FUNCTIONAL AND STRUCTURAL PLASTICITY OF SYNAPTIC NETWORKS

Dominique Muller

Introduction

When Ramon y Cajal described the morphological complexity of cortical neurones with their numerous dendritic spines, small protrusions that are the site of communication between neuronal cells, he proposed several ideas about their function that are still the subject of intense investigations. He proposed for example that these small biological connections could be reinforced by exercise, thus contributing to learning and that new connections could be established through an increased ramification and growth of dendritic arborisations (Ramon y Cajal, 1911). This was purely speculative at the time, but he established in this way the first conceptual bases for the notion of synaptic plasticity. This still holds true nowadays and synaptic plasticity is still considered as one of the fundamental properties of brain networks accounting for many aspects of higher brain functions, including memory and cognition. Despite this importance the molecular mechanisms underlying these properties are still far from being understood. Major progress has been realized within the last 5–10 years, essentially through the development and refinement of imaging and transfection technologies that now allow us to visualize and investigate the function of identified synaptic boutons while altering the expression of specific molecular constituent. It has become possible in this way to start understand at the molecular level the mechanisms that contribute to the various aspects of synaptic plasticity.

Modulation of synaptic efficacy

One of the main concepts about synaptic plasticity has been formulated by D. Hebb. (Hebb, 1949). He proposed a learning mechanism based

on lasting changes in the efficacy of synaptic communication between two neurons and triggered by a coordinated activity of these neurons. Later physiological studies by Bliss and colleagues revealed the existence of a synaptic property, referred to as long-term potentiation or LTP that closely matched the predictions of D. Hebb (Bliss and Lomo, 1973; Bliss and Collingridge, 1993). It was found in many cortical regions that activation of a synapse with high frequency trains mimicking the firing of neurons observed during learning, lead to an increase in the efficacy of neurotransmission. The change was very fast; it resulted in larger synaptic responses in the target cell (figure 1) and thus increased the probability that this cell would also be activated and fire. Overall, induction of LTP changes the population of neurons that respond to a stimulus, a phenomenon that may be considered as a learning mechanism for a neuronal network. A key feature of this property is its stability: the increase in transmission efficacy is generated within a few seconds, but the change remains effective for extended periods of time, up to several weeks for the longest experiments carried out in living animals (Abraham, 2003). It thus represents a perfect mechanism for the long-term storage of information. In addition to this, LTP has also been shown to be synapse-specific, to exhibit associative properties and to be required for many behavioral situations involving learning tasks (Malenka, 2003). This property is thus currently considered as the most important mechanism allowing neuronal networks to adapt their behavior and function in an activity-dependent manner. Its involvement in memory has been demonstrated in many examples of transgenic animals unable to perform in learning or memory tasks due to a selective blockade of LTP, or conversely that learned better due to enhanced LTP (Tsien et al., 1996; Tang et al., 1999).

Molecular mechanisms underlying synaptic potentiation

A fascinating and highly controversial issue about this property has been to understand the mechanisms responsible for the long-lasting increase in synaptic transmission (Malenka and Nicoll, 1999; Luscher et al., 2000). While the debate is still going on (Emptage et al., 2003), it is generally agreed that a major role is played by postsynaptic recept-

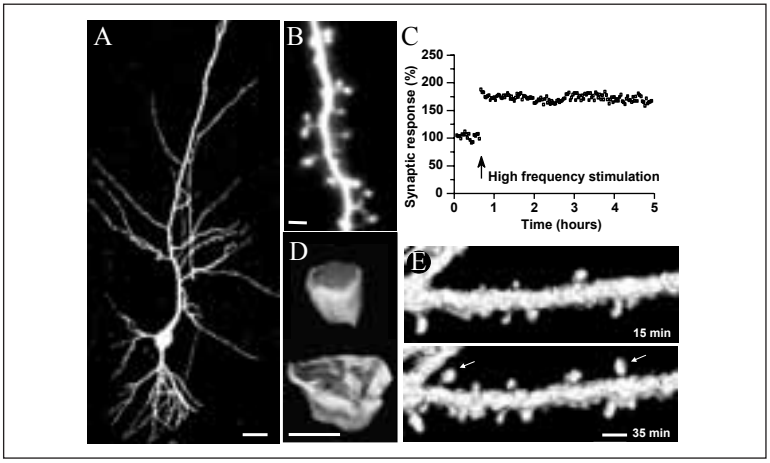

Figure 1:
Illustration of properties of synaptic plasticity. A. Hippocampal CA1 neuron in an organotypic slice cultures expressing EGFP following transfection using a biolistic approach (bar: 20 μm). B. Higher magnification of a dendritic segment illustrating the presence of numerous dendritic spines characterized by various shapes, length and morphologies (bar: 2 μm). C. Illustration of the increase in synaptic efficacy (LTP) induced by high frequency stimulation of a group of CA3 neurons in a hippocampal organotypic slice culture. D. Illustration of the heads of dendritic spines with their postsynaptic densities (gray zones) reconstructed from serial EM sections. The top image is characteristic of a thin spine while the bottom image represents a mushroom-type spine with a segmented postsynaptic density (perforated synapse). This type of spines was found to increase following induction of LTP in slice cultures. E. Formation of two new dendritic spines, as observed with 2-photon confocal imaging in organotypic slice cultures following application of a short hypoxia/hypoglycaemia protocol that triggers a lasting synaptic potentiation.

ors (Malinow and Malenka, 2002). There are two main types of excitatory glutamate receptors at these synapses referred to as NMDA and AMPA receptors according to their selective agonists. NMDA receptors have been shown to be responsible for triggering the synaptic changes. They are activated only under conditions where firing of the two connected neurons is synchronous and blocking these receptors prevents induction of LTP. Once activated, they allow calcium to enter the postsynaptic spine, which in turn activates various signaling messengers that lead to the lasting increase in synaptic efficacy. How this is pro-

duced remains controversial, but again, much evidence points to an involvement of AMPA type of receptors. We provided the first evidence that enhancement of AMPA transmission was involved in the increase in synaptic efficacy (Muller et al., 1988; Muller and Lynch, 1988). This has now been confirmed in many different ways and the current view of how LTP occurs proposes that the increase in AMPA receptor mediated transmission results from two main mechanisms: i) the phosphorylation by the enzyme calcium/calmodulin dependent protein kinase II (CaMKII) of the GluR1 subunit of AMPA receptors which increases the conductance of the ionic channel associated with the receptor (Barria et al., 1997; Benke et al., 1998; Lisman et al., 2002); and ii) an increased turnover rate of AMPA receptors that brings additional receptors to the synapse, producing in this way larger signals (Malinow and Malenka, 2002). An important finding brought into light by these studies has been the highly dynamic aspect of receptor mobility and insertion in neuronal membranes. It appears that receptors such as AMPA receptors continuously cycle through constitutive and activity-dependent mechanisms between synaptic or dendritic membranes and intracellular compartments. The number of receptors present at the synapse can be thus rapidly and tightly controlled, probably through the presence of scaffold proteins that determine the size of the postsynaptic density containing the receptors, and through the existence of a recycling machinery (Park et al., 2004). The possibility to transfect cells with vectors that express specific proteins or with antisense oligos or siRNAs that prevent expression of wild type proteins has made possible to start analyze the role of specific molecules in these mechanisms. In a recent collaborative work carried out with Dr. H. Hirling from EPFL in Lausanne, we contributed to the identification of one of the proteins that regulate the recycling of AMPA receptors. In a screen for proteins involved in vesicle trafficking, Dr. Hirling discovered the protein NEEP21, a protein specifically expressed in neurons and highly enriched in early endosomes (Steiner et al., 2002). He found that suppression of this protein using an antisense approach significantly altered the expression of AMPA receptors at the membrane. This prompted a more careful analysis of the participation of NEEP21 in the control of synaptic transmission and plasticity at excitatory synapses. By analyzing synaptic transmission, receptor recycling and synaptic plasticity in cells

transfected with a NEEP21 antisense, S. Alberi, in the laboratory, found that elimination of NEEP21 reduced synaptic transmission and prevented synaptic plasticity, probably by diminishing the number of AMPA receptors expressed at the synapse and altering the recycling of the receptor (Alberi et al., 2005). He could also show that this effect specifically concerned the GluR2 subunit of the AMPA receptor, as the conductance properties of the receptor were altered by interference with NEEP21 function (Steiner et al., 2005). Together these studies provided further evidence that the trafficking of receptors tightly controls the efficacy of synaptic transmission and allowed to identify one of the first molecules participating in this control.

Structural plasticity

In addition to changes in receptor numbers or properties, another mechanism often considered to account for the stability of synaptic plasticity involved a structural remodeling of the synapse and eventually also the creation of new synaptic contacts. To address this issue, we developed several years ago an electron microscopic analysis of activated synapses that revealed the occurrence of major morphological changes at activated synapses (Buchs and Muller, 1996; Toni et al., 1999; Toni et al., 2001; Nikonenko et al., 2002). These concerned two major aspects: changes of existing synapses and appearance of new types of synapses.

Induction of synaptic potentiation was found to be associated with an enlargement of the size of the dendritic spines, an increase in size of the postsynaptic density, modifications of its distribution and formation of perforated synapses characterized by multiple postsynaptic densities (Buchs and Muller, 1996). These morphological changes could represent the structural correlate of the increased trafficking of receptors. The increase in size of dendritic spines was recently confirmed using sophisticated imaging techniques (Matsuzaki et al., 2004) and might reflect several interesting mechanisms. It could result from membrane insertion associated with receptor recycling, or from a reorganization of the actin cytoskeleton in order to adapt the spine to the new synaptic structure, or it might also reflect the activation of local mechanisms of

protein synthesis together with the migration of polyribosomes within the spine (Ostroff et al., 2002). A current view of synaptic plasticity mechanisms is that the long-lasting stability would depend upon the synthesis of new proteins, including receptors, constituents of the post-synaptic density or signaling molecules that would be responsible for maintaining and stabilizing the contact with the presynaptic structure. Several recent studies have indeed directly demonstrated the capacity of neurons to locally regulate the synthesis of several important synaptic proteins (Kang and Schuman, 1996; Ostroff et al., 2002; Ju et al., 2004; Sutton and Schuman, 2005). According to this view, synchronous activity would stimulate the growth of dendritic spines and the formation of large, mushroom-type spines that would be more effective, due

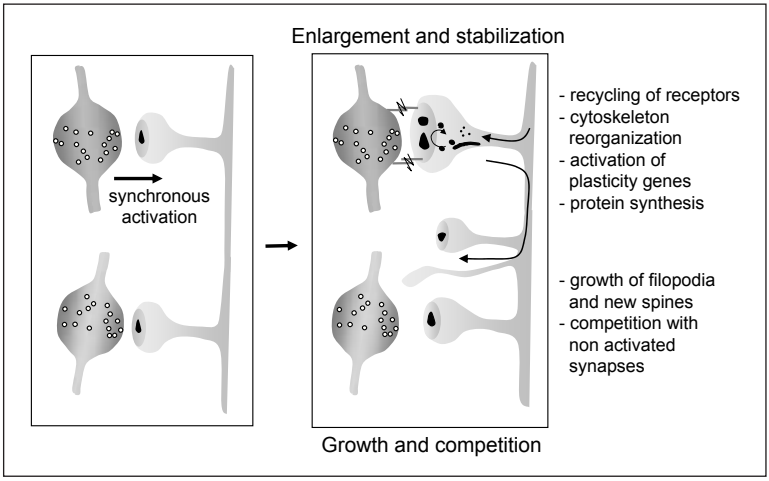

Figure 2
Schematic diagram summarizing the different mechanisms contributing to activity-induced synaptic plasticity. The diagram represents the possible changes that take place on a small dendritic segment with two spine synapses following synchronous activation of the top synapse. The activated synapse becomes strengthened through expression of new receptors associated with an enlargement of the spine head and transformation in a more stable mushroom-type spine. Activity further triggers the growth of filopodia or new spines that will enter in competition with the non-activated synapse for further stabilization.

to a larger number of receptors and, at the same time, become stabilized through the synthesis of specific proteins (Kasai et al., 2003). In contrast, synapses that would not be used regularly or activated in synchrony would rather remain immature, with a small number of receptors and probably also a shorter life time. An interesting idea that emerges from this proposal is the notion of competition between synapses: those synapses that are used in a coordinated, synchronous manner, because they would refer to coherent information, would become strengthened and stabilized at the expense of those that would not participate in neuronal communication.

Synaptogenesis

Implicit with this notion of competition between synapses is the idea that there must be a continuous process that eliminates and forms new synapses throughout life, even if this process concerns only a small fraction of synaptic contacts. This mechanism would allow synaptic networks to adapt to new experiences and new situations and would thus form the basis for learning processes. In contrast the more stable and larger synapses would constitute the backbone of the network architecture and account for properties such as long-term memory.

The idea of synapse competition and turnover has been very difficult to test experimentally essentially for technical reasons. However, recent improvements in confocal imaging techniques and particularly the introduction of 2-photon confocal approaches has open new possibilities to observe over prolonged periods of time the behavior and function of identified synapses, even under in vivo conditions (Trachtenberg et al., 2002). These approaches revealed important new information about synaptic properties. First, they demonstrated the highly dynamic nature of dendritic spines. These are highly enriched in actin and they appear to continuously change shape and organization in an activity-dependent manner (Matus, 2000; Yuste and Bonhoeffer, 2001). The function of this intrinsic motility remains unclear, but it certainly highlights the important capacity for plasticity of dendritic spines. Second, they allowed to demonstrate that the activity patterns that induced LTP

not only resulted in strengthening of synapses, but also promoted the growth of new protrusions, filopodia and spines (Maletic-Savatic et al., 1999; Engert and Bonhoeffer, 1999; Toni et al., 1999; Jourdain et al., 2002). The phenomenon occurred quite quickly following stimulation leading within 10–30 minutes to the appearance of new synaptic structures. We studied these mechanisms particularly under conditions of brief ischemia, which through an important release of glutamate, results in a synchronous activation of many synapses and thus mimics synaptic potentiation. Under this condition as following induction of LTP, we could identify growth mechanisms that affected both the pre- and postsynaptic structures (Jourdain et al., 2002; Jourdain et al., 2003; Nikonenko et al., 2003).

At the postsynaptic level, activity triggers the growth of filopodia that extend over the course of tens of minutes and then usually retract and stabilize as new dendritic spines. Alternatively, in many instances, activity directly stimulates the appearance of new dendritic spines that appear after about 15–30 minutes, but form very quickly within 1–2 minutes. Whether these new spines are functional and how long they persist remains unclear, but several examples, including observations from 3D-EM reconstructions suggested that they could represent the formation of new synaptic contacts (Toni et al., 1999). At the EM level, we even found a marked increase in images in which a single nerve terminal contacted two distinct, adjacent spines on the same dendrite, a phenomenon that clearly suggested a process of synapse duplication as contributing to the potentiation effect (Toni et al., 1999).

At the presynaptic level, we could also detect growth mechanisms induced by activity: they were seen as filopodia-like protrusions arising from stimulated varicosities or sometimes also directly from the axon (Nikonenko et al., 2003; Muller and Nikonenko, 2003). These protrusions appeared rapidly, within 10–20 minutes after stimulation, and could be studied at the EM level using 3D reconstruction. In this way we could analyze the characteristics of the contacts that were established by these protrusions. We found that within 20 minutes, 80% of them were engaged in a synaptic contact detectable by the presence of

a postsynaptic density. More interesting even, we found that the vast majority of these protrusions contacted first a dendritic shaft, while after 30 minutes almost 90% of them were in fact involved in a synaptic contact with a dendritic spine. These observations thus clearly supported the idea that activity can also trigger presynaptic growth mechanisms that may be involved in the formation of new dendritic spines, following establishment of an initial synaptic contact on the dendritic shaft (Muller and Nikonenko, 2003). Together these various studies provided clear evidence that neuronal and synaptic activity are able to promote synaptogenesis and that remodeling of synaptic networks is likely to be part of the mechanisms that contribute to information processing. The possibility to image even under in vivo conditions the behavior of identified cortical neurons and their dendritic spines further showed that this continuous process of synapse formation and elimination occurs in different cortical regions; it varies as a function of age and can be modulated by sensory stimulation (Trachtenberg et al., 2002; Shepherd et al., 2003; Holtmaat et al., 2005). It is thus only now really that this whole idea of synapse competition will start to be amenable to experimental tests.

Molecular control of cognition

It follows from the different concepts described above, consistent with the original ideas formulated by Ramon y Cajal, that higher brain functions critically depend upon a tight control and balance between mechanisms that, on the one hand, allow a plasticity of the function and organization of synaptic networks, in order to ensure continuous learning and adaptation, and, on the other hand, mechanisms that stabilize and maintain the connections critically involved in behaviors that must be conserved or memorized. Failure to operate correctly one or the other mechanism is likely to be detrimental to cognitive function. By studying the role of specific molecules involved in these aspects of plasticity, one should therefore gain information on important mechanisms for cognitive processing and, conversely, studies of selective deficits in cognition should highlight signaling pathways that might be critical for synaptic plasticity. We recently started this kind of approaches by anal-

yzing the mechanisms underlying specific, genetic forms of mental retardation in which the mental handicap is the only deficit and due to a mutation of a specific gene. Many different genes have now been identified as responsible for X-linked forms of mental retardation (Ramakers, 2002; Ropers et al., 2003). We focused in our work on the gene PAK3, the expression of which is modulated by induction of synaptic plasticity (Boda et al., 2002). Using a transfection approach through which we could express various mutants of PAK3 gene or suppress the expression of PAK3 gene in neurons, we found that this kinase does indeed contribute to synaptic plasticity mechanisms (Boda et al., 2004). Suppression of PAK3 resulted in the formation of aberrant, elongated spines and a decrease in the number of stable, mushroom-type spines; it also interfered with the formation of synaptic contacts, since many of these aberrant spines were de-afferented and since, in the remaining spines, the size of the postsynaptic densities was also markedly reduced. Furthermore, these morphological alterations were associated with defects of synaptic transmission and an impairment of LTP. As PAK3 belong to a family of molecules involved in Rho-GTPase signaling (Luo, 2000), it is likely that PAK3 produces these effects by interfering with cytoskeleton regulations that underlie spine and synapse dynamics (Edwards et al., 1999; Bokoch, 2003). Several other recent studies of other mental retardation genes or even of autism have started to unravel the importance of numerous molecular constituents of synapses as well as of unsuspected mechanisms for the function and plasticity of synaptic networks (Kaufmann and Moser, 2000; Zhang et al., 2003; Govek et al., 2004). Together the molecular complexity of the machinery that regulates synaptic mechanisms is thus probably directly related to its functional importance for the brain.

Conclusion and perspectives

While some of the general ideas about synaptic network plasticity have been proposed about a century ago, the fine details and importance of these concepts for brain function start only now to be better understood and their molecular mechanisms to be accessible to experimental analysis. The results of recent studies lead to the conclusion that both

modifications of synaptic function and structure and mechanisms of synapse formation and elimination contribute to the processing of information by synaptic networks. These two aspects are probably tightly inter-related to allow at the same time adaptation of brain networks to new situations and stabilization through competition of effective connections. These mechanisms are controlled by a complex machinery that starts only to be unravelled. It involves the numerous proteins localized in the postsynaptic density, the various receptors and molecules that regulate their trafficking, cell-cell signalling molecules that allow communication between pre- and postsynaptic structures, all second messenger pathways that regulate cytoskeleton organization and dynamics and the mechanisms that control gene transcription and local protein synthesis. Understanding the specific role of each of these molecules in the various aspects of plasticity will represent a major challenge for the coming years in view of the critical importance that these mechanisms play in functions as diverse as learning and memory, rehabilitation, mental retardation or even psychiatric diseases such as schizophrenia.

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