

MOLECULAR GENETICS OF ACTIVITY-DEPENDENT STRUCTURAL CHANGES AT THE SYNAPSE

JEAN MARC DEVAUD^a and 5
ALBERTO FERRÚS^b

^a*Laboratoire de Génomique Fonctionnelle, CNRS,
UPR-2580, Montpellier, France*

^b*Instituto Cajal, CSIC, Madrid 28002, Spain*

(Received ■; accepted ■) 10

INTRODUCTION

Santiago Ramón y Cajal discovered that nervous systems at all stages of development are composed of neurons as independent cells, rather than neurons fused into a network. In his Croonian Lecture of 1894, he showed images where axons terminated in club-shaped endings later referred to as boutons (Fig. 1A). Sir Charles Sherrington envisioned the need for a specialized structure that would transmit signals between neurons in a most efficient way as reflexes indicated, and coined the term “synapse” in 1897 to mean a tight embracement. However, both fathers of Neurobiology were unable to actually see a synapse because the technical limitations of that time. It was not until 1954 when this mysterious structure was seen for the first time, using the electron microscope, thus terminating the agonizing saga of the few last defenders of the reticular theory (Palade & Palay, 1954). Beyond their electron-dense aspect and the variety of forms in which synapses can be found in nervous systems (Fig 1B,C), the constituents and their assemblage mechanisms are only now beginning to be unraveled. A correspondence between the electron-dense structure and the neurotransmitter release site detected by electrophysiological methods is generally accepted.

Address correspondence to Alberto Ferrús, Instituto Cajal, CSIC, Ave. Dr. Arce 37, Madrid 28002, Spain. E-mail: aferrus@cajal.csic.es

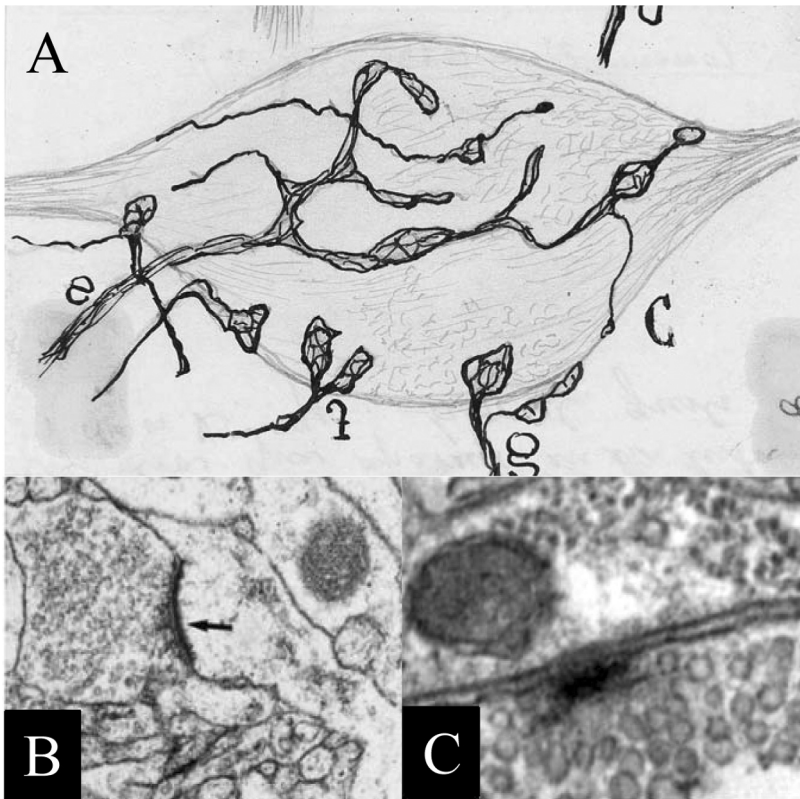


FIGURE 1 Synaptic boutons and active zones. A) Drawing by S. Ramón y Cajal showing axonal varicosities covering the soma of a neuron in the ventral cochlear nucleus of the dog. Note the boutons and the fine neurofibrils inside each axon as revealed by the silver impregnation method. ©Legado Cajal. Instituto Cajal.CSIC. Madrid. B-C) Electron-dense specializations at synapses in the human brain cortex (B), and the *Drosophila* neuromuscular junction (C). Note the abundant small clear vesicles in the presynaptic component (average ϕ 25 nm). Bar in C = 50 nm. Image in B is a courtesy of Prof. De Felipe (See Color Plate I at the end of this issue).

Neurotransmitter-containing vesicles accumulate around the so-called active zone, and vesicles fused to the plasma membrane, either completely into omega figures or in a short transient (“kiss-and-run”) state, have been documented (Aravanis et al., 2003; Gandhi & Stevens, 2003; Palfrey & Artalejo, 2003; Morgan et al., 2002). The early boutons of Cajal, also known today as varicosities, can harbor one to several electron-dense active zones. Similarly, active zones can be found either within or outside

30

35

a varicosity. Notwithstanding the structural differences among synaptic active zones of different species or tissues (Fig. 1B,C), the synapse appears a rather conserved mechanism for rapid (millisecond range) communication between neurons (Atwood & Karunanithi, 2002; Harlow et al., 2001; Sakaba & Neher, 2001). 40

Ever since the first observations, including those with the light microscope, it was clear that the number of synapses in any nervous system would be an exceedingly large number. In effect, the number is so large that it has never been counted in any organism. There is a good reason, indeed, for not attempting this colossal task. As all biological traits, synapses are dynamic structures. Their number changes at various rates, along the postembryonic development of an organism, within the hormonal oestrus cycle in female mammals, with the light/dark periodicity, etc. (McEwen et al., 2001; Naftolin et al., 1996; Hegstrom et al., 2002; Balkema et al., 2001). We will address here the current status of molecular mechanisms involved in synaptic changes that occur as a consequence of neural activity. That is, mainly at a rate of hours or days. Rather than give a complete catalogue of all molecular interactions involved in structural changes at the synapse, we would like to present an overview of the mechanisms at work, with a particular focus on results obtained from genetic approaches. 45 50 55

SYNAPSES AS DYNAMIC STRUCTURES

Since Hebb's postulate for a regulation of synaptic strength by neuronal activity, experimental evidence of rapid changes affecting synapses in mature neurons have been accumulated (for reviews: Segal & Andersen, 2000; Yuste & Bonhoeffer, 2001). Individual synapses undergo quick, sometimes long-lasting, functional alterations that affect the information they transmit. In addition, the size and number of synapses in a given neuron or circuit are also subject to modifications, and provide a more permanent way to modify information processing by that neuron or circuit. Such structural changes can result from a variety of processes involving long-lasting changes in the organism's response to sensory stimuli: several forms of learning and memory, as well as long-time exposure to sensory stimulations. It should be noted, however, that real-time observations of synaptic changes in adult animals subject to these conditions is a technically difficult observation to make. Although some authors have been successful (Trachtenberger et al., 2002) the overall available data remain scant and, to a certain extent, not conclusive as far as the role of these new synapses is concerned (Moser, 1999). It is clear, however, that the number of synapses shows major changes at critical developmental periods or following changes of sensory stimuli (see references above). 60 65 70 75

According to the dominant view, structural changes occur at synapses as a consequence of previous functional modifications, the most studied of which are long-term potentiation (LTP) and depression (LTD) in the rodent hippocampus (Paulsen & Sejnowski, 2000; but see Agnihotri et al., 1998). Experimental manipulation of incoming activity to identified synapses, neurons or circuits, has led to the observation of synaptic growth in some models, both in vitro and in vivo. The time-course of such changes is very rapid: morphological changes of hippocampal synapses have been reported as early as within the first hour following LTP induction (Geinisman et al., 1991; 1993; Engert and Bonhoeffer, 1999; Weeks et al., 2000; Toni et al., 2001; Kadota & Kadota, 2002), and significant variations in synaptic density are documented within a few hours after learning (O'Malley et al., 1998). Also, correlations between enhanced activity and dendritic sprouting have been documented in humans. For example, the size of the hippocampus in taxi drivers is significantly larger than in controls, suggesting that the frequent use of spatial representations of city maps stimulates the growth of this brain center which is clearly involved in this task (Maguire et al., 2000).

Reduction in the number of synapses can also be a result of particular sensory environments. For example, long-term exposure to restricted sensory stimuli (Rehn et al., 1988) or drugs (Lundqvist et al., 1994), can reduce synapse number, presumably through long-term depression. Experiments in *Drosophila* eliciting long-term exposure to specific odors show that selective olfactory glomeruli are reduced in size along with a decrease of synapse number (Devaud et al., 2001). In the opposite direction, individuals with mutant antennae where the cells enlarge forming three times more synapses upon genetically normal postsynaptic neurons, exhibit up to three orders of magnitude increased sensitivity to odorant perception (Acebes et al., 2001). These experiments are a direct demonstration that the number of synapses is a relevant feature in sensory perception and processing. It is interesting to note that in the latter experiment, the increment of synapse number is produced only in the primary sensory neurons since the animals are mosaics, and yet, the increase of sensory input is normally processed by the rest of the brain yielding a proportionally augmented behavioral response. The importance of the observation relies in the demonstration that changes in a sensory organ (e.g., cell size in this case) can result in profound behavioral changes that might lead to ecological niche diversification and, ultimately, reproductive isolation.

New synapses can form through budding of axonal or dendritic shafts (Engert and Bonhoeffer, 1999) or splitting of existing synapses (Weeks et al., 2001). Electronic microscopy observations suggest that both processes can take place in the dendrite (Toni et al., 1999), but whether they represent responses to different conditions, or at different times, remains to be determined (see Lüscher et al., 2000). Still, several recent studies

have provided convergent evidence for the remodeling of already formed synapses, probably leading to their splitting (Muller et al., 2000). Characteristic morphologies, such as perforated synapses usually associated with synaptic activation (Marrone & Petit, 2002) and presynaptic boutons with multiple postsynaptic elements, are more frequent after LTP induction (Geinisman et al., 1991; 1993; Jones et al., 1997; Neuhoff et al., 1999; Weeks et al., 1999; 2000; 2001). Similarly, experience (Geinisman et al., 2000), as well as LTP induction (Desmond and Levy, 1986; Geinisman et al., 1991; 1993; Weeks et al., 1999; Toni et al., 2001; Weeks et al., 2000; 2001), can lead to activity-dependent variations in, composition first and later the size, of the post-synaptic density (PSD), a structure involved in the coordinated reception of information at the postsynaptic element (Okabe et al., 1999; Geinisman et al., 2000) (see below). Recently, expression of fluorescent-tagged PSD proteins in cultured hippocampal neurons allowed visualizing over time their accumulation during spine formation (Okabe et al., 1999; Bresler et al., 2001; Mehta et al., 2001; Qin et al., 2001; Okabe et al., 2001; Ebihara et al., 2003; reviewed in Inoue & Okabe, 2003). The use of this technique in combination with LTP induction should be extremely helpful to determine the precise time-scale of PSD modifications in combination with activity-induced spine remodeling, and would provide a means to test the role of different molecules in this process.

Recent observations have provided support to the conception of perforated synapses as intermediate structures during splitting. First, smooth endoplasmic reticulum, which is believed to be involved in membrane synthesis, is more abundant in perforated synapses (Spacek & Harris, 1997). Second, perforated synapses express more AMPA receptors than non-perforated synapses (Desmond & Weinberg, 1998). Thus, this model establishes a link between neuronal activity and structural remodeling, although it does not explain how presynaptic elements grow, or form. Whether this can be extrapolated to non-glutamatergic synapses remains to be seen.

MOLECULAR MECHANISMS THAT MODIFY SYNAPSES

In the past few years it has become increasingly clear that structural synaptic alterations induced by input modulation share at least some of the basic cellular and molecular events that underlie functional synaptic plasticity (Nestler, 2002; Wolf & Heberlein, 2003). The available data obtained from studies of the hippocampus has been integrated in a model of the initial steps that lead to the formation of new (glutamatergic) synapses (Lüscher et al., 2000). This model proposes that the increase in intracellular calcium following the stimulation of postsynaptic glutamate receptors (those of the N-methyl-D-aspartate [NMDA] type) activates

several signaling cascades (Fig. 2). One of the first events is the translocation of glutamate receptors of the amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) type to PSD (Antonova et al., 2001). Other PSD components are also aggregated, often in a cooperative way through diverse molecular interactions. Different modes of targeting appear to be involved, either dependent or independent of interactions with actin and tubulin cytoskeleton (Allison et al., 1998; 2000).

Among such components is PSD-95, a membrane-associated guanylate kinase that interacts with AMPA receptors (AMPA-Rs). Genetic manipulations that increase (El-Husseini et al., 2000; Schnell et al., 2002) or decrease (El-Husseini et al., 2002) PSD-95 amounts result in correlated variations of AMPAR density at the membrane and corresponding changes in synaptic size. Accordingly, hypomorphic mutants for *Discs-large (Dlg)*, the *Drosophila* homologue of PSD95, have reduced postsynaptic specializations (Guan et al., 1996). Similar genetic

170

175

180

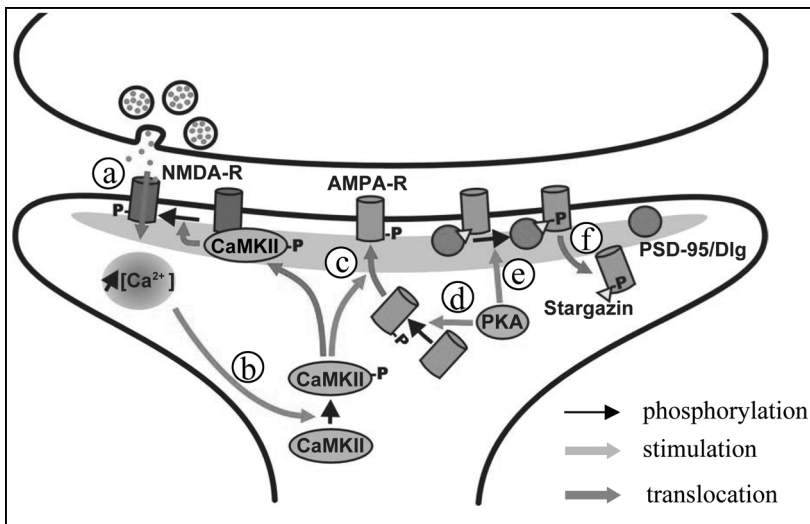


FIGURE 2 Initial events during synaptic growth. Some of the main molecular events underlying this process are shown: after activation of NMDARs by released glutamate (a), the resulting increase in postsynaptic calcium concentration activates CaMKII (b). In its active state, it can then regulate the translocation of AMPARs to the PSD (c). PKA also control AMPAR targeting to the synapse, both directly through their phosphorylation (d) and indirectly, by modulating Interactions between PSD-95 and AMPAR through the phosphorylation of Stargazin (e): when phosphorylated, Stargazin no longer binds PSD-95, and its complex with NMDAR does not remain targeted at the PSD (f). (See Color Plate II at the end of this issue).

approaches have provided further evidence for a causal link between PSD enlargement and accumulation of PSD proteins such as Shank (Sala et al., 2001; 2003; Usui et al., 2003), SPAR (Pak et al., 2001) and Homer (Sala et al., 2003). Many of these and other PSD proteins interact with each other in a cooperative way (Kennedy, 1997; Tu et al., 1999; Ehlers, 2002; Boeckers et al., 2002), so that altering the quantity of a single component is generally sufficient to induce a change in PSD size through cooperative targeting. Increased levels of PSD-95 have been detected during a long-lasting conditioning procedure sufficient to induce changes in connectivity (Skibinska et al., 2001).

The phosphorylated state of many of these PSD components appears to be a key factor in the control of their density at the synapse by activity. Calcium/calmodulin-dependent kinase II (CaMKII), also present in the PSD, plays a crucial role in this process (Toni et al., 1999; Hayashi et al., 2000), and thus provides a link between synaptic changes of activity and structure. Under strong stimulation, incoming calcium through the activated NMDA receptors stimulates the activity of CaMKII, which in turn results in increased delivery of AMPARs to the synapse (Hayashi et al., 2000). Surprisingly, available data on the *Drosophila* neuromuscular junction (a glutamatergic synapse) suggest a CaMKII-dependent regulation of Dlg/PSD-95 localization in the opposite direction. Its aggregation is disrupted when rendering the CaMKII enzyme constitutively active and its localization at perisynaptic sites is favored when it is dephosphorylated (Koh et al., 1999).

It should be noted that this model can serve also to understand synaptic loss, as the reverse process resulting from insufficient intracellular calcium. Its generalization to non-glutamatergic synapses still awaits firm experimental bases. However, that other synapses may be regulated in the same way is likely. For example, the identified association of PSD-95 and other PSD proteins with nicotinic receptors argues in favor of this hypothesis (Conroy et al., 2003).

RELEASE FROM CONSTRAINTS OF ADHESION MOLECULES

Once the post-synaptic density has enlarged, the following step appears to be its fragmentation, leading to the typical perforated morphology of post-synaptic elements. Fragmentation of the PSD is likely to be a limiting step before splitting can occur. The molecular mechanisms that allow this process still need to be understood, but some clues are already available. Not surprisingly, perforation, as shown in hippocampal neurons, depends on NMDA-R activation (Neuhoff et al., 1999). It also requires a proteolytic cascade that would act downstream, since inhibiting tissue type plasminogen activator (tPA, an extracellular

protease) impairs the increase in perforated synapses induced by activity (Neuhoff et al., 1999). Along the same lines, mice lacking tPA display impaired activity-dependent reorganization of visual projections following sensory deprivation, which can be restored by activation of the gene (Mataga et al., 2002). Such alterations can be related to reduced LTP/LTD and memory impairments in those mutants (Calabresi et al., 2000).

This role of tPA indicates that control of adhesion molecules is a putative means for destabilizing the PSD. Indeed, a coordinated regulation of adhesion molecules is required for the consequences of activity on synapse morphology and/or number. Such molecules are present either at the neuron surface or in the extracellular matrix (ECM). They maintain the shape and contacts of a given neuron with its cellular and extracellular environment. Thus, it is easily conceivable that tight contacts between synapses would severely restrict synaptic changes, by keeping axon terminals and dendritic spines in a rigid state. The recent work by Togashi and colleagues (2002) is a good illustration of this. These authors examined the consequences of blocking the adhesion molecule N-cadherin by transfecting cultured hippocampal neurons with a dominant-negative form of the protein. Interestingly, this treatment disrupted dendritic spine morphology (inducing particular bifurcation events reminiscent of splitting) and altered the distribution of PSD-95. Thus, reduced levels of cadherin *in vivo* might be permissive for synaptic changes to occur, as suggested by the enhanced LTP in cadherin-11 mutants (Manabe et al., 2000). However, previous studies had shown that cadherin was required for LTP (Tang et al., 1998; Bozdagi et al., 2000), as many adhesion molecules (see below). An interpretation of these contradictory results is that cadherin activity could be partially inactivated during early LTP, so that synaptic remodeling could take place without disrupting the establishment of LTP (Togashi et al., 2002). A possible model for this process would be the destabilization of adhesive bonds due to the transient depletion of calcium in the synaptic cleft (Murasé & Schuman, 1999) (Fig. 3).

Thus, a decrease in membrane adhesion should be required to release this constraint and enable the elaboration of new synapses, or the elimination of existing ones (Benson et al., 2000). Experimental data supporting this view is still rather scarce, but give already some insights into the processes at work. Two adhesion molecules, Nerve Cell Adhesion Molecule (NCAM) and L1, have been shown to be down-regulated following LTP. Both are cleaved, so that the cell membrane is no longer attached to the ECM through their extracellular domains (Fazeli et al., 1994; Matsumoto-Miyai et al., 2003). The *Aplysia* NCAM homologue, apCAM, has also been extensively studied for its role in synapse plasticity. Its regulation involves another mechanism: when long-term facilitation is induced at sensory-motor synapses, apCAM is internalized

225

235

240

245

250

255

260

265

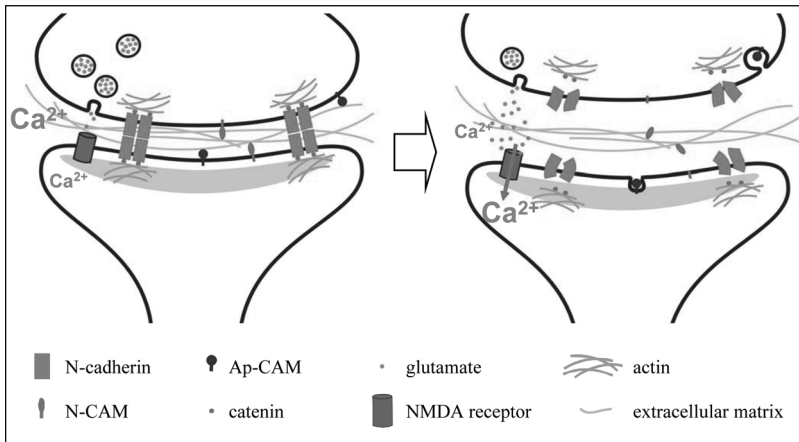


FIGURE 3 Down-regulation of adhesion molecules. During early LTP, adhesion between pre- and postsynaptic compartments and with the extracellular matrix may be decreased through several processes. *Inactivation*: cadherins (green) are inactivated, which results in the destabilization of their bonds with cytoskeleton. This inactivation may be due to the fall of extracellular concentration in the synaptic cleft. *Cleavage*: N-CAM (in red) loses its adhesive properties as its extracellular domain, which interacts with the extracellular matrix (pink), is cleaved. *Internalization*: ApCAM (in blue) is internalized (adapted from Murase & Schuman, 1999) (See Color Plate III at the end of this issue).

and degraded (Mayford et al., 1992; Zhu et al., 1995; Bailey et al., 1997). In *Drosophila* also, down-regulation of the homologue FasII is required for structural remodeling of synapses in the larval neuro-muscular junction (Schuster et al., 1996a,b), but the underlying mechanism is unknown.

How do changes in adhesion enable synapse remodeling? Adhesion molecules provide a link between cell surface and the cytoskeletal apparatus, involved in morphogenetic changes. Thus, interactions, either direct or indirect, between adhesion molecules and proteins of the cytoskeleton, are probably crucial for activity-dependent synaptic remodeling. For example, cadherins bind to intracellular scaffolding proteins, catenins, that interact with cytoskeletal elements, in particular actin (Rimm et al., 1995). Several studies have demonstrated the importance of actin regulation for spine morphogenesis (for review: Bonhoeffer & Yuste, 2002; Luo, 2002). Key actors of this regulation are members of the Ras superfamily, particularly the Rho family of small GTPases. Purkinje cells of transgenic mice expressing a constitutively active form of one of these GTPases (Rac1) had an overproduction of dendritic spines (Luo et al., 1996), and rat hippocampal slices transfected

270

275

280

285

with the same construct had also additional spines, while the dominant negative had the reverse effect (Nakayama et al. 2000). These findings establish the role of Rac1 in promoting the formation and stabilization of spines. Another protein of this family, Rho, has opposite effects (Tashiro et al., 2000; Wong et al., 2000).

290

KINASES AND PHOSPHATASES AS MODULATORS OF SYNAPTIC ORGANIZATION

Synaptic plasticity, either functional or structural, is highly dependent on the phosphorylation state of many proteins, such as AMPA receptors and associated PSD proteins (Scannevin & Huganir, 2000), members of the MAP kinase pathway (Sweatt, 2001a) or cAMP response element-binding (CREB) (Lonze & Ginty, 2002). Incoming levels of activity control the balance between competing activities of kinases and phosphatases (Wang & Kelly, 1996; Sweatt, 2001b). At least two Ca^{2+} - and cAMP-sensitive pathways are implicated (Waltereit & Welter, 2003). Both involve chains of phosphorylation reactions that contribute to prolong and amplify the initial response to depolarization. In this regard, CaMKII autophosphorylating activity plays an important role by achieving an activity state independent of calcium as it is translocated to the PSD (Lisman et al., 2002). This “molecular switch” is necessary for maintaining long-lasting functional and behavioral changes, as shown by the defects displayed by transgenic mice expressing a constitutively active form of the kinase (Bach et al., 1995; Mayford et al., 1995). More recently, evidence for a control by CaMKII of activity-driven structural plasticity has been obtained as well. Remodeling in the visual cortex following monocular deprivation was impaired in transgenic mice expressing a mutant CaMKII unable to autophosphorylate (Taha et al., 2002). In *Drosophila*, the role of CaMKII in long-term plasticity remains to be explored, although mutants are already known to display learning and memory deficits (Griffith et al., 1993; Joiner & Griffith, 1997; Griffith, 1997).

295

300

305

310

315

Changing cAMP levels in neurons affects drug response (Heyne et al., 2000; Wolf & Heberlein, 2003), and learning and memory, as well as functional plasticity at synapses, both in vertebrates and invertebrates (Waltereit & Weller, 2003). The consequences on structural plasticity have been less studied so far. An increase in cAMP levels in response to stimulation induces the down-regulation of the adhesion molecule FasII in the *Drosophila* neuromuscular junction, leading to activity-dependent sprouting of additional boutons (Davis et al., 1996; Schuster et al., 1996b). Along the same lines, mutants with deregulated cAMP levels do not undergo the specific adjustments of connectivity normally observed in olfactory centers in response to long-term adaptation to an odorant

320

325

(Devaud et al., 2001; 2003). These effects may be mediated by protein kinase A (PKA), that is activated by cyclic AMP and can phosphorylate numerous substrates, among which some are common to CaMKII like NMDARs, AMPARs and CREB. PKA has been shown to be necessary for reorganization of the visual cortex in response to monocular deprivation (Beaver et al., 2001) and for late LTP-associated synaptogenesis (Bozdagi et al., 2000; Tominaga-Yoshino et al., 2002). Recently, it has been shown to control AMPAR targeting (either through phosphorylation of Stargazin [Chetkovitch et al., 2002] or through direct phosphorylation of AMPAR [Esteban et al., 2003]). Its activity is also linked with synapse stabilization through the induction of N-cadherin synthesis during late-phase LTP (Bozdagi et al., 2000).

The phosphorylation level of synaptic proteins appears to be crucial for whether the synapse undergoes LTP or LTD, as shown in particular by several recent genetic approaches (Malleret et al., 2001; Woo et al., 2002). Thus, presumably it is also determinant for inducing either formation or elimination of synapses. While kinase activities are generally associated with strengthening connectivity by increasing synapse number (see above, Kotak & Sanes, 2002), phosphatases counteract this effect, and would therefore promote a decrease in synapse number. Several genetic approaches support this view. For example, PP1A and calcineurin (the most abundant phosphatases at the synapse) counterbalance the action of PKA on common targets, and their activity is necessary to sustain LTD (e.g., Zeng et al., 2001). Also, in *Drosophila*, abnormal levels of PP1 activity were shown to impair associative learning (Orgad et al., 1989). Conversely, as shown by the inducible expression of its auto-inhibitory domain in transgenic mice, calcineurin inhibition is associated with LTP induction and memory formation (Malleret et al., 2001). It also contributes to control F-actin stability (Halpain et al., 1998). PP1 is controlled by inhibitor 1 (I1), which is a substrate of both calcineurin and PKA. Thus, competition between calcineurin and PKA determines the activity level of PP1.

Mitogen-activated protein kinases (MAPKs), also known as extracellular signal regulated protein kinases (ERKs), are essential elements of phosphorylation cascades in the postsynaptic element (Patterson et al., 2001; Purcell et al., 2003). Their activity is modulated both by CaMKII and PKA pathways, and link with the regulation of gene expression (see below). MAPK/ERK activation has been shown to be required for long-term memory in different species (e.g. Kelly et al., 2003; Sharma et al., 2003), and is likely to be involved in long-term synaptic effects of drug exposure (Sanna et al., 2002; Ukike et al., 2002; Roberto et al., 2003). Its role in the regulation of synapse formation/stabilization is suggested by several lines of evidence. For example, it mediates the reciprocal regulation of NCAM levels and tPA activity by antagonistic actions, as it stimulates both NCAM proteolysis by tPA and NCAM-dependent tPA

synthesis (Son et al., 2002). Moreover, phosphorylation of a dendrite-specific MAP2 isoform (by either CaMKII or MAPK/ERK pathways) was recently shown to be required for dendritic spine stability in cultured neurons (Vaillant et al., 2002). These authors also showed that this function is regulated by CaMKII.

375

PROTEIN SYNTHESIS AND DEGRADATION AT THE SYNAPTIC TERMINAL

Long-term changes that involve building, or destruction, of synaptic elements (membranes, cytoskeleton microtubules, PSD and so on) are dependent on protein synthesis or degradation, respectively. It is well established that long-term memory (LTM) and its associated synaptic changes are impaired after blockade of protein synthesis (Bailey et al., 1996; Beaumont et al., 2001; Matsushita et al., 2001; Barco et al., 2002). Control at both transcriptional and translational levels are involved. One the one hand, as mentioned earlier, the signaling cascades activated by activity in the post-synaptic element ultimately lead to the regulation of gene expression. On the other hand, 'silent' mRNAs are present in the cytoplasm, and their translation can be activated in an activity-dependent manner.

380

385

390

Accordingly, in *Drosophila* several genes that are part of a pathway controlling mRNA localization and translation have been identified as candidate LTM genes. They were selected on the ground of either variations in their expression levels during LTM formation, or a specific LTM impairment when mutated (Dubnau et al., 2003). So far, quantitative PCR experiments have confirmed the changes in expression levels detected with microarrays for one such gene, *pumilio*, that encodes a translational repressor. Other elements of this pathway have been identified for their role in long-term memory in a screen for LTM-impaired mutants, and interestingly show expression in the brain centers involved in olfactory memory (Dubnau et al., 2003). Thus, this data provide convergent arguments for the control of mRNA targeting and translation as essential for the formation and/or maintenance of LTM.

395

400

A recently identified, important regulation pathway of protein turnover involved in synaptic plasticity is that of ubiquitination. Ubiquitins are small protein tags added to proteins destined to degradation in the proteasome (Hershko & Ciechanover, 1998). The control of this signaling pathway is thus crucial in determining the stability of those molecular components necessary to maintain long-term plasticity (Hegde & DiAntonio, 2002). As shown recently by Ehlers (2003), activity-dependent remodeling of synapses is associated with changes in protein turnover that are mediated by ubiquitin signaling. This has direct consequences on the postsynaptic response to stimulation, as NMDA-mediated activation of MAP kinases and CREB is also altered.

405

410

**TRANSITION FROM PRE- TO POST-SYNAPTIC
MODIFICATIONS: SEARCHING FOR THE
RETROGRADE SIGNALS**

415

As presented in this review, we now begin to understand the regulation along the network of processes that lead to the growth (and to a lesser extent, elimination) of a postsynaptic element. Remarkably, although synaptic transmission clearly triggers the initial postsynaptic changes detailed so far, a new dendritic spine presumably does not necessarily need a presynaptic input to develop fully. Data from cultured neurons even indicate that AMPA and GABA receptors, as well as PSD-95, can aggregate without neurotransmitter input (Rao et al., 2000). These observations are consistent with the model of a sequential building of new synapses that can be initiated, at least in some cases, by the presynaptic partner (Ziv & Garner, 2001). It is clear though, that coordinated changes should then occur on the presynaptic side to adjust connectivity. Thus, reorganization of circuits in the mature nervous system can be conceived as a sequential process, in which postsynaptic formation/elimination precedes equivalent presynaptic changes. How is this sequence achieved? In order to adjust, the presynaptic element should receive information coming from the postsynapse, as indicated by the increase of presynaptic function after postsynaptic overexpression of PSD-95 (El-Husseini et al., 2000) or Shank/Homer (Sala et al., 2003). However, the molecular substrate of such information is still unknown, but there are already some candidates for retrograde messengers.

ECM/adhesion molecules are in the ideal position for this role. They might intervene to form a physical link between pre- and post-synaptic membranes, either through homophilic interactions or indirectly by anchoring on the extracellular matrix. (Benson et al., 2000; Dityatev & Schachner, 2003). In addition to functional interference with antibodies or peptides, genetical manipulation of expression levels of many ECM/adhesion molecules has provided strong evidence for their promoting functional plasticity at synapses (reviewed in Dityatev and Schachner, 2003). Direct evidence of their control of structural changes is lacking (except during development: e.g., O'Brien et al., 2002; Togashi et al., 2002). However, consistently with a stabilization model, some ECM/adhesion molecules are up-regulated in an activity-dependent manner (Tsui et al., 1996; Nakic et al., 1998; Schuster et al., 1998), and at least one study reports such an increase to be associated with increased synaptic number (Bozdagi et al., 2000). More recently, β -catenin was shown to redistribute from the dendritic shaft to spines in an activity-dependent manner, through its dephosphorylation (Murase et al., 2002). These observations contrast with their expected down-regulation while synapses change. As discussed above, a precise timing and control of the level of

inhibition could account for remodeling during early LTP and stabilization during late LTP. Accordingly, most genetic manipulations of ECM/adhesion molecules have revealed specific alterations of late-phase LTP, while early-phase LTP and basal transmission were poorly or not affected (Murase & Schuman, 1999; Benson et al., 2000; Dityatev & Schachner, 2003). Thus, synaptic adhesion appears to undergo a complex regulation through both decrease and increase in the levels of ECM/adhesion molecules, probably at early and late phases of synaptic remodeling. Whether each step involves different molecules, or whether the same molecule can be regulated in both directions at different moments of synapse formation remains unclear.

Several recent studies point at CaMKII as a regulator of retrograde signaling. In the developing *Drosophila* neuromuscular junction, contrasting results have been obtained. On the one hand, an increase in the number of vesicle-associated active zones was obtained by selectively expressing an inhibitor of CaMKII in the muscle (Haghighi et al., 2003). On the other hand, muscular expression of a constitutively active variant of this enzyme was found to promote the same effect on active zones (Kazama et al., 2003). The reasons for this discrepancy remain unclear. Consistently with this second observation, cortical neurons expressing the same mutant protein also received a greater number of synaptic contacts (Pratt et al., 2003). Thus, in any case, postsynaptic CaMKII activity seems to be crucial for retrograde signaling to presynaptic afferents. How this effect is mediated remains to be clarified, but at least one possible way would be to regulate amounts of adhesion molecules such as FasII/NCAM, through the activation of Dlg/PSD-95 (Thomas et al., 1997; Koh et al., 1999).

LONG-TERM MAINTAINENCE OF SYNAPSES: THE PROBLEM OF SPECIFICITY

Although all the mentioned changes in the postsynaptic compartment can take place without the need for gene transcription, the maintenance of newly formed synapses on the long term requires new gene products. It is well established in different animal models that the signaling cascades activated by sustained activity ultimately lead to promote gene expression (Impey et al., 1999; Platenik et al., 2000). The mitogen-activated protein kinase (MAPK) pathway plays a central role in this process, through its major target CREB. CREB is activated (by phosphorylation) in a MAPK-dependent manner, and then can bind to several genes containing a CRE (cAMP response element) domain. Among these are the genes encoding the ubiquitin hydrolase (involved in the recruitment of the proteasome) (Hegde et al., 1994) and the transcription factor C/EBP (CCAAT enhancer binding protein). This pathway can be activated by a

postsynaptic increase of either cAMP, via PKA, or calcium, via small GTPases of the Ras family (Waltereit & Weller, 2003). 500

Gene expression is regulated at the scale of the whole neuron, while long-term modifications usually concern only a fraction of its synapses. Nonetheless, local enrichment in mRNAs and translation has been reported (Ostroff et al., 2002; Richter and Lorenz, 2002). Also, selective protein transport may yield regional synapse heterogeneity within a given neuron. A recent study by Miller et al. (2002) showed that interfering with the dendritic localization of CaMKII resulted in reduced amounts of the protein in the PSD, together with impaired LTP and spatial memory. It is also likely that this manipulation would have consequences on structural synaptic modifications. In any case, the strengthening of activated synapses by products of gene expression poses a problem of specificity (Kandel, 2001). The dominant explanation is that only those synapses that contain persistent molecular markers of activation can be strengthened by capturing those products. Several candidates exist for such synaptic ‘tags’ (reviewed in Martin & Kosik, 2002). Any molecule activated in a local, somehow persistent, but reversible manner may play the role, provided its activation can lead to variations in changed expression. Virtually any synaptic component cited here fulfill these criteria, but the most likely candidates include kinases (CaMKII, PKA), adhesion molecules (N-CAM, cadherins) and regulators of protein translation and degradation (Martin & Kosik, 2002). The ‘tag’ needs not be unique, and it is likely that a combination of molecular markers is responsible for the specificity of synaptic reinforcement. Their identification is one of the major challenges in neuroscience research in the years to come. 510 515 520

Future avenues of research will benefit from the realization that synapses are dynamic structures that, at any point in time, represent the equilibrium between a network of protein interactions that promote their growth and a counterpart that leads to their regression. As with living organisms, synapses seem to grow, reproduce, and die.

REFERENCES 530

- Agnihotri, N., Lopez-Garcia, J.C., Hawkins, R.D., & Arancio, O. (1998). Morphological changes associated with long-term potentiation. *Histol. Histopathol.* **13**, 1155–1162.
- Allison, D.W., Gelfan, V.I., Spector, I., & Craig, A.M. (1998). Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. *J. Neurosci.* **18**, 2423–2436. 535
- Allison, D.W., Chervin, A.S., Gelfan, V.I., & Craig, A.M. (2000). Postsynaptic scaffolds of excitatory and inhibitory synapses in hippocampal neurons: maintenance of core components independent of actin filaments and microtubules. *J. Neurosci.* **20**, 4545–4554.
- Antonova, I., Arancio, O., Trillat, A.C., Wang, H.G., Zablow, L., Udo, H., Kandel, E.R., & Hawkins, R.D. (2001). Rapid increase in clusters of presynaptic proteins at onset of long-lasting potentiation. *Science* **294**, 1547–1550. 540

- Aravanis, A.M., Pyle, J.L., & Tsien, R.W. (2003). Single synaptic vesicles fusing transiently and successively without loss of identity. *Nature* **423**, 643–647.
- Atwood, H.L., Govind, C.K., & Wu, C.F. (1993). Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in *Drosophila* larvae. *J. Neurobiol.* **24**, 1008–1024. 545
- Atwood, H.L. & Karunanithi, S. (2002). Diversification of synaptic strength. Presynaptic elements. *Nat. Rev. Neurosci.* **3**, 497–516.
- Bach, M.E., Hawkins, R.D., Osman, M., Kandel, E.R., & Mayford, M. (1995). Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency. *Cell* **81**, 905–915. 550
- Bailey, C.H., Bartsch, D., & Kandel, E.R. (1996). Toward a molecular definition of long-term memory storage. *Proc. Natl. Acad. Sci. USA* **93**, 13445–13452.
- Bailey, CH, Kaang, B.K., Chen, M., Martin, K.C., Lim, C.S., Casadio, A., & Kandel, E.R. (1997). Mutation in the phosphorylation sites of MAP kinase blocks learning-related internalization of ApCAM in *Aplysia* sensory neurons. *Neuron* **18**, 913–924. 555
- Balkema, G.W., Cusik, K., & Nguyen, T.H. (2001). Diurnal variation in synaptic ribbon length and visual threshold. *Vis. Neurosci.* **18**, 789–797.
- Barco, A., Alarcon, J.M., & Kandel, E.R. (2002). Expresión of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* **108**, 689–703. 560
- Beaumont, V., Zhong, N., Fletcher, R., Froemke, R.C., & Zucker, R.S. (2001). Phosphorylation and local presynaptic protein synthesis in calcium- and calcineurin-dependent induction of crayfish long-term facilitation. *Neuron* **32**, 489–501.
- Benson, D.L., Schnapp, L.M., Shapiro, L., & Huntley, G.W. (2000). Making memory stick: cell-adhesion molecules in synaptic plasticity. *Trends Cell Biol.* **10**, 473–482. 565
- Boeckers, T.M., Bockmann, J., Kreutz, M.R., & Gundelfinger, E.D. (2002). ProSAP/Shank proteins – a family of higher order organizing molecules of the postsynaptic density with an emerging role in human neurological disease. *J. Neurochem.* **81**, 903–910.
- Bonhoeffer, T., & Yuste, R. (2002). Spine motility: phenomenology, mechanisms, and function. *Neuron* **35**, 1019–1027. 570
- Bozdagi, O., Shan, W., Tanaka, H., Benson, D.L., & Huntley, G.W. (2000). Increasing numbers of synaptic puncta during late-phase LTP: N-cadherin is synthesized, recruited to synaptic sites, and required for potentiation. *Neuron* **28**, 245–259.
- Bresler, T., Ramati, Y., Zamorano, P.L., Zhai, R., Garner, C.C., & Ziv, N.E. (2001). The dynamics of SAP90/PSD-95 recruitment to new synaptic junctions. *Molec. Cell Neurosci.* **18**, 149–167. 575
- Calabresi, P., Napolitano, M., Centonze, D., Marfia, G.A., Gubellini, P., Teule, M.A., Bernardi, G., Frati, L., Tolu, M., & Gulino, A. (2000). Tissue plasminogen activator controls multiple forms of synaptic plasticity and memory. *Eur. J. Neurosci.* **12**, 1002–1012. 580
- Conroy, W.G., Liu, Z., Nai, Q., Coggan, J.S., & Berg, D.K. (2003). PDZ-containing proteins provide a functional postsynaptic scaffold for nicotinic receptors in neurons. *Neuron* **38**, 759–771.
- Davis, G.W., Schuster, C.M., & Goodman, C.S. (1996). Genetic dissection of structural and functional components of synaptic plasticity. III. CREB is necessary for presynaptic functional plasticity. *Neuron* **17**, 669–679. 585
- Desmond, N.L., & Levy, W.B. (1986). Changes in the postsynaptic density with long-term potentiation in the dentate gyrus. *J. Comp. Neurol.* **253**, 476–482.

- Desmond, N.L. & Weinberg, R.J. (1998). Enhanced expression of AMPA receptor protein at perforated axospinous synapses. *Neuroreport* **30**, 857–860. 590
- Devaud, J.-M., Acebes, A., & Ferrus, A. (2001). Odor exposure causes central adaptation and morphological changes in selected olfactory glomeruli in *Drosophila*. *J. Neurosci.* **21**, 6274–6282.
- Devaud, J.-M., Acebes, A., & Ferrus, A. (2003). Structural and functional changes in the olfactory pathway of adult *Drosophila* take place at a critical age. *J. Neurobiol.* **56**, 13–23. 595
- Dityatev, A. & Schachner, M. (2003). Extracellular matrix proteins and synaptic plasticity. *Nat. Rev. Neurosci.* **4**, 456–468.
- Dubnau, J., Chiang, A.-S., Grady, L., Barditch, J., Gossweiler, S., McNeil, J., Smith, P., Buldoc, F., Scott, R., Certa, U., Broger, C., & Tully, T. (2003). The *staufen/pumilio* pathway is involved in *Drosophila* long-term memory. *Curr. Biol.* **13**, 286–296. 600
- Ebihara, T., Kawabata, I., Usui, S., Sobue, K., & Okabe, S. (2003). Synchronized formation and remodeling of postsynaptic densities: long-term visualization of hippocampal neurons expressing postsynaptic density proteins tagged with green fluorescent protein. *J. Neurosci.* **23**, 2170–2180. 605
- Ehlers, M.D. (2002). Molecular morphogens for dendritic spines. *Tr. Neurosci.* **25**, 64–67.
- El-Husseini, A.E., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., Gauthier-Campbell, C., Aguilera-Moreno, A., Nicoll, R.A., & Brecht, D.S. (2002). Synaptic strength regulated by palmitate cycling on PSD-95. *Cell* **108**, 849–863. 610
- El-Husseini, A.E., Schnell, E., Chetkovitch, D.M., Nicoll, R.A., & Brecht, D.S. (2000). PSD-95 involvement in maturation of excitatory synapses. *Science* **290**, 1364–1368.
- Engert, F., & Bonhoeffer, T. (1999). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**, 66–70.
- Esteban, J.A., Shi, S.H., Wilson, C., Nuriya, M., Huganir, R.L., & Malinow, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nat. Neurosci.* **6**, 136–143. 615
- Fazeli, M.S., Breen, K., Errington, M.L., & Bliss, T.V. (1994). Increase in extracellular NCAM and amyloid precursor protein following induction of long-term potentiation in the dentate gyrus of anaesthetized rats. *Neurosci. Lett.* **169**, 77–80. 620
- Gandhi, S.P. & Stevens, C.F. (2003). Three modes of synaptic vesicular recycling revealed by single-vesicle imaging. *Nature* **423**, 607–613.
- Geinisman, Y., de Toledo-Morrell, L., & Morrell, F. (1991). Induction of long-term potentiation is associated with an increase in the number of axospinous synapses with segmented postsynaptic densities. *Brain Res.* **566**, 77–88. 625
- Geinisman, Y., Disterhoft, J.F., Gundersen, H.J., McEchron, M.D., Persina, I.S., Power, J.M., van der Zee, E.A., & West, M.J. (2000). Remodeling of hippocampal synapses after hippocampus-dependent associative learning. *J. Comp. Neurol.* **417**, 49–59.
- Geinisman, Y., de Toledo-Morrell, L., Morrell, F., Heller, R.E., Rossi, M., & Parshall, R.F. (1993). Structural synaptic correlate of long-term potentiation: formation of axospinous synapse with multiple, completely partitioned transmission zones. *Hippocampus* **3**, 435–445. 630
- Griffith, L.C. (1997). *Drosophila melanogaster* as a model system for the study of the function of calcium/calmodulin-dependent protein kinase in synaptic plasticity. *Invert. Neurosci.* **3**, 93–102. 635
- Griffith, L.C., Verselis, L.M., Aitken, K.M., Kyriacou, C.P., Danho, W., & Greenspan, R.J. (1993). Inhibition of calcium/calmodulin-dependent protein kinase in *Drosophila* disrupts behavioral plasticity. *Neuron* **10**, 501–509.

- Guan, B., Hartmann, B., Kho, Y.H., Gorczyca, M., & Budnik, V. (1996). The *Drosophila* tumor suppressor gene, *dlg*, is involved in structural plasticity at glutamatergic synapses. *Curr. Biol.* **6**, 695–706. 640
- Haghighi, A.P., McCabe, B.D., Fetter, R.D., Palmer, J.E., Hom, S., & Goodman, C.S. (2003). Retrograde control of synaptic transmission by postsynaptic CaMKII at the *Drosophila* neuromuscular junction. *Neuron* **39**, 255–267.
- Halpain, S., Hipolito, A., Saffer, L. (1998). Regulation of F-actin stability in dendritic spines by glutamate receptors and calcineurin. *J. Neurosci.* **18**, 9835–9844. 645
- Harlow, M.L., Ress, D., Stoschek, A., Marshall, R.M., & MacMahan, U.J. (2001). The architecture of active zone material at the frog's neuromuscular junction. *Nature* **409**, 479–484.
- Hayashi, Y., Shi, S.H., Esteban, J.A., Poncer, J.C., & Malinow, R. (2000). Driving AMPA receptors into synapses by LTP and CaMKII: requirement of GluR1 and PSZ domain interaction. *Science* **287**, 2262–2267. 650
- Hegstrom, C.D., Jordan, C.L., & Breedlove, S.M. (2002). Photoperiod and androgens act independently to induce spinal nucleus of the bulbocavernosus neuromuscular plasticity in the Siberian hamster, *Phodopus sungorus*. *J. Neuroendocrinol.* **14**, 368–374. 655
- Hershko, A. & Ciechanover, A. (1998). The ubiquitin system. *Ann. Rev. Biochem.* **67**, 425–479.
- Impey, S., Obrietan, K., & Storm, D.R. (1999). Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. *Neuron* **23**, 11–14.
- Inoue, A., & Okabe, S. (2003). The dynamic organization of postsynaptic proteins: translocating molecules regulate synaptic function. *Curr. Opin. Neurobiol.* **13**, 332–340. 660
- Joiner, M.A. & Griffith, L.C. (1997). CaM kinase II and visual input modulate memory formation in the neuronal circuit controlling courtship conditioning. *J. Neurosci.* **17**, 9384–9391.
- Jones, T.A., Klintsova, A.Y., Kilman, V.L., Sirevaag, A.M., & Greenough, W.T. (1997). Induction of multiple synapses by experience in the visual cortex of adult rats. *Neurobiol. Learn. Mem.* **68**, 13–20. 665
- Kadota, T. & Kadota, K. (2002). Rapid structural remodeling of shaft synapses associated with long-term potentiation in the cat superior cervical ganglion in situ. *Neurosci. Res.* **43**, 135–146. 670
- Kandel, E.R. (2001). The molecular biology of memory storage: a dialogue between genes and synapses. *Science* **294**, 1030–1038.
- Kazama, H., Morito-Tanifuji, T., & Nose, A. (2003). Postsynaptic activation of calcium/calmodulin-dependent protein kinase II promotes coordinated pre- and post-synaptic maturation of *Drosophila* neuromuscular junctions. *Neuroscience* **117**, 615–625. 675
- Kelly, A., Laroche, S., & Davis, S. (2003). Activation of mitogen-activated protein kinase/extracellular signal regulated kinase in hippocampal circuitry is required for consolidation and reconsolidation of recognition memory. *J. Neurosci.* **23**, 5254–5360.
- Kennedy, M.B. (1997). The postsynaptic density at glutamatergic synapses. *Trends Neurosci.* **20**, 264–268. 680
- Koh, Y.H., Popova, E., Thomas, U., Griffith, L.C., & Budnik, V. (1999). Regulation of DLG localization at synapses by CaMKII-dependent phosphorylation. *Cell* **98**, 353–363.
- Kotak, V.C., & Sanes, D.H. (2002). Postsynaptic kinase signaling underlies inhibitory synaptic plasticity in the lateral superior olive. *J. Neurobiol.* **53**, 36–43.
- Lisman, J., Schulman, H., & Cline, H., (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. *Nature Rev. Neurosci.* **3**, 175–190. 685

- Lonze, B.E., & Ginty, D.D. (2002). Function and regulation of CREB family transcription factors in the nervous system. *Neuron* **35**, 605–623.
- Lundqvist, C., Volk, B., Knoth, R., & Alling, C. (1994). Long-term effects of intermittent versus continuous ethanol exposure on hippocampal synapses of the rat. *Acta Neuro-pathol.* **87**, 242–249. 690
- Luo, L. (2002). Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. *Ann. Rev. Cell Devel. Biol.* **18**, 601–635.
- Luo, L., Hensch, T.K., Ackerman, L., Barbel, S., Jan, L.Y., & Jan, Y.N. (1996). Differential effects of the Rac GTPase on Purkinje cell axons and dendritic trunks and spines. *Nature* **379**, 837–840. 695
- Lüscher, C., Nicoll, R.A., Malenka, R.C., & Muller, D. (2000). Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nat. Neurosci.* **3**, 545–550.
- Maguire, E.A., Gadian, D.G., Johnsrude, I.S., Good, C.D., Ashburner, J., & Frackowiak, R.S. (2000). Navigation-related structural change in the hippocampi of taxi drivers. *Proc. Natl. Acad. Sci. USA* **97**, 4398–403. 700
- Malleret, G., Haditsch, U., Genoux, D., Jones, M.W., Bliss, T.V., Vanhose, A.M., Weitlauf, C., Kandel, E.R., Winder, D.G., & Mansuy, IM (2001). Inducible and reversible enhancement of learning, memory and long-term potentiation by genetic inhibition of calcineurin. *Cell* **104**, 675–686. 705
- Manabe, T., Togashi, H., Uchida, N., Suzuki, S.C., Hayakawa, Y., Yamamoto, M., Yoda, H., Miyakawa, T., Takeichi, M., & Chisaka, O. (2000). Loss of cadherin-11 adhesion receptor enhances plastic changes in hippocampal synapses and modifies behavioral responses. *Molec. Cell. Neurosci.* **15**, 534–546.
- Marrone, D.F. & Petit, T.L. (2002). The role of synaptic morphology in neural plasticity: structural interactions underlying synaptic power. *Brain Res. Rev.* **38**, 291–308. 710
- Martin, K.C., & Kosik, K.S. (2002). Synaptic tagging—who's it ? *Nat. Rev. Neurosci.* **3**, 813–820.
- Mataga, N., Nagai, N., & Hensch, T.K. (2002). Permissive proteolytic activity for visual cortical plasticity. *Proc. Natl. Acad. Sci. USA* **99**, 7717–7721. 715
- Matsumoto-Miyai, K., Ninomiya, A., Yamasaki, H., Tamura, H., Nakamura, Y., & Shiosaka, S. (2003). NMDA-dependent proteolysis of presynaptic adhesion molecule L1 in the hippocampus by neuropsin. *J. Neurosci.* **23**, 7727–7736.
- Matsushita, M., Tomizawa, K., Moriwaki, A., Li, S.-T., Terada H., & Matsui, H. (2001). A high-efficiency protein transduction system demonstrating the role of PKA in long-lasting long-term potentiation. *J. Neurosci.* **21**, 6000–6007. 720
- Mayford, M., Barzilai, A., Keller, F., Schacher, S., & Kandel, E.R. (1992). Modulation of an NCAM-related adhesion molecule with long-term synaptic plasticity in *Aplysia*. *Science* **256**, 638–644.
- Mayford, M., Wang, I., Kandel, E.R., & O'Dell, T.J. (1995). CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. *Cell* **81**, 891–904. 725
- McEwen, B., Akama, K., Alves, S., Brake, W.G., Bulloch, K., Lee, S., Li, C., Yuen, G., & Milner, T.A. (2001). Tracking the estrogen receptor in neurons: implications for estrogen-induced synapse formation. *Proc. Natl. Acad. Sci. USA.* **98**, 7093–7100. 730
- Mehta, S., Wu, H., Garner, C.C., & Marshall, J. (2001). Molecular mechanisms regulating the differential association of kainate receptor subunits with SAP90/PSD-95 and SAP97. *J. Biol. Chem.* **27**, 16092–16099.

- Miller, S., Yasuda, M., Cotas, J.K., Jones, Y., Martone, M.E., & Mayford, M. (2002). Disruption of dendritic translation of CaMKIIalpha impairs stabilization of synaptic plasticity and memory consolidation. *Neuron* **36**, 507–519. 735
- Morgan, J.R., Augustin, G.J., & Lafer, E.M. (2002). Synaptic vesicle endocytosis: the races, places and molecular faces. *Neuromolec. Med.* **2**, 101–114.
- Moser, M.B. (1999). Making more synapses: a way to store information? *Cell Molec. Life Sci.* **55**, 593–600. 740
- Muller, D., Toni, N., & Buchs, P.A. (2000). Spine changes associated with long-term potentiation. *Hippocampus* **10**, 596–604.
- Murase, S., Mosser, E., & Schuman, E.M. (2002). Depolarization drives beta-Catenin into neuronal spines promoting changes in synaptic structure and function. *Neuron* **35**, 91–105. 745
- Murase, S. & Schumann, E.M. (1999). The role of cell adhesion molecules in synaptic plasticity and memory. *Curr. Opin. Cell Biol.* **11**, 549–553.
- Naftolin, F., Mor, G., Horvath, T.L., Luquin, S., Fajer, A.B., Kohen, F. & García-Segura, L.M. (1996). Synaptic remodeling in the arcuate nucleus during the estrous cycle is induced by estrogen and precedes the preovulatory gonadotropin surge. *Endocrinology* **137**, 5576–5580. 750
- Nakayama, A.Y. & Luo, L. (2000). Intracellular signaling pathways that regulate dendritic spine morphogenesis. *Hippocampus* **10**, 582–586.
- Nestler, E.J. (2002). Common molecular and cellular substrates of addiction and memory. *Neurobiol. Learn. Mem.* **78**, 637–647. 755
- Neuhoff, H., Roeper, J., & Schweizer, M. (1999). Activity-dependent formation of perforated synapses in cultured hippocampal neurons. *Eur. J. Neurosci.* **11**, 4241–4250.
- Nacic, M., Manahan-Vaughan, D., Reymann, K.G., & Schachner, M. (1998). Long-term potentiation in vivo increases rat hippocampal tenascin-C expression. *J. Neurobiol.* **37**, 393–404. 760
- O'Brien, R., Xu, D., Mi, R., Tang, X., Hopf, C., & Worley, P. (2002). Synaptically targeted Narp plays an essential role in the aggregation of AMPA receptors at excitatory synapses in cultured spinal neurons. *J. Neurosci.* **22**, 4487–4498.
- Okabe, S., Kim, H.D., Miwa, A., Kuriu, T., & Okado, H. (1999). Continuous remodeling of postsynaptic density and its regulation by synaptic activity. *Nat. Neurosci.* **2**, 804–811. 765
- Okabe, S., Urushido, T., Okado, H., & Sobue, K. (2001). Rapid distribution of the post-synaptic density protein PSD-Zip45 (Homer 1c) and its differential regulation by NMDA receptors and calcium channels. *J. Neurosci.* **21**, 9561–9571.
- O'Malley, A., O'Connell, C., Murphy, K.J., & Regan, C.M. (1998). Ultrastructural analysis reveals avoidance conditioning to induce a transient increase in hippocampal dendate spine density in the 6 hour post-training period of consolidation. *Neuroscience* **87**, 607–613. 770
- Ostroff, L.E., Fiala, J.C., Allwardt, B., & Harris, K.L. (2002). Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**, 535–545. 775
- Pak, J.H., Huang, F.L., Li, J., Balschun, D., Reymann, K.G., Chiang, C., Westphal, H., & Huang, K.P. (2001). Involvement of neurogranin in the modulation of calcium/calmodulin-dependent protein kinase II, synaptic plasticity and spatial learning: a study with knockout mice. *Proc. Natl. Acad. Sci. USA* **97**, 11232–11237.
- Palade, G.E. & Palay, S.L. (1954). Electron microscope observations of interneuronal and neuromuscular synapses. *Anat. Rec.* **118**, 335–336. 780

- Palfrey, H.C. & Artalejo, C.R. (2003). Secretion: kiss-and-run caught on film. *Curr. Biol.* **13**, R397–399.
- Patterson, S.L., Pittenger, C., Morozov, A., Martin, K.C., Scanlin, H., Drake, C., & Kandel, E.R. (2001). Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron* **32**, 123–140. 785
- Paulsen, O. & Sejnowski, T.J. (2000). Natural patterns of activity and long-term synaptic plasticity. *Curr. Opin. Neurobiol.* **10**, 172–179.
- Platenik, J., Kuramoto, N., & Yoneda, Y. (2000). Molecular mechanisms associated with long-term consolidation of the NMDA signals. *Life Sci.* **67**, 335–364. 790
- Pratt, K.G., Watt, A.J., Griffith, L.C., Nelson, S.B., & Turrigiano, G.G. (2003). Activity-dependent remodeling of presynaptic inputs by postsynaptic expression of activated CaMKII. *Neuron* **39**, 268–291.
- Purcell, A.L., Sharma, S.K., Bagnall, M.W., Sutton, M.A., & Carew, T.J. (2003). Activation of a tyrosine kinase-MAPK cascade enhances the induction of long-term facilitation and long-term memory in *Aplysia*. *Neuron* **37**, 473–484. 795
- Qin, L., Marrs, G.S., McKim, R., & Dailey, M.E. (2001). Hippocampal mossy fibers induce assembly and clustering of PSD95-containing postsynaptic densities independent of glutamate receptor activation. *J. Comp. Neurol.* **440**, 284–298. 800
- Rehn, B., Panhuber, H., Laing, D.G., & Breipohl, W. (1988). Spine density on olfactory granule cell dendrites is reduced in rats reared in a restricted olfactory environment. *Brain Res.* **468**, 143–147.
- Richter, J.D. & Lorenz, L.J. (2002). Selective translation of mRNAs at synapses. *Curr. Opin. Neurobiol.* **12**, 300–304. 805
- Roberto, M., Nelson, T.E., Ur, C.L., Brunelli, M., Sanna, P.P., & Gruol, D.L. (2003). The transient depression of hippocampal CA1 LTP induced by chronic intermittent ethanol exposure is associated with an inhibition of the MAP kinase pathway. *Eur. J. Neurosci.* **17**, 1646–1654.
- Sakaba, T. & Neher, E. (2001). Quantitative relationship between transmitter release and calcium current at the calyx of Held synapse. *J. Neurosci.* **21**, 462–476. 810
- Sala, C. (2001). Molecular regulation of dendritic spine shape and function. *Neurosignals* **11**, 213–223.
- Sala, C., Futai, K., Yamamoto, K., Worley, P.F., Hayashi, Y., & Sheng, M. (2003). Inhibition of dendritic spine morphogenesis and synaptic transmission by activity-inducible protein Homer1a. *J. Neurosci.* **23**, 6327–6337. 815
- Sanna, P.P., Simpson, C., Lutjens, R., & Koob, G. (2002). ERK regulation in chronic ethanol exposure to and withdrawal. *Brain Res.* **948**, 186–191.
- Scannevin, R.H. & Huganir, R.L. (2000). Postsynaptic organization and regulation of excitatory synapses. *Nat. Rev. Neurosci.* **1**, 133–141. 820
- Schnell, E., Sizemore, M., Karimzadegan, S., Chen, L., Brecht, D.S., & Nicoll, R.A. (2002). Direct interactions between PSD-95 and stargazing control synaptic AMPA receptor number. *Proc. Natl. Acad. Sci. USA* **99**, 13902–13907.
- Schuster, C.M., Davis, G.W., Fetter, R.D., & Goodman, C.S. (1996a). Genetic dissection of structural and functional components of synaptic plasticity. I. Fasciclin II controls presynaptic stabilization and growth. *Neuron* **17**, 641–654. 825
- Schuster, C.M., Davis, G.W., Fetter, R.D., & Goodman, C.S. (1996b). Genetic dissection of structural and functional components of synaptic plasticity. II. Fasciclin II controls presynaptic structural plasticity. *Neuron* **17**, 655–667.

- Schuster, T., Krug, M., Hassan, H., & Schachner, M. (1998). Increase in proportion of hippocampal spine synapses expressing neural cell adhesion molecule NCAM 180 following long-term potentiation. *J. Neurobiol.* **37**, 359–372. 830
- Segal, M. & Andersen, P. (2000). Dendritic spines shaped by synaptic activity. *Curr. Opin. Neurobiol.* **10**, 582–586.
- Sharma, S.K., Sherff, C.M., Shobe, J., Bagnall, M.W., Sutton, M.A., & Carew, T.J., (2003). Differential role of mitogen-activated protein kinase in three distinct phases of memory for sensitization in *Aplysia*. *J. Neurosci.* **23**, 3899–3907. 835
- Skibinska, A., Lech, M., & Kossut, M. (2001). PSD95 protein level rises in murine somatosensory cortex after sensory training. *Neuroreport* **12**, 2907–2910.
- Spacek, J. & Harris, K.M. (1997). Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J. Neurosci.* **15**, 190–203. 840
- Sweatt, J.D. (2001a). The neural MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J. Neurochem.* **76**, 1–10.
- Sweatt, J.D. (2001b). The yin and yang of protein phosphorylation. *Curr. Biol.* **11**, R391–R394. 845
- Taha, S., Hanover, J.L., Silva, A.J., & Stryker, M.P. (2002). Autophosphorylation of alphaCaMKII is required for ocular dominance plasticity. *Neuron* **36**, 483–491.
- Tang, L., Hung, C.P., & Schuman, E.M. (1998). A role for the cadherin family of cell adhesion molecules in hippocampal long-term potentiation. *Neuron* **20**, 1165–1175. 850
- Togashi, H., Abe, K., Mizogushi, A., Takaoka, K., Chisaka, O., & Takeichi, M. (2002). Cadherin regulates dendritic spine morphogenesis. *Neuron* **35**, 77–89.
- Tominaga-Yoshino, K., Kondo, S., Tamotsu, S., & Ogura, A. (2002). Repetitive activation of protein kinase A induces slow and persistent potentiation associated with synaptogenesis in cultured hippocampus. *Neurosci. Res.* **44**, 357–367. 855
- Toni, N., Buchs, P.A., Nikonenko, I., Bron, C.R., & Muller, D. (1999). LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrites. *Nature* **402**, 421–425.
- Toni, N., Buchs, P.A., Nikolenko, I., Povilaitite, P., Parisi, L., & Muller, D. (2001). Remodeling of synaptic membranes after induction of long-term potentiation. *J. Neurosci.* **21**, 6245–6251. 860
- Trachtenberg, J.T., Chen, B.E., Knott, G.W., Feng, G., Sanes, J.R., Welker, E., & Svoboda, K. (2002). Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* **26**, 788–794.
- Tsui, C.C., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Barnes, C., & Worley, P.F. (1996). Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity. *J. Neurosci.* **16**, 2463–2478. 865
- Tu, J.C., Xiao, B., Naisbitt, S., Yuan, J.P., Petralia, R.S., Brakeman, P., Doan, A., Aakalu, V.K., Lanahan, A., Sheng, M., & Worley, P.F. (1999). Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* **23**, 583–592. 870
- Vaillant, A.R., Zanassi, P., Walsh, G.S., Aumont, A., Alonso, A., & Miller, F.D. (2002). Signaling mechanisms underlying reversible, activity-dependent synaptic formation. *Neuron* **34**, 985–998.
- Waltereit, R. & Weller, M. (2003). Signaling from cAMP/PKA to MAPK and synaptic plasticity. *Molec. Neurobiol.* **27**, 99–106. 875

- Weeks, A.C., Ivanco, T.L., Leboutillier, J.C., Racine, R.J., & Petit, T.L. (1999). Sequential changes in the synaptic structural profile following long-term potentiation in the rat dentate gyrus. I. The intermediate maintenance phase. *Synapse* **31**, 97–107.
- Weeks, A.C., Ivanco, T.L., Leboutillier, J.C., Racine, R.J., & Petit, T.L. (2000). Sequential changes in the synaptic structural profile following long-term potentiation in the rat dentate gyrus. II. Induction/early maintenance phase. *Synapse* **36**, 286–296. 880
- Weeks, A.C., Ivanco, T.L., Leboutillier, J.C., Racine, R.J., & Petit, T.L. (2001). Sequential changes in the synaptic structural profile following long-term potentiation in the rat dentate gyrus. III. Long-term maintenance phase. *Synapse* **40**, 74–84. 885
- Wolf, F.W. & Heberlein, U. (2003). Invertebrate models of drug abuse. *J. Neurobiol.* **54**, 161–178.
- Wong, W.T., Faulkner-Jones, B.E., Sanes, J.R., & Wong, R.O. (2000). Rapid dendritic remodeling in the developing retina: dependence on neurotransmission and reciprocal regulation by Rac and Rho. *J. Neurosci.* **20**, 5024–5036. 890
- Woo, N.H., Abel, T., & Nguyen, P.V. (2002). Genetic and pharmacological demonstration of a role for cyclic AMP-dependent protein kinase-mediated suppression of protein phosphatases in gating the expression of late LTP. *Eur. J. Neurosci.* **16**, 1871–1876.
- Yuste, R. & Bonhoeffer, T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Ann. Rev. Neurosci.* **24**, 1071–1089. 895
- Zhu, H., Wu, F., & Schachner, M. (1995). Changes in expression and distribution of Aplysia cell adhesion molecules can influence synapse formation and elimination in vitro. *J. Neurosci.* **15**, 4173–4183.
- Ziv, N.E. & Garner, C.C. (2001). Principles of glutamatergic synapse formation: seeing the forest for the trees. *Curr. Opin. Neurobiol.* **11**, 536–543. 900