OPINION

A clustered plasticity model of long-term memory engrams

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Abstract | Long-term memory and its putative synaptic correlates the late phases of both long-term potentiation and long-term depression require enhanced protein synthesis. On the basis of recent data on translation-dependent synaptic plasticity and on the supralinear effect of activation of nearby synapses on action potential generation, we propose a model for the formation of long-term memory engrams at the single neuron level. In this model, which we call clustered plasticity, local translational enhancement, along with synaptic tagging and capture, facilitates the formation of long-term memory engrams through bidirectional synaptic weight changes among synapses within a dendritic branch.

A key function of a neuron is to modify its response to synaptic input in an experience-dependent fashion. In most models of neuronal function, changes in synaptic weight are the cellular foundation of these experience-dependent alterations. This is supported by extensive evidence that shows long-lasting increases and decreases in synaptic weights, known as long-term potentiation (LTP) and long-term depression (LTD), respectively[1]. Importantly, LTP and LTD, as well as memory, can be divided into at least two phases. Long-term memory (LTM) formation requires enhanced protein synthesis (translation), whereas short-term memory (STM) formation does not[2]. Analogously, in synaptic plasticity, although the induction of the early phase of LTP (E-LTP) and the early phase of LTD (E-LTD) does not require enhanced translation, this process is essential for induction of the late phase of LTP (L-LTP) and the late phase of LTD (L-LTD), which are thought to be synaptic correlates of LTM engram formation[3–11]. Transcriptional activation is also required for the maintenance of some, but not all, forms of L-LTP and L-LTD; importantly, the products of transcription are required only some time after plasticity is induced, whereas the protein products of enhanced translation are used immediately after the induction of plasticity[4,6–15]. Here, we focus on only the translation-dependent, transcription-independent phase of LTM engram formation.

The requirement for LTM engram formation for enhanced translation, which has not been a key parameter in most computational models of neuronal function proposed so far, can impose new computational constraints on LTM engram formation and reactivation. Here, we summarize recent findings that shed light on the regulatory mechanisms underlying neuronal activity-induced translation, and discuss how this mechanism provides a molecular basis for an electrophysiologically identified associativity between L-LTP and L-LTD, a phenomenon known as 'cross-tagging'[16]. We also explain how translation-dependent plasticity leads to forms of synaptic cooperativity and associativity that act over a timescale of minutes, as opposed to the traditional synaptic cooperativity and associativity that act over a timescale of milliseconds, and are optimal only when the stimulated synapses are located near each other. In addition, we propose a new model for the formation and reactivation of LTM engrams at the individual cell level, which we refer to as 'clustered plasticity'. To determine the computational value of our model, we compare it with an alternative model[17], which we refer to as the 'dispersed plasticity' model, that suggests that the LTM engram is stored randomly at synapses throughout the neuron. Our clustered plasticity model extends the model of LTM engram storage proposed by Frey[18] — who proposes that localized protein synthesis within dendrites facilitates biochemical economy and local autonomy of neuronal response rules when inputs are segregated — by incorporating several computational advantages that apply to non-segregated neuronal input. It also extends the model of Mel *et al.*[19–21], which describes the advantages of clustered input to a neuron in action potential generation; by contrast, the clustered plasticity model discusses plasticity, as opposed to action potential generation, and is not constrained by the requirement for clustered inputs. It is to be noted that the model described here applies to the storage of information within single cells, and so the mechanisms of neuronal-ensemble engram storage, which are superimposed on the mechanisms of engram storage within single cells, will not be discussed here.

Neuronal activity-induced translation

L-LTP and L-LTD are manifestations of opposite synaptic weight changes, but the induction of both requires upregulated translation[4–9]. Interestingly, data suggest that both sets of stimuli that induce L-LTP and L-LTD cause enhanced synthesis of largely overlapping sets of diverse proteins, through the activation of common biochemical signalling pathways. Specifically, both L-LTP- and L-LTD-inducing stimuli enhance mitogen-activated protein kinase (MAPK)- and mammalian target of rapamycin (mTOR)-dependent translation of a diverse set of mRNAs[9,10,22]. Mechanistically, this increase in translation is characterized by MAPK- and mTOR-dependent increases in phosphorylations of eukaryotic translation initiation factor 4E (eIF4E), its repressors eIF4E-binding protein (4EBP1, 4EBP2), small ribosomal protein 6 (S6) (REFS 9,10,22–27), as well as increases in the concentration of eukaryotic elongation factor 1α (eEF1α)[28,29] (FIG. 1). As these events lead to a general enhancement of translation[9,22], synthesis of a predominantly overlapping set of proteins is probably upregulated

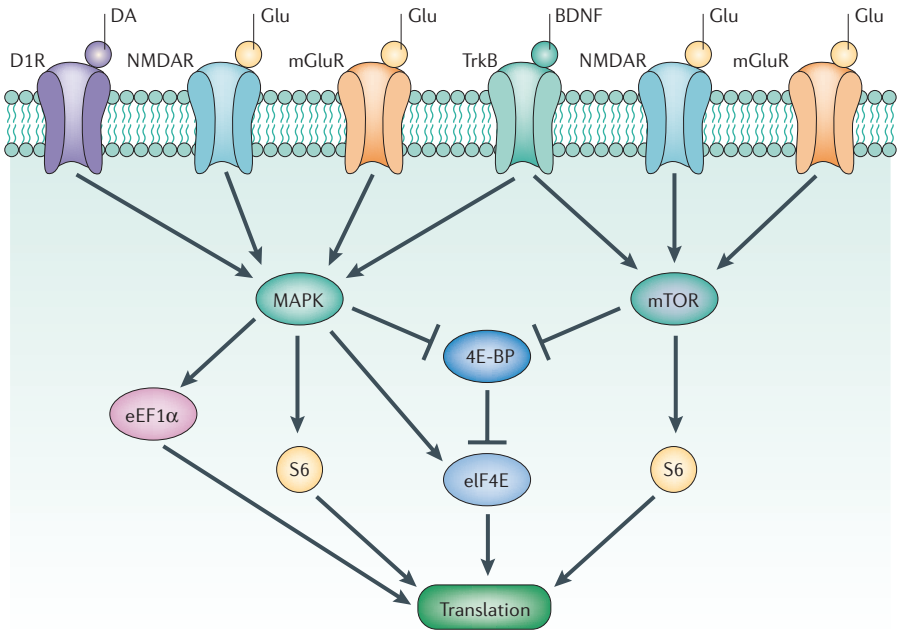

Figure 1 | **The MAPK and mTOR pathways regulate neuronal activity-induced translation.** This schematic shows the molecular mechanisms behind general translational enhancement. Neuromodulators (dopamine (DA), acetylcholine and noradrenaline), glutamate (via both NMDA (*N*-methyl-D-aspartate) and metabotropic glutamate (mGlu) receptors; NMDAR and mGluR, respectively) and neurotrophins (for example, brain-derived neurotrophic factor; BDNF) activate the mitogen-activated protein kinase (MAPK) pathway. Glutamate and neurotrophins also activate the mammalian target of rapamycin (mTOR) pathway. In turn, these pathways activate translation of most mRNAs present by stimulating phosphorylation of eukaryotic translation initiation factor 4E (eIF4E), small ribosomal protein 6 (S6) and eukaryotic elongation factor 1α (eEF1α), as well as by phosphorylation of eIF4E-binding protein (4EBP), which negatively regulates eIF4E. D1R, dopamine D1 receptor; TrkB, tyrosine receptor kinase B.

by both L-LTP- and L-LTD-inducing stimuli[9,22]. Furthermore, the MAPK and mTOR pathways must be active for induction of L-LTP and L-LTD[9,10,22,27,30–36].

Given that L-LTP and L-LTD represent opposite synaptic weight changes, how is it that the stimuli that induce them might trigger the synthesis of similar sets of proteins? It is possible that this could simply be because L-LTP and L-LTD require similar proteins for their expression. However, we favour another possibility — the concept of synaptic cross-tagging suggested by Sajikumar and Frey[16], which we elaborate on below. The authors propose that L-LTD/L-LTP-inducing stimuli trigger the synthesis of proteins necessary for L-LTD/L-LTP expression not only at the stimulated synapse but also for L-LTP/L-LTD expression at nearby synapses that receive E-LTP/E-LTD-inducing stimuli, which does not lead to translational activation.

Spatial aspects of translational activation. The discovery by Steward and colleagues of synaptically localized ribosomes[37,38], and the finding that synapses are capable of synthesizing proteins[39–41], suggests that activity-induced translation occurs at or near stimulated synapses[37–47]. Studies have also shown that brain-derived neurotrophic factor (BDNF) and dihydroxyphenylglycine (DHPG), which induce L-LTP[48] and L-LTD[6], respectively, without the need for somatic protein synthesis, trigger dendritic protein synthesis[9,46,49–52]. Furthermore, tetanus-induced L-LTP does not require somatic translation[53,54]. Recent evidence also points to protein synthesis occurring only at sites near stimulated synapses in response to dopamine[55]. This localized translation could be mediated by biochemical signalling cascades that are activated locally by synaptic input[56]. In addition, Ras activation (which occurs upstream of MAPK activation) in response to stimulation with epidermal growth factor in COS cells is localized to the stimulated sites[57].

One piece of evidence against localized translation is that the MAPK pathway can be activated throughout the neuron through the patterned generation of antidromic action potentials[58,59], although translational upregulation has not been measured after such stimulation. This is important because MAPK pathway activation is not likely to be sufficient to induce translation: there is evidence that the MAPK pathway can also be activated by E-LTP-inducing stimuli[30] and is necessary in many cases for E-LTP induction[31,60]. It is possible that higher levels of MAPK pathway activation are necessary for translational induction, but to achieve this level of activation requires L-LTP/L-LTD-inducing stimuli rather than E-LTP/E-LTD-inducing stimuli[61,62]. Alternatively, other pathways such as the mTOR pathway, which is also required for L-LTP/L-LTD induction[32,33,35,36], could be activated preferentially by L-LTP/L-LTD-inducing stimuli[36]. In addition, synaptic plasticity can occur in the absence of action potential generation, for example, through local dendritic spike generation[63–65], which would result in local activation of the MAPK pathway. We therefore believe that there is compelling evidence supporting local activity-induced translational upregulation, which forms an integral part of our proposed clustered plasticity model.

Synaptic integration in translational activation. Once activated, the MAPK and mTOR pathways remain active over a timescale of the order of minutes[10,61]. Therefore, the reliance of L-LTP/L-LTD-dependent translational upregulation on kinase activation — that is, the MAPK and mTOR kinase pathways — allows for the integration of inputs that are separated by many minutes as translational stimulators. In addition, these molecular pathways are not only activated by kinases that are downstream of glutamatergic action but also by signalling cascades stimulated by neurotrophins[10] and neuromodulators, such as noradrenaline, dopamine and acetylcholine[66], and so both ionotropic and metabotropic inputs[66] can be integrated (FIGS 1,2). This idea is supported by evidence that the activation of noradrenergic receptors[67] or D1/D5 dopamine receptors[68,69] can facilitate the induction of L-LTP, and that electrically induced L-LTP requires dopaminergic function[70,71]. Importantly, neuromodulators are thought to be released in a diffuse manner[72], which would lead to nonspecific upregulation of translation, and therefore we predict that neuromodulatory input can only facilitate translational activation when coupled with synapse-specific neurotransmitter activation, such as through glutamate. This is consistent with data from several laboratories that indicate that the modulatory effects of dopaminergic[68,69,73,74] and β-adrenergic activation[67] on hippocampal LTP and LTD are dependent on glutamate receptor activation. We refer to this long-term (timescale

of minutes) kinase-dependent integration as 'synaptic integration in translational activation' (SITA; FIG. 2) to distinguish it from short-term (millisecond timescale) dendritic integration and electrical cooperativity. In this mechanism, dendritic membranes and NMDA (*N*-methyl-D-aspartate) receptors (NMDARs) mediate the cooperation of synapses with subthreshold activation levels to induce E-LTP and E-LTD[75,76].

Synaptic tagging and capture

The phenomenon of proteins synthesized in response to strong stimulation at one set of synapses facilitating plasticity at other stimulated synapses was first shown by Frey and Morris[5]. Specifically, when one set of Schaffer collateral synapses was tetanized with an L-LTP-inducing stimulus, and another set of synapses was tetanized with an E-LTP-inducing stimulus within ~1 h before or after application of the L-LTP-inducing stimulus at the first set of synapses, both sets of synapses expressed L-LTP[5]. These data have been explained by the 'synaptic tag and capture' model[5]. It has been proposed that E-LTP- and L-LTP-inducing stimuli create immobile 'tags', a process that is translation-independent, at the stimulated synapse. Because L-LTP-inducing stimuli enhance protein synthesis, and the new proteins are available to nearby synapses[5], tagged synapses close to synapses receiving L-LTP-inducing stimuli can capture the required proteins from the protein pool to express L-LTP themselves[5]. An analogous phenomenon is seen for E-LTD and L-LTD[16].

Strikingly, if an E-LTP-inducing stimulus was applied to one set of synapses before or after an L-LTD-inducing stimulus was applied to another set of synapses, L-LTP was observed at the first set of synapses[16]. This process has been termed 'cross-tagging' by the authors[16], although it is our opinion that the term 'cross-capture' is more appropriate. Moreover, we also believe that the general enhancement of translation in response to either L-LTP- or L-LTD-inducing stimuli outlined above provides the molecular basis for synaptic capture and cross-capture. As bidirectional plasticity has been postulated to be a key mechanism for efficient memory storage[77], one advantage of this associativity between L-LTP and L-LTD is that synaptic weight changes will be stabilized without altering the direction of the change.

Spatiotemporal features of synaptic capture. The associativity resulting from synaptic tagging and capture occurs between synapses stimulated on a timescale of ~1 h measured *in vitro*: the synthesized proteins have lifetimes of many minutes to hours, and the tags seem to endure for roughly the same period of time[5,78–81]. Therefore, synapses that are tagged many minutes before or after the onset of translation can capture protein, and express L-LTP or L-LTD. This is in contrast to the previously described electrical associativity between synapses, which relies on the membrane properties of the neuron and the properties of the NMDAR to associate two synapses activated within milliseconds of each other in the induction of E-LTP and E-LTD[75,76]. We refer to this longer-term associativity[81] as 'capture associativity' (FIG. 2).

Notably, many postsynaptic proteins are transported at a fairly slow rate, including the NMDAR subunit NR1 and the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor (AMPAR) subunit GluR1, which move along the dendrite at rates of 240 μm h^{-1} and 120 μm h^{-1}, respectively[82]. As AMPARs are likely to be involved in the expression mechanism of L-LTP and L-LTD[1], and the tag's lifetime seems to be ~1 h *in vitro*[5,78], it can be argued that capture associativity cannot occur

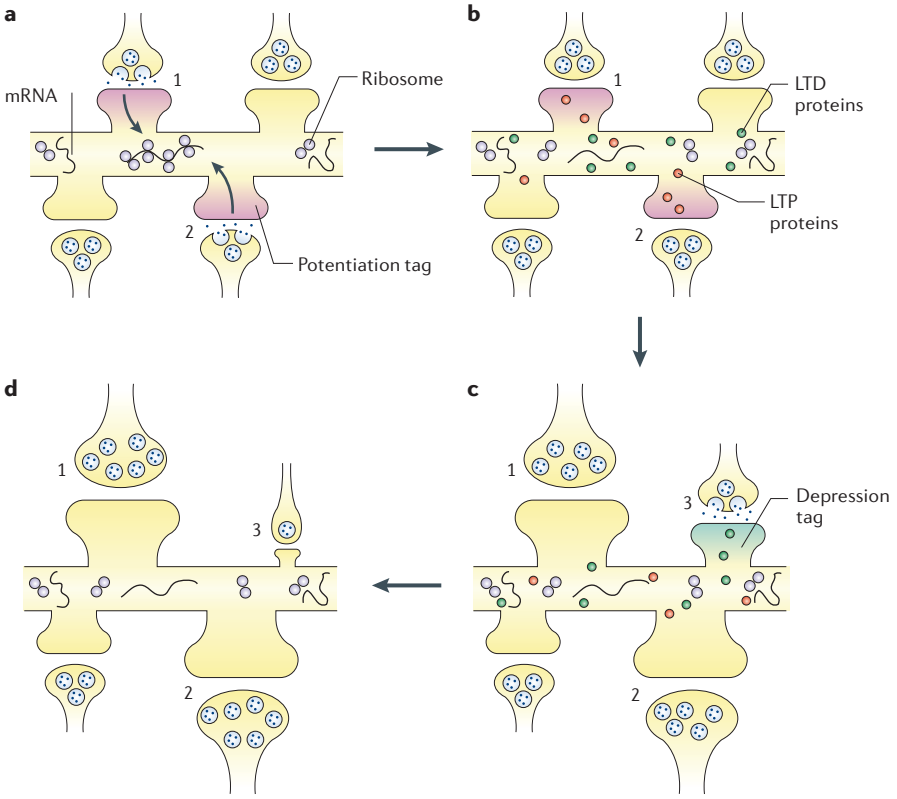

Figure 2 | **General translational upregulation, and synaptic tagging and capture, enable synaptic integration in translational activation and capture associativity. a** | In this example, two long-term potentiation (LTP) stimuli, which are insufficient for the induction of the late phase of LTP (L-LTP), arrive at the synapses (1,2), marking them with potentiation tags[5,16] (pink shading). They also cooperate, via synaptic integration in translational activation (SITA), to activate translation. Importantly, there could be an interval of many minutes between the stimuli[5,16,78]. **b** | The enhanced translation results in the production of the proteins required for the expression of both L-LTP and the late phase of long-term depression (L-LTD), represented by red and green circles, respectively. The proteins required for the expression of L-LTP are then captured by the synapses marked with the potentiation tag (1,2)[5,16]. **c** | The potentiation-tagged synapses therefore express L-LTP (depicted as expanded synapses). In addition, an early-phase LTD (E-LTD) stimulus arrives at synapse 3, and marks it with a depression tag (green shading)[16]. Although the input to the synapse is too weak to activate translation, the synapse captures proteins required for the expression of L-LTD from the pool of proteins synthesized in response to the previous stimuli to synapses 1 and 2[16]. This form of associativity between the input to synapse 3 and the original inputs to synapses 1 and 2 is known as capture associativity. **d** | Capture associativity leads to the expression of L-LTD (depicted as a shrunken synapse). Note that in panel **a**, one of the synaptic inputs could be neuromodulatory via SITA. However, in such a case, there would be no tag, and so that synapse would remain the same. Also, the exact locus of translation (spine versus base of spine versus dendritic shaft) is an open question, and is not important for our model.

more than ~120 μm away from the site of translation, which is approximately equal to the average length of a dendritic branch[83,84]. Furthermore, neither synaptic tagging[85] nor competition during synaptic capture[80] occurs when the inputs reach dendrites that are not in close proximity to each other, making the case for localized capture associativity.

The clustered plasticity model

At the single cell level, an LTM engram is thought to be composed of a pattern of synapses with stable synaptic weight changes[86]. The evidence presented above points to a model of LTM storage that depends on behaviourally relevant stimuli inducing either L-LTP- or L-LTD-like mechanisms, leading to the activation of the MAPK and mTOR signalling pathways. This, in turn, leads to the locally enhanced synthesis of a set of diverse proteins that is available to all nearby synapses and contains the entire repertoire of proteins necessary for the expression of both L-LTP and L-LTD. Also, both E-LTP- and L-LTP-inducing stimuli create 'potentiation tags' at the stimulated synapses[5,16], which then capture proteins required for expression of LTP. By contrast, E-LTD- and L-LTD-inducing stimuli create 'depression tags'[16], and these tagged synapses capture locally synthesized proteins required for the expression of L-LTD[5,9,16] (FIG. 2). Importantly, the tags remain at the synapses at which they were formed[5,16]. Unstimulated synapses do not get tagged, and therefore cannot capture protein, even if the necessary proteins are available, and so their synaptic weights remain unchanged. In agreement with previous models[78,81], we posit that both the strength of the tag and the amounts of the different proteins available are variable, with the amount of protein(s) captured by a synapse being proportional to both the strength of the tag and the local concentration of essential proteins[78,81].

Electrical cooperativity and electrical associativity[75,76] facilitate E-LTP and E-LTD induction, and concomitant setting of tags[5,16], at synapses with subthreshold stimulation. As multiple excitatory inputs onto synapses within a dendritic branch can summate supralinearly (generating a total effect that exceeds their linear sum)[19,65,87–89], we postulate that there is a greater probability of tag formation at stimulated synapses located close together within a dendritic branch compared with those dispersed throughout the dendritic arbor[64,90] (FIGS 2,3). Furthermore, our model predicts that, at a subset of the synapses in some cells, sufficient SITA occurs to induce translation. Within such dendritic branches, capture associativity will convert nearby expressions of E-LTP and E-LTD into expressions of L-LTP and L-LTD, respectively, and the information encoded by those synapses will be bound with the information conveyed by the set of synapses that originally received the L-LTP- or L-LTD-inducing stimuli. It is the combination of all these synapses with weight changes that have been stabilized that constitutes the LTM engram (FIG. 3).

As SITA and capture associativity occur most efficiently when synapses in close proximity to each other are stimulated within minutes of each other, those dendritic branches in which sufficient input occurs to stimulate tag formation and enhanced translation are effectively 'selected' as the locus of persistent memory storage. The various synaptic weight changes that form an LTM engram are therefore more likely to take place at synapses that are clustered in a few dendritic branches. We have named this model the clustered plasticity model. To analyse the computational benefits of such a model, we compare it with the model reviewed in Yuste and Urban[17]: we refer to this model as the dispersed plasticity model,

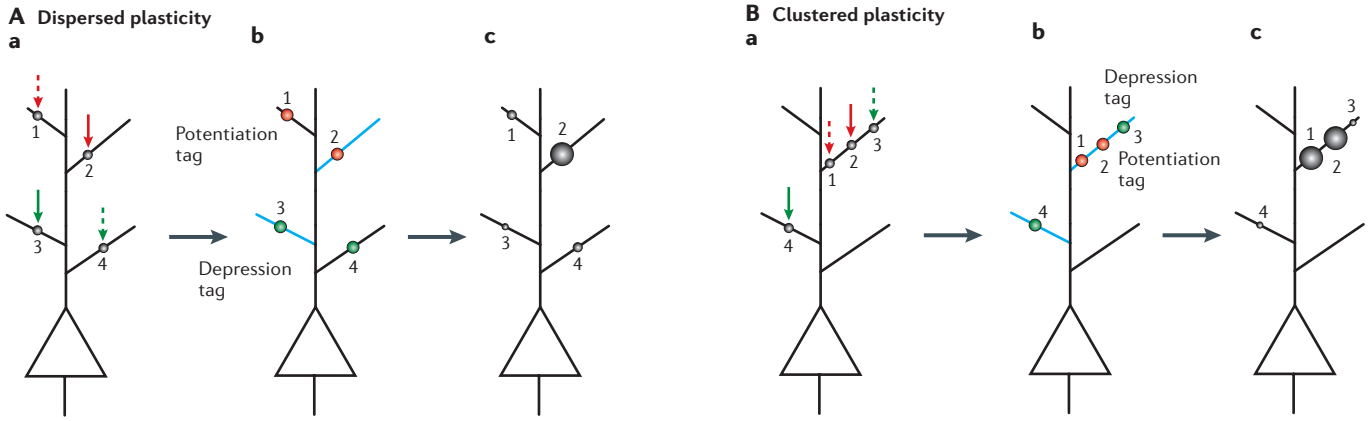

Figure 3 | **Formation of long-term memory engrams in dispersed plasticity and clustered plasticity models.** In both dispersed and clustered plasticity models, inputs arrive at four synapses (black circles marked 1–4) in the neuron. **Aa** | Early-phase long-term potentiation (E-LTP)-inducing stimuli (dashed red arrow) arriving at synapse 1 and late-phase LTP (L-LTP)-inducing stimuli (solid red arrow) arriving at synapse 2 mark the synapses with potentiation tags (red synapses in panel **Ab**). Analogously, late-phase long-term depression (L-LTD)-inducing stimuli (solid green arrow) at synapse 3 and early-phase LTD (E-LTD)-inducing stimuli (dashed green arrow) at synapse 4 mark the synapses with depression tags (green synapses in panel **Ab**). **Ab** | In addition, the L-LTP- and L-LTD-inducing stimuli at synapses 2 and 3, respectively, stimulate local translation. The proteins produced will be available to synapses in the dendritic branches (blue). **Ac** | The single, activated synapse in each branch will express L-LTP (synapse 2) or L-LTD (synapse 3). **Ba** | E-LTP-inducing stimuli (dashed red arrow) and L-LTP-inducing stimuli (solid red arrow) result in potentiation tags being set at synapses 1 and 2 (red synapses in panel **Bb**). Similarly, E-LTD-inducing stimuli (dashed green arrow) and L-LTD-inducing stimuli (solid green arrow) result in depression tags (green synapses in panel **Bb**) being set at synapses 3 and 4. **Bb** | The L-LTP- and L-LTD-inducing stimuli at synapses 2 and 4, respectively, activate local translation. The newly synthesized proteins, which comprise the proteins necessary for the expression of both L-LTP and L-LTD, are available to all synapses in the dendritic branches (blue). **Bc** | Synaptic capture from this pool of proteins leads to all four synapses acquiring the proteins required for either L-LTP (synapses 1 and 2) or L-LTD (synapses 3 and 4), which, in turn, leads to expression of L-LTD and L-LTP. In this case, translation could occur even if the input at synapse 2 was not strong enough to activate local translation, due to synaptic integration in translational activation (SITA) between the inputs at synapses 1 and 3. So, SITA and capture associativity facilitate long-term memory engram formation when inputs occur to clustered synapses, and therefore there will tend to be more clustered long-term memory engrams compared with dispersed long-term memory engrams.

which proposes that the synaptic changes that form an LTM engram are randomly scattered throughout the neuron[17].

Clustered plasticity versus dispersed plasticity. Clustered plasticity allows for the binding of some neutral or less salient information with more relevant information into a single LTM engram[18,81]. This is possible because of synaptic tagging and capture — through these processes E-LTP/E-LTD-expressing synapses can be converted to L-LTP/L-LTD-expressing synapses by capturing proteins that have been synthesized in response to L-LTP/L-LTD induction at a nearby synapse. By contrast, this process is inherently competitive if the amounts of proteins available are limiting. In such cases, the strengths of the tags and local availability of proteins will determine to what extent E-LTP/E-LTD is converted to L-LTP/L-LTD. In turn, the strength of the tag will depend on the strength of the initial input, which could reflect saliency of the information encoded by the input[81], as well as the amount of time elapsed between the initial input and the availability of newly synthesized proteins[81]. Moreover, the availability of proteins at a synapse will be determined by the distance between the synapse and the site(s) of activity-induced translation. Indeed, Bonhoeffer *et al.*[80] recently showed this type of competition during synaptic tagging and capture. This competition among tagged synapses allows cells to set a balance between information that is to be stored as part of the engram and information that is to be discarded. This enables optimum use of memory capacity, because only the most salient subset of available information is stored. Although many other processes are also likely to be involved, we propose that the mechanisms behind protein synthesis-dependent synaptic plasticity also have a role in this binding process.

The clustered plasticity model also allows for easier engram reactivation. As input arriving at potentiated synapses contributes preferentially to the output of the neuron, and because the engram is stored preferentially at spatially clustered synapses, stimulated synapses contributing to the neuron's output will tend to be located close to each other. Considering engram reactivation, the clustering of such stimulated synapses has two advantages over dispersal of stimulated synapses throughout the dendritic arbor. First, there is considerable evidence, both experimental and computational, that the stimulation of synapses that are close together on the same dendritic branch can cooperate synergistically in inducing an action potential, something more distant synapses on different dendritic branches cannot achieve[19–21,65,87–89]. This means that the input arriving as part of engram reactivation can make a greater contribution to the neuron's output if potentiated synapses activated by that input are spatially clustered within the same dendritic branch. Therefore, fewer stimulated synapses would be needed for action potential generation, and, consequently, a smaller number of potentiated synapses needs to be stimulated for engram reactivation (FIG. 4), which would facilitate memory retrieval.

Second, during engram reactivation or recall, the clustering of stimulated synapses allows neurons to increase the number of patterns that can be differentiated. When an engram is stored at synapses dispersed throughout the dendritic arbor, inputs onto potentiated synapses summate linearly[19–21,65,87–89]. This means that the number of synapses reactivated in response to recall cues and their strengths are the only determinants of whether the neuron fires an action potential. When an engram is stored at synapses clustered within a few dendritic branches, inputs onto potentiated synapses summate using a sigmoidal function within a dendritic branch and linearly between dendritic branches[19–21,65,87–89]. The number of different possible summated potentials therefore depends not only on the number of stimulated synapses and their strengths, but also on the spatial distribution of the stimulated synapses. The number of possible summated potentials is accordingly greater in the clustered plasticity model than in the dispersed plasticity model, thereby allowing for an increase in the number of patterns that the neuron can differentiate[21].

The advantages of clustered plasticity during engram reactivation described above significantly extend models put forward by Frey, who describes the role of synaptic tagging in a model of cellular consolidation[18], and Mel and colleagues[19–21], who describe the active properties of a dendrite in transmitting electrical signals and synapses being stimulated in a spatially clustered manner. In the Frey model, protein synthesis within dendritic domains would be a mechanism by which cells could integrate information from many afferents over an extended period of time, while economizing on protein production by having proteins produced locally[18]. Furthermore, the authors argue that many dendritic arbors have various afferents in different layers, and therefore dendritic domains would allow for local decision-making based on local connectivity[18]. Using data relating to local synthesis and limited protein diffusion, we have extended this model to provide other advantages, as described above, of dendritic branch-specific localized protein synthesis and capture. Also, we argue that having branch-specific engram formation would be beneficial even in an arbor in which the connectivity is more uniform.

Compared with the Mel model[19–21], which is based on the active membrane properties of dendrites, our clustered plasticity model uses the properties of translational regulation and protein movement, and does not require any spatial organization to the

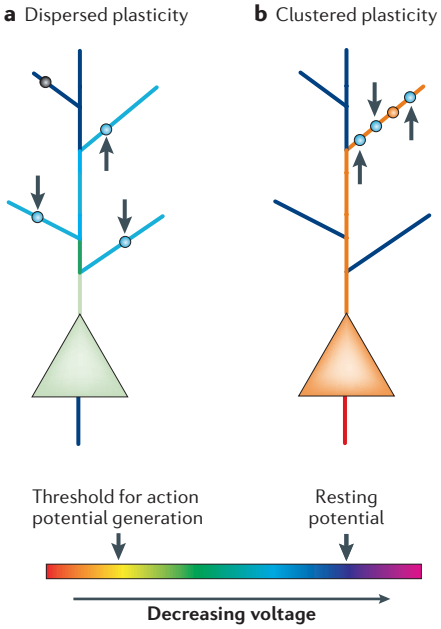

Figure 4 | **Clustered plasticity allows for more efficient action-potential firing during recall compared with dispersed plasticity.** The potentiated synapses that are components of the long-term memory engram that is being reactivated are depicted as circles. The colour indicates the voltage across the cell membrane during this reactivation. **a** | In the dispersed plasticity model, reactivation of the engram by recall cues (arrows) stimulates synapses that are dispersed across many dendritic branches, which, in turn, results in linear summation of inputs and the appropriate voltage change at the cell soma. In the case shown in the figure, this summed potential is insufficient to generate an action potential. **b** | In the clustered plasticity model, the recall cues activate the engram, which stimulates synapses clustered in a few dendritic branches (only one is shown). The potentials will therefore summate supralinearly[19–21,65,88], resulting in a higher integrated potential at the cell soma, which, in turn, leads to a higher probability of action-potential firing with clustered plasticity than with dispersed plasticity.

stimulation itself during engram formation. Instead, it argues that, during engram formation, spatially random stimulation will result in an STM engram composed of synapses dispersed throughout the neuron. Subsequently, SITA and capture associativity will favour LTM engram formation at synapses clustered close together, as described in the previous sections. Furthermore, Mel's model is based on naive synapses, whereas our model applies to potentiated synapses during LTM engram reactivation. However, as described in this section, the advantages of the model proposed by Mel and colleagues also apply to our model, when engram reactivation is considered.

Predictions and perspectives

The clustered plasticity model makes several testable predictions. First, the model predicts an inverse relationship between the effectiveness of synaptic capture and the distance between stimulated synapses, such that synaptic capture is most efficient when the interacting synapses are located on the same dendritic branch. Second, it posits that the enhancement of dendritic translation that occurs in response to L-LTP-or L-LTD-inducing stimuli takes place only within the branch containing the stimulated synapses. Third, assuming that connectivity between the set of presynaptic neurons and postsynaptic neurons is random, clustered plasticity would be advantageous compared with dispersed plasticity only if the density of active inputs is high enough to enable the setting of potentiation and depression tags at multiple synapses within at least one dendritic branch in the postsynaptic neuron. In support of this, 30–50% of hippocampal cells are active in a given environment[91,92], hippocampal activity resembles theta-burst stimulation, which has been used as a robust inducer of plasticity[93–96], and 45–75% of synapses are capable of undergoing plasticity[97]. Therefore, in an episode (a sequence of related events; the hippocampus is important for acquiring memory of such sequences), it is probable that there is sufficient activation so as to result in many dendritic branches in the hippocampus containing multiple tagged synapses. The probability of this being the case is even higher when sharp waves are considered. In rats, it has recently been shown that sharp wave activity during exploration carries information about the environment explored by the animal[98,99]. Furthermore, sharp wave-type activity would lead to high enough activity to enable activation of multiple synapses in a dendritic branch[65,87,89].

Finally, clustered plasticity would only be favoured over dispersed plasticity if the density and amount of activity in a hippocampal neuron did not result in activation of translation in most of the branches of the neuron. Although data directly addressing this issue are not available, it has been shown that transcription, as determined by observations of the immediate-early gene *Arc*, is activated in ~35% of CA1 neurons when an animal is placed in an unusual environment[100]. Despite the large amount of input arriving at hippocampal CA1 neurons, transcription is activated in less than a half of the cells, implying that a given input has a low probability of activating transcription. Similarly, as transcription and translation are activated by similar stimuli *in vitro* (that is, L-LTP- and L-LTD-inducing stimuli), it is likely that the probability of a given neuronal input activating translation is also low, and so it is unlikely that translation will be activated in many branches in a neuron.

It is probable that synaptic activity in the hippocampus is sufficiently high to set multiple tags on many dendritic branches in the hippocampus, while upregulating translation in a minority of the branches — under these conditions, SITA and capture associativity will facilitate clustered plasticity. This means that neurons will experience enhanced reactivation of engrams, and have the capacity to differentiate between patterns. However, future experiments are required to determine directly the spatiotemporal spread of translational activation and the diffusion rates of the slowest moving proteins required for the expression of L-LTP/L-LTD, as well as the number and spatiotemporal distribution of synapses that undergo E-LTP, E-LTD, L-LTP and L-LTD *in vivo* after behavioural training. We predict

Glossary

Antidromic
Conduction of an action potential in the opposite direction to normal — that is, towards the cell soma.

Associativity
When stimulation at one synapse is too weak to induce LTP, the simultaneous strong stimulation of another synapse can be sufficient to trigger LTP at both.

Cooperativity
When multiple synaptic inputs that are individually insufficient to induce LTP (or LTD) can collectively produce a postsynaptic depolarization that is sufficient to trigger LTP (or LTD).

Dedepression
A reversal of LTD by high-frequency synaptic stimulation. Dedepression shares some characteristics with LTP — both are induced by high-frequency stimulation, and both require NMDAR and protein kinase activity. However, there is evidence that LTP and dedepression are different processes.

Depotentiation
A reversal of LTP by low-frequency synaptic stimulation. Depotentiation shares some characteristics with LTD — both are induced by low-frequency stimulation, and both require NMDAR and protein phosphatase activity. However, there is evidence that LTD and depotentiation are different processes.

Engram
A persistent change in the brain that is formed in response to a stimulus, and is the neuronal substrate for a memory (also known as a memory trace).

Immediate-early gene
Genes that are induced within minutes of intense neuronal activity, even in the absence of protein synthesis. They are often induced by behavioural training. Examples include *Zif268*, c-*fos* and *Arc*.

Mammalian target of rapamycin
(mTOR). An evolutionarily conserved kinase, originally found to be stimulated by nutrients, that is a component of one of two key pathways in general translational regulation.

Mitogen-activated protein kinase
(MAPK). Any member of a family of evolutionarily conserved kinases (consisting of multiple isoforms of extracellular signal-regulated kinases, c-Jun N-terminal kinases, p38 MAPKs), originally found to be stimulated by growth factors, that are important in relaying signals from the cell membrane to various parts of a cell, including the nucleus, translational machinery, ion channels and cytoskeleton. The MAPK pathway is one of two key pathways in regulating general translation.

Sharp waves
Large amplitude electroencephalogram potentials that are the result of coherent neuronal discharges observed in the hippocampus and are accompanied by high-frequency (~200 Hz) oscillations during certain behavioural states.

Synaptic tag
Stimulated synapses are tagged in a protein synthesis-independent manner to distinguish them from other synapses on the same neuron that have not been activated. This mechanism enables tagged synapses to capture proteins required for, and to express, late-phase forms of plasticity, even when they receive stimuli that would normally result in early-phase forms of plasticity.

Synaptic weight
The relative amplitude of the postsynaptic response that is generated by the activity of the presynaptic neuron (also known as synaptic strength).

Theta-burst stimulation
Rhythmic neural activity with a frequency of 4–8 Hz that is present in several parts of the brain during certain behavioural states.

that imaging technologies[101–104] that are currently being developed will be useful in addressing these issues.

Translation is also required for a process known as immunity from depotentiation. This means that synapses in which L-LTP — but not E-LTP — has been induced cannot be depotentiated with low-frequency stimulation[105–107]. Analogous to L-LTP and L-LTD, this immunity from depotentiation can also be captured[107]. The phenomena of depotentiation and immunity from depotentiation are consistent with our model, as they simply accelerate the decay of E-LTP without affecting L-LTP. However, there are no similar reports of immunity from dedepression, and future studies will be required to address this issue. In addition, although transcription, unlike translation, is not required for the initial phases of L-LTP and L-LTD, it is essential for the persistent maintenance of L-LTP, and some forms of L-LTD[9,13–15]. Furthermore, transcription is required for heterosynaptic capture of immunity from depotentiation, although it is not necessary for homosynaptic immunity from depotentiation[107]. The relationship between activity-induced transcription and translation must be better defined with respect to their roles in the formation and maintenance of LTM engrams.

In addition to the general translational enhancement described here, some transcript-specific mechanisms that rely on sequences in the untranslated regions of transcripts are also triggered by neuronal activity, and regulate the translation of genes that are essential for either the expression of L-LTP or L-LTD, but not both[9,22]. This seems irreconcilable with cross-capturing, but it is possible that these mechanisms, although they regulate genes necessary for either L-LTP or L-LTD, are activated by both L-LTP and L-LTD-inducing stimuli. As the necessity of such process-specific mechanisms has only been shown by stimulating naive synapses, it is also possible that the induction of L-LTP and L-LTD leads to the production of other proteins that can take the place of the proteins that are normally synthesized by these process-specific mechanisms. Which of these two possibilities occurs in neurons remains to be established.

Summary

Computational models have generally relied on associational LTP and LTD as the predominant learning mechanism, but the dependence of learning and LTM engram formation on enhanced local protein synthesis has not been previously considered. We have argued that, far from being inconsequential to the algorithm implemented by a neuron, protein synthesis-dependent plasticity, along with the temporal and spatial characteristics of inputs to a neuron, has a crucial role in maximizing the memory capacity of a neuron, determining which information is stored and the binding of stimuli that occur over a period of minutes into single LTM engrams. The clustered plasticity model could subserve a multitude of different translation-dependent computational functions through the distinct morphologies of dendritic arbors seen in different neuronal types, the locations of synapses in the dendritic arbors, the widely varying densities of connectivity between neurons in different parts of the brain[108] and the diverse spatial and temporal patterns of synaptic activation in different brain regions.

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1. Malenka, R. C. & Bear, M. F. LTP and LTD: an embarrassment of riches. *Neuron* **44**, 5–21 (2004).
2. Davis, H. P. & Squire, L. R. Protein synthesis and memory: a review. *Psychol. Bull.* **96**, 518–559 (1984).
3. Krug, M., Lossner, B. & Ott, T. Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. *Brain Res. Bull.* **13**, 39–42 (1984).
4. Frey, U., Krug, M., Reymann, K. G. & Matthies, H. Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region *in vitro*. *Brain Res.* **452**, 57–65 (1988).
5. Frey, U. & Morris, R. G. Synaptic tagging and long-term potentiation. *Nature* **385**, 533–536 (1997).
6. Huber, K. M., Kayser, M. S. & Bear, M. F. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* **288**, 1254–1257 (2000).
7. Huber, K. M., Roder, J. C. & Bear, M. F. Chemical induction of mGluR5- and protein synthesis-dependent long-term depression in hippocampal area CA1. *J. Neurophysiol.* **86**, 321–325 (2001).
8. Scharf, M. T. *et al*. Protein synthesis is required for the enhancement of long-term potentiation and long-term memory by spaced training. *J. Neurophysiol.* **87**, 2770–2777 (2002).
9. Kelleher, R. J., Govindarajan, A. & Tonegawa, S. Translational regulatory mechanisms in persistent forms of synaptic plasticity. *Neuron* **44**, 59–73 (2004).
10. Kelleher, R. J., Govindarajan, A., Jung, H. Y., Kang, H. & Tonegawa, S. Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* **116**, 467–479 (2004).
11. Manahan-Vaughan, D., Kulla, A. & Frey, J. U. Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. *J. Neurosci.* **20**, 8572–8576 (2000).
12. Otani, S., Marshall, C. J., Tate, W. P., Goddard, G. V. & Abraham, W. C. Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. *Neuroscience* **28**, 519–526 (1989).
13. Nguyen, P. V., Abel, T. & Kandel, E. R. Requirement of a critical period of transcription for induction of a late phase of LTP. *Science* **265**, 1104–1107 (1994).
14. Frey, U., Frey, S., Schollmeier, F. & Krug, M. Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons *in vivo* and *in vitro*. *J. Physiol. (London)* **490**, 703–711 (1996).
15. Kauderer, B. S. & Kandel, E. R. Capture of a protein synthesis-dependent component of long-term depression. *Proc. Natl Acad. Sci. USA* **97**, 13342–13347 (2000).
16. Sajikumar, S. & Frey, J. U. Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. *Neurobiol. Learn. Mem.* **82**, 12–25 (2004).
17. Yuste, R. & Urban, R. Dendritic spines and linear networks. *J. Physiol. (Paris)* **98**, 479–486 (2004).
18. Frey, J. U. Long-lasting hippocampal plasticity: cellular model for memory consolidation? *Results Probl. Cell Differ.* **34**, 27–40 (2001).
19. Poirazi, P., Brannon, T. & Mel, B. W. Pyramidal neuron as two-layer neural network. *Neuron* **37**, 989–999 (2003).
20. Poirazi, P., Brannon, T. & Mel, B. W. Arithmetic of subthreshold synaptic summation in a model CA1 pyramidal cell. *Neuron* **37**, 977–987 (2003).
21. Poirazi, P. & Mel, B. W. Impact of active dendrites and structural plasticity on the memory capacity of neural tissue. *Neuron* **29**, 779–796 (2001).
22. Klann, E. & Dever, T. E. Biochemical mechanisms for translational regulation in synaptic plasticity. *Nature Rev. Neurosci.* **5**, 931–942 (2004).
23. Takei, N., Kawamura, M., Hara, K., Yonezawa, K. & Nawa, H. Brain-derived neurotrophic factor enhances neuronal translation by activating multiple initiation processes: comparison with the effects of insulin. *J. Biol. Chem.* **276**, 42818–42825 (2001).
24. Takei, N. *et al*. Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. *J. Neurosci.* **24**, 9760–9769 (2004).
25. Banko, J. L., Hou, L. & Klann, E. NMDA receptor activation results in PKA- and ERK-dependent Mnk1 activation and increased eIF4E phosphorylation in hippocampal area CA1. *J. Neurochem.* **91**, 462–470 (2004).
26. Banko, J. L. *et al*. The translation repressor 4E-BP2 is critical for eIF4F complex formation, synaptic plasticity, and memory in the hippocampus. *J. Neurosci.* **25**, 9581–9590 (2005).
27. Banko, J. L., Hou, L., Poulin, F., Sonenberg, N. & Klann, E. Regulation of eukaryotic initiation factor 4E by converging signaling pathways during metabotropic glutamate receptor-dependent long-term depression. *J. Neurosci.* **26**, 2167–2173 (2006).
28. Tsokas, P. *et al*. Local protein synthesis mediates a rapid increase in dendritic elongation factor 1A after induction of late long-term potentiation. *J. Neurosci.* **25**, 5833–5843 (2005).
29. Huang, F., Chotiner, J. K. & Steward, O. The mRNA for elongation factor 1α is localized in dendrites and translated in response to treatments that induce long-term depression. *J. Neurosci.* **25**, 7199–7209 (2005).
30. English, J. D. & Sweatt, J. D. Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. *J. Biol. Chem.* **271**, 24329–24332 (1996).
31. English, J. D. & Sweatt, J. D. A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *J. Biol. Chem.* **272**, 19103–19106 (1997).
32. Tang, S. J. *et al*. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proc. Natl Acad. Sci. USA* **99**, 467–472 (2002).
33. Hou, L. & Klann, E. Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. *J. Neurosci.* **24**, 6352–6361 (2004).

34. Gallagher, S. M., Daly, C. A., Bear, M. F. & Huber, K. M. Extracellular signal-regulated protein kinase activation is required for metabotropic glutamate receptor-dependent long-term depression in hippocampal area CA1. *J. Neurosci.* **24**, 4859–4864 (2004).
35. Gong, R., Park, C. S., Rezaei Abbassi, N. & Tang, S. J. Roles of glutamate receptors and the mTOR signaling pathway in activity-dependent dendritic protein synthesis in hippocampal neurons. *J. Biol. Chem.* 1 May 2006 [epub ahead of print].
36. Cammalleri, M. *et al*. Time-restricted role for dendritic activation of the mTOR-p70S6K pathway in the induction of late-phase long-term potentiation in the CA1. *Proc. Natl Acad. Sci. USA* **100**, 14368–14373 (2003).
37. Steward, O. & Levy, W. B. Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *J. Neurosci.* **2**, 284–291 (1982).
38. Steward, O. & Fass, B. Polyribosomes associated with dendritic spines in the denervated dentate gyrus: evidence for local regulation of protein synthesis during reinnervation. *Prog. Brain Res.* **58**, 131–136 (1983).
39. Rao, A. & Steward, O. Evidence that protein constituents of postsynaptic membrane specializations are locally synthesized: analysis of proteins synthesized within synaptosomes. *J. Neurosci.* **11**, 2881–2895 (1991).
40. Torre, E. R. & Steward, O. Demonstration of local protein synthesis within dendrites using a new cell culture system that permits the isolation of living axons and dendrites from their cell bodies. *J. Neurosci.* **12**, 762–772 (1992).
41. Torre, E. R. & Steward, O. Protein synthesis within dendrites: glycosylation of newly synthesized proteins in dendrites of hippocampal neurons in culture. *J. Neurosci.* **16**, 5967–5978 (1996).
42. Burgin, K. E. *et al*. *In situ* hybridization histochemistry of Ca^{2+}/calmodulin-dependent protein kinase in developing rat brain. *J. Neurosci.* **10**, 1788–1798 (1990).
43. Lowenstein, P. R. *et al*. Polarized distribution of the trans-Golgi network marker TGN38 during the *in vitro* development of neocortical neurons: effects of nocodazole and brefeldin A. *Eur. J. Neurosci.* **6**, 1453–1465 (1994).
44. Tiedge, H. & Brosius, J. Translational machinery in dendrites of hippocampal neurons in culture. *J. Neurosci.* **16**, 7171–7181 (1996).
45. Gardiol, A., Racca, C. & Triller, A. Dendritic and postsynaptic protein synthetic machinery. *J. Neurosci.* **19**, 168–179 (1999).
46. Job, C. & Eberwine, J. Identification of sites for exponential translation in living dendrites. *Proc. Natl Acad. Sci. USA* **98**, 13037–13042 (2001).
47. Steward, O. & Schuman, E. M. Protein synthesis at synaptic sites on dendrites. *Annu. Rev. Neurosci.* **24**, 299–325 (2001).
48. Kang, H. & Schuman, E. M. A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science* **273**, 1402–1406 (1996).
49. Kacharmina, J. E., Job, C., Crino, P. & Eberwine, J. Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. *Proc. Natl Acad. Sci. USA* **97**, 11545–11550 (2000).
50. Aakalu, G., Smith, W. B., Nguyen, N., Jiang, C. & Schuman, E. M. Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* **30**, 489–502 (2001).
51. Martin, K. C. & Kosik, K. S. Synaptic tagging — who's it? *Nature Rev. Neurosci.* **3**, 813–820 (2002).
52. Ju, W. *et al*. Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nature Neurosci.* **7**, 244–253 (2004).
53. Bradshaw, K. D., Emptage, N. J. & Bliss, T. V. A role for dendritic protein synthesis in hippocampal late LTP. *Eur. J. Neurosci.* **18**, 3150–3152 (2003).
54. Vickers, C. A., Dickson, K. S. & Wyllie, D. J. Induction and maintenance of late-phase long-term potentiation in isolated dendrites of rat hippocampal CA1 pyramidal neurones. *J. Physiol. (London)* **568**, 803–813 (2005).
55. Smith, W. B., Starck, S. R., Roberts, R. W. & Schuman, E. M. Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron* **45**, 765–779 (2005).
56. Augustine, G. J., Santamaria, F. & Tanaka, K. Local calcium signaling in neurons. *Neuron* **40**, 331–346 (2003).
57. Sawano, A., Takayama, S., Matsuda, M. & Miyawaki, A. Lateral propagation of EGF signaling after local stimulation is dependent on receptor density. *Dev. Cell* **3**, 245–257 (2002).
58. Dudek, S. M. & Fields, R. D. Mitogen-activated protein kinase/extracellular signal-regulated kinase activation in somatodendritic compartments: roles of action potentials, frequency, and mode of calcium entry. *J. Neurosci.* **21**, RC122 (2001).
59. Dudek, S. M. & Fields, R. D. Somatic action potentials are sufficient for late-phase LTP-related cell signaling. *Proc. Natl Acad. Sci. USA* **99**, 3962–3967 (2002).
60. Selcher, J. C. *et al*. A role for ERK MAP kinase in physiologic temporal integration in hippocampal area CA1. *Learn. Mem.* **10**, 26–39 (2003).
61. Wu, G. Y., Deisseroth, K. & Tsien, R. W. Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. *Nature Neurosci.* **4**, 151–158 (2001).
62. Wu, G. Y., Deisseroth, K. & Tsien, R. W. Activity-dependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proc. Natl Acad. Sci. USA* **98**, 2808–2813 (2001).
63. Rhodes, P. A. in *Cerebral Cortex* (eds Ulinski, P., Jones, E. G. & Peters, A.) 139–200 (Plenum, New York, 1999).
64. Golding, N. L., Staff, N. P. & Spruston, N. Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature* **418**, 326–331 (2002).
65. Gasparini, S., Migliore, M. & Magee, J. C. On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. *J. Neurosci.* **24**, 11046–11056 (2004).
66. Sweatt, J. D. Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr. Opin. Neurobiol.* **14**, 311–317 (2004).
67. Gelinas, J. N. & Nguyen, P. V. β-Adrenergic receptor activation facilitates induction of a protein synthesis-dependent late phase of long-term potentiation. *J. Neurosci.* **25**, 3294–3303 (2005).
68. Huang, Y. Y. & Kandel, E. R. D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. *Proc. Natl Acad. Sci. USA* **92**, 2446–2450 (1995).
69. Mockett, B. G., Brooks, W. M., Tate, W. P. & Abraham, W. C. Dopamine D1/D5 receptor activation fails to initiate an activity-independent late-phase LTP in rat hippocampus. *Brain Res.* **1021**, 92–100 (2004).
70. Frey, U., Matthies, H., Reymann, K. G. & Matthies, H. The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region *in vitro*. *Neurosci. Lett.* **129**, 111–114 (1991).
71. Frey, U., Schroeder, H. & Matthies, H. Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. *Brain Res.* **522**, 69–75 (1990).
72. Schultz, W. & Dickinson, A. Neuronal coding of prediction errors. *Annu. Rev. Neurosci.* **23**, 473–500 (2000).
73. Otmakhova, N. A. & Lisman, J. E. D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. *J. Neurosci.* **16**, 7478–7486 (1996).
74. Swanson-Park, J. L. *et al*. A double dissociation within the hippocampus of dopamine D1/D5 receptor and β-adrenergic receptor contributions to the persistence of long-term potentiation. *Neuroscience* **92**, 485–497 (1999).
75. Lee, K. S. Cooperativity among afferents for the induction of long-term potentiation in the CA1 region of the hippocampus. *J. Neurosci.* **3**, 1369–1372 (1983).
76. Bennett, M. R. The concept of long term potentiation of transmission at synapses. *Prog. Neurobiol.* **60**, 109–137 (2000).
77. Bear, M. F. A synaptic basis for memory storage in the cerebral cortex. *Proc. Natl Acad. Sci. USA* **93**, 13453–13459 (1996).
78. Frey, U. & Morris, R. G. Weak before strong: dissociating synaptic tagging and plasticity-factor accounts of late-LTP. *Neuropharmacology* **37**, 545–552 (1998).
79. O'Carroll, C. M. & Morris, R. G. Heterosynaptic co-activation of glutamatergic and dopaminergic afferents is required to induce persistent long-term potentiation. *Neuropharmacology* **47**, 324–332 (2004).
80. Fonseca, R., Nagerl, U. V., Morris, R. G. & Bonhoeffer, T. Competing for memory; hippocampal LTP under regimes of reduced protein synthesis. *Neuron* **44**, 1011–1020 (2004).
81. Frey, U. & Morris, R. G. Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. *Trends Neurosci.* **21**, 181–188 (1998).
82. Washbourne, P., Bennett, J. E. & McAllister, A. K. Rapid recruitment of NMDA receptor transport packets to nascent synapses. *Nature Neurosci.* **5**, 751–759 (2002).
83. Bannister, N. J. & Larkman, A. U. Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: I. Branching patterns. *J. Comp. Neurol.* **360**, 150–160 (1995).
84. Trommald, M., Jensen, V. & Andersen, P. Analysis of dendritic spines in rat CA1 pyramidal cells intracellularly filled with a fluorescent dye. *J. Comp. Neurol.* **353**, 260–274 (1995).
85. Alarcon, J. M., Barco, A. & Kandel, E. R. Capture of the late phase of long-term potentiation within and across the apical and basilar dendritic compartments of CA1 pyramidal neurons: synaptic tagging is compartment restricted. *J. Neurosci.* **26**, 256–264 (2006).
86. Martin, S. J. & Morris, R. G. New life in an old idea: the synaptic plasticity and memory hypothesis revisited. *Hippocampus* **12**, 609–636 (2002).
87. Gasparini, S. & Magee, J. C. State-dependent dendritic computation in hippocampal CA1 pyramidal neurons. *J. Neurosci.* **26**, 2088–2100 (2006).
88. Polsky, A., Mel, B. W. & Schiller, J. Computational subunits in thin dendrites of pyramidal cells. *Nature Neurosci.* **7**, 621–627 (2004).
89. Losonczy, A. & Magee, J. C. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. *Neuron* **50**, 291–307 (2006).
90. Mehta, M. R. Cooperative LTP can map memory sequences on dendritic branches. *Trends Neurosci.* **27**, 69–72 (2004).
91. Wilson, M. A. & McNaughton, B. L. Dynamics of the hippocampal ensemble code for space. *Science* **261**, 1055–1058 (1993).
92. Nakazawa, K., McHugh, T. J., Wilson, M. A. & Tonegawa, S. NMDA receptors, place cells and hippocampal spatial memory. *Nature Rev. Neurosci.* **5**, 361–372 (2004).
93. Otto, T., Eichenbaum, H., Wiener, S. I. & Wible, C. G. Learning-related patterns of CA1 spike trains parallel stimulation parameters optimal for inducing hippocampal long-term potentiation. *Hippocampus* **1**, 181–192 (1991).
94. Stewart, M., Luo, Y. & Fox, S. E. Effects of atropine on hippocampal theta cells and complex-spike cells. *Brain Res.* **591**, 122–128 (1992).
95. Jeffery, K. J., Donnett, J. G. & O'Keefe, J. Medial septal control of theta-correlated unit firing in the entorhinal cortex of awake rats. *Neuroreport* **6**, 2166–2170 (1995).
96. Holscher, C. Synaptic plasticity and learning and memory: LTP and beyond. *J. Neurosci. Res.* **58**, 62–75 (1999).
97. Petersen, C. C., Malenka, R. C., Nicoll, R. A. & Hopfield, J. J. All-or-none potentiation at CA3–CA1 synapses. *Proc. Natl Acad. Sci. USA* **95**, 4732–4737 (1998).
98. O'Neill, J., Senior, T. & Csicsvari, J. Place-selective firing of CA1 pyramidal cells during sharp wave/ripple network patterns in exploratory behavior. *Neuron* **49**, 143–155 (2006).
99. Foster, D. J. & Wilson, M. A. Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature* **440**, 680–683 (2006).
100. Guzowski, J. F., McNaughton, B. L., Barnes, C. A. & Worley, P. F. Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. *Nature Neurosci.* **2**, 1120–1124 (1999).
101. Tsien, R. Y. Imagining imaging's future. *Nature Rev. Mol. Cell Biol.* **4** (Suppl.), SS16–SS21 (2003).
102. Brecht, M. *et al*. Novel approaches to monitor and manipulate single neurons *in vivo*. *J. Neurosci.* **24**, 9223–9227 (2004).
103. Mehta, A. D., Jung, J. C., Flusberg, B. A. & Schnitzer, M. J. Fiber optic *in vivo* imaging in the

mammalian nervous system. *Curr. Opin. Neurobiol.* **14**, 617–628 (2004).
104. Miyawaki, A. Innovations in the imaging of brain functions using fluorescent proteins. *Neuron* **48**, 189–199 (2005).
105. Barco, A., Alarcon, J. M. & Kandel, E. R. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell* **108**, 689–703 (2002).
106. Woo, N. H. & Nguyen, P. V. 'Silent' metaplasticity of the late phase of long-term potentiation requires protein phosphatases. *Learn. Mem.* **9**, 202–213 (2002).
107. Woo, N. H. & Nguyen, P. V. Protein synthesis is required for synaptic immunity to depotentiation. *J. Neurosci.* **23**, 1125–1132 (2003).
108. Fiala, J. C. & Harris, K. M. in *Dendrites* (eds Stuart, G., Spruston, N. & Häusser, M.) 376 (Oxford Univ. Press, New York, 1999).

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
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OPINION

Gene–environment interactions in psychiatry: joining forces with neuroscience

Avshalom Caspi and Terrie E. Moffitt

Abstract | Gene–environment interaction research in psychiatry is new, and is a natural ally of neuroscience. Mental disorders have known environmental causes, but there is heterogeneity in the response to each causal factor, which gene–environment findings attribute to genetic differences at the DNA sequence level. Such findings come from epidemiology, an ideal branch of science for showing that a gene–environment interactions exist in nature and affect a significant fraction of disease cases. The complementary discipline of epidemiology, experimental neuroscience, fuels gene–environment hypotheses and investigates underlying neural mechanisms. This article discusses opportunities and challenges in the collaboration between psychiatry, epidemiology and neuroscience in studying gene–environment interactions.

Gene–environment interactions occur when the effect of exposure to an environmental pathogen on a person's health is conditional on his or her genotype. The first evidence that genotype moderates the capacity of an environmental risk to bring about mental disorders was reported in 2002 (REF. 1). Although mental health research into gene–environment interactions0 is new, it seems to be gathering momentum. We argue that, to fulfill its potential, gene–environment interaction research must integrate with neuroscience. Moreover, the gene–environment interaction approach brings exciting opportunities for extending the range and power of neuroscience. Here, we examine opportunities for collaboration between experimental neuroscience and research on gene–environment interactions. Successful collaboration can solve the biggest mystery of human psychopathology: how does an environmental factor, external to the person, get inside the nervous system and alter its elements to generate the symptoms of a disordered mind? Concentrating the considerable resources of neuroscience and gene–environment research on this question will bring discoveries that advance the understanding of mental disorders, and increase the potential to control and prevent them.

Psychiatric genetic approaches

The recent history of psychiatric research that has measured genetic differences at the DNA sequence level can be divided into three approaches, each with its own logic and assumptions. The first approach assumes direct linear relations between genes and behaviour (FIG. 1a). The goal of this approach has been to correlate psychiatric disorders with individual differences in DNA sequence. This has been attempted using both linkage analysis and association analysis, with regard to many psychiatric conditions such as depression[2], schizophrenia[3] and addiction[4]. Although a few genes have accumulated replicated evidence of association with disorder, replication failures are routine and overall progress has been slow[5]. Because of inconsistent findings, many scientists have despaired of the search for a straightforward association between genotype and diagnosis[6], that is, for direct main effects.

The second approach has sought to make more progress by replacing the disorder outcomes with intermediate phenotypes, called 'endophenotypes' (FIG. 1b). Endophenotypes are heritable neurophysiological, biochemical, endocrinological, neuroanatomical or neuropsychological constituents of disorders[7]. Endophenotypes are assumed to have simpler genetic underpinnings than disorders themselves. Therefore, this research approach pursues the hypothesis that it will be easier to identify genes associated with endophenotypes than genes associated with their correlated disorders. Although this approach substitutes the psychiatric diagnosis with an intermediate brain measure, it still searches for direct main effects.

The third approach to psychiatric genetics, unlike the first two approaches, seeks to incorporate information about the environment (FIG. 1c). This gene–environment interaction approach differs fundamentally from the 'main-effect approaches', with regard to the assumptions about the causes of psychiatric disorders. Main-effect approaches assume that genes cause disorder, an assumption carried forward from early work that identified single-gene causes of rare Mendelian conditions. By contrast, the gene–environment interaction approach assumes that environmental pathogens cause disorder, and that genes influence susceptibility to pathogens. In contrast to main-effect studies, there is no necessary expectation of a direct gene-to-behaviour association in the absence of the environmental pathogen. The gene–environment interaction approach has grown out of two observations: first, that mental disorders have environmental causes; second, that people show heterogeneity in their response to those causes[8].