals were far from single-AP resolution. A comparison of the resolution between Mono-, Double-, and Triple-versions of synaptophysin-Phluorins showed an increased signal strength, but not an improved signal-to-noise-ratio with the multiply labelled versions.

Given the experience with various phluorins, we decided to concentrate on synaptophysin-Phluorins and to work towards two knock-in mouse strains, one carrying the plain syphy-phluorin sequence at the synaptophysin locus and another one, which would allow one to conditionally replace a wild-type-like synaptophysin by the fluorescent SyPhy. ES-cells for the unconditional knock-in have been generated. While the transgenic lines could not be established anymore within the duration of the project, they are underway and will be available probably by the end of 2010.

### Genetic and biochemical techniques for the study of protein function in synapses

The goal was to develop new techniques for studying the function of selected proteins in synapses. To achieve this, proteins need to be selectively manipulated in identified nerve terminals. Subsequently, the functional and structural consequences of this manipulation need to be determined. As model, a special type of synapse was used, the calyx of Held. This is a glutamatergic giant synapse in the brainstem involved in auditory information processing in mammals. The synapse is unique since it allows for simultaneous recordings from the presynaptic and postsynaptic side, giving unprecedented access to the physiological performance of a CNS synapse. We made new viruses as gene shuttles to establish selective genetic manipulations and developed stereotaxic delivery protocols to inject these gene shuttles into the brain of newborn rats. This resulted in genetic manipulations of the living organism. Subsequently, the determination of structure and function was done in acute brain slices. We successfully tested several virus systems including adeno-associated virus (AAV) and adenovirus. These were used to overexpress presynaptic proteins in the calyx of Held. The functional consequences of overexpression was then determined for several of these proteins. One of these perturbations was analysed in detail, revealing a new role of the synaptic $Ca^{2+}$-sensor synaptotagmin in controlling release probability. The knockdown of proteins using RNA interference was established successfully as well, although many difficulties had to be overcome. Two proteins could be knocked down in the calyx.

Regarding new immunological tools we could selectively label GABAergic nerve terminals with antibodies recognizing the C-terminus of the vesicular GABA-transporter VGAT. This tool opens the possibility of studying novel GABA release sites, characterizing inhibitory vesicle trafficking, and establishing their contribution to inhibitory neurotransmission. Using the same antibody a VGAT-saporin immunotoxin was developed. The immunotoxin efficiently and specifically eliminates inhibitory neurons in culture and also *in vivo* after stereotactic injection. Furthermore, we successfully established an ELISA-based assay that allows to quantitatively define changes in expression levels of a set of presynaptic proteins including transmembrane receptors that cannot be measured by conventional immunosorbent assays. The acute genetic perturbation approach successfully developed here rests on educated guesses regarding the selection of proteins to study. To provide a more unbiased approach, we successfully identified the gene expression profile of the calyx of Held at three postnatal stages of maturation. This was possible by selectively labelling neurons giving rise to calyces using stereotaxic tracer injections. This profile revealed new candidate proteins that can be explored in future work. Therefore, the approaches established here provide a powerful strategy to study presynaptic protein function, and hence, to a better understanding of synaptopathies.



**Labelling of recycling vesicles in cultured hippocampus neurons with the polyclonal antibody against the lumenal domain of VGAT. (Synaptic Systems, Cat. No. 131 103)**

### Significance

Many diseases of the central nervous system (such as Schizophrenia, Mood Disorders, Epilepsies) are a consequence of misregulation of synaptic transmission. Being able to alter synaptic function by specifically modifying a protein involved in this process will enable researchers to understand better and manipulate the dysfunctions involved. The fact that we now can do this in a synapse (the calyx of Held), which is particularly suitable for quantitative studies, adds to the impact of our work. Furthermore, the synapse is part of the auditory pathway. Understanding its function and its role in signal processing (as it manifests itself in specific forms of short term plasticity) contributes to improve hearing aids.

Our work mainly utilized *in vivo* gene transfer into the nervous system of rodents using viral vectors. We explored several different viral systems for their suitability of delivering neuron-specific, long-term, non-toxic expression of proteins in defined areas of the central nervous system. From the systems tested, the adeno-associated virus (AAV) provided the most powerful set of features. In particular, AAV did not reveal any effects on the integrity and health of the animals injected with it, even after long-term expression lasting for more than a year. The protocol for expression of large genes through high-capacity adenoviral vectors may well become a standard for heterologous expression in neurons, and they may open new avenues for the use of viral gene transfer in human gene therapy.

The knock-in mouse, which expresses a fluorescent marker for synaptic activity, carries great promise to become a valuable tool for neuroscientists in the European Research Area.

## *Subproject 2: Analysis of the molecular mechanisms of synapse assembly and synapse dynamics.*

### Overall goals

During human development, synapses are formed at very high rates to generate a network of $10^{14}$ synapses between some $10^{11}$ nerve cells. Operationally, synaptogenesis can be

subdivided into two successive key processes, both of which are thought to be mediated by transsynaptic cell adhesion proteins: (i) the initial contact formation between an approaching axon and its target cell and (ii) the subsequently pre- and postsynaptic protein recruitment processes that lead to the formation of a mature synapse. The massive synaptogenesis that takes place during late brain development is paralleled and followed by a phase of synapse elimination, which is essential for the fine-tuning of neuronal networks and the formation of specific neural connections. In the adult central nervous system, a similar interplay between synaptogenesis and synapse elimination is thought to play a key role in memory formation. To guarantee appropriate network properties, the processes of synapse formation and synapse elimination are exquisitely regulated during development as well as in memory processes. The molecular and cell biological mechanisms underlying these processes are still poorly understood.

Studies in subproject 2 were aimed at elucidating the cell biological and molecular processes involved in synaptogenesis and synapse elimination during development, memory processes, and under pathological conditions. For this purpose specifically designed mouse models were developed that allow *in vitro* and *in vivo* imaging of synaptogenesis and synapse elimination. In addition to analyses under normal physiological conditions, selected disease-relevant mouse mutants were analyzed in which these processes are disturbed.

## Major results

### Assembly and dynamics of presynaptic and postsynaptic specializations

- A novel knock-in mouse line was generated that expresses the active zone protein Munc13-1 with a fluorescent protein tag, thus providing for the first time a minimally perturbed mouse genetic tool for live imaging of active zone formation as a hallmark of CNS synaptogenesis. A similar knock-in mutant mouse line that expresses a Munc18-1-Venus from the endogenous Munc18-1 locus was generated later. The latter mouse model allows for the analysis of synaptic trafficking and dynamics of one of the most important regulators of presynaptic function. Studies employing the two mouse lines mice have revealed (i) that Munc13-type active zone components turn over rather quickly at the synapse and that their turnover is severely affected by activity, and (ii) that Munc18-1 localizes to patches at the synapse, which may represent active release sites.
- Imaging studies showed that growth cone mobility is dependent upon transmitter release activity, indicating the presence of an autocrine feedback loop that regulates neuronal differentiation.
- Studies in mouse mutants lacking gamma-protocadherins showed that these multifaceted adhesion proteins play a key role in establishing specificity of synaptic connectivity in the olfactory system and in the regulation of functional synapse properties.
- Studies on N-cadherin showed that this abundant synaptic adhesion protein regulates the ultrastructure and functional characteristics of the presynaptic compartment.
- A novel AMPA receptor interaction protein, AIP47/CKAMP44, was identified, which controls AMPA receptor function.
- Studies on knock-out mice showed that neuroligins are key regulators of synapse assembly. Depending on the isoform, they regulate receptor recruitment to excitatory and inhibitory synapses by forming tripartite complexes with scaffold proteins and glutamate, GABA, and glycine receptor subunits

### Autism spectrum disorders

Research on the genetic foundations of autism spectrum disorders (ASD) was one of the focal points of EUSynapse research. ASD are diagnosed on the basis of three behaviourally

altered domains, namely social deficits, impaired language and communication, and stereotyped and repetitive behaviours. Beyond this unifying definition lies an extreme degree of clinical heterogeneity, ranging from severe impairments to mild personality traits. Hence autism is not a single disease entity, but rather a complex phenotype encompassing multiple "autistic conditions". ASD affect 0.6 % of children. Our previous studies pointed at one synaptic pathway, including synaptic cell adhesion molecules (neuroligins and neurexins) in the susceptibility to ASD. Nevertheless, for the majority of the cases, the cause of the disorder remains unknown and no efficient therapy is available. Our aim within the EUSynapse project was to identify new susceptibility genes for ASD. We therefore used a candidate gene strategy as well as a high-throughput genotyping to detect single nucleotide polymorphisms (SNP) or copy number variations (CNVs) associated with ASD.

During the EUSynapse project we explored the genetic variability of different genes (RPL10, SHANK1, SHANK2, SHANK3, NLGN1, NLGN2, ASMT) and could identify the synaptic scaffolding protein SHANK3 as a new gene for ASD. These results were confirming that a synaptic complex NLGN-NRXN-SHANK3 is crucial for synapse formation/maintenance as well as correct balance between GABA and glutamate synaptic currents. Following these results, we performed a high-throughput genotyping of 250 patients with ASD and could confirm the involvement of the NRXN-NLGN-SHANK pathway in the susceptibility to ASD and detect new synaptic genes associated with ASD such as SHANK2 (unpublished results). In parallel, we could show that mutations within the ASMT gene, encoding the last enzyme of melatonin synthesis, lead to melatonin deficiency in a subset of patients with ASD. Melatonin is known to play a key role in the regulation of circadian rhythms such as sleep-wake cycles and was shown to modulate GABAergic currents, as well as neurite and memory formation.

Based on these results, we propose that ASD could be the consequence of an alteration in the homeostasis of the synaptic currents in specific regions of the brain. In some cases, imbalance of excitatory/inhibitory currents could be revealed or amplified by an alteration of the melatonin pathway and/or abnormal sleep homeostasis. Consistent with this hypothesis, a better characterization of the interplay between synaptic and clock genes may shed light on new pathways associated with ASD and hopefully new therapeutic strategies. Genetic studies on autism patients showed that genes encoding key components of glutamatergic synapses (neuroligin 3, neuroligin 4, SHANK3, neurexin 1) are mutated in certain heritable forms of autism. These studies establish the functional postsynaptic protein network at glutamatergic synapses as a major autism susceptibility focus. Indeed, we found that mice with corresponding neuroligin 4 mutations show behavioural aberrations (reduced social interaction and ultrasonic communication) that are reminiscent of autism. These mouse mutants are ideal model systems for future research on experimental autism therapies.

**Synapse stability and protein ubiquitylation**

A novel ubiquitination-dependent signalling pathway was discovered that regulates dendrite growth in neurons. The pathway involves the E3 ubiquitin ligase Nedd4-1, which operates by ubiquitination of the small GTPase Rap2, which, in turn, controls protein kinases of the TNIK family. The latter are key regulators of dendrite growth, and their activity is inhibited by the Nedd4-1/Rap2/TNIK signalling pathway.

AMPA receptors in postsynaptic densities mediate the majority of fast synaptic excitatory neurotransmission. Over the course of the EUSynapse project it became clear that the functional properties of AMPA receptors, as predicted from an analysis of these receptors assembled from the known four AMPA receptor subunits, GluA1-A4 in reduced systems, such as HEK293 cells or *Xenopus* oocytes, differ from the properties of native synaptic AMPA receptors. One main reason for this is that native AMPA receptor complexes contain additional proteins, auxiliary subunits, as exemplified by the TARPs (work of Roger Nicoll and David Bredt), whose association with the core AMPA receptor subunits modulates most of the electrophysiological and pharmacological properties of synaptic AMPA receptors.

In mid-course of the EUSynapse project, we had identified a new AMPA receptor interacting protein by a proteomics approach, and focused our efforts on characterizing the effects of this protein, a type I transmembrane protein with an N-terminal cysteine-rich region and a C-terminal postsynaptic density zone (PDZ) interaction motif, on AMPA receptor function. This brain specific protein, now termed CKAMP44 (Cys-knot AMPA receptor modulating protein of 44 Kd), was shown to inhibit steady-state AMPA currents, but not NMDA or kainate receptor-mediated currents in Xenopus oocytes. Detailed electrophysiological studies in hippocampal slices of CKAMP44 knock-out mice and mice overexpressing CKAMP44 by recombinant adeno-associated virus demonstrated that CKAMP44 leads to a more rapid desensitization and slower recovery from desensitization of AMPA EPSCs. CA1 pyramidal cells express normally little CKAMP44, but dentate gyrus granule cells express high amounts. As a consequence, Schaffer collateral/commissural fiber stimulation by a paired-pulse protocol in hippocampal slices of wild-type and CKAMP44 ko mice shows AMPA EPSC facilitation. Upon CKAMP44 overexpression in CA1 pyramidal cells, however, the CA3-to-CA1 connections show depressing EPSCs by the same paired-pulse stimulation. Conversely, perforant path-to-dentate gyrus granule cell connections show normally short-term depression, which converts to facilitation in the CKAMP44 knockout. Thus, CKAMP44 attenuates at the postsynaptic side short-term facilitatory presynaptic properties at select synapses in the brain.

**Analysis of the activity-dependence of synaptogenesis in normal development and memory formation**

The overall goal of the subproject was to characterize activity-dependent and activity-independent components of synapse formation, organization and remodelling in cultured hippocampal neurons. The specific aims were two-fold: first, to analyze the activity-dependence of early synaptogenic events, and second, to examine the presynaptic molecular organization associated with activity-dependent remodelling of more mature synapses. The first aim was addressed by comparing axon outgrowth onto control target neurons from either wild type mice or mice in which neurotransmitter release was silenced by loss of Munc13-1/2 or Munc18-1, using live imaging in organotypic hippocampal cultures. In mutant neurons deficient in neurotransmitter release, axonal growth cone advance was reduced whereas presynaptic components showed enhanced motility; therefore secretion was required to maintain balanced directed outgrowth of axons and targeted delivery of presynaptic components. The second aim was investigated by studying the activity-dependent behaviour of fluorescently tagged active zone components Munc13-1 and Munc13-2 using photoconductive stimulation of neurons overexpressing these marker proteins. Stimulation did not alter the movements of these active zone components, which suggested that the overall molecular organization of the active zone could be maintained in spite of active synaptic vesicle recycling.

## Significance

Autism is a rather frequent psychiatric disorder with a prevalence of 0.6 %. So far, there is no effective autism treatment available; the only treatment that consistently shows positive effects is behavioural therapy.

A key aspect of our work focused on the functional role of neuroligins and their involvement in psychiatric disorders. This approach was based on the fact that two members of the neuroligin family of cell adhesion proteins, neuroligin 3 and neuroligin 4, play a key role in autism; their genes (NLGN3 and NLGN4X) are mutated in monogenic heritable forms of autism. In more general terms, we showed that the main function of neuroligins is to recruit the postsynaptic transmitter receptor apparatus to synapses. With regard to autism, we showed that neuroligin 4 KO mice, which genetically mimic monogenic heritable forms of autism in involving NLGN4X loss-of-function mutations, show two main behavioural deficits that are reminiscent of two key symptoms of autism in humans, i.e. aberrant social

interaction and aberrant (ultrasonic) communication. Thus, the neuroligin 4 KO mouse model has construct and face validity and can be used to develop novel autism therapies in an experimental approach. We developed one of the two first genetically defined mouse models of autism worldwide.

The novel neuroligin 4 KO mouse model can be used for experimental approaches aimed at the development of pharmacotherapies or for the search for biomarkers of the disorder. If successful, the mouse model will aid in the therapy of a frequent psychiatric disorder that causes a significant burden to the public health systems in Europe.

## *Subproject 3: Presynaptic calcium dynamics and the synaptic vesicle cycle*

### Overall goals

Due to the poor accessibility of nerve terminals and the fact that classical drug targets are typically postsynaptic, the understanding of *pre*synaptic contributions is particularly underdeveloped. However, an increasing body of evidence links malfunctioning presynaptic proteins to neurological disease. The first examples came from the detection of circulating antibodies against presynaptic proteins in various neurodegenerative autoimmune diseases. More recently, different presynaptic genes have been associated to major psychiatric disorders like autism, Parkinson's disease, ADHD and schizophrenia. Finally, one of the first systematic studies of gene expression in schizophrenia showed that expression of presynaptic genes is specifically altered. Hence, these studies provide strong indications that major brain diseases are (in part) synaptopathies and that their pathogenesis may be presynaptic. However, they do not provide clues to the underlying pathogenic mechanisms.

Here we set out to characterize the protein cascades and signal transduction pathways that orchestrate presynaptic function in the normal (healthy) brain using the best available methodologies and new methodologies. Furthermore, compromised systems were analyzed including mutant mice and disease models in order to address the question how impaired function of given presynaptic genes affects synaptic processing.

In this subproject, studies in mature synapses were carried out on the signal transduction that couples input to output, i.e., action potential arrival to secretion and the gene cascade that accounts for the different steps of the vesicle cycle. This information has then been integrated into use-dependent changes in synaptic processing and into small network performance to address the question how molecular changes in the nerve terminal translate into synaptic plasticity and network properties.

### Major results

#### Presynaptic calcium dynamics

We showed that the calcium current through voltage-gated calcium channels (VDCC) is modulated by SNAP25 levels in glutamatergic, but not GABAergic neurons and this modulation may be relevant to prevent epileptic-like network activity. In line with previous data from giant synapses, we showed that these calcium currents also cause fast action potential-evoked transients in small synaptic terminals and simulated how gradients occur after calcium diffusion and binding within the terminal. Finally, we showed that even in the absence of an action potential, central synapses contain high-affinity calcium sensors that can couple calcium signals of submicromolar amplitudes to secretory events, known as spontaneous fusion. Taken together the amplitude of synaptic calcium transients is regulated by the synaptic protein apparatus, and different amplitudes trigger different modes of synaptic secretion through specialized calcium sensor proteins.

**The synaptic vesicle cycle**

In a series of experiments in mouse adrenal chromaffin cells, we showed that the minimal docking machinery comprises syntaxin, Munc18, SNAP25 and synaptotagmin-1. Surprisingly synaptobrevin was not required for docking and instead appears to act downstream in the fusion step of the synaptic vesicle cycle.

**Protein-protein interactions in the presynapse**

Novel platforms were developed to shed light on protein-protein interactions that govern presynaptic function. First, a new protocol was developed for the isolation of synaptic vesicles from mouse brain that results in vesicle fractions comparable in purity to the classical standard protocol but with a 6-fold increased yield. Thus, a full biochemical and proteomic characterization can now been carried out of synaptic vesicles isolated from a single mouse brain or even from part of a mouse brain. Furthermore, we have developed quantitative proteomics in order to compare the protein composition of vesicles from wildtype and mutant mouse brains. The protocols have been standardized in numerous repetitions and refinements and are now being made available to the scientific community.

To study protein-protein interactions with an independent approach, we have utilized an automated and rigorously tested yeast two-hybrid screening platform to screen approximately 100 protein domains of presynaptic proteins against a library of 14,000-17,000 gene products. This screen has been completed. The data analysis resulted in a confirmation of many previously described interactions and uncovered several novel binding partners that can now be studied in-depth. One of these interactions identified a new adaptor protein that links members of the presynaptic fusion machinery (Munc18, syntaxin) to kinesin in a phosphorylation-dependent manner, thus shedding light on the biogenesis of the synaptic plasma membrane and the synaptic release apparatus.

### Significance

The adaptation of synaptic strength dependent on its usage is of key importance for learning and memory. While our understanding of presynaptic plasticity was underdeveloped so far, it is now clear that many steps in excitation-secretion coupling are subject to regulation. The molecular signalling cascades identified in presynaptic regulation in the healthy nervous system provides a basis for the identification of potential drug targets in the treatment of synaptopathies. The detailed analysis of protein-protein interactions also facilitates to better predict the specificity of selected drug targets.

## *Subproject 4: Short-term plasticity*

### Overall goals

Chemical synapses are not static transmitting devices, but dynamic links, displaying plastic behaviours on various time scales. Different types of neurons deploy synapses with different types of plasticity when they form connections. The type of short-term plasticity (STP) may vary from strong synaptic depression to strong synaptic facilitation. The way in which information is processed by synaptically interconnected networks of nerve cells is strongly influenced by the reliability and the type of short-term plasticity of synapses. Therefore, alterations in the short-term plasticity patterns of synapses will allow a neuronal circuit to process different features of action potential trains. Thus, understanding what determines the type of short-term plasticity of synapses, the locus of alterations during the train, and how the plasticity pattern can be changed are essential for deciphering the cellular and molecular basis of neuronal information processing.

Despite a consensus about the significance of short-term plasticity, understanding the mechanisms of short-term plasticity has been difficult. One of the main difficulties

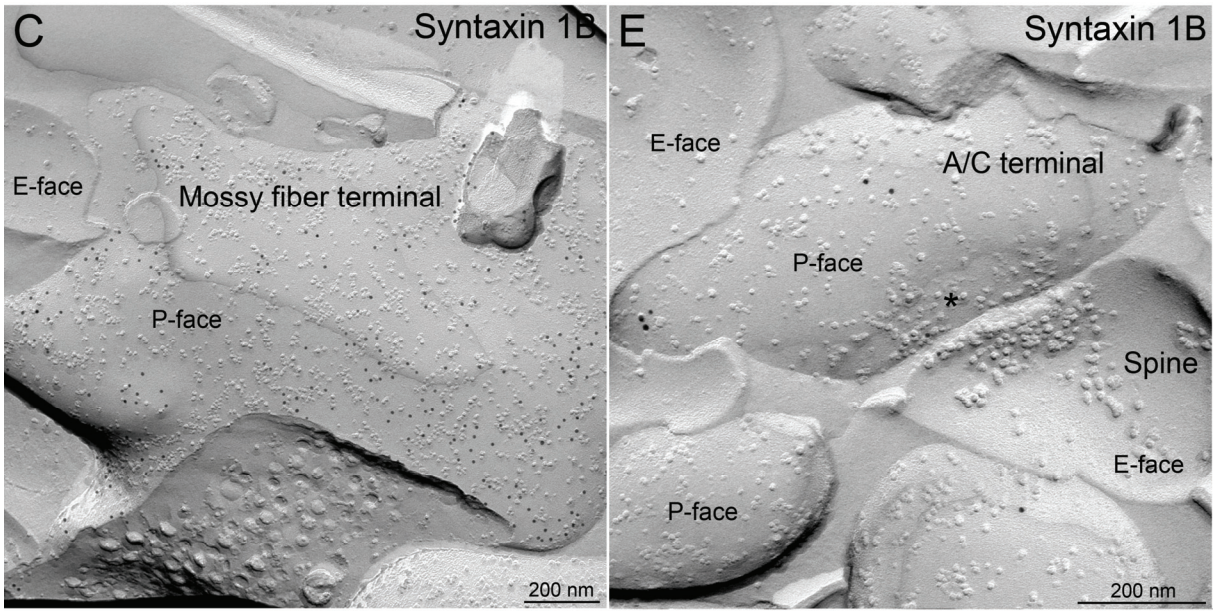
encountered when studying synaptic plasticity is quantifying the contributions made by pre and postsynaptic factors. Presynaptic alterations in short-term plasticity could arise from changes in the readily releasable pool supply, the release probability of individual synaptic vesicles, or the recruitment of new release-ready synaptic vesicles following neurotransmitter release. Postsynaptic factors include a change in neurotransmitter receptors, or effects downstream from neurotransmitter binding.

This subproject used two model preparations for studying synaptic plasticity: the hippocampus, and the calyx of Held. Each model system has its own advantages for studying mechanisms of short-term plasticity. Cultured hippocampal synapses can be easily transfected and are highly suitable for imaging. They can be used for studies of synaptic transmission in mouse mutants with a lethal phenotype. To understand the significance of short-term plasticity for neuronal networks, we use the hippocampal slice preparation, which is a relatively well understood neuronal network. At the giant, axosomatic synapse formed by the calyx of Held with neurons of the medial nucleus of the trapezoid body (MNTB), voltage and composition of the cytoplasm can be well controlled, both pre- and postsynaptically. To understand common mechanisms and to identify key cellular proteins, selected mutants were investigated in multiple preparations and at multiple levels of complexity.

## Major results

### Short-term plasticity in the hippocampus

In the initial set of experiments, *in vitro* electrophysiological and modelling approaches were applied to provide a quantitative characterization of CA1 pyramidal cell to mGluR1α positive O-LM cell synapses of the CA1 area of the hippocampus. The results demonstrated that the initial release probability at these glutamatergic synapses is very low (~0.1) and that during short-term facilitation, an increase in release probability underlie the increase in the postsynaptic responses. No evidence for multivesicular release was found. When the CA1 pyramidal cell synaptic inputs were examined onto parvalbumin positive basket cells, a low initial release probability and short-term depression was found. In order to identify the molecular basis for the differences in release probability and short-term plasticity, the molecular constituents of the release machinery of synapses made onto O-LM were compared with those of basket cells using high-resolution immunohistochemistry. Light microscopic triple labelling experiments have indicated that that these synapses differ in the expression of RIM1/2 proteins. This original light microscopic finding was expanded at the electron microscopic level using a newly developed, highly sensitive electron microscopic immunogold localization technique, called SDS-digested freeze-fracture replica labelling (SDS-FRL). Axon terminal-dependent quantitative differences in the density of syntaxin1B in mossy vs. associational/commissural terminals, and of RIM1/2 between glutamatergic and GABAergic terminals were observed.




C: High density immunogold labelling (black dots) of Syntaxin 1B was observed on mossy fiber axon terminals. E: Investigated A/C axon terminals in the stratum radiatum show that immunogold particle density in these terminals is much lower. These results clearly demonstrate a quantitative difference in the density of syntaxin 1B in distinct glutamatergic axon terminals of the hippocampus.

Furthermore, it was determined whether short-term synaptic plasticity is represented by a reciprocal relationship between morphological and functional changes. To this end, initial set of experiments examined how actin and/or organization of synaptic vesicles is affected in synapses that show altered short-term synaptic plasticity using Munc13-1 and Munc13-2 knock-out mice. No discernable differences in the behaviour of stimulus-induced actin remodeling were observed between wild type and mutant neurons. Next, it was investigated whether Munc18-1 regulates synaptic actin cytoskeleton and affects actin-sensitive component of neurotransmitter release. In contrast to chromaffin cells where overexpression of Munc18-1 strongly reduced F-actin levels, in cultured hippocampal neurons, overexpression of Munc18-1 caused no visible change on presynaptic actin distribution nor did we detect any change in actin-dependent modulation of neurotransmitter release. Finally, the relationship between synapse structure and synaptic efficacy by studying the trans-synaptic effect of postsynaptic perturbation of the N-cadherin/beta-catenin synapse adhesion complex on presynaptic efficacy were examined. Comparison of synaptic vesicle turnover at identified synapses from axons of control neurons formed onto dendrites of neurons overexpressing control GFP, a dominant negative form of N-cadherin, or wild type N-cadherin revealed a reduced recycling synaptic vesicle pool size and release probability when N-cadherin function was impaired postsynaptically. Therefore, N-cadherin synapse adhesion proteins play a role in trans-synaptic coordination of synapse integrity and efficacy.

**Short term plasticity in the calyx of Held**

The presynaptic contribution to short-term plasticity can be understood as an interplay between changes in release probability and the availability of vesicles for release, as originally proposed by Katz. Release probability increases during repetitive activity, while availability decreases due to the consumption of release-ready vesicles. A major problem in the analysis of these events lies in the fact that the postsynaptic signal reports only the net effect. The Calyx of Held offers unique possibilities to tease apart some aspects of these mechanisms. During the course of the EUSynapse project the group of Erwin Neher first characterized the changes in availability of release-ready vesicles and started a quantitative description of the process of vesicle recruitment. They established a unique relationship between recruitment rate and the global intracellular $Ca^{2+}$-concentration. Subsequently, they developed a model, which describes most of the features of interest in short-term plasticity. These are: Calcium-current facilitation and effects of “residual calcium” on short-term

facilitation; effects of postsynaptic desensitization and vesicle depletion/recycling on short-term depression. This model – unlike previous models on the Calyx of Held – includes explicit modelling of global [$Ca^{2+}$] changes. A 'graphical user interface' was developed in Matlab, which conveniently allows one to visualize the influence of various model parameters on the release time course and also on the 'internal' quantities, such as release probability, filling-state of the vesicle pool, Calcium current etc. The model nicely reproduces characteristic features of STP in the Calyx of Held, such as a transient facilitation of release, when switching from a low stimulation frequency to a high one.

The impact of short-term plasticity (STP) was studied *in vivo*, both in pre-hearing and in young-adult calyx of Held synapses. Surprisingly, it was observed that the prominent STP observed *in vivo* during development disappeared after the onset of hearing. This was accompanied by evidence that the decay of facilitation became much faster and that release probability was strongly reduced. The STP observed in pre-hearing animals may help to propagate bursts of activity originating from the cochlea to more central auditory structures. The decrease in release probability and the speeding up of facilitation may contribute to the relay function of this synapse.

A specific CaMKII phosphorylation site on the GABA-B1 subunit was identified. Point mutations of this site prevented both the phosphorylation of GABA-B1 and Ca2+-mediated inhibition of GABA-B receptors. These data suggest that excitatory glycine receptors (GlyRs) on the calyx of Held produce Ca2+ signals leading to activation of CaMKII, which in its turn can inhibit presynaptic GABA-B receptors. Using immunoelectron microscopy they next showed that the GlyRs showed a strong spatial relationship with location of glycine-releasing zones, which nicely matches the previously reported activation of these receptors by glycine spillover. The presynaptic GABA-B receptors were shown to colocalize at the light-microscopic level with a new modulator called GIP2.

## Significance

Auditory synapses in the ventral brainstem show unique specializations that allow them to preserve the timing information that provides the clue for finding out where sounds are coming from. Our research within the EUSynapse consortium has studied how these synapses form and what the contribution of short-term plasticity is for auditory functioning.

Adult auditory neurons show very high firing rates, but the firing patterns and the source of the activity at early development had not been studied in detail. We showed that auditory brainstem neurons have a very specific firing pattern, which originates from the cochlea. This specific pattern may help in the formation of auditory synapses.

We also studied the way in which auditory synapses can sustain very high rates. Previous studies in slices had suggested that at these very high firing rates, the synapses are rapidly exhausted. In contrast, we observed that during *in vivo* conditions, the synapses are much more economical with their resources, and there were no signs of exhaustion.

While working on these research problems, we have established new ways to study synaptic transmission, synaptic development and synaptic pharmacology. As almost all brain diseases manifest themselves as disorders in synaptic communication and since almost all centrally-acting drugs act on synaptic transmission, we expect these assays to have a positive impact on the study of animal models for human brain diseases, including the evaluation of new drugs for treatment.

# *Subproject 5: Assembly, trafficking, and signal transduction mechanisms for glutamate receptors*

## Overall goals

Ionotropic glutamate receptors (iGluRs) are the main mediators of excitatory synaptic transmission. They also play a role in the regulation of synaptic activity and the establishment of mature neuronal networks. In addition, iGluRs are involved in numerous neurological diseases, including excitotoxic brain damage in ischemia and epilepsy. More recently, genetic and pathophysiological studies have identified links between iGluRs and their protein partners and several brain disorders such as developmental disorders (autism or mental retardation), neuropathic pain or schizophrenia. The role of GluRs in both healthy and diseased brain depends on the composition, distribution and density of iGluRs and mGluRs in specific neuronal cell domains.

It is thus important to study the mechanisms that lead to the regulated expression and localization of glutamate receptors in order to fully understand their physiological and pathological roles. In addition, the variety of functions for iGluRs has recently extended from a pure ionotropic action to also include properties that rely on non-ionotropic signal transduction, through mechanisms involving association with cytoplasmic protein partners that are yet largely unknown. These non-ionotropic functions could appear as new promising therapeutic targets, and thus need to be elucidated.

The objective of this workpackage was to determine the rules of assembly, polarized trafficking, and signal transduction of iGluRs during physiologically relevant network activity.

## Major results

Following our goal to understand both the mechanisms and regulation of receptor assembly, trafficking to and from the synaptic plasma membrane, and signal transduction of ionotropic glutamate receptors, we have combined *in vitro* work in which mutated and/or tagged receptor subunits are expressed in cultured cells and *in vivo* analysis using transgenic mice, supported by the use of viral vectors. For this we have generated tools for the study of glutamate receptor assembly and trafficking. For instance, we have produced AAV vectors expressing GluR6 and GluR7 which are very efficient in infecting and expressing KARs in cultured neurons, and should be valuable tools *in vivo*. We have been able to see expression of GluR7 in the dentate gyrus, which appeared to be mainly dendritic, contrary to our expectations. We have implemented a virus delivery system into the neonate mouse brain. This rapid and reproducible system could serve as a standard for expressing proteins of interest in select regions of the mouse brain.

We have also identified molecular and cellular signals underlying the apparent selective subunit compositions within native AMPA and KA receptors. Using electrophysiological and biochemical assays to specifically quantify heteromeric assemblies of recombinant AMPA receptors expressed in *Xenopus* oocytes, we found that GluA2 is preferentially incorporated in heteromeric assemblies with either GluA1 or GluA3, and these complexes better traffic to the cell surface compared to the corresponding homomeric assemblies. Conversely, GluA1/A3 assemblies are less favoured than their counterpart homomers, and these complexes are mostly retained in the ER. The AMPAR interacting proteins TARP/gamma2 and cornichon 3 had little effect on the assembly process. Using chimeric subunits and deletion mutants we further found that the subunit amino-terminal domain (particularly lobe-2) constitutes the major determinant for the apparent preferred AMPAR subunit composition. Therefore, subunit protein assembly cues combined with differential expression of the subunit genes in the different neuronal types are the major factors that underlie the apparent composition and density of native AMPA receptors. Excitatory neurotransmission in cortex and hippocampus underlies learning and memory, and contributes to pathological functions,

such as occur in epilepsy, autism and schizophrenia. It is therefore essential to characterize the native complexes containing the major neurotransmitter receptors mediating fast glutamatergic transmission. We have found a novel synaptic, brain specific type I transmembrane protein that binds to AMPA receptors and modulates receptor function.

Because ionotropic glutamate receptors can also be located in a presynaptic position, we sought to characterize signalling pathways for presynaptic AMPA and KA receptors. We have previously demonstrated that AMPA receptors endowed with metabotropic functions are localized in the axonal growth cone, where they control synaptic vesicle dynamics through the activation of the MAPK pathway. Using a combination of immunocytochemistry, assays for synaptic vesicle recycling and FRET experiments, we have now shown that that, in developing hippocampal neurons, activation of presynaptic kainate receptors leads to the generation of cAMP and PKA activation, which is followed by phosphorylation of synapsin at PKA sites. These data indicate that distinct glutamate receptors types converge, through distinct signalling pathways, on the same molecular target at the presynaptic level.

We also identified the mechanisms by which presynaptic KARs activated by endogenously released glutamate inhibit the release of GABA, and found that this involved the endocannabinoid system.The role of presynaptic glutamate receptors in presynaptic plasticity GluR7 has been identified at mossy fibres (Mfs) to CA3 synapses by the electrophysiological comparison of kainate receptor mutant mice. We have performed pilot experiments with calcium imaging of presynaptic Mf boutons in the slice preparation. In parallel, we have sought new pharmacological tools to study presynaptic kainate receptors, and we have identified several ligands for GluR7 which were previously known as GluR5 antagonists or specific for AMPARs. Finally, we have analyzed the biophysical properties of recombinant kainate receptors, which are putative presynaptic receptors, and have described atypical kinetic and ion permeation properties.

A key focus of our work has concerned the contribution of an NMDA receptor subtype in excitatory synapses of the forebrain. These synapses operate with glutamate as the neurotransmitter, which activates AMPA receptor channels for fast synaptic communication and two functionally distinct NMDA receptor subtypes that mediate the setting of synaptic strength commensurate with the use of excitatory synapses. The individual contribution of these two subtypes is unknown. We developed a genetically altered mouse in whose principal forebrain neurons one of these NMDA receptor subtypes was deleted. We could determine that the lack of this very subtype in forebrain had severe consequences on all cognitive aspects tested in the mutant mice, whereas lack of the other subtype generated only mild impairments. We have also developed mouse lines to identify the presence and possibly localization of NR2D in hippocampal neurons. Using an anatomical and electrophysiological approach in hippocampal slices from wildtype, EGFP-NR2D and NR2D knockout mice we could show that early in development NR2D is expressed in hippocampal interneurons and CA1 pyramidal cells and is synaptically localized. NR2D mediated currents decrease in size with development and are lost in both cell types at adult stages.

We have described a novel form of long term potentiation (LTP) of NMDA-EPSCs at hippocampal mossy fiber synapses, that relies on the activation of adenosine A2A receptors in combination with mGluR5 and NMDA receptors. To follow up on the analysis of the mechanisms, we have adressed the question of the subtypes of NMDA receptors involved and whether there is a switch in receptor composition aftre LTP (Srikumar et al, manuscript in preparation). Finally we have analyzed the functional consequences of LTP of NMDA-EPSCs in terms of spike transfer at hippocampal mossy fiber synapses, and found that LTP of NMDA-EPSCs serves as a metaplastic switch which endows these synapses with additional mechanisms of plasticity.

### Significance

Excitatory synaptic transmission clearly depends on the variety of function and subcellular localization of glutamate receptors. Overall this sub-project has contributed significantly to a better understanding of the role of glutamate receptors in synaptic function and plasticity. Knowledge of the precise assembly and of the molecular complexes that underlie proper trafficking and targeting of glutamate receptors is fundamental to understand synaptic modifications during learning-related synaptic plasticity or during pathological forms of changes of synaptic function. Because we think that these mechanisms should ultimately explored in the brain, the work package has provided new tools and methods for the study of receptor trafficking in situ that should be of use to a wide community.

A significant outcome of the work by several partners to the work package relies in the original discoveries concerning presynaptic glutamate receptors. This bears important consequences, because we propose that presynaptic ionotropic glutamate receptors are up to now poorly explored targets for interventions in the regulation of synaptic circuits, in the normal and pathological brain.

Finally, we have provided links between specific receptor subtypes and forms of synaptic plasticity with behavioural traits by the use of appropriate engineered mouse mutants

## *Subproject 6: The role of synaptic transmission in neural networks of healthy and diseased brain*

### Overall goals

An important objective in neuroscience is to understand how information is processed in various brain regions, thereby laying a basis for understanding brain function and how neural networks are disrupted during disease. Each brain region has unique sets of neurons, which perform distinct computational tasks. Very little is known about how groups of excitatory and inhibitory neurons interact to process information under normal conditions either in sensory systems or in the cortex. The main difficulties in studying network function are: 1) the identification of different functional cell types (this is particularly problematic for interneurons which are highly diverse) and 2) inferring the properties of the network from single cell recordings. In this work package, the intrinsic electrical properties, the precise operation of chemical and electrical synapses was examined in identified neurons of small neural networks (a sensory system and two cortical regions). We investigated how these properties influence network behaviour, with special reference to GABAergic interneurons. We used transgenic mice with cell type-specific expression of EGFP to identify interneuron subtypes (tg Px1::Pannexin 1 EGFP; tg Parv::EGFP tg Calr:EGFP; tg CCK::EGFP; tg 5HT3A::EGFP; Tab. 1) and made multiple patch-electrode recordings to examine their intrinsic properties and their synaptic transmission. Since it is difficult to determine the complex spatio-temporal dynamic properties of network activity directly from single- or even multi-cell measurements, we also used biologically realistic computer simulations of the network to gain a deeper insight into large-scale network activity. An additional milestone of this work package was to compare the properties of healthy cortex with cortex from three different animal models of autism that cover both genetic and environmental factors. It is hoped that we will begin to understand how neural networks processes information in health, and identify the important changes in network function that could underlie the autistic condition.

### Major results

Relatively little is known about how groups of excitatory and inhibitory neurons interact to process information under normal conditions either in sensory systems or in the cortex. This has been addressed in this work package, by studying the intrinsic electrical properties, the

precise operation of chemical and electrical synapses in identified neurons of small neural networks. The group of Zoltan Nusser has successfully characterized the morphological, neurochemical and electrophysiological properties of retrogradely labelled non-principal cells of the main olfactory bulb. These results reveal new principles in the cellular elements and synaptic connectivity of this sensory brain area. The group of Hannah Monyer has carried out a morphological and electrophysiological characterization of 5HT3 positive interneurons. They have also investigated the basis of hippocampal ripple oscillations in mice with reduced fast excitation onto parvalbumin-positive cells. The group of Henry Markram has completed an extensive characterization of Martinotti cell inhibition in the somatosensory and other neocortical regions. They have also characterized the *layer 6* inhibitory neurons and their synaptic pathways. Lastly, during the project they have they have made an extensive study of the changes in synaptic connectivity that occur in the valproic acid (VPA) model of autism. The groupof Angus Silver has recently published a study in Nature examining the role of synaptic short-term plasticity in gain modulation in cerebellum and cortex using electrophysiological and modelling methods. They showed that when excitation is mediated by synapses with short-term depression (STD), neuronal gain is controlled by an inhibitory conductance in a noise independent manner, allowing driving and modulatory inputs to be multiplied together. The nonlinearity introduced by STD transforms inhibition-mediated additive shifts in the input–output relationship into multiplicative gain changes. On the software development front they have extended the software application neuroConstruct (http://www.neuroconstruct.org), which enables network models with a high degree of biological realism to be built, to automatically generate code for parallel computer architectures and have developed NeuroML, a simulator independent model description language for defining single neuron and network models.

### Significance

In addition to making significant contributions to our knowledge base on networks in the cortex, olfactory bulb and cerebellum we have also made significant progress in understanding an animal model of autism (VPA). We have also provided new tools for modelling brain function in health and disease and contributed new information about the properties of synapses, computational properties of neurons and the properties of networks. More specifically we have released a software application called neuroConstuct that enables the automated construction of 3D models of neural networks with a high degree of biological detail. This freely available software also provides, for the first time, a simulator independent description of single cell and network models allowing cross-simulator validation, greater interoperability and model transparency. This, together with the development of the related XML language NeuroML, has facilitated standardization of the description of single cell and network anatomy and the description of kinetic models of ionic conductances and synapses. During this project we have also standardized the statistical approach used to extract quantal parameters with variance –mean analysis.

## *Subproject 7: Dissemination and training*

### Overall goals

In order to make the research progress and the results available to the public and the scientific community, the first step was to install a public website. Offered training courses should be listet there as well as conferences. Access to the consortium-internal communicaion platform was provided, too.

In line with the work plan of the project, 25 courses should be offered, including public conferences and meeting to spread the gained knowledge.

## Major results

The project website was set up as planned and is available at http://www.eusynapse.mpg.de. It features a publications database and general information about the project as well. The *Publishable executive summaries* of period one to four can be downloaded. The conferences (European Synapse Meetings) and EUSynapse training offers were announced. An online-registration tool was provided for the courses.

Our initial plan to offer 25 courses had to be changed in course of the project based on a enquiry to find out which of the courses are the most interesting. Unused funds have been made available for lab-research.

| Year | Title | No. of Partic. | Organiser |
|---|---|---|---|
| 2005 | Workshop on „Synaptopathies and mental disorders" (Baeza) | 75 | Guillermo A. de Toledo |
| 2006 | In vivo intracellular recordings (Eilat) | 10 | Yael Stern-Bach |
| 2006 | In vivo imaging (Rotterdam) | 4 | Gerard Borst |
| 2006 | European Synapse Summer School (Bordeaux) | 20 | Christophe Mulle |
| 2007 | In vivo phenotyping of mutant rodents: integrating neural activity, heart rate and behaviour (Amsterdam) | 16 | Matthijs Verhage |
| 2007 | European Synapse Summer School (Bordeaux) | 22 | Christophe Mulle |
| 2007 | Flash Photolysis of caged-$Ca^{2+}$ and exocytosis (Göttingen) | 6 | Erwin Neher |
| 2007 | Photoconductive Stimulation (London) | 6 | Yukiko Goda |
| 2008 | ESM2008: 1$^{st}$ European Synapse Meeting (Bordeaux) | 200 | Christophe Mulle |
| 2008 | Virus-mediated gene delivery into the neonatal and adult mouse brain (Heidelberg) | 8 | Peter Seeburg |
| 2009 | In vivo patch clamping (Rotterdam) | 4 | Gerard Borst |
| 2009 | Viral gene transfer and stereotaxic delivery (Heidelberg) | 6 | Thomas Kuner |
| 2009 | ESM2009: 2$^{nd}$ European Synapse Meeting (Göttingen) | 250 | Reinhard Jahn |
| 2009 | European Synapse Summer School on Synaptic mechanisms and synaptopathies (Bordeaux) | 22 | Christophe Mulle |
| 2009 | In vivo phenotyping of mutant rodents: integrating neural activity, heart rate and behaviour (Amsterdam) | 16 | Matthijs Verhage |

## Significance

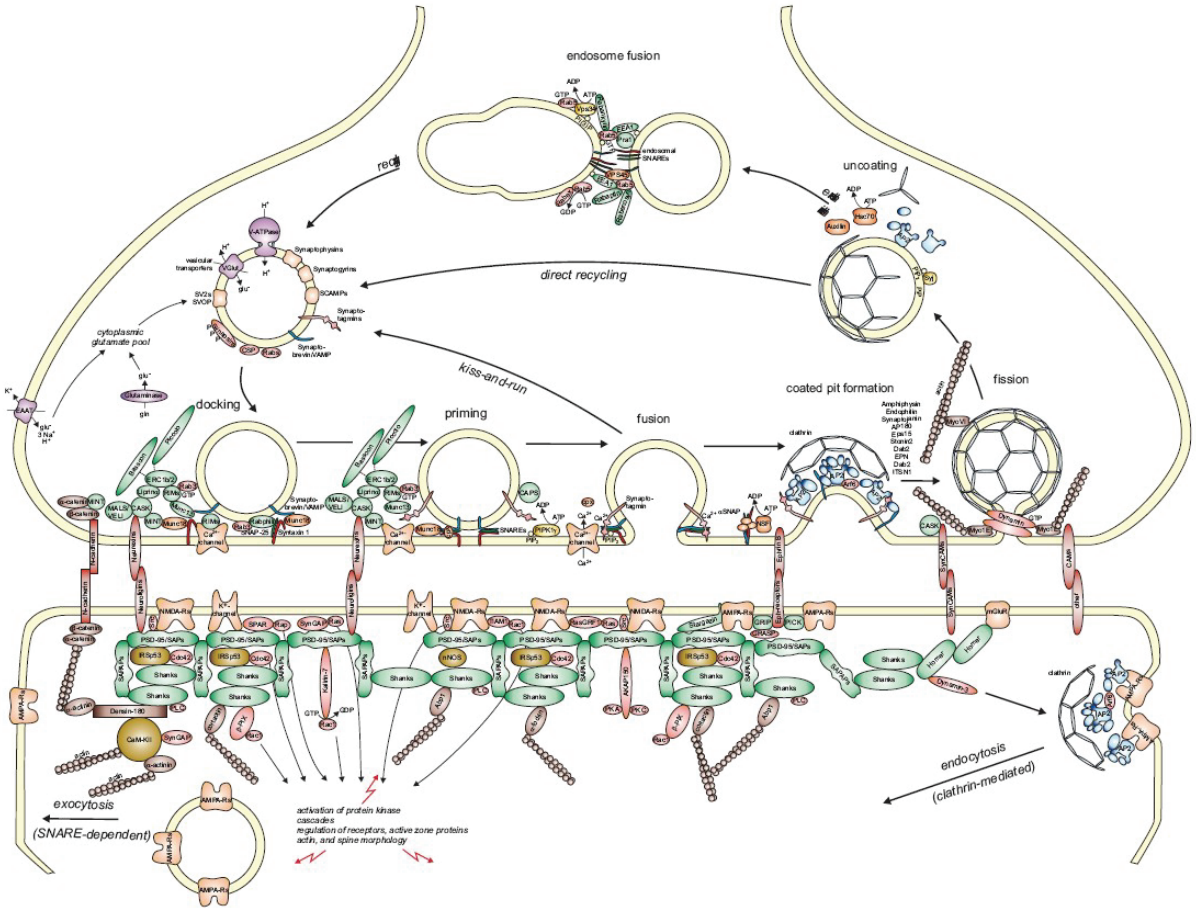
The EUSynapse website was moderately accessed with an average of 500 page-visits/month. Peaks with up to 2000 page-visits could be observed during the reporting-periods and in advance of announced conferences.

With regard to the training courses we always received good feedback from the participating scientists. Returning back to their host institutions they could translate the acquired methodologies and techniques to their experiments and spread the serving group's knowledge within the consortium (and abroad). Moreover, the intercultural and social components of such courses (get-togethers, dinners, coffee-breaks etc.) helped in building up a trusted atmosphere among the scientists and therefore laying the ground for further collaboration between different labs. This also lead to enriched exchange of research staff e.g. via site-visits and follow-up employments.

# *The Architecture of an Excitatory Synapse*

John J. E. Chua, Stefan Kindler, Janina Boyken and Reinhard Jahn

Download the high-resolution-version at http://www.eusynapse.mpg.de/architecture.pdf




**Key events occurring at an excitatory synapse**: Synaptic vesicles are formed and recycled at the presynaptic side (steps in this process are numbered in sequential order). Synaptic vesicles containing neurotransmitter (NT; glutamate, the main excitatory neurotransmitter, is shown here as an example) are recruited to specialized release sites known as active zones. NT diffuses across the synaptic cleft and binds to postsynaptic NT receptors that alter the membrane potential and trigger signal transduction cascades. Multiprotein complexes that make up a region known as the postsynaptic density mediate clustering of receptors and cell-adhesion molecules, and orchestrate the coupling of diverse signalling components.

## Publications

The EUSynapse-project, resulted in 78 publications in peer reviewed journals. The collection of scientific publications is available on the EUSynapse website and will be extended as long as project-related papers are released. Please visit us at http://www.eusynapse.mpg.de.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## *Glossary*

| | |
|---|---|
| ADHD | Attention-deficit hyperactivity disorder |
| Calyx of Held | A giant synapse in the auditory system of rodents, very useful in presynaptic experimental studies |
| ELISA | Enzyme-linked immunosorbent assay, a technique to detect the presence of an antibody or an antigen in a sample. |
| EPSC | Excitatory postsynaptic current, a temporary depolarization of postsynaptic membrane potential caused by the flow of positively charged ions into the postsynaptic cell as a result of opening of ligand-sensitive channels |
| FRET | Förster resonance energy transfer, a mechanism describing energy transfer between two chromophores |
| GABA | gamma-Aminobutyric acid, chief inhibitory neurotransmitter in the mammalian central nervous system |
| GFP | Green fluorescent protein, a standard tool in molecular biology |
| knock-in | Technique to add genetic information (e.g. modified genes) to an organism, e.g. a mouse |
| Knock-out | Technique to remoove a gene or temporarily switch off (conditional knock-out) the function of a gene in an animal, e.g. mouse. Afterwards the consequences for the cells or the whole animal are studied. |
| metaplastic | Metaplasticity refers to the plasticity of synaptic plasticity. The idea is that the synapse's previous history of activity determines its current plasticity. |
| Munc-18 | Member of the munc protein family with essential component of the synaptic vesicle fusion protein complex |
| NMDA | N-methyl-D-aspartic acid, acts as a specific agonist of the NMDA-receptor, mimics the action of glutamate on this receptor |
| Plasticity | Ability of the synapse to change its response to signals by e.g. changes in the quantity of neurotransmitters released. Synaptic plasticity is one of the important neurochemical foundations of learning and memory |
| postsynapse | That part of the synapse that carries neurotransmitter receptors and generates an electric current when stimulated by the chemical signal |
| presynapse | The part of the synapse that sends the signal by release of neurotransmitters |
| proteomics | large-scale study of proteins, particularly their occurrence, structure and function |
| saporin | A ribosome inactivating protein (RIP), toxic molecule to (specifically) kill cells |
| Stereotaxic … | A technique to use a three-dimensional coordinate system for e.g. brain surgeries in mice. Specific landmarks are used to achieve reproducible application of stimuli to brain regions. |
| synaptobrevin | small integral membrane protein of secretory vesicles, a SNARE protein |
| synaptopathies | a made-up-word for synapse-related brain diseases like Alzheimer's disease, Parkinson's disease, autism, schizophrenia, epilepsy, dementia etc. |

| | |
|---|---|
| synaptophysin | vesicle membrane protein |
| synaptotagmin | $Ca^{2+}$ binding membrane protein |
| transfection | Here: modification of the cells genetic information e.g. by using viruses as transporters for mutated genes |
| Venus | A fluorescent component, can be genetically coupled to proteins to be studied |
| Xenopus | African Clawed Frog, a model system for gene-, protein expression and knock-down studies |

# Dissemination and use

Publishable results of the Final plan for using and disseminating the knowledge: None