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Plasticity of GABAergic synapses in the neonatal rat hippocampus

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Abstract

While the development and plasticity of excitatory synaptic connections have been studied into detail, little is known about the development of inhibitory synapses. As proposed for excitatory synapses, recent studies have indicated that activity-dependent forms of synaptic plasticity, such as long-term potentiation and long-term depression, may play a role in the establishment of functional inhibitory synaptic connections. Here, I review these different forms of plasticity and focus on their possible role in the developing neuronal network.

Keywords: synaptic plasticity - GABA - glycine - calcium

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Introduction

The development of highly organized structures in the CNS is a complex process that will determine the functional integrity at adult stage. Neuronal circuits are constructed over an extended period, both before and after birth, during which both activity-independent and activity-dependent mechanisms interact. During the last two decades, investigations have revealed that synaptic activity plays a role in shaping several aspects of circuits development including cell migration and differentiation, axonal path finding and the establishment of functional synaptic connections. The mechanisms by which synaptic activity participates to the refinement of initially coarse patterns of synaptic connections is presently not fully understood, but several studies have proposed that long-term changes in synaptic efficacy, such as long-term depression or potentiation (LTD and LTP) might participate in this process [1, 2].

Most of our understandings on activity-dependent maturation of synaptic connections and the possible contribution of long-term changes in synaptic efficacy in this process largely derived from studies on excitatory synaptic transmission [3-5]. However, several lines of evidence support the idea that spontaneous neuronal activity is also necessary for the full development of the inhibitory circuitry. In the auditory system, the topographic organization of glycinergic projections is achieved through synapses elimination [6], a process involving activity-dependent mechanisms [7]. In organotypic slice cultures of postnatal hippocampus [8] and cerebellum [9], synaptic activity regulates the density of inhibitory synapses. Moreover, sensory deprivations from birth lead to profound alterations in the number of GABA-imunopositive synaptic contacts or neurons in rat somatosensory [10] and visual [11-13] cortex. These changes provide the morphological substrate for the alterations in the spontaneous GABAergic synaptic activity observed both *in vitro* [14, 15] and *in vivo* [16] following manipulations of neuronal activity. The mechanisms underlying activity-depend maturation of inhibitory synapses are unknown. However, it is tempting to speculate that they are similar to those required for long-term plasticity, as proposed for excitatory synapses.

The validity of this hypothesis however depends on several assumptions that are discussed in the present review.

Developing inhibitory synapses undergoes long-term changes in synaptic strength

The major aim of this review is to discuss the possible role of long-term synaptic plasticity in the establishment of functional inhibitory synapses in the developing brain. However, the characterization of their induction and expression processes could help to understand the relevance of any form of synaptic plasticity. In this chapter, I will therefore briefly summarize the minimal requirements necessary for the induction and expression of long-term plasticity at developing GABAergic and glycinergic synapses.

Both LTP and LTD of inhibitory synapses have been described in different developing brain regions including the lateral superior olive [17], the cortex [18] and the hippocampus [19]. In all these structures, attention had been paid to demonstrate that GABAergic and glycinergic synapses themselves undergo long-term changes in synaptic efficacy. Not surprisingly, several mechanisms have been reported to contribute to the induction and maintenance of long-term plasticity at developing inhibitory synapses. A rise in intracellular Ca^{2+} concentration appears to be the common trigger for inducing the synaptic plasticity, although the source of Ca^{2+} and the underlying consequences on inhibitory synaptic efficacy may differ from one structure to another (reviewed in [20]). The rise in intracellular Ca^{2+} concentration can be produced by the opening of voltage-gated calcium channels (VGCCs) [21] or NMDA-gated channels [22, 23], or by the release of Ca^{2+} from $InsP_3$-sensitive stores [24] or Ca^{2+} -induced Ca^{2+} release stores [25]. In the neonatal rat hippocampus, the same conditioning protocol, i.e. high frequency stimulation, can lead to either LTP or LTD of GABAergic synapses (Fig. 1). The polarity of the plasticity induced depends on whether or not NMDA receptors have been activated during the conditioning protocol [19]. Given that both forms of plasticity require a postsynaptic rise in calcium [19], these observations suggest that the direction of synaptic changes is determined by the source of the calcium influx (*i.e.* VGCCs for LTP [21] versus NMDA for LTD [23]) (Fig. 1).

The mechanisms underlying the expression of long-tem plasticity at inhibitory synapses have been

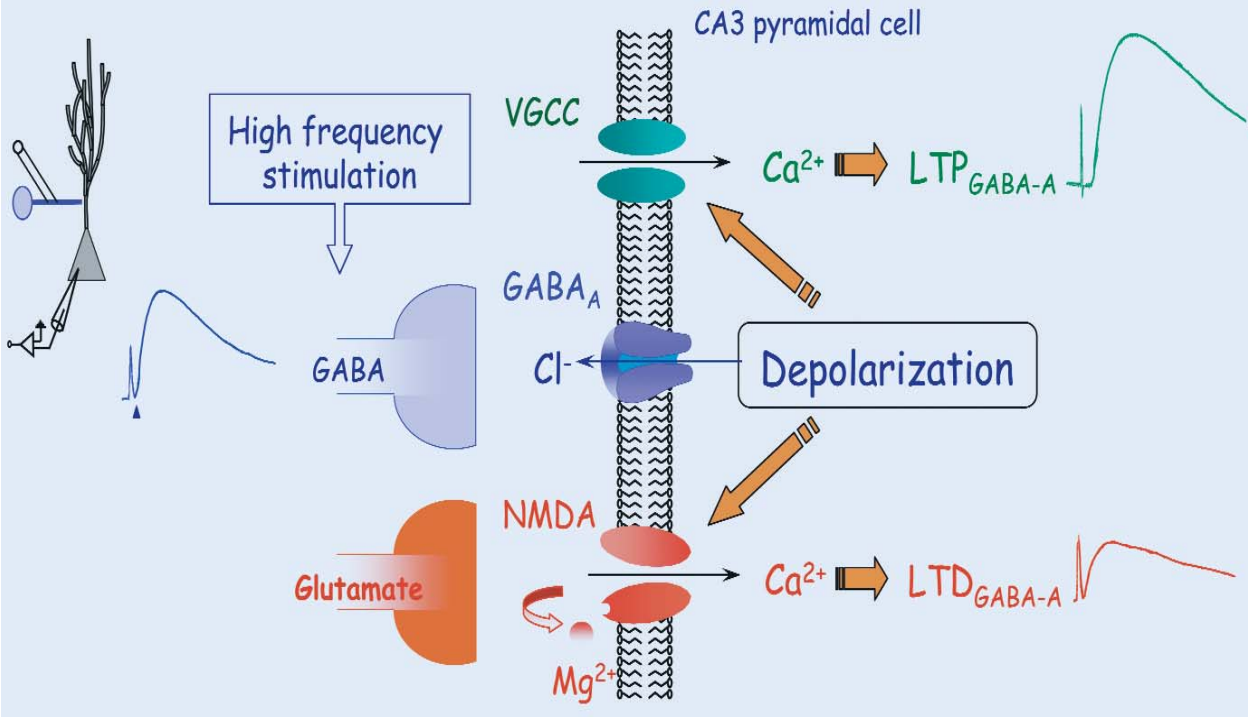

Fig. 1 Long-term changes in GABAergic synaptic efficacy in the developing rat hippocampus. During the first postnatal week of life, direct stimulation of the GABAergic interneurons, in the presence of ionotropic glutamatergic receptor antagonist, induces a depolarizing GABAergic potential. High frequency stimulation induces either a long-term potentiation (LTPGABA-A) or depression (LTDGABA-A) of GABAergic synaptic potentials. Both LTP and LTD required a membrane depolarization, mediated by the activation of GABAA receptors. This depolarization induces the opening of voltage gated Ca^{2+} channels (VGCCs) and allows the removal of Mg^{2+}-block from NMDA channels. The calcium influx through VGCCs leads to an LTP of GABAergic synapses, while the calcium influx trough NMDA channels lead to an LTD.

investigated. Long-term changes in the strength of synaptic efficacy can be accounted for by at least three non exclusive mechanisms: (i) modifications in the number or properties of receptors at functional synapses, (ii) modifications in the probability of transmitter release, and (iii) modifications in the number of functional synapses though either pre- or post-synaptic mechanisms. In the neonatal rat hippocampus, long-term plasticity of GABAergic synapses is expressed as modifications in the number of functional synapses or changes in the probability of transmitter release. Thus GABAergic synaptic plasticity is associated with a modification in the frequency, but not amplitude, of quantal inhibitory postsynaptic currents (IPSCs) [21, 23] and in the coefficient of variation of evoked IPSCs [23]. If the induction has a postsynaptic origin while the locus of expression is presynaptic, then a retrograde messenger should travel backward across the synapses to modulate the probability of transmitter release. Retrograde messengers have been reported to participate in long-lasting plasticity of GABAergic synapses in the adult hippocampus [26, 27]. Whether similar synaptic feedback also contributes to the induction of long-term plasticity at developing inhibitory synapses remained to be determined.

Long-term plasticity is generated by physiologically relevant stimuli

In most studies tetanic stimulation has been used to induce long-term changes in the efficacy of inhibitory synaptic transmission. Although such conditioning protocol provides a good tool to study the mechanisms by which plasticity is triggered and expressed, it is less obvious that inhibitory synapses are stimulated at such rates with the exception of

ripples and sharp waves [28]. Therefore, one always has to take into account the relevance of the conditioning protocol to physiological conditions to gain insight into the role that this plasticity may serve.

There are now compelling evidences suggesting that conditioning protocols relevant to physiological or pathological conditions can induce plasticity at GABAergic and glycinergic synapses. The first demonstration was provided by Korn and collaborators, in the goldfish [29]. They showed that auditory stimulation can trigger an LTP of inhibitory synapses *in vivo* that shares similar properties with the LTP induced by tetanic stimulation [30-32]. Although less direct, other evidences have been provided in the neonatal rat hippocampus. Thus, a Ca^{2+}-dependent LTP at GABAergic synapses can be induced by repeated bursts of action potential applied at a low frequency to CA3 pyramidal cells [21, 33]. This conditioning protocol is clearly related to the sort of activity that neonatal CA3 pyramidal cells experience *in vivo* [34]. In neonates, burst of action potentials can result from endogenous bursting properties of CA3 pyramidal cells [35] or from the network-driven synaptic activity present at early stages of development [36]. To further address the relevance of the conditioning protocol, it will be of interest in future studies to determine whether action potentials generated by the ongoing spontaneous activity are also able to trigger long-term plasticity at GABAergic and glycinergic synapses.

Long-term plasticity and the establishment of appropriate inhibitory synaptic connections

Activity-dependent long-term changes in inhibitory synaptic efficacy are believed to be an important phenomenon in learning and memory formation, and pathological states of neuronal excitability in the adult central nervous system (reviewed in [37]). A number of studies now suggest that long-term changes in synaptic efficacy, such as long-term depression or potentiation, might also play a crucial role in the establishment of functional inhibitory synapses in the developing brain.

First, in all developing structures where inhibitory synaptic plasticity has been described, the induction is restricted to a limited period of development. Moreover, although the period during which the plasticity can be induced differ in these structures, this period always closely matches the period of functional maturation of neuronal network as shown in the visual cortex [18], the hippocampus [19, 33] and the auditory system [17]. In the neonatal rat hippocampus, for instance, the GABAergic synaptic plasticity induced by postsynaptic firing of pyramidal cells is restricted to the first postnatal week of life [33]. This developmental window coincides with the period of functional maturation of GABAergic synapses [38].

Second, the conditioning protocol leading to long-term changes in synaptic plasticity also leads to functional maturation of inhibitory synapses. The developing rat hippocampus is an interesting model to test this hypothesis. Thus, at birth the hippocampal pyramidal cells are synaptically "silent": that is, they show neither spontaneous, nor evoked synaptic responses. The synaptic maturation follows a sequence with the GABAergic synapses being functional before the glutamatergic one [39, 40]. In our study, we have recorded such "silent" neurons, and asked whether a relevant conditioning protocol can trigger the functional maturation of GABAergic synapses. Indeed, repeated bursts of action potentials, leading to long-term plasticity of GABAergic synapses when applied to "active" cells", also lead to the appearance of evoked and spontaneous GABAergic synaptic activity when applied to "silent" cells [33].

Finally, to further strengthen the link between activity-dependent maturation and long-term plasticity, it is necessary to show that the same cellular mechanisms are involved in both phenomena. In this context, neurotrophins and related Trk receptor-coupled protein tyrosine kinases (PTKs) have been implicated in synapse development and plasticity. In cerebellar and hippocampal cultures, activity deprivation decreases the number of GABAergic synapses, an effect reversed by the application of brain-derived neurotrophic factor (BDNF) or neurotrophin-4 [8, 41]. Moreover, overexpression of BDNF [42] or chronic treatment with different neurotrophins [43-45] accelerates the functional maturation of GABAergic synapses. Several studies have also shown that neurotrophins can modulate the efficacy of inhibitory synaptic transmission [46-48]. Interestingly, it was reported that NT-3 enhances

GABAergic synaptic transmission during the development period when $GABA_A$ receptors activation induced a depolarization and a postsynaptic Ca^{2+} rise, but not later when GABA leads to a membrane hyperplarisation [49]. Neurotophins may therefore be the signal linking long-term plasticity and activity-dependent maturation of inhibitory synapses. In agreement with this hypothesis, a recent study suggests that Trk receptors participate in the induction of long-term depression of glycinergic synapses in the developing auditory brain stem [50]. Similarly, in the developing neonatal rat hippocampus, our data show that the long-term potentiation of GABAergic synapses induced by the postsynaptic firing of CA3 pyramidal neurons is prevented by the application of PTK inhibitors (Gaiarsa and Gubellini, unpublished results).

Conclusion and perspectives

The data reviewed here indicate that inhibitory synapses undergo long-term change in synaptic efficacy in the developing brain, and that this form of plasticity might be instrumental in the establishment of appropriate synaptic connections. In future studies, a key issue to further link long-term plasticity and activity-depend maturation of synaptic transmission will be to characterize the synaptic activity underlying functional maturation of inhibitory synapses, and to determine whether this activity is able to induce long-term plasticity at inhibitory synapses. A hallmark of developing network is the presence of spontaneous patterned synaptic activity (for review see [51]). In spite of differences in the mechanisms underlying their generation, these patterned activities share several interesting features with regard to activity-dependent maturation of the neuronal networks. They are present during a restricted period of development corresponding to the period of synaptic maturation. In the neonatal rat hippocampus, this activity disappeared at a time when long-term plasticity can no longer be induced by postsynaptic firing of CA3 pyramidal cells. Moreover, they are always associated with postsynaptic variation in intracellular Ca^{2+} concentration, although the mechanisms leading to this Ca^{2+} transient differ from one structure to another. These activities may therefore represent the physiological pattern of activity leading to the functional maturation of inhibitory synapses through LTP/LTD like mechanisms. Further studies are needed to directly address this hypothesis.

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