Mechanisms of heterosynaptic metaplasticity

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Synaptic plasticity is fundamental to the neural processes underlying learning and memory. Interestingly, synaptic plasticity itself can be dynamically regulated by prior activity, in a process termed ‘metaplasticity’, which can be expressed both homosynaptically and heterosynaptically. Here, we focus on heterosynaptic metaplasticity, particularly long-range interactions between synapses spread across dendritic compartments, and review evidence for intracellular versus intercellular signalling pathways leading to this effect. Of particular interest is our previously reported finding that priming stimulation in stratum oriens of area CA1 in the hippocampal slice heterosynaptically inhibits subsequent long-term potentiation and facilitates long-term depression in stratum radiatum. As we have excluded the most likely intracellular signalling pathways that might mediate this long-range heterosynaptic effect, we consider the hypothesis that intercellular communication may be critically involved. This hypothesis is supported by the finding that extracellular ATP hydrolysis, and activation of adenosine A2 receptors are required to induce the metaplastic state. Moreover, delivery of the priming stimulation in stratum oriens elicited astrocytic calcium responses in stratum radiatum. Both the astrocytic responses and the metaplasticity were blocked by gap junction inhibitors. Taken together, these findings support a novel intercellular communication system, possibly involving astrocytes, being required for this type of heterosynaptic metaplasticity.

1. Introduction

It is now commonly accepted that synaptic plasticity in the form of long-term potentiation (LTP) and long-term depression (LTD) is a fundamental component of the neural processes underlying learning and memory. As memory mechanisms, however, LTP and LTD present a number of challenges to the neuronal network. In particular, unregulated synaptic plasticity has the potential to produce runaway effects that lead to functional maladaptation and impaired cognitive function. For example, if the strength of connections reaches saturated floor or ceiling levels, synaptic plasticity becomes unavailable for further change in that same direction, and previously stored memories may become impaired [1]. Moreover, removing restrictions on synaptic potentiation also impairs learning and memory [2]. At more extreme levels, there could be dramatic pathological effects such as synapses being potentiated to a level that causes excitotoxicity, or depressed to non-function. A key issue then is to understand how synaptic plasticity is regulated in order to optimise encoding and storage of information.

One class of plasticity regulatory mechanisms is metaplasticity, which refers to activity-dependent and persistent changes in the state of synapses or neurons that alter the magnitude or duration of subsequent synaptic plasticity [3]. Metaplasticity is a particularly attractive regulatory mechanism, because it dynamically links the history of neuronal activity with the current response. While the term metaplasticity was first formally coined in 1996 [4], the preceding Bienenstock, Cooper and Munro (BCM) theory of synaptic plasticity incorporated a metaplasticity-type feature that adjusts (‘slides’) the threshold for LTP according
to the cell’s previous history of activity [5] (figure 1). The purpose of this feature is to address the instability problem noted above from unregulated plasticity.

A particular feature of the BCM sliding threshold is that it applies simultaneously to all synapses spread across the cell. That is, for a given postsynaptic neuron, both the synapses participating in the activity that induces the metaplastic state (the homosynaptic pathway) and those that do not participate (heterosynaptic pathways) are proposed to undergo the same changes in subsequent plasticity. We refer to such regulation as heterosynaptic metaplasticity. Overall, the BCM theory has been hugely influential in the plasticity field by formalizing testable predictions not only about the direction and degree of plasticity arising from conjunctive pre- and postsynaptic activity, but also about the activity-dependent regulation of the LTP threshold. These predictions have been confirmed, at least in part, by studies in the visual cortex and hippocampus [6–10].

Not all experimentally described heterosynaptic metaplasticity effects conform to BCM principles however [8], raising questions about the mechanisms that underlie these various phenomena. For example, how do they vary with respect to their mechanisms of induction, their expression mechanisms that alter subsequent plasticity, and very importantly, their mechanisms that coordinate the metaplasticity effect between synapses scattered across the dendritic arbour? Here, we review and discuss these issues, with a particular focus on metaplasticity that is cell-wide, or at least spread broadly across dendritic compartments.

2. Intracellular pathways

Most studies of heterosynaptic metaplasticity have focused naturally on intracellular mechanisms. A key question is the cellular location that governs the setting of plasticity thresholds. In other words, is synaptic/cellular activity integrated by a central mechanism to direct a cell-wide state change, or is metaplasticity triggered directly at the over- or under active synapses and then transmitted to heterosynaptic locations? Equally, is the expression of the metaplasticity at synapses or at other neuronal elements such as dendrites?

(a) Central integration of neuronal activity

A central metaplasticity integrator proposed by the BCM theory is the time-averaged history of cell firing, although whether the expression of the metaplastic state is via synaptic or non-synaptic mechanisms was not defined. In support of the theory, neuronal discharge generated by either antidromic stimulation or intra-somatic current injections can modify

![Figure 1. BCM-like heterosynaptic metaplasticity.](image-url)
subsequent synaptic plasticity in a BCM-like manner [9,11]. However, under other conditions, cell firing is neither necessary nor sufficient for BCM-like heterosynaptic metaplasticity [8,12]. In fact, cell firing in these cases facilitated rather than inhibited subsequent LTP. The more recent calcium-dependent plasticity (CaDP) model, derived from the BCM model, also suggests a central mediator of metaplastic induction, i.e. time-integrated membrane voltage [13]. Moreover, the CaDP model specifically proposes that this integrator regulates a distributed synaptic expression mechanism, namely the modification of synaptic $N$-methyl-$D$-aspartate (NMDA) receptor conductances. Indeed, during visual cortex development, BCM-like metaplasticity is governed by activity-dependent regulation of the NMDA receptor subunit composition [14,15], an expression mechanism that also underlies certain metaplasticity effects in the hippocampus [16]. However, others have proposed that heterosynaptic metaplasticity can be mediated by a widespread modification of cellular excitability through changes in ion channels that control the discharge properties of neurons such as $h$, or the slow after-hyperpolarization, for which there is experimental support [17–21]. It should be noted that these various potential expression mechanisms are not mutually exclusive.

One well-studied type of heterosynaptic metaplasticity that does not conform to the BCM theory is encapsulated by the synaptic tag and capture (STC) model of plasticity. Here, the duration of LTD/LTP is centrally regulated by protein synthesis such that prior neural activity facilitates rather than impairs LTP heterosynaptically [22]. In this model, a pool of proteins required for consolidation of LTD and LTP can be made available by suitably strong afferent stimulation, or even by simply postsynaptic spiking [23,24]. Crucially, the capture and usage of these proteins can occur at heterosynaptic locations where there is only relatively weak synaptic activity that is normally insufficient to induce the late phase of synaptic plasticity. Interestingly, proteins that are produced by strong stimulation of one pathway can contribute to ‘cross-capture’, allowing LTP-inducing stimulation to reinforce LTD at heterosynaptic locations and vice versa [25,26].

On the surface this mechanism appears to suggest a simple, uniform cell-wide regulation of synaptic plasticity duration; however, the nature of these interactions is complicated. Although capture between basal and apical dendritic compartments can occur, it happens under limited conditions [27–30]. Instead STC interactions are more usually confined to specific dendritic compartments. Moreover, differences even exist in the effectiveness of STC mechanisms between proximal and distal synapses on the apical dendrite [31]. An interesting aspect of the STC model is that the mechanism requires additional neuromodulatory input [25,32], highlighting an interesting route to heterosynaptic metaplasticity; that is, while the postsynaptic induction pathway is exclusively intracelluar, more than one coincident presynaptic input plus non-glutamatergic transmission can be involved. In line with this, prior stimulation of other brain areas, particularly the basolateral amygdala, can also transform decremental LTP to a more stable form through a protein synthesis-dependent mechanism [33]. Interestingly, negative interactions that impair plasticity duration can also arise, particularly through competition for newly synthesized proteins. This occurs either when protein synthesis is limited or when strong induction protocols are used [30,34]. This competitive mechanism may represent an important brake on the extent to which STC mechanisms can enhance LTP, helping to avoid some of the positive-feedback instability on synaptic efficacy that the sliding threshold feature of the BCM theory was also designed to avoid.

(b) Metaplasticity triggered at and spreading between synapses

Can a metaplastic state be triggered directly at synapses and then spread heterosynaptically? There is little experimental evidence to indicate that this can occur to any significant degree. While metaplasticity consistent with most BCM principles has been demonstrated or modelled, this is typically confined to the active (or inactive) synapses [35,36]. However under some conditions, it is clear that synaptic plasticity at adjacent synapses can be metaplasticity regulated through the diffusion of intracellular factors. For example, inducing LTD at a single synapse can spread an LTP-permissive metaplastic state across the dendrite to other spines approximately 10 $\mu$m away [37]. While this effect is in the opposite direction to BCM predictions, it represents proof of principle for the intracellular spread of a metaplasticity state, lending credence to the possibility that similar molecules could spread other metaplasticity effects heterosynaptically, at least to immediately adjacent synapses. Inositol trisphosphate (IP$_3$) and cyclic adenosine monophosphate are two diffusible messengers that could act in such a way, as G-protein signalling culminating in the formation of these molecules triggers metaplastic states favouring LTD and LTP, respectively [38].

3. Intercellular pathways

The extensive spatial distribution of change that characterizes heterosynaptic metaplasticity in some experimental models raises the possibility that such long-range communication employs intercellular mechanisms beyond just the postsynaptic neurons (figure 2). For example, we recently described a heterosynaptic metaplasticity that spread from basal to apical dendrites in CA1 pyramidal neurons, without requirement for action potential generation or even somatic depolarization [8]. We identified activation of M1-acheterol receptors (M1-AChRs) and IP$_3$-mediated release of Ca$^{2+}$ from intracellular stores as contributing mechanisms. However, the fact that extracellularly released molecules contribute to the induction of this form of metaplasticity suggest it is not mediated purely by an intracellular signalling pathway within CA1 pyramidal cells (see below). Thus, we have considered the possibility that intercellular signalling may play a crucial role in inducing this metaplastic state [40].

Already there is good reason to consider intercellular signalling as a mechanism for heterosynaptic metaplasticity. Under normal circumstances, extrinsic input such as from inhibitory interneurons plays a key role in gating the induction of synaptic plasticity. If the efficacy of such inputs could be regulated in an activity-dependent manner, this would be a locus of expression for a metaplastic state. Indeed, retrograde endocannabinoid signalling from excitatory synapses induces both transient and persistent suppressions of gamma-aminobutyric acid (GABA) release that can locally facilitate LTP [39,41]. Extrinsic input from other brain areas can also mediate heterosynaptic metaplasticity as prior stimulation of the basolateral amygdala can persistently modify subsequent plasticity in the hippocampus [42]. Moreover, the prior history of amygdala activity influences
the induction of such effects in both the hippocampus [43] and prefrontal cortex [44], albeit thus far only when the timing between amygdala stimulation and plasticity induction is short.

Interestingly, regulation of GABAergic transmission was not a mediator of the cell-wide heterosynaptic plasticity we have recently described [8]. Instead, we identified two other signalling mechanisms, both of which are commonly implicated in intercellular communication. First, the effect requires a purinergic signalling cascade involving the extracellular hydrolysis of adenosine triphosphate (ATP) to adenosine by ectonucleotidases [40], and activation of adenosine A2 receptors (figure 3). ATP exerts diverse and diffuse effects through its numerous sites of release, whether from presynaptic terminals, nodes of Ranvier or gap junction hemichannels [45–47]. Following hydrolysis, adenosine acts on several receptor subtypes both homo- and heterosynthetically to modulate network activity (for a review, see [48]). In reduced prepa rations like the hippocampal slice, nodal Ranvier or gap junction hemichannels [45–47]. Following hydrolysis, adenosine acts on several receptor subtypes both homo- and heterosynthetically to modulate network activity (for a review, see [48]). In reduced preparations like the hippocampal slice, nodal Ranvier or gap junction hemic hannels [45–47].

Figure 2. Intercellular pathways for heterosynaptic metaplasticity. (a) GABAergic interneurons in the hippocampus can mediate local heterosynaptic facilitation of LTP following presynaptic activity (red axon), glutamate (Glu) release and activation of postsynaptic glutamate receptors (GluR), retrograde endocannabinoid (eCB) signalling from the postsynaptic neuron to presynaptic type 1 cannabinoid receptors (CB1R) on GABAergic interneurons persistently reduces GABA release and thus activation of GABA$_A$ receptors (GABA$_A$R), thereby facilitating LTP at nearby synapses (green halo, [39]). (b) Another potential intercellular pathway for heterosynaptic metaplasticity involves astrocytes. Long-range signalling through the astrocytic network may alter subsequent synaptic plasticity at distant synapses, including those in different dendritic compartments (left-hand side of figure). Here, for example, activation of inputs to basal dendrites (the red presynaptic axon) results in heterosynaptic metaplasticity (orange halos) in the apical dendrites. Activation of a single astrocyte may also produce heterosynaptic metaplasticity, albeit likely over a more limited spatial extent (right-hand side of figure). The illustrated pathways for intercellular mediation of heterosynaptic metaplasticity are almost certainly not exhaustive. (a) GABAergic interneurons in the hippocampus can mediate local heterosynaptic facilitation of LTP. Following presynaptic activity (red axon), glutamate (Glu) release and activation of postsynaptic glutamate receptors (GluR), retrograde endocannabinoid (eCB) signalling from the postsynaptic neuron to presynaptic type 1 cannabinoid receptors (CB1R) on GABAergic interneurons persistently reduces GABA release and thus activation of GABA$_A$ receptors (GABA$_A$R), thereby facilitating LTP at nearby synapses (green halo, [39]).

Figure 3. Heterosynaptic metaplasticity in the hippocampal slice is dependent on activation of adenosine A2 receptors. In field potential recordings from CA1 of acute hippocampal slices (refer for methods [40]), LTP (2 × 100 Hz) in stratum radium of CA1 is inhibited in slices which first receive priming stimulation (3 × 100 Hz, repeated after 5 min) delivered to stratum oriens afferents (Or.prime). This effect is inhibited by co-administration of the A2AR antagonist ZM241385 and the A2BR antagonist MRS1754 (50 nM each, bar), bath applied prior to and during priming (control: n = 5, 144 ± 4%; primed: n = 8, 122 ± 2%; drug: n = 5, 139 ± 6%, F$_{2,15}$ = 10.12, p = 0.002). Data are expressed as a percentage of the averaged baseline responses. Arrows denote time-points of oriens priming or radium HFS. Connexin hemichannels allow for a dynamic and highly plastic mode of fast intercellular communication [49], especially for non-neuronal cells such as astrocytes. For example, diffusible messengers such as Ca$^{2+}$ and IP$_3$ travel between cells via gap junctions.
The above considerations raise the possibility that hippocampal astrocytes can in fact communicate widely enough across the CA1 layers to be able in principle to mediate long-range heterosynaptic plasticity that spreads from basilar to apical dendritic compartments. To address this, we have undertaken calcium imaging of CA1 astrocytes filled with Fluo-4 and labelled with sulforhodamine-101 by injection of these compounds directly into the hippocampus prior to slice preparation (see electronic supplementary material). Using the same stratum oriens stimulation parameters that inhibit LTP and promote LTD in stratum radiatum (6 × 100 Hz, 1 s trains [8]), we observed that each high-frequency train of priming stimulation reliably induced a calcium elevation not only in stratum oriens astrocytes (not shown), but also in stratum radiatum astrocytes as far from the cell body layers as was imaged (297 μm; mean ± 10 μm; figure 4b,d). The greatest increase occurred in response to the first high-frequency stimulation (HFS) in a set of three trains (20 s apart). A second set of trains 5 min later gave a nearly identical set of responses. To test the hypothesis that this communication between CA1 layers was mediated by gap junctional communication, we repeated the experiments in the presence of carbenoxolone. This drug, at the same concentration used to inhibit the metaplasticity, greatly inhibited the calcium response in radiatum astrocytes, often completely eliminating the responses altogether, particularly to the second and third trains in a burst of stimulation (figure 4c).

If astrocytes do mediate the heterosynaptic plasticity, which glio
transmitter(s) might be signalling back to neurons to modulate plasticity? Astrocytic ATP, converted to adenosine and acting on A1Rs, is one candidate as it is implicated in heterosynaptic depression [73–75] (but also see [66]). Astrocytic glutamate is another candidate, as cholinergic activation of astrocytes induces mGluR or NMDAR-dependent LTP in neurons in vivo [60,79,80]. Moreover, astrocytic activation of neuronal A1Rs and NMDARs regulates plasticity thresholds [81,82]. However, these receptors do not contribute to heterosynaptic plasticity in our model [8,40].
Interestingly, A2Rs are already implicated in an inhibitory form of metaplasticity [83], although the precise mechanism of action remains unknown. HFS and activation of A2BRs can trigger the release of cytokines from astrocytes [84,85], and this has been proposed as a metaplastic mechanism for inhibiting LTP both homo- and heterosynaptically [86], and indeed, we have recently shown that A2BR activation can generate a cell-wide inhibition of LTP [40]. It is therefore possible that priming stimulation in stratum oriens modulates plasticity in stratum radiatum by eliciting widespread cytokine release from astrocytes, a hypothesis we consider worthy of future investigation.

Taken together, the pattern of results we have obtained so far is strongly suggestive of an intercellular signalling pathway mediating BCM-like long-range heterosynaptic metaplasticity in the hippocampus. We have also established proof of principle that the participating intercellular network may include astrocytes, which are activated extensively by afferent stimulation and which are capable of regulating LTP and LTD induction. However, to fully test this hypothesis, experiments are needed that directly manipulate astrocyte function both during and after priming stimulation.

(a) Functional implications of heterosynaptic metaplasticity

Looking across studies, there is a clearly a wide range of mechanisms mediating heterosynaptic metaplasticity, perhaps reflecting the range of preparations used (in vivo versus in vitro, specific brain regions, etc.), but also perhaps reflecting the diverse capability of neural networks for this class of metaplasticity. Regardless, heterosynaptic metaplasticity has the potential to powerfully modulate local network function, particularly when mediated by third-party cells such as astrocytes. It is possible that such network metaplasticity acts to enhance the signal-to-noise ratio between active and quiescent inputs, thus maximizing the distinction between salient and non-salient information. Such a distinction may be augmented by the synaptic release of agents that also regulate plasticity. For example, the facilitation of LTP by A2AR activation may be balanced by heterosynaptic depression mediated by astrocytic activation of A1Rs [48]. Furthermore, whereas endocannabinoids can induce homeosynaptic depression they can also activate astrocytes which mediate heterosynaptic facilitation via glutamate release [63]. Another function of BCM-like metaplasticity may be to prolong the duration of synaptic plasticity by rendering newly established synaptic weightings resistant to change for a period of time. Alternatively, where dramatic perturbations of normal neural activity occur, such metaplasticity may serve a neuroprotective role by braking subsequent plasticity, and facilitating homeostatic synaptic scaling in the opposite direction to normalize overall synaptic weights. In this scenario, metaplasticity could promote functionally adaptive responses such as preventing potentiation of synaptic inputs to the point of excitation. It is interesting to note in this context the considerable mechanistic overlap between neuroprotective preconditioning effects which prevent excitotoxicity and metaplasticity [3,87,88].

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