Resolution of this important issue must await further experimentation.

The extensive similarities between the Cbln1 and Grid2 knockout mice, including deficits in cerebellar long-term depression[4,9] and genetic evidence that these two genes act in the same pathway, suggested that Cbln1 acts with Grid2 in the postsynaptic density of PC spines. However, Hirai and colleagues show—using a combination of *in vitro* studies, hybridization *in situ* and a transgenic approach—that Cbln1 is a glycoprotein secreted by granule cells, and not by Purkinje cells. Thus, Cbln1 and Grid2 are part of a transsynaptic pathway controlling the stability and plasticity of the PF-PC synapse.

The transsynaptic action of Cbln1 is particularly interesting given the biochemical properties of other C1q/TNFα family proteins[10]. These proteins form large complexes, which interact with a variety of molecules through their globular C1q domains. They often are important in tissue remodeling. In some cases, this action requires associated protease activities—certainly a handy feature if one hopes to remodel extracellular structures that promote synapse stability. Furthermore, the demonstration that Grid2 is tethered to a molecular complex regulating autophagy[11] and that activation of Grid2 receptors in *Lurcher* mice results in stimulation of this important catabolic pathway[11,12] suggests an intracellular mechanism through which the Cbln1/Grid2 pathway could effect changes in PF-PC synaptic structure.

A very interesting parallel can be made with the neuromuscular junction. Agrin is a glycoprotein secreted by motoneurons at the neuromuscular junction[13]. It was thought to promote synapse formation by clustering acetylcholine receptors. Indeed, agrin knockout mice have very few acetylcholine receptor clusters and lack neuromuscular junctions. However, this phenotype can be rescued by inactivating the gene encoding choline acetyltransferase in those same mice, thus preventing acetylcholine receptor activation. These results, together with *in vitro* studies, have indicated that the role of agrin is to inhibit the destabilizing effect of the activation of acetylcholine receptors[14,15].

Given the results of Hirai *et al.*, and previous studies of Grid2, a similar model (**Fig. 1**) can be proposed for the roles of the Cbln1/Grid2 signaling pathway at the PF-PC synapse. Grid2 seems to have a dual function. Its regulation of autophagy suggests a destabilizing role for this receptor in PF-PC synapses, whereas the phenotype of the Grid2 knockout suggests that it is necessary for synapse stabilization and maintenance. One role for Cbln1 secretion from PF boutons may be to locally inhibit the destabilizing action of Grid2 receptors, as agrin inhibits the destabilizing effects of nAChR activity at the neuromuscular junction. This would promote stabilization of PC-PF contacts immediately adjacent to the active zone. Outside of the active zone, which is presumably free of Cbln1 owing to its limited ability to diffuse out of the cleft, Grid2 would retain its destabilizing actions. This would explain the phenotypes observed by Hirai *et al.* as well as the role of this new pathway in promoting both stabilization of the synaptic contact and matching of the pre- and postsynaptic specializations.

Although a great deal of additional information will be required to understand in detail the mechanisms regulating the structural integrity of central synapses, Hirai *et al.* have made an important step in identifying a mechanism for synapse stabilization that operates transsynaptically in the brain. Their studies reveal a common logic for this important process at the neuromuscular junction and at central synapses, and suggest that the transsynaptic actions of large, secreted glycoproteins on neurotransmitter receptors may provide a key function for structural remodeling of these critical CNS structures.

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Synaptic plasticity and self-organization in the hippocampus

György Buzsáki & James J Chrobak

A new paper reports that long-term potentiation in the hippocampus, a model of learning and memory, can induce sharp wave-ripple complexes, which are thought to be critical for the stabilization of memory traces in cortex.

After buying a new cell phone, we quickly transfer our phone book to the new gadget and relegate the old instrument to the recycling bin. Likewise, sometime after an event, memories that initially depend on the activity of hippocampal neuronal assemblies are transferred to and consolidated in the neocortex and no longer depend on the hippocampus. How does this change take place, and how do patterns of activity within hippocampal cell assemblies transfer information to the neocortex and consolidate it there? And how can cell assemblies produce the patterns of neuronal discharge required to induce synaptic change? Self-organized population discharges in the hippocampus such as hippocampal sharp wave-ripple (SPW-R) complexes, mainly seen *in vivo*, are thought to represent stored information that is then transferred to the neocortex. Nonetheless, the mechanisms responsible for the induction of these SPW-R complexes are unclear.

Now Behrens and colleagues[1] report that they can induce *in vitro* SPW-R complexes,

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similar to the fast-frequency ensemble patterns commonly seen *in vivo*. Moreover, SPW-R can be induced with stimulation protocols known to induce LTP, a popular neurophysiological model of learning and memory. The authors also demonstrate that these events induce synaptic change among CA3 neurons. Thus, they have established a link between the induction of LTP and the emergence of a physiological network pattern believed to be involved in shaping memories. Their report takes one big step in bridging the chasm separating synaptic mechanisms studied *in vitro* and the consolidation of memory traces in the intact brain.

The hippocampal SPW-R complex has features that make it a candidate pattern for the consolidation of synaptic plasticity and the transfer of neuronal patterns[2]. Importantly, it also has a widespread effect. In the approximately 100-ms time window of a hippocampal SPW, between 50,000 and 100,000 neurons—10–20% of the total neuronal population of the rat hippocampus—discharge simultaneously in the CA3-CA1–subicular complex–entorhinal axis, qualifying it as the most synchronous network pattern in the brain[3] (**Fig. 1**). The SPW-R complex arises in the recurrent collateral system of the CA3 region and spreads downstream. Although its recruitment dynamics are delicately controlled by various classes of interneurons[4], a three- to fivefold gain of network excitability is achieved transiently[5]. The observation that the SPW-R is shaped by previous experience[6] gives further support for the fundamental role of these population patterns. However, there are several missing links in the story, including how SPW-R complexes emerge and whether this pattern is actually accompanied by changes in synaptic connectivity. Behrens and colleagues[1] demonstrate that stimuli that induce LTP lead to the generation of SPW-R complexes in slices of the dorsal hippocampus of the rat. Further, the induction, but not the expression, of SPW-R complexes is NMDA receptor–dependent. They also provide evidence that the induction of SPW-R complexes is paralleled by changes in both excitation and inhibition in the CA3 region.

SPW-R complexes *in vitro* have been observed only in the mouse hippocampus and from the ventral hippocampus of the rat[7,8] and the mouse[9,10]. One possible explanation for the absence of spontaneous SPW-R in slices of the rat dorsal hippocampus is that the density of recurrent axon collaterals and the mutual excitation are simply not sufficient to bring about a self-organized population burst. If so, strengthening the surviving synapses could rescue the compromised circuit. This is precisely what Behrens and colleagues[1] have found. During repeated stimulus trains (400 ms at 100 Hz), repeated every 40 s, SPW-R complexes gradually increased in incidence and amplitude after the third to fifth stimulus. Trains that failed to induce LTP failed to induce SPW-R. After approximately the fifth LTP train, the incidence of SPW-Rs reached a plateau, perhaps because the synapses involved in the generation of the spontaneous patterns were 'saturated'—that is, they reached their maximum possible strength.

Both MK-801 (a noncompetitive antagonist of the NMDA subtype of glutamate receptor) and D-AP5 (a competitive NMDA receptor antagonist) could prevent the induction of SPW-R complexes. However, once these complexes were established, these drugs did not prevent SPW-Rs. Indeed, the incidence of established SPW-R incidence increased in the presence of these drugs, probably via mechanisms that involve decreased calcium influx through NMDA receptors and a subsequent reduction in the activation of SK2 calcium-activated potassium channels[11]. Importantly, the established SPW-R events were reduced or abolished by low-frequency stimulation, a protocol that induces long-term depression of synapses. The blockade of gap junctions with carbenoxolone also attenuated ripple occurrence, in accordance with earlier observations of SPW-R complexes *in vitro*[12]. In short, the spontaneously emerging SPW-R complexes show a strong parallel with synaptic changes observed *in vivo*[13].

Using combined intracellular and extracellular recordings, Behrens and colleagues also examined intracellular responses in pyramidal cells during the development of SPW-R complexes. They observed extracellular post-synaptic potential–intracellular postsynaptic potential (EPSP-IPSP) sequences, IPSP-EPSP sequences and prominent IPSPs, but they never found isolated EPSPs. Thus, inhibitory inputs are prominent in the development of ripple complexes and may contribute to the temporal precision of EPSP-spike coupling[14,15]. Importantly, the pattern of inhibition-excitation in any particular neuron remained stable during the development of SPW-Rs in individual neurons; this indicated that the discharge sequence of the participating neurons during the SPW-R complexes is determined largely by a unique distribution of synaptic strengths at both excitatory and inhibitory connections[2,13].

Although these findings show substantial homology between a hippocampus-generated event *in vivo* and population events *in vitro*, some differences are worth pointing out. First, SPW-Rs are associated with large population events and ripple oscillations in both the CA1 and CA3 pyramidal layer *in vitro*. In contrast, the *in-vivo* ripple event in CA1 reflects a convergence of small-amplitude excitatory inputs from a large area of the CA3 region, without synchronous ripple oscillations. This difference may be interpreted as an artificial augmentation of excitation of the truncated CA3 collateral circuitry, reminiscent of epileptiform activity. Second, in contrast to the regularly occurring and uniformly sized SPW-Rs in the slice, their *in-vivo* counterparts are very irregular in both their temporal distribution and magnitude.

Nevertheless, the replication of an endogenous brain pattern *in vitro* allows for the investigation of a number of important

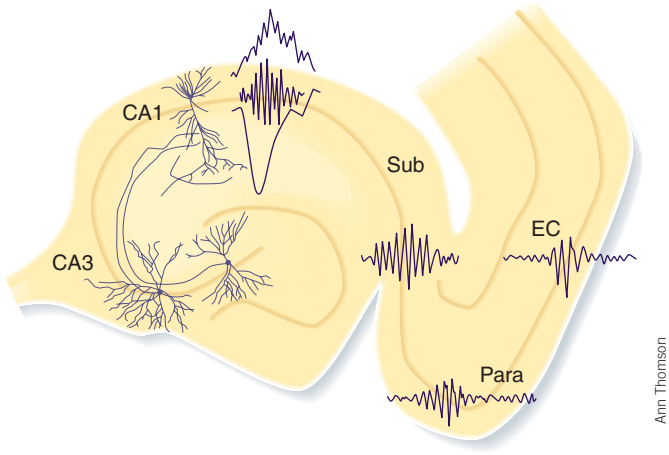

Figure 1 Self-organized burst of activity in the hippocampal CA3 region produces a field potential in the dendritic layer of CA1 and a short-lived fast-frequency field oscillation (200-Hz ripple) within stratum pyramidale, as well as a phase-related discharge of the neurons. Hippocampal output, in turn, produces similar sharp wave-ripple complexes in the subiculum ('Sub'), parasubiculum ('Para') and deep layers of the entorhinal cortex ('EC'). Behrens and colleagues[1] show that the rules of synaptic plasticity govern the emergence and the recruitment of particular cell groups in these hippocampal output events.

mechanisms that are difficult to explore *in vivo*. For example, using simultaneous intracellular recordings from two or more neurons, future experiments will be able to reveal if the induced but otherwise self-generated patterns involve stable recruitment mechanisms. If so, such findings would provide evidence for the hypothesis that endogenous patterns preserve the information about the perturbations that gave rise to the patterns. Monitoring large numbers of neurons and modifying targeted parts of the circuit may identify the elementary mechanisms involved in the consolidation of network patterns. The finding that such modifications can be brought about by stimulation protocols that induce LTP and can be altered by those that produce long-term depression is even more exciting. Memories may really be made in the hippocampus, and SPW-R complexes may contribute to the process after all.

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Glial cells under remote control

Klaus-Armin Nave & Markus H Schwab

Not all axons in a peripheral nerve are myelinated. A recent study shows that the expression of neuregulin-1 on an axon membrane determines whether immature Schwann cells will differentiate into myelinating Schwann cells.

Like the insulation on electrical wires in your house, myelin sheaths are essential for rapid impulse propagation throughout the vertebrate nervous system. The multilayered myelin membranes are synthesized by highly specialized glial cells, termed oligodendrocytes, in the CNS and Schwann cells in the PNS. In the PNS, the decision of Schwann cells to myelinate resembles a cell lineage decision that is triggered by axonal signals, but the nature of these signals has remained unclear. Recently in *Neuron*[1], Taveggia *et al.* demonstrated that axonal neuregulin-1 type III, a regulator of myelin growth, is required to induce the differentiation of myelinating Schwann cells in dorsal root ganglion (DRG) and superior cervical ganglion (SCG) explant cultures.

Ever since electron microscopists demonstrated the complexity of compact myelin sheaths, cellular neurobiologists have been hooked on the myelin-forming glia, their complex interaction with axons and their spectacular membrane growth. Numerous proteins of myelin and the axon-glia junction have been identified; nevertheless, major questions remain unanswered. We do not know the driving force of glial ensheathment. Cross-sections suggest that myelin assembly is a spiral membrane growth process, but this has not been verified. It is unclear why axons larger than 1 μm are myelinated, but small-caliber axons are only ensheathed, and dendrites seem not to interact at all with oligodendroglia. We do not know what signals provide specificity of axon-glia interaction and instruct glia to myelinate, or whether these mechanisms are the same for oligodendrocytes and Schwann cells. One would expect neurons and myelinating glia to communicate through a complex assembly of developmentally regulated signaling proteins. It is therefore surprising that, at least in the PNS, one signaling system, comprised of axonal neuregulin-1 and glial ErbB receptors, seems to operate at all stages of Schwann cell development and myelination.

Neuregulin-1 (Nrg1) comprises a family of more than 15 membrane-associated and secreted growth factors that are derived from a single gene by alternative splicing and promotor use. Major subgroups are Nrg1 type I (including proteins named NDF, heregulin, and ARIA) and type II ('glial growth factor' or GGF) isoforms, both of which are potentially secreted or shed upon proteolytic cleavage. In contrast, Nrg1 type III has a second transmembrane domain and remains a membrane-associated ligand[2]. Common to all isoforms is an EGF-like domain that activates ErbB receptor tyrosine kinases. In the developing nerve, ErbB2 and ErbB3 are expressed at the cell surface of Schwann cell progenitors and are essential for their survival and subsequent differentiation[3]. Nrg1 signaling also contributes to synaptogenesis (at least *in vitro*), the migration of cortical interneurons and cardiac development in the embryo. The latter has greatly hampered the conventional genetic analysis of Nrg1 function in the postnatal nervous system.

Taveggia *et al.*[1] now provide experimental evidence that neuregulin-1 type III is necessary for myelination in the PNS and can instruct immature ensheathing cells to become true myelin-forming Schwann cells. These *in vitro* findings close a gap between related *in vivo* findings by other groups, including the requirement of neuregulin-1 for the survival of precursor cells and immature Schwann cells[4], the requirement of glial ErbB2 receptors for normal myelination in conditional mouse mutants[5], and the identification of neuregulin-1 type III as an axonal signal that regulates myelin sheath thickness in mice with altered neuregulin gene dosage[6].

Mice that selectively lack Nrg1 type III die perinatally and have a marked decrease in Schwann cells and degenerating motor and sensory nerves[7]. To study the competence of these mutant axons to be myelinated, Taveggia *et al.* used a coculture system in which *in vitro* myelination is initiated by the addition of ascorbate[8]. Sensory DRG neurons from wild-type and Nrg1 type III–deficient mice were cocultured with Schwann cells from normal rats. The Nrg1 type III mutant axons never became myelinated, even in the presence of a fivefold excess of Schwann cells. Lentiviral expression of Nrg1 type III was sufficient to restore myelination competence. In agreement with a report of hypermyelination in mice that overexpress Nrg1 type III, but not type I (ref. 6), multiple examples of unusually thick myelin profiles were seen in 'rescued' cocultures.

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