Synaptic plasticity, astrocytes and morphological homeostasis

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Abstract

Recent discoveries suggest that astrocytes are an integral part of synaptic connections, as they sense and modulate synaptic activity. Moreover, there is evidence that astrocytes change the number of synaptic connections directly via synaptogenic signals or indirectly, by modifying the morphology of axons and dendrites. Here, we formulate the hypothesis that astrocytes mediate the morphological homeostasis of nerve cells, which is any adaptation of the morphology of a neuron to preserve its ability to respond to and generate synaptic activity during learning and memory-induced changes. We argue that astrocytes control neuronal morphology locally and across long-ranging assemblies of neurons and that on the other hand, astrocytes are part of the engram with plasticity-related changes affecting their morphology.

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1. Introduction

One of the most intensely investigated topics in neuroscience are the cellular and molecular mechanisms of learning and memory. It is now largely accepted that the formation of long-term memory involves changes in the number and strength of synaptic connections between neurons (Bailey and Kandel, 1993; Greenough and Chang, 1988; Lamprecht and LeDoux, 2004). These changes are considered to be implemented solely by the neuronal network due to its ability to generate and process electrical signals. However, research within the last decade provokes the hypothesis that the neuronal network is not autonomous, but depends on the dialogue with a parallel network, which consists of astrocytes (Fig. 1). In a visionary article, Smith (1992) asked whether astrocytes participate in the generation and processing of information. The idea that astrocytes contribute to cognitive processes such as learning and memory is in line

with an evolutionary increase in the glia-neuron index (Bass et al., 1971; Kuffler et al., 1984) and with differences in the organization and morphology of cortical astrocytes between primates and other mammals (Colombo and Reisin, 2004; Reisin and Colombo, 2002). Here, we propose that astrocytes are crucial elements for the homeostatic regulation of plasticity (Fig. 1). We suggest first that these cells regulate the number of synaptic connections between neurons to balance the level of activity in the whole network and second, that learning-induced structural changes in the astroglial network are part of memory traces. Comprehensive reviews on other aspects neuron–glia interactions can be found elsewhere (Araque et al., 1999; Bacci et al., 1999; Bezzi et al., 2001; Carmignoto, 2000; Castonguay et al., 2001; Hatton and Parpura, 2004; Haydon, 2001; Hertz et al., 2001; Kettenmann and Ransom, 2004; Laming et al., 2000; Slezak and Pfrieger, 2003; Ullian et al., 2004; Volterra et al., 2002).

2. The concept of morphological homeostasis

For many years, research on the mechanisms of learning and memory formation concentrated on changes in the

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Fig. 1. Diagram illustrating the concept of morphological homeostasis. Under baseline conditions, astrocytes maintain and modulate synaptic function. Activity-dependent synaptic plasticity changes the synaptic input to a neuron, which then may cause hyperactivity or silencing. In response to this, astrocytes induce local and distant compensatory changes in the number of synapses directly or by changing axonal and dendritic morphology to reset neuronal activity.

efficiency of individual synapses. The most frequently postulated mechanism for synaptic plasticity is based on Hebb's rule, which states that strengthening of a connection between two neurons occurs, if both cells are simultaneously and repeatedly active (Hebb, 1949). To avoid an intrinsic positive feedback that follows from Hebb's rule, theoreticians postulated compensatory mechanisms to adjust the total synaptic input of a neuron to local changes in efficacy (Fregnac, 1995; Viana Di Prisco, 1984; von der Malsburg, 1973). However, such mechanisms have only recently been described in real neurons. One of them is multiplicative synaptic scaling, a phenomenon that was discovered first in cultured cortical neurons (Turrigiano et al., 1998) and then described in cortical slices (Desai et al., 2002). In this process, which proceeds within hours to days, the strengths of all synaptic inputs to a given neuron are scaled up or down by a multiplicative factor to preserve the relative intersynaptic differences in efficacy and to keep the firing rate of the neuron in an optimal range. Without such compensatory scaling, an increase in synaptic efficiency would saturate the firing rate of neurons and, in consequence, ablate their ability to respond to changing stimulation patterns, whereas a decrease in synaptic strength could lower the probability for reaching the threshold for action potential initiation and silence neurons (Feldman, 2002; Fregnac, 1998; Turrigiano, 1999). The discovery of synaptic scaling raises the question whether the mechanisms that balance the overall activity of a neuron also comprise structural or morpholog-

ical changes. This may involve the formation of new and elimination of existing connections and changes in the morphology of axons and dendrites. It is known that the shape of dendrites influences the firing rates of neurons (Duijnhouwer et al., 2001; Mainen and Sejnowski, 1996; Nasuto et al., 2001) and their ability for coincidence detection (Schaefer et al., 2003). This implies that neuronal morphology is under some form of homeostatic control. In fact, Greenough and co-workers have shown that learning-induced changes in synapse number are subject to homeostatic control: Synapse formation in rats housed in a complex environment, an experimental paradigm to test learning-induced changes (van Praag et al., 2000), was accompanied by a proportional increase in dendritic length thus keeping synaptic density constant (Jones and Greenough, 2002; Wallace et al., 1992). We will further refer to mechanisms that maintain the ability of a neuron to sense and generate meaningful outputs during plasticity-induced changes as "morphological homeostasis" (Fig. 1). Although it is conceivable that morphological homeostasis is implemented exclusively by neuron-intrinsic properties, we will argue that glial cells, namely astrocytes, play a significant role in this process.

3. Evidence for astrocyte-mediated morphological homeostasis

If astrocytes contribute to morphological homeostasis, they must be in close proximity to synapses and be able

to sense their activity and to induce compensatory changes in neuronal connectivity. We will present evidence that these requirements are fulfilled.

3.1. Astrocytes are in close contact to synapses and can sense synaptic activity

It has been shown that in the rat CA1 hippocampal area, a single astrocyte can contact up to 140,000 synapses (Bushong et al., 2002) through its “domain of influence”, i.e. the volume delimited by its ramifying processes (Antanitus, 1998). On the other hand, in this region about 60% of mainly glutamatergic synapses are in contact with astrocyte processes (Ventura and Harris, 1999). A large number of studies indicates that these processes can sense synaptic activity by expression of receptors for neurotransmitters (Pasti et al., 1997; Porter and McCarthy, 1996; Verkhratsky et al., 1998). A common astroglial reaction to synaptic activity is a rise in the intracellular concentration of calcium ions (Fellin and Carmignoto, 2004). It has been shown that astrocytes respond to synaptic activity in a graded manner and distinguish between different types of activity: Astrocytes *in situ* show an increase in the intracellular calcium concentration after stimulation with trains of action potentials, similar to those that induce long-term potentiation (LTP) (Porter and McCarthy, 1996; Zhang et al., 2003). Furthermore, the form of calcium transients depends on the frequency and intensity of the stimulus (Cooper, 1995; Cornell-Bell et al., 1990; Gallagher and Salter, 2003; Pasti et al., 1995, 2001).

3.2. Astrocytes control neuronal morphology

Astrocytes can influence neuronal morphology in two ways. First, by releasing factors that enhance or inhibit synaptic transmission (Kang et al., 1998; Zhang et al., 2003). Such modifications in synaptic efficacy can then lead to activity-dependent formation or elimination of connections. A particularly interesting substance in this context is D-serine, which is produced and released by astrocytes and acts as co-agonist of the NMDA receptor (Mothet et al., 2000). This glutamate receptor plays a crucial role in LTP, and D-serine may thus represent an astrocyte-derived factor that regulates long-term plasticity. Indeed, Yang and coworkers (Yang et al., 2003) have shown that in culture and in hippocampal slices, LTP requires the presence of glia-derived D-serine. Moreover, application of D-serine helped to restore retrograde memory after lesion of rat perirhinal cortex (Andersen et al., 2003) suggesting a crucial role of this “gliotransmitter” in memory formation.

Second, astrocytes may change the number of synaptic connections (Fig. 1). These cells are known to release a variety of factors that influence axonal and dendritic growth and thus the potential for synapse formation like for example laminins (Liesi et al., 1983), neurotrophins (Althaus and Richter-Landsberg, 2000), S100B (Donato, 2001; Muller et al., 1993) and activity-dependent neurotrophic factor

(Blondel et al., 2000). In addition, astrocytes secrete factors that directly stimulate synaptogenesis without affecting neuronal survival and growth (Slezak and Pfrieger, 2003; Ullian et al., 2004). Studies on highly purified retinal ganglion cells showed that astroglia-derived soluble factors induce the formation of new synapses (Nagler et al., 2001; Pfrieger and Barres, 1997; Ullian et al., 2004; Ullian et al., 2001). Two synaptogenic factors have been identified as cholesterol transported via apolipoprotein E-containing lipoproteins (Goritz et al., 2005; Mauch et al., 2001) and thrombospondins (Christopherson et al., 2005), but it appears likely that astrocytes secrete other factors with synapse-promoting activity (Elmariash et al., 2005). Furthermore, there is evidence that contact of neurons to astrocytes promotes their ability to form synapses via integrin-dependent signaling pathways (Hama et al., 2004). Experiments on organotypic cultures provided evidence for a crucial role of astrocytes in the structural maturation of cerebellar Purkinje cells, and particularly, in the regulation of their dendritic spine density (Meshul et al., 1987; Seil, 1997, 2001). More recently, Murai and coworkers revealed an influence of ephrin signaling on the regulation of spine morphology in mouse hippocampal pyramidal cells *in situ* (Murai et al., 2003). They showed that interaction between ephrin-A3, localized on astrocytic processes, and its EphA4 receptor on dendritic spines leads to retraction of spines and that inhibition of this interaction caused distortions of spine morphology. These studies, together with recent evidence that spines and their synapses are highly dynamic (Grutzendler et al., 2002; Trachtenberg et al., 2002), raise the question, whether astrocytic processes surrounding spines show a similar degree of mobility and whether they actually actively modulate spine turnover. Interestingly, a recent study on mice, where cells expressing glial fibrillary acid protein (GFAP) are labeled by a fluorescent protein, revealed a highly dynamic morphology of astroglial processes *in situ* (Hirrlinger et al., 2004).

The maintenance of a signaling-competent neuronal network may also involve elimination of synapses, and there is evidence that astrocytes can participate in this process. The selective stabilization or elimination of synapses may depend on a balance of proteases and protease inhibitors (Nelson et al., 1995) and it has been shown that astrocytes release both components (Rosenberg, 2002; Slezak and Pfrieger, 2003). Recent *in vivo* studies revealed that pruning of axons in the peripheral nervous system of mice (Bishop et al., 2004) and mushroom bodies of *Drosophila* involves glial cells (Awasaki and Ito, 2004; Watts et al., 2004) suggesting that astrocytes may also remove synapses physically.

3.3. Remote control by astrocytes

As outlined above, astrocytes are equipped to adapt the neuronal morphology and connectivity to changing levels of activity. Their extensions can influence the number of synapses by contact-dependent and secreted signals in their

immediate surroundings thus creating microdomains (Gro-sche et al., 1999; Nett et al., 2002) that ensure local homeostatic regulation. A main characteristic of the neuronal network is, however, its distributed function, i.e. that the creation and processing of information depends on electrical activity in distant assemblies of neurons. Consequently, plasticity-related changes involve heterosynaptic modifications in widely distributed cells, which in turn require compensatory changes in the connectivity of the neuronal assembly to guarantee its responsiveness. This raises the question, whether astrocytes can also implement homeostatic regulation across an assembly of neurons that are located in different brain areas. There is experimental evidence that astrocytes mediate activity-dependent heterosynaptic modifications by releasing neuromodulatory substances on neighboring neurons (Kang et al., 1998; Serrano et al., 2003; Zhang et al., 2003). This suggests that they cause indirectly compensatory morphological changes. A feature that may enable astrocytes to directly exert a sort of “remote control” over synaptic input in distant neurons is their ability to generate calcium waves. These oscillatory changes in intracellular calcium concentration have been shown to travel across entire cells and syncytia of electrically coupled astrocytes (Cornell-Bell et al., 1990; Dani et al., 1992) with the number of activated cells and the intensity of their response depending on neuronal activity (Aguado et al., 2002; Hirase et al., 2004; Sul et al., 2004). The velocity of astrocytic calcium waves (about 0.00002 m/s; (Cornell-Bell et al., 1990)) is lower than that of neuronal action potentials, but this should be sufficient to support homeostatic changes, which occur along with long-term plasticity-related modifications. Calcium waves exhibit a plethora of patterns (Cornell-Bell et al., 1990), the ability of self-renewing (Cooper, 1995) and the potential for all-or-nothing responses (Shao and McCarthy, 1995) rendering them functionally comparable to neuronal signals. It has been suggested that calcium waves carry encoded information (Crunelli et al., 2002; Smith, 1992; Zonta and Carmignoto, 2002), but the relevance and nature of such a code remains speculative and requires further studies.

4. Are engrams encoded by astrocytes?

The fact that learning and memory formation induce structural changes in the neuronal network, and the idea that astrocytes contribute to these changes raise the question whether learning and memory formation change the morphology of astrocytes leaving somehow an astrocytic engram. There is evidence that neural activity induces morphological changes in astrocytes: Greenough and coworkers showed alterations in astroglial morphology, when animals were exposed to a complex environment (Jones and Greenough, 2002, 1996; Jones et al., 1996). Furthermore, a correlation between synaptogenesis and astroglial hypertrophy was observed after motor skill learning in rats (Anderson et al., 1994). Electron microscopic studies

revealed an increased apposition of astrocytic processes to synapses within the visual cortex of animals housed in the complex environment (Jones and Greenough, 1996) and in the hippocampal dentate gyrus after LTP-induction by high-frequency stimulation of the perforant path (Wenzel et al., 1991). These ultrastructural observations indicate changes in the extent of astrocytic coverage and thus the volume of the extracellular space. Such changes profoundly alter the extracellular concentration of neuroactive substances and thus modulate synaptic transmission (Oliet et al., 2001; Piet et al., 2004; Smith, 1992; Sykova and Chvatal, 2000).

How can synaptic activity change the morphology of an astrocyte? The morphology of these cells depends on the phosphorylation and polymerization status of GFAP, a characteristic astroglial cytoskeletal protein and a component of intermediate filaments. It has been shown that different neurotransmitters including glutamate (Kommers et al., 2002, 1999; Wofchuk and Rodnight, 1995), GABA (Runquist and Alonso, 2003), and serotonin (Chang et al., 2005) influence the phosphorylation of GFAP, and thus in turn its degree of polymerization (Inagaki et al., 1994). A secreted glial protein, S100B, whose role in memory formation has been suggested some time ago (Hyden and Lange, 1970), inhibits polymerization of GFAP (Bianchi et al., 1995; Ziegler et al., 1998) and may therefore regulate astrocyte morphology in an autocrine fashion. There is evidence that serotonin induces the release of S100B from astrocytes (Chang et al., 2005; Whitaker-Azmitia et al., 1990) suggesting a link between neuronal (serotonergic) activity, release of S100B and astroglial morphology. A correlation between the astroglial cytoskeleton and memory is indicated, albeit indirectly, by the observation that mice lacking GFAP display alterations in eyeblink conditioning and in forms of synaptic plasticity that are thought to be associated with memory formation (McCall et al., 1996; Shibuki et al., 1996). Mice devoid of S100B develop normally and do not show defects in the brain cytoarchitecture, but they display enhanced LTP in the hippocampal CA1 area (Nishiyama et al., 2002). On the other hand, transgenic mice with additional copies of S100B gene show defects in LTP and in spatial memory tests (Gerlai et al., 1995; Winocur et al., 2001). So far, it is unclear whether the observed changes in synaptic plasticity are due defects in S100B-dependent astrocytic morphology.

Notably, most studies on the morphology of astrocytes rely on immunostaining for GFAP. However, staining for this protein does not allow to label the fine astrocytic processes. In fact, Bushong and coworkers (Bushong et al., 2002) combined immunohistochemistry with intracellular injections of fluorescent dyes and showed that staining for GFAP reveals only the basic stellate cytoskeleton and thus delineates only about 15% of the total astrocytic volume, whereas the remaining volume consists of dense spongiform ramifications. These fine processes, which contain mainly an actin-based cytoskeleton, appear to ensheath synapses (Derouiche et al., 2002) and mediate

communication by gap-junctions (Theiss and Meller, 2002).

Cornell-Bell et al. (1990) observed that glutamate induces the formation of filopodia in cultured hippocampal astrocytes, a reaction that was later named infotropism (Antanitus, 1998). Changes in the actin-based cytoskeleton are fast, requiring minutes (Cornell-Bell et al., 1990) and reversible. On the other hand, changes in the GFAP-based cytoskeleton are slow and probably relatively irreversible compared to actin-mediated motility. It is well known that expression of GFAP increases progressively with aging (Amenta et al., 1998; Goss et al., 1991; Nichols et al., 1993) suggesting that the age-related decline in learning performance results from the reduced plasticity of astrocytes. Evidence supporting this idea comes from a recent study (Wei et al., 2003) on primary cultures of embryonic neurons and young or adult astrocytes, which showed that neuronal sprouting depends on the GFAP level in glial cells. A diminution of GFAP in old astrocytes by RNA interference caused an increase in neural plasticity, whereas an increase in the GFAP level in young astrocytes induced the reverse effect. These observations correspond well with earlier observations that housing old rats in a complex environment improved memory performance and simultaneously decreased astrocytes number and size (Soffie et al., 1999).

5. Conclusion and outlook

Our goal was to formulate hypotheses on astrocyte function that may spark new efforts to understand these enigmatic cells. Some of the ideas presented here are supported by experimental data, whereas others need rigorous validation. In any case, fundamental questions about the mechanisms of learning and memory formation are unsolved as yet (see (Dudai, 2004) for excellent review), in spite of more than a century of research. Taking astrocytes into account may in fact help to advance in this area. First, we need to learn more about astrocytes: So far, their identification relies on a few markers despite increasing evidence for their molecular and physiological diversity (D'Ambrosio et al., 1998; Kimelberg, 2004; Matthias et al., 2003; Oderfeld-Nowak et al., 2003; Ogata and Kosaka, 2002; Soltys et al., 2003; Walz and Lang, 1998; Zhou and Kimelberg, 2000, 2001), which may reflect heterogeneity of the astroglial population, different functional states or distinct stages of maturation. A major challenge is therefore to establish new tools to label these cells, to assess their diversity and to determine how it develops. Second, nearly all evidence for functions of astrocytes comes from in vitro studies and from neuropathological observations, but it is not known whether they are relevant in a living and healthy animal. This could be addressed in transgenic mouse models, but so far, there are essentially only two mouse lines designed for astrocyte-specific gene ablation and they are based on the human (Zhuo et al., 2001) and mouse (Garcia et al., 2003) GFAP promoter,

which unfortunately also drive expression in neuronal precursors. Thus, there is an urgent need to develop animal models for temporally controlled elimination of gene expression exclusively in astrocytes. Last, not least, it appears timely to incorporate astrocytes in computational approaches to understand brain function, for example by creating artificial neuro-glial networks (Wallace and Bluff, 1995). Such models, even simplified, could be useful to explore the full functional range of neural networks. All in all, it appears that a complex machine like the brain can only be understood, if all the building blocks are taken into account.

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