The magic of synaptogenesis

Meeting on Spinogenesis and Synaptic Plasticity

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The first symposium on spinogenesis and synaptic plasticity was held in Westerburg, Germany, between 23 and 26 August 2004, and was organized by M. Kreutz from the Leibniz Institute for Neurobiology, Magdeburg, Germany, with the help of C. Spilker, K.-H. Smalla, S. Staak and U. Thomas. See <http://neptun.ifn-magdeburg.de/ssp> for more information

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Introduction

The romantic castle of Westerburg in Germany is near the Harz Mountains, where legend has it that witches gather on Walpurgis nights to dance and to practice their magic skills. Earlier this year, approximately 100 scientists from all over the world gathered at the castle and were submerged in the magic of synaptogenesis. The fact that none of the participants could escape across the moat during the symposium, but instead spent their evenings in the castle drinking beer with their fellow scientists, ensured lively post-session discussions. The meeting included presentations on the assembly of synaptic contacts and on many interesting molecules the manipulation of which affects the number, shape or plasticity of synapses. Although the meeting covered both excitatory and inhibitory

synapses, the title of the symposium refers mainly to excitatory synapses, which were the focus of most of the presentations. Whereas inhibitory synapses are mainly localized on the somata and shafts of dendrites, excitatory synapses are commonly associated with dendritic spines (which were depicted on the poster for the meeting). At the spine heads, which are found opposite the glutamatergic-axon terminals, the complex postsynaptic network of transmitter receptors and their associated signalling proteins is assembled in the so-called postsynaptic density (PSD; Fig 1). The composition of this network is dynamically regulated in an activity-dependent manner; for example, in response to the intensive synaptic stimulation that induces long-term potentiation (LTP), which is regarded as an experimental model for learning and memory. The activity-dependent changes in synaptic efficacy are associated with changes in spine morphology. Here, we give a brief overview of the recent advances in this field that were discussed during the meeting.

Cell-adhesion molecules and synaptic function

During the formation of synaptic connections, neurons extend their processes to specific locations where they find appropriate targets and form contacts through transcellular adhesive interactions. These are mediated by cell-adhesion molecules (CAMs), which contribute to the later stages of synaptic differentiation and signalling, as well as to guidance, recognition and adhesion. Y. Yamaguchi (San Diego, CA, USA) presented data on the significance of signalling that is mediated by the ephrin family of CAMs and their receptors: the Eph family of receptor tyrosine kinases (Yamaguchi & Pasquale, 2004). The transfection of hippocampal neurons with a dominant-negative form of EphB2 causes dendritic protrusions to remain filopodial, rather than forming mature spines. A similar reduction in the number of spines is found in triple-knockout (TKO) mice that lack EphB1–3 receptors. Yamaguchi showed that EphB receptors are required for the tyrosine phosphorylation of the postsynaptic heparin sulphate proteoglycan (HSPG) syndecan 2, the clustering of which might induce the formation of spines.

The importance of trans-synaptic adhesive interactions that are mediated by nectins was highlighted by T. Sakisaka from the laboratory of Y. Takai (Osaka, Japan). During development, presynaptic nectin-1 and postsynaptic nectin-3 colocalize in the synapses and the *puncta adherentia* (adhesion sites between the axon terminals and their target cells) on CA3 pyramidal cells. The inhibition of

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Fig 1 | Postsynaptic density proteins as scaffolds for small G-protein-mediated signalling. Exchange factors are depicted in dark green, effector proteins in red, cell-adhesion molecules in turquoise, transmitter receptors in yellow and scaffold proteins in dark blue. AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; β PIX, β -PAK-interactive exchange factor; GKAP, guanylate-kinase-associated protein; Glu, glutamate; IRSp53, insulin-receptor substrate of 53 kDa; kal-7, kalirin-7; NMDA, N-methyl-D-aspartate; N-WASP, neural Wiskott–Aldrich syndrome protein; PAK, p21-activated kinase.

nectin-based adhesion decreases the size and number of synaptic contacts in cultured neurons (Mizoguchi *et al*, 2002). In adult animals, nectin-1 and -3 colocalize exclusively in the *puncta adherentia* junctions, and mice that are deficient in nectin-1 show a significant reduction in this type of intercellular contact.

A. Dityatev (Hamburg, Germany) presented data showing that a polysialylated form of the neural CAM (PSA-NCAM) in complex with as yet unidentified HSPGs promotes the formation of synapses during early synaptogenesis. PSA-NCAM and heparan sulphates are also required for the formation of perforated spine synapses after the induction of the N-methyl-D-aspartate (NMDA) receptor-dependent form of LTP. Preferential formation and stabilization of presynaptic boutons on postsynaptic cells that express PSA-NCAM involves signalling via NMDA and fibroblast-growth-factor receptors (Dityatev *et al*, 2004). The importance of PSA-NCAM for synaptic dynamics is further supported by impairment of LTP and long-term depression (LTD) in mice that are deficient in NCAM or PSA.

M. Missler (Göttingen, Germany) reported severe changes in synaptic properties in TKO mice that are deficient in three α -neurexins (Missler *et al*, 2003). Presynaptic neurexins bind to postsynaptic CAMs, such as neuroligins and syndecans. Neurons from these TKO mice show a manifold reduction in spontaneous transmitter release and a modest reduction in the number of inhibitory synapses. Furthermore, the amplitude and use-dependent modulation of evoked excitatory postsynaptic currents (EPSCs) is affected in these TKO mice. An inhibitor of N-type Ca^{2+} channels, ω -conotoxin, does

not reduce evoked EPSCs as strongly in TKO mice as in control mice, which indicates that α -neurexins might couple N-type channels to synaptic-vesicle exocytosis.

In summary, the presented data imply that CAMs are engaged in the regulation of presynaptic release and the postsynaptic machinery, as well as their activity-dependent modulation.

Functional assembly of active zones

At the pre- and postsynaptic sites, large protein complexes are assembled that depend on multidomain scaffold proteins. For the presynapse, E. Gundelfinger (Magdeburg, Germany) presented evidence that a preassembled dense-core transport vesicle forms part of a large complex, which is known as the cytomatrix of the active zone (CAZ), at axon terminals. This transport vesicle contains two large scaffold proteins, Piccolo and Bassoon, which serve as markers for the so-called Piccolo–Bassoon transport vesicle (PTV; Shapira *et al*, 2003). PTVs carry several components of the CAZ and their vesicular nature implies that maturation of the presynaptic terminals occurs in a quantal manner, with between three and five PTVs being sufficient to form a functional presynapse. The significance of Bassoon is underlined by the fact that only 30%–40% of the central synapses are active in mice that lack a large fragment of this protein (Altrock *et al*, 2003; Dick *et al*, 2003). In the absence of Bassoon, the photoreceptor synapse ribbon structure, which serves as a kind of conveyor belt to transport synaptic vesicles to the active zone, is dislocated or ‘floating’. The

presence of preassembled vesicles raises the questions of where they are assembled and how they are stabilized in nascent synapses. Gundelfinger presented evidence to indicate that assembly occurs in the Golgi apparatus. With regard to their stabilization, Dityatev reported that NCAM accumulates in nascent synapses within minutes of the initial contact-formation and that this molecule serves as a 'synaptic trap' for β -spectrin-coated trans-Golgi-derived organelles (Sytnyk *et al*, 2002).

Functional assembly of postsynaptic scaffolds

Transmitter receptors are associated with postsynaptic densities (PSDs). How and where the protein complexes that form the PSD are assembled is unclear, but vesicular transport, local protein synthesis and complex assembly under the control of local signalling molecules are probably all involved in parallel. It is also clear that at least some assembly of the PSD occurs in dendrites through the gradual accumulation of components. M. Kneussel (Hamburg, Germany) reported that in the inhibitory synapses, the accumulation of γ -aminobutyrate A (GABA_A) receptors depends on gephyrin, which interacts with dynein light-chain 1 and is possibly transported to the synapses by dynein motors.

Large multidomain scaffold proteins, such as the Discs large (Dlg)/PSD-95 or shank/proline-rich synapse-associated protein (ProSAP) families, form the framework of the PSD. Several talks focused on the control of the assembly and synaptic targeting of these large macromolecules in the dendrite. In *Drosophila*, PDZ domains are important for targeting Dlg to the neuromuscular junction (U. Thomas, Magdeburg, Germany). This protein binds to, and is essential for the proper synaptic localization of, K^+ channels and a homologue of NCAM, fasciclin II. Dlg-deficient flies have larger synaptic boutons owing to the mislocalization of fasciclin II. An alternatively spliced form of Dlg that contains the so-called S97 domain indirectly binds to the guanylate kinase (GUK) Dlin-7 and directs it, along with the associated epidermal growth factor (EGF) receptor and syndecan, into the synaptic boutons.

Besides the myriad of protein interactions between PSD members, the targeting of proteins to the postsynaptic membrane can also be achieved by lipid modification. For example, PSD-Zip70 (K. Sobue, Osaka, Japan) and the caldendrin-associated protein Jacob (M. Kreutz, Magdeburg, Germany) require amino-terminal myristoylation for synaptic targeting. The synaptic localization of PSD-95, which associates with NMDA receptors and the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor/stargazin complex, is dynamically regulated by palmitoylation. This reaction is mediated by palmitoyl-acyl-transferases (PATs) that are characterized by an Asp-His-His-Cys (DHHC) motif (M. Fukata, San Francisco, CA, USA). Out of 23 DHHC proteins, Fukata identified one PAT that palmitoylates PSD-95 and regulates the membrane anchoring of PSD-95 in spines.

C. Sala (Milano, Italy) highlighted the fact that shank proteins are of particular interest for spine generation as they significantly enhance spine maturation by recruiting other PSD components. In transfected COS cells, as well as neurons, he showed that none of the scaffold proteins, such as PSD-95 or shank, can act alone; rather, the intricate network of intra- and intermolecular interactions—for example between PSD-95, guanylate-kinase-associated protein (GKAP) and shank—is required to induce the synaptic clustering of shank.

Morphogenic signalling by small G proteins

The shape and function of spines are determined by small G proteins of the Rho family (including Rho, Rac and Cdc42), as well as by Rap. As Yamaguchi and T. Sakisaka (Osaka, Japan) pointed out, post-synaptic morphology is controlled by specific guanine nucleotide-exchange factors (GEFs) for either Rac or Cdc42. For example, EphB2 activates the Cdc42-GEF intersectin. Signalling from intersectin to the neural Wiskott–Aldrich syndrome protein (N-WASP), which is an actin-modulating protein that is controlled by Cdc42, is necessary for the generation of spines. The β -PAK-interactive exchange factor (β PIX; E. Kim, Daejeon, South Korea) and kalirin 7 (R. Mains, Farmington, CT, USA) are further postsynaptic GEFs for Rac and Cdc42 that are anchored at the PSD by PDZ (PSD-95/Discs large/ZO-1) domains. Mains presented data on spinogenesis in dissociated and organotypic hippocampal cultures, but also ventured away from these prototypical models and showed that kalirin 7 contributes to dopamine signalling after chronic cocaine exposure in the area of the brain that is known as the nucleus accumbens, which leads to an increased number of spines in dopamine target neurons.

Data presented by Kim, H.-J. Kreienkamp (Hamburg, Germany) and T. Böckers (Ulm, Germany) indicate that shank proteins provide scaffolds for G-protein signalling. The shank proteins target the Rac/Cdc42-specific exchange factor β PIX and the p21-associated kinase (PAK) to the synapses (Park *et al*, 2003). In addition, the shank proteins also bind to the insulin-receptor substrate of 53 kDa (IRSp53), which is another effector of Cdc42. IRSp53 promotes actin rearrangements that enhance the number of dendritic spines or filopodia-like protrusions. Therefore, as well as providing a scaffold, shank proteins might also be viewed as downstream effectors of Cdc42/IRSp53 because an interaction of IRSp53 through its carboxy-terminus with PSD-95 connects two important PSD scaffolds: PSD-95 and shank proteins (Soltau *et al*, 2004). In addition, Böckers described a link between shank proteins and Spar, which is a GTPase-activating protein for Rap small G proteins. This interaction involves the intermediate ProSAP-interacting protein (ProSAPiP). As highlighted by Sobue, Spar interacts with actin filaments, regulates spine actin dynamics and is necessary for spine maturation.

The intricate connection between NMDA receptors and the regulation of the spine cytoskeleton was further clarified by A. Matus (Basel, Switzerland), who showed that receptor stimulation leads to a loss of actin motility in spines owing to a translocation of the actin-regulatory protein profilin from the dendrite to the spine heads.

Activity-dependent synaptic plasticity

As synaptogenesis is activity dependent, A. Konnerth (Munich, Germany) investigated patterns of synaptic activity during early brain development. Ca^{2+} imaging in cortical slices showed the regular appearance of Ca^{2+} waves propagating through the cortex. Similar recordings in freely moving mice confirmed that these waves are generated *in vivo* during sleep-like episodes.

Synaptic activity might induce bidirectional changes—that is, LTP or LTD. The leading hypothesis states that the magnitude and duration of changes in intracellular Ca^{2+} levels determine the outcome of synaptic activity. M. Sheng (Cambridge, MA, USA) challenged this view by showing that the pharmacological blocking of NMDA receptors that contain the subunit NR2B prevents LTD, but not LTP, whereas the inhibition of NR2A-containing receptors has the opposite effect (Liu *et al*, 2004). Although some reported data contradict these results (as discussed by R. Malenka, Stanford, CA,

USA), recent reports have provided further support for differential roles of NR2A versus NR2B in LTP and LTD. Sheng suggested that downstream signals involve molecules, such as PSD-95 and synapse-associated protein 102 (SAP-102), which preferentially associate with intracellular segments of NR2A or NR2B. Interestingly, signalling by NR2A, but not NR2B, induces cAMP-response-element-binding protein (CREB) dephosphorylation, which is inhibited by the deletion of PSD-95.

The list of molecules that are involved in synaptic plasticity is growing and now includes extracellular matrix (ECM) proteins. C. Seidenbecher (Magdeburg, Germany) presented data from double-knockout mice that are deficient in the proteoglycans neurocan and brevican, and which show a complete loss of LTP. She also reported that deficits in ECM proteins lead to abnormalities in the organization of perineuronal nets, rhythmic activity and diffusion in some brain regions.

Synaptic control of gene expression

Long-term changes in synaptic efficiency eventually require changes in synaptic structure that are dependent on protein synthesis. Transcription might be regulated through the nuclear translocation of the transcription factor CREB after its phosphorylation by Ca^{2+} /calmodulin-dependent (CaM) kinase IV, which is activated by Ca^{2+} influx during synaptic activity. Expression of an active kinase results in an increase in glutamate-receptor-mediated currents, LTP and the number of dendritic spines (Malenka). Another mechanism was presented by Kreutz, who identified an interaction between the postsynaptic Ca^{2+} -sensor caldendrin and the protein Jacob. Activation of NMDA receptors releases Jacob from caldendrin and the protein then accumulates in the nucleus.

As the putative transcriptional events that are triggered by these factors provide messenger RNAs (mRNAs) for a whole neuron, it is unclear how they might affect the function of individual activated synapses. One possible mechanism is that the mRNAs are transported into dendrites and are locally translated beneath the synapses (D. Kuhl, Berlin, Germany, and S. Kindler, Hamburg, Germany). Kindler showed that the transport of mRNAs into dendrites is mediated by their 3' untranslated regions (UTRs). RNA-binding proteins of the far-upstream element (FUSE)-binding protein family specifically recognize such a dendritic-targeting element in the 3' UTR of the microtubule-associated protein 2 (MAP2) mRNA. Furthermore, the RNA-binding protein Staufien interacts with motor proteins, as well as with ribosomes in the dendrites, and might link dendritic transport to translational control. Kuhl highlighted the role of the dendritically synthesized Arg3.1 protein in synaptic plasticity: transcription of the *Arg3.1* gene is induced by synaptic activity, which leads to the rapid accumulation of *Arg3.1* mRNA and Arg3.1 protein in the dendrites. Mice that lack Arg3.1 can no longer maintain LTP, which correlates with subtle performance deficits in a spatial-learning model.

Complex signalling from presynaptic boutons to the nucleus of postsynaptic cells was presented by V. Budnik (Amherst, MA, USA). Presynaptically secreted Wnt proteins (Wingless in *Drosophila*) might bind to the postsynaptic receptor Frizzled, which leads to the phosphorylation of Dishevelled. Phosphorylated Dishevelled then prevents the formation of the axin-conducin-Gsk3b complex, which would otherwise promote the degradation of β -catenin/Armadillo. Therefore, Wnt stabilizes β -catenin, which is required for the transcription of Wnt-responsive genes by lymphoid-enhancer

factor (Lef)/T-cell factor (Tcf) transcription factors. Using an atypical protein kinase C (aPKC) as an example, J. Yin (Madison, WI, USA) further elaborated on what can be learned from *Drosophila*, as aPKC might have a function in local translational control as well as in the generation of polarity in certain cell types.

Lateral diffusion and recycling of transmitter receptors

Synaptic activity might regulate the synaptic expression of neurotransmitter receptors. Exo- and endocytosis occur outside the synaptic zones, and so receptors need to be transported between the synaptic and endo/exocytic regions. D. Choquet (Bordeaux, France) labelled single AMPA receptors with fluorescent antibodies and visualized their lateral diffusion. He found that synaptically located AMPA receptors have, on average, significantly lower coefficients of diffusion than extrasynaptic receptors, which move long distances before they become trapped at synaptic sites (Tardin *et al.*, 2003). The low mobility of synaptic GluR2 receptors is owing to their interaction with the stargazin-PSD-95 complex.

Complementary data on the development of neuromuscular junctions in *Drosophila* were presented by S. Sigrist (Göttingen, Germany). By analysing glutamate receptors that were tagged with photo-activated green fluorescent protein (GFP), he found that new glutamatergic synapses that are formed close to preformed ones are mostly assembled from new receptors, rather than from receptors derived from preformed postsynaptic regions. Therefore, in this system, strengthening of the synapses does not require splitting of the PSDs. Consistent with the *in vitro* data presented by Choquet, Sigrist detected a large pool of diffuse receptors *in vivo*. Interestingly, he also found that the overexpression of different subunits of GluRs differentially affects the size of junctions, whereas the loss of GluRs leads to the loss of synapses.

S. Moss (Philadelphia, PA, USA) investigated the lateral mobility of GABA_A receptors by tagging them with a pH-sensitive fluorescent dye. The depletion of gephyrin by RNA interference reduced the clustering of GABA_A receptors, and Moss therefore concluded that gephyrin limits their lateral mobility. GABA_A receptors are rapidly endocytosed and their association with the Huntingtin-associated protein HAP1 determines whether they recycle back to the cell surface or are degraded in lysosomes (Kittler *et al.*, 2004).

To maintain synaptic transmission effectively, neurotransmitters need to be removed from the extracellular space and synaptic vesicles must be refilled. In glycinergic synapses, these functions are performed by the glial and presynaptic transporters GlyT1 and GlyT2, as shown by V. Eulenburg (Frankfurt, Germany). The loss of these proteins leads to severe phenotypes and death in mutant mice.

Concluding remarks

Despite the relatively small number of participants, this was an intense meeting, which reflected the rapid progress that is being made in the field. We returned to everyday life not only knowing more about synapses, but also feeling that a few new synapses had been formed in our brains in the magic atmosphere of Westerborg. We hope that this meeting will become a regular event to consolidate development of the synaptogenic field in Germany and abroad (and to allow us to sample the good food again). We expect these meetings to stimulate a molecular analysis of functional and structural synaptic plasticities and, hopefully, to lead us to a better understanding of how mutations in synaptic proteins lead to neurological and mental diseases.

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Alexander Dityatev & Hans-Jürgen Kreienkamp