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Functional and structural underpinnings of neuronal assembly formation in learning

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Learning and memory are associated with the formation and modification of neuronal assemblies: populations of neurons that encode what has been learned and mediate memory retrieval upon recall. Functional studies of neuronal assemblies have progressed dramatically thanks to recent technological advances. Here we discuss how a focus on assembly formation and consolidation has provided a powerful conceptual framework to relate mechanistic studies of synaptic and circuit plasticity to behaviorally relevant aspects of learning and memory. Neurons are likely recruited to particular learning-related assemblies as a function of their relative excitabilities and synaptic activation, followed by selective strengthening of pre-existing synapses, formation of new connections and elimination of outcompeted synapses to ensure memory formation. Mechanistically, these processes involve linking transcription to circuit modification. They include the expression of immediate early genes and specific molecular and cellular events, supported by network-wide activities that are shaped and modulated by local inhibitory microcircuits.

Mechanistic studies of learning and memory have mostly focused on specific local correlates such as synaptic physiology and its plasticity, molecular mechanisms of synaptic and neuronal plasticity, or the relative roles of identified neurons and microcircuits in defined forms of learning. Important progress in these major areas of learning and memory research has been summarized in excellent recent reviews[1,2]. On the other hand, while each specific focus has provided invaluable insights, learning and memory ultimately involve complex neuronal network phenomena, which historically could not be addressed explicitly by studies focusing mainly on neuronal and synaptic plasticity. Indeed, until recently, it has not been clear how insights from mechanistic studies of synapses, neurons and microcircuits could be leveraged to develop a network-level understanding of learning and memory processes. These premises might be changing with the recent advent of technology to investigate and genetically control neuronal cell assemblies involved in learning and memory (see below)[3–6].

Neuronal assemblies have been defined as groups of neurons that can be recruited together and activated synchronously, through synaptic connections between them[5–9] (see **Box 1**). They can be viewed as the smallest physical counterparts of representations in the brain, whereby neurons belonging to a particular assembly can be located within several brain areas. Many, and possibly most, individual neurons are thought to belong to several different assemblies, greatly expanding potential coding space in the brain. In learning and memory, neuronal assemblies are thought to form, modify and dissolve dynamically. Assemblies that account for memories must thereby be specified through learning processes. They would then provide access to what was learned upon appropriate recall cues and would support further learning.

Each feature of neuronal assemblies mentioned above raises important unresolved issues, and indeed much remains to be learned about organizational principles of neuronal memory assemblies, the network and synapse dynamics that drive them, and how they relate to coding principles in learning and memory[1]. However, and as outlined below, recent progress has been remarkable. This review focuses on possible links between studies of neuronal assemblies and those on the plasticity of synapses, neurons, microcircuits and networks to elucidate mechanisms of learning and memory. Topics covered include the roles of neuronal assemblies in learning and memory, mechanistic challenges in assembly formation and remodeling upon learning, the role of inhibitory microcircuits and how structural and functional synaptic plasticity mechanisms might drive memory assembly formation.

Insights into learning from a neuronal assembly perspective

Neuronal assemblies in learning and memory. It seems likely that assemblies of neurons recruited during learning must ultimately be related to the ensembles of neurons encoding the corresponding memory (see **Box 1**). Indeed, sequences of hippocampal neurons recruited during learning are reactivated, in the same or opposite order but in a temporally compressed way (about tenfold), during quiet wakefulness and during slow-wave sleep, providing evidence that at least some of the neurons engaged during learning are re-engaged, in the same or in opposite order, during offline network processes known to be associated with memory formation[10–16]. In these experiments, neurons with the same place field must be part of a neuronal assembly, but how such assemblies are modified between acquisition and long-term memory formation remains to be determined. Interestingly, coupling the reactivation of a single hippocampal place cell during sleep to a reward signal from the medial forebrain bundle using a brain–machine interface was sufficient to produce goal-oriented learning—i.e., mice aimed for the particular

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Box 1 Terminology

Neuronal assembly: a group of neurons that can be recruited together due to synaptic connections between them, usually as a consequence of a learning process. Neurons belonging to one assembly can be distributed between several interconnected brain areas. Neuronal assemblies can be viewed as the smallest counterparts of representations in the brain and might represent the physical bases of memories. This term is mostly used in the context of learning and memory.

Neuronal ensemble: a population of neurons involved in a particular computation. The notion of ensemble implies that coding is produced by populations of neurons whose individual contributions are noisy but that together produce coherent outputs. The term is mostly used within systems and computational neuroscience to describe a neural network with a particular function.

Engram: the hypothetical physical means through which memories are stored in the brain. Engrams are thought to reflect biochemical and biophysical reactions in the brain induced upon learning, which are maintained as latent traces to allow subsequent memory retrieval.

Preplay and replay: preplay reflects the possibility that spontaneous patterns of neuronal activation occurring just before learning might be recruited through associative mechanisms to represent a learned relationship. Replay involves the reoccurrence of learning-related neuronal activation sequences subsequent to learning. Replay is thought to be an important process in memory consolidation.

Memory consolidation: the processes that stabilize a memory trace after its initial acquisition. Long-term memory consolidation is thought to involve long-lasting changes in the efficacy of pre-existing synaptic connections, as well as formation of new synapses and elimination of pre-existing synapses.

Functional synaptic plasticity: changes in the strength of preexisting synapses. These can be short-term or long-lasting (short- and long-term potentiation or short- and long-term depression). Mechanisms underlying long-term plasticity involve expression of plasticity-related genes, specific retention of plasticity-related components at synapses primed during acquisition (tagging) and changes in the contents of key receptors at synapses, particularly at AMPA-type glutamate receptor subunits. Changes in the contents of AMPA receptors and/or scaffold proteins can be accompanied by corresponding changes in synapse size (expansion or shrinkage).

Structural plasticity: changes in local synaptic connectivity involving the formation of new synapses and/or loss of pre-existing synapses. Such structural plasticity is thought to provide a physical basis for the notion of savings in learning, i.e., the observation that a learned function can be rapidly redeployed at any subsequent time through reactivation of latent traces of the original learning process.

place field on the next day—providing evidence that strong place cell reactivation offline can be decoded to produce explicit memories[17]. Hippocampal place cells can therefore be viewed as examples of neurons in which coding, memory and learning overlap (**Fig. 1**). Whether this is a particular attribute of hippocampal principal neurons and their prominent roles in memory or whether learning-related principal neurons in other brain areas have similar features remains to be seen. Nonetheless, tuning of pyramidal neurons in hippocampal CA1 is not confined to positions in space. For example, studies carried out on epileptic patients have provided evidence that individual temporal lobe neurons can be tuned to particular objects (for example, a famous building) and even to whole concepts including the face, cartoon, written name and spoken name of an actor[18]. In view of the fact that such tuning must result from learning, it seems likely that pyramidal neurons in the hippocampus provide the physical basis for a very large number of cell assemblies tuned to features ranging from spatial coding to percepts in episodic learning and memory.

Because their functions can be interrogated through recall experiments, and because powerful methods have recently become available to genetically label and control their activation, neuronal memory assemblies have become a central focus of studies on learning and memory. The current key operational criteria that define neuronal memory assemblies are (i) their selective reactivation is sufficient to produce behaviorally effective memory recall and (ii) their inactivation prevents memory recall[19–24]. Nevertheless, it is important to bear in mind that neuronal assemblies that are experimentally accessible and are therefore defined as representing the memory are not necessarily identical to the entire ensemble of neurons and synapses representing a learned relationship. Furthermore, some of these assemblies might represent partial aspects of a memory (for example, a relevant cue) whose reactivation is sufficient to elicit a behavioral response. Alternatively, activating a partial aspect of the memory might lead to reactivation of the entire memory assembly (i.e., the engram; see **Box 1**) distributed among several brain systems. Such uncertainties at the interfaces between neuronal coding, partial assemblies and full neuronal memory assemblies likely underlie important yet poorly understood features of coding in learning and memory.

Experimental access to neuronal memory assemblies

As discussed above, the current impact of neuronal assembly research on studies of learning and memory relates to the powerful ways through which assemblies can be targeted and interrogated functionally. In most studies discussed in this review, neuronal memory assemblies in rodents have been defined and accessed genetically by virtue of learning- and recall-induced expression of the immediate early genes *cFos* or *Arc* (also called *Arg3.1*; ref. 3). Expression of these genes is necessary for long-term memory consolidation (see **Box 1**) in several types of learning, suggesting general roles in memory processes[4,25–27]. As discussed in excellent recent reviews, cFos and Arc neuronal assemblies account for many of the features that have been assigned to memory engrams, the structural counterparts of memory traces in the brain discussed initially by Richard Semon and later by Donald Hebb[5–8,28]. However, and as discussed in this review, elucidating the precise relationships between cFos- and/or Arc-defined assemblies and memory engrams will require further research.

Expression of cFos and Arc does not simply reflect previous activity in neurons but instead appears to reflect induction of activity-related plasticity in a subset of active neurons[5,6]. What exactly constitutes that plasticity and how cFos and Arc-expressing neuronal subsets are delineated are important but unresolved questions. Both proteins are subject to complex transcriptional, post-transcriptional and post-translational controls that link their expression to plasticity processes in neurons. The relationship between neuronal activity during learning and expression of cFos and Arc has remained unclear, but current evidence is consistent with the notion that their expression is triggered selectively in highly active neurons that have also triggered signaling pathways involving calcium, cAMP and the MAP kinase ERK, as well as plasticity-related growth factors such as BDNF (brain-derived

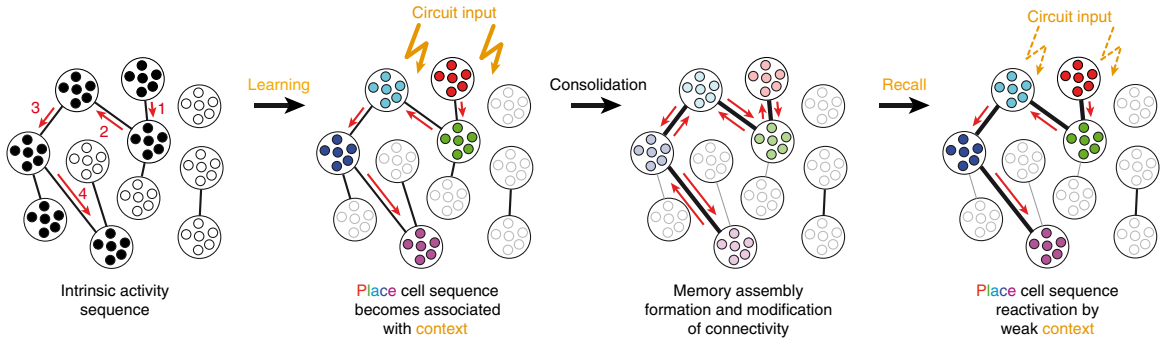

Figure 1 Relationship between neuronal tuning and learning-related assembly formation in hippocampal place cells. A set of content-neutral assemblies (large circles) of individual interconnected hippocampal neurons (small circles) that exhibits a spontaneous sequence (arrows 1–4) of activity (filled black cells) is converted into a place-cell assembly sequence (colored circles) during learning of a spatial navigation task. This activation pattern is driven from external circuits (yellow lightning bolts) representing a context association. During memory consolidation, when external input is absent and the memory sequence is replayed in the same order, connectivity within and between the assemblies is strengthened (thick black lines), and connectivity with unrelated assemblies is weakened (faint gray lines) such that a subsequent brief or weak presentation of the context (dotted yellow lightning bolts) during memory recall restarts the same sequence of place cell assemblies.

neurotrophic factor), all leading to the activation of key transcription factors such as CREB (cAMP response element-binding protein)[29]. The latter is a central point of convergence for neuronal signaling pathways involved in plasticity and has been causally related to memory in organisms ranging from *Aplysia* to humans[2,30]. CREB activation can enhance neuronal excitability, a process important for memory formation[31–33]. Although signaling pathways impinging on CREB have clearly been associated with plasticity and memory, there is no simple signaling sequence that appears to predict cFos expression at the level of individual neurons. Furthermore, the circuit mechanisms required to link learning-related neuronal activity to cFos expression remain to be identified. As discussed below, these might include competitive processes among activated neurons implemented by local inhibitory networks.

Allocating neurons to memory assemblies

Recent groundbreaking studies have provided compelling evidence that enhancing the excitability of small subsets of randomly selected neurons (by overexpressing CREB, manipulating potassium channels or by chemogenetics) just before learning greatly increases the likelihood that those neurons will become part of the corresponding neuronal memory assemblies. At the same time it reduces the likelihood that other neurons in the same brain structure will become part of that memory engram[9,21,31–36]. Remarkably, these manipulations of small and random subsets of neurons greatly enhanced the strength of fear memories, as assessed by freezing to context, suggesting that activation thresholds for assembly recruitment are correlated to memory strength.

In conceptually related experiments, optogenetic activation of random subsets of mouse piriform cortex or basolateral amygdala principal neurons shortly before aversive or appetitive odor-associated Pavlovian learning led to efficient incorporation of those optogenetically activated neurons into corresponding functional memory assemblies[37,38]. In another striking example of how assembly recruitment relates to learning, contextual fear conditioning paired with the activation of neurons that had previously been tagged by cFos under neutral conditions led to a 'false' association memory, in that mice learned to fear the otherwise neutral context[22]. Similar protocols were applied to produce binding of unrelated memories[21,24,39]. Taken together, these studies provide evidence that those neurons that are most efficiently activated during learning dominate memory allocation processes (**Fig. 2**). Although these results demonstrate sufficiency and sometimes also necessity, as discussed above, they do not necessarily indicate that these experimentally created assemblies are identical to a complete neuronal assembly associated with a memory.

Coding and memory in hippocampal cell assemblies

What might be physiological counterparts of experimentally induced highly excitable neurons in memory assembly formation? Key studies have provided evidence for preplay of place cell sequences in naive mice before spatial exploration (see **box 1**). Mice that never ran through a maze could produce 10–15 different place cell sequences several hours before the first navigation protocol, and selected one or a few of those hippocampal sequences during navigation[40,41]. These findings suggest a scenario in which network dynamics intrinsic to the hippocampus, and possibly unrelated to previous experience (but see a recent diverging view[42]), might prefigure assemblies recruited for place-related coding in episodic memories[43–45]. Such preconfigured assemblies might be physiological counterparts of highly excitable neurons in the artificial memory studies described in the previous section (**Fig. 2**). Indeed, and consistent with the notion that place-cell activity might reflect intrinsic excitability features of neurons in addition to the strength of their synaptic input, silent cells in hippocampal CA1 can be rapidly converted into spatially tuned cells by lowering their activation thresholds through somatic current injections[46].

Overall, and as discussed in more detail below, the findings from studies of hippocampal place cells and artificial memories suggest that coding and memory in neuronal networks might reflect selection of particularly excitable neurons or of sequences of excitable neurons from much larger pools of appropriately connected cells (**Fig. 2**). Such excitability might depend on intrinsic membrane properties of these neurons and/or be the result of synchronous synaptic input[47,48]. In most cases (but see ref. 21), experimentally biased memory assemblies are of sizes comparable to those forming in the absence of excessive activation, arguing for the existence of memory allocation processes operating on a competitive basis. According to this influential notion, only a subset of the neurons that could possibly become part of a memory assembly based on their connectivity and learning-related activation is in fact recruited to a particular neuronal memory assembly[4,9,33,34].

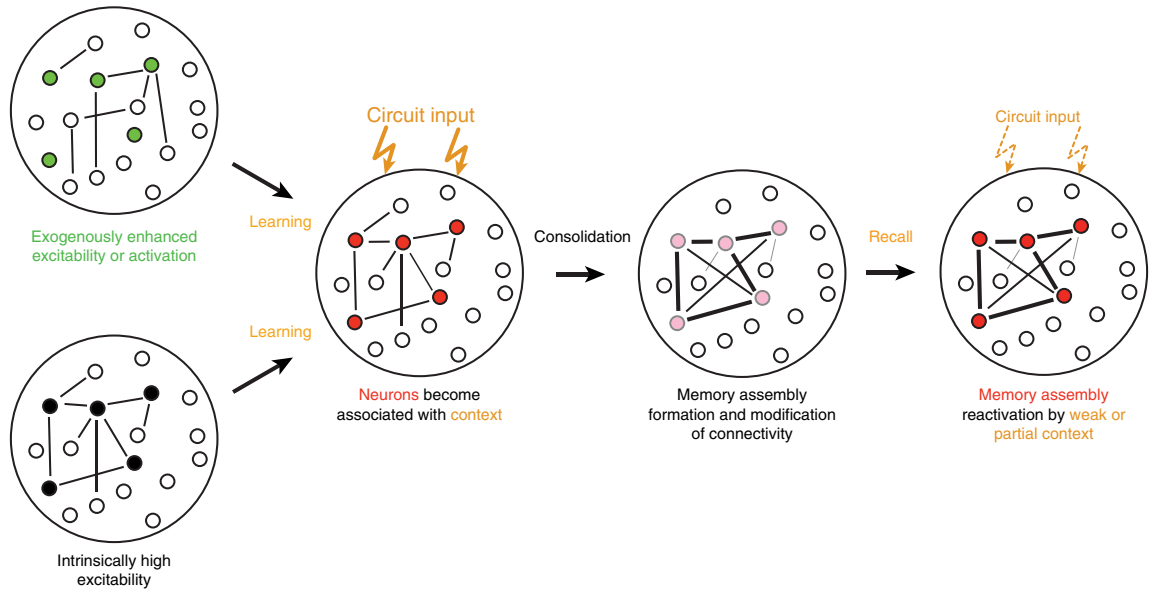

Figure 2 Mechanisms of assembly formation during learning. The excitability of neurons is a main determinant for their recruitment to a memory assembly. Increased excitability, either through experimental (exogenous, green circles) activation of a random subset of neurons (for example, by manipulation of CREB function or optogenetics) or due to intrinsic neuronal properties, enhances the likelihood for those neurons to become activated (red circles) during associational learning driven by context (yellow lightning bolts). During learning and consolidation, synaptic connections (black lines) within the neuronal assembly that is representing learning-related context are modified (stronger connections are symbolized by thicker lines). Even weak or partial exposure to context (dotted yellow lightning bolts) will then reactivate the assembly upon recall.

Mechanistic challenges in memory assembly formation and consolidation

Molecular mechanisms from learning to neuronal memory assembly. A major experimentally tractable challenge is to elucidate molecular mechanisms that link activity and signaling during learning to assembly formation as defined by cFos and/or Arc expression. Immediate early gene products that currently serve as markers of neuronal memory assemblies are detectably elevated from about 45 min after learning or memory recall[49,50]. This delay likely reflects transcription and translation processes required for memory consolidation[25]. However, it is not clear whether only a fraction of the neurons in which appropriate signaling is initiated at the time of learning end up expressing proteins such as cFos, Arc and Zif268, which are known to be essential for long-term memory consolidation. cFos and Arc levels in memory neurons peaked at 60–90 min after learning, but elevated levels of cFos or Arc could still be detected 4 h after acquisition. Moreover, in a fear conditioning model, a second peak of cFos and Arc expression occurred in the hippocampus at around 12–15 h after acquisition, i.e., during the time window that has been associated with completion of long-term memory consolidation[49–54] (**Fig. 3**). The specific role of the second cFos and Arc expression peak in memory assembly formation remains to be determined. Nonetheless, interfering with cFos expression immediately after memory acquisition prevented cFos expression at 12–15 h, suggesting that cFos-dependent processes in memory neurons during the first hours after acquisition are essential for the second peak of plasticity in those neurons, as well as for long-term memory consolidation. Which synaptic plasticity processes are targeted by cFos and Arc is still unclear, but likely candidates include the strengthening of pre-existing synapses, as well as the formation of new synapses in memory neurons[53]. Cellular and molecular pathways that might interface with immediate early genes in memory assembly formation include epigenetic changes, modulation of gene expression (for example, by miRNAs) and changes in the expression of key synaptic proteins such as scaffold proteins and glutamate receptor subunits.

Structure–function relationships in neuronal memory assemblies

A second challenge in understanding memory assembly formation and function relates to the relative roles of individual neurons and their synaptic connections within a given assembly. Highly excitable neurons might function as nodes within memory networks, facilitating binding and association processes among memories, but this may come at the risk of producing interference among memories. Global homeostatic mechanisms that indiscriminately scale down all inputs to such neurons would reduce the risk of interference, but this might not be consistent with their putative function as network nodes. Instead, local dendritic mechanisms could preferentially increase the synaptic weight of particular inputs, such as those belonging to a particular memory assembly. This might provide additional and effective ways to selectively enhance subsets of inputs in such node neurons[9,55–57]. Detailed functional analyses of local networks have indeed revealed the existence of strongly interconnected neurons within larger networks of more weakly interconnected neurons[45,58–62]. In principle, such node neurons might function as a memory assembly core, defining the identity of a particular memory. Related information could then be added, combined or removed flexibly upon subsequent learning processes[45]. However, the roles of such substructures in memory assembly function and specificity remain to be determined.

Assembly remodeling in learning and memory

A further challenge involves elucidating the mechanisms involved in assembly reorganization, from learning to long-term memory consolidation. Establishment of a specific memory assembly implies that a set of neurons selected during learning can be recruited as a group, together with its upstream and downstream neurons to recreate aspects of the particular learning event[9,63]. That, in turn, implies that learning-related connectivity within the neuronal assembly needs to be selectively strengthened[2,9,53,64]. Consistent with these basic requirements, cFos-expressing neurons involved in learning aversive associations underwent robust functional and structural synaptic

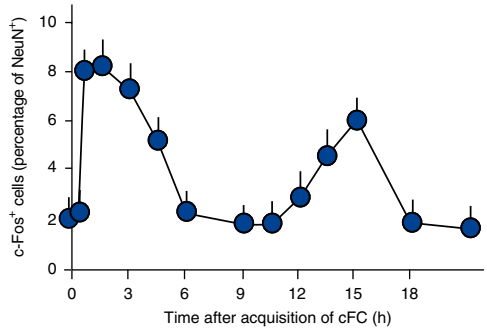

Figure 3 Time course of cFos induction upon contextual fear conditioning. Relative numbers of cFos-positive pyramidal neurons in ventral hippocampus CA3b exhibit two waves of induction after acquisition of contextual fear conditioning (cFC). A first wave is detectable from 45 min on. cFos contents return to baseline levels 6 h after acquisition. A second wave is detectable between 12 h and about 15 h, when it coincides with a long-term memory consolidation time window. Error bars represent s.e.m. Adapted from ref. 54, Nature Publishing Group.

plasticity for many hours after learning (**Fig. 2**)[65–68]. The underlying mechanisms might involve synaptic tagging and capturing processes at pre-existing synapses, as well as cooperative plasticity processes within spatially restricted dendritic domains (see below)[9,55–57,69]. In addition, learning-related replay processes within the neuronal assemblies might serve to strengthen and stabilize new assembly-specific synapses through Hebbian mechanisms[9,70] (see **Box 1**).

While assembly formation during learning might primarily be influenced by intrinsic network properties such as excitability and synaptic connectivity, it probably also involves specific strengthening or remodeling of synaptic circuits. For example, odor or whisker perceptual learning in go/no-go licking tasks strengthened assembly responses and increased the temporal correlation between neuronal pairs of similar response types in motor cortex[71,72]. Instructive inputs from neurons that are involved in the percept likely drive this process[71,72]. Furthermore, a whisker-based texture discrimination task promoted the recruitment of additional cortical sensory neurons that project to motor cortex, suggesting that the assembly involved in relaying the percept to motor cortex increased in size[73]. Interestingly, a population of neurons that projected to secondary somatosensory areas and whose activity correlated with touch did not expand but seemed to improve their discrimination[73]. A similar pattern of expansion and refinement of cortical responses was seen in a visual discrimination learning task[74], a purely lever-pressing motor task[75] and in associative fear learning[76]. How these network-remodeling processes interface with assembly selection in learning remains to be determined, but one possibility is that learning-associated remodeling might unfold subsequent to the emergence of an initial learning-related core assembly. In such a scenario, remodeling would operate to refine an initial memory assembly through further learning.

Toward a mechanistic understanding of memory assembly formation and consolidation

Shaping assembly formation through inhibition and disinhibition. Distinct classes of inhibitory neurons, each with unique molecular, morphological and connectivity features, have central roles in shaping network activity[77,78], which make them prime candidates for shaping neuronal assembly formation. Disinhibition could shape plasticity in sensory cortices during development[79] and in adults[80–85]. Furthermore, various forms of inhibition and disinhibition may regulate learning processes[86–89] and structural plasticity[90,91] (see **Box 1**).

An important disinhibitory microcircuit consists of vasoactive intestinal polypeptide (VIP)-expressing neurons, which inhibit somatostatin (SOM)-or parvalbumin (PV)-expressing interneurons that subsequently target their inhibitory activity to excitatory cell dendrites or perisomatic regions, respectively[92]. This microcircuit might in turn be modulated by attentional signals such as acetylcholine[93]. As suggested by the examples described below, inhibitory and disinhibitory microcircuits likely have instrumental roles in learning-related assembly formation and remodeling.

PV basket cells are prominent fast-spiking local GABAergic neurons that provide powerful feedforward and feedback perisomatic inhibition to principal neurons[94]. As such, they are optimally suited to locally restrict the size of neuronal cell assemblies through recurrent inhibition processes, suppressing all but the most effectively recruited neurons during learning and memory formation[77,94]. PV basket cell recruitment enhances network activity by synchronizing principal neurons to support coordinated, fast network activities such as ripples, spindles and gamma-range oscillations[95]. These network activities are critically important for long-term memory consolidation[15]. PV basket cells themselves also exhibit dramatic plasticity upon learning[87]. This consists of elevated (high-PV plasticity) or reduced (low-PV plasticity) levels of PV and GAD67 (an enzyme important for GABA production), accompanied by an increase or decrease, respectively, in the ratio of afferent excitatory over inhibitory synaptic input onto their dendrites. Both types of plasticity are detected from about 6 h upon learning[96]. Late-born PV basket cells implement low-PV plasticity in incremental learning (for example, during early phases of water maze learning), whereas early-born PV basket cells implement high-PV plasticity[96]. Regardless of the type of learning, this PV plasticity is specifically required within a time window 12–14 h after acquisition for enhanced ripple densities, cFos expression and long-term memory consolidation[54]. Failure to stabilize new excitatory synapses onto PV neurons in hippocampal CA3 upon learning led to a deficit in memory precision as shown by the freezing of animals in an unrelated context in a contextual fear-conditioning model[97].

Trial-and-error types of learning, such as water maze navigation and rotarod running, increase VIP-inhibitory inputs onto PV cells and reduce their PV and GAD67 levels (low-PV plasticity)[87]. As trial-and-error learning is completed, the number of excitatory inputs onto PV cells increases (high-PV plasticity), and the network gradually switches to a state dominated by inhibition. Similarly to the completion of trial-and-error learning, rapid associational fear learning increases inhibitory states. Fear-learning further increases inhibition through SOM cells in hippocampus CA1, a process mediated by cholinergic inputs from the medial septum[98]. A similar response was seen in the dentate gyrus in a contextual fear-learning model[99]. Here dentate granule cells activated SOM cells, which provided lateral inhibition to surrounding granule cells, limiting the size of the assembly. In the basolateral amygdala, Pavlovian fear learning involved general disinhibition through the inhibition of both PV and SOM cells[88]. Upon the cue alone, only SOM cells were inhibited, which led to selective gating of sensory inputs onto excitatory cell dendrites, promoting the association. Altogether, these studies suggest a scenario in which incremental learning processes cause long-lasting disinhibition (through VIP inputs), which allows protracted forms of plasticity and facilitates the recruitment of weak memory assemblies. On the other hand, rapid associative learning such as fear conditioning installs immediate strong memories through disinhibition of dendrites (through SOM inputs) during acquisition, followed by increased inhibition of principal neuron somas and proximal dendrites (through PV inputs; **Fig. 4**).

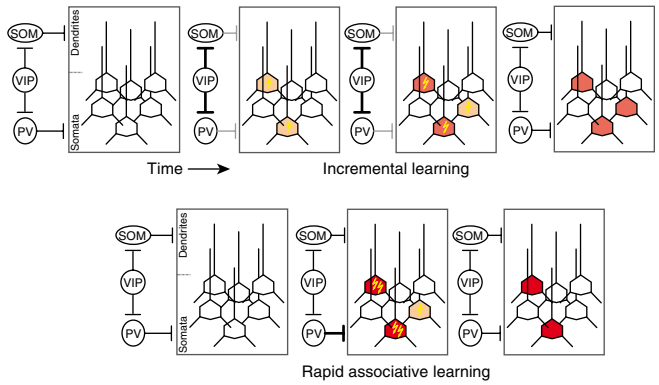

Figure 4 Inhibition and disinhibition as mechanisms to shape memory assembly formation. Activation of VIP interneurons (thicker black lines) during incremental learning increases inhibition (gray lines) of SOM and PV cells, which in turn reduces the inhibition of principal neuron dendrites and somata (upper panels). Under a regime of relatively weak assembly activation (pale red polygons in the second panel), this allows recruitment of other neurons into the assembly, which is thereby readily modified and grows stronger (red polygons in the third and fourth panel). As long as such learning continues, disinhibition keeps gating assembly modifications. At learning completion, the relative impact of VIP cells becomes weak again, and the activity of PV and SOM cells returns to normal, which precludes further modifications to the assembly. In rapid associative learning (lower panels), under a regime of relatively strong assembly induction, high levels of inhibition through PV cells allows only the most strongly activated neurons to be recruited (red polygons) to the assembly, whereas weakly activated neurons are excluded.

Synaptic mechanisms for neuronal assembly formation

Ultimately, neuronal assembly formation must involve synaptic plasticity mechanisms. A key candidate mechanism for recruiting neurons to an assembly is through strengthening of appropriate but weak synaptic connections. Synaptic long-term potentiation (LTP), a mechanism for synaptic strengthening widely believed to play a major role in learning and memory formation, is expressed by the addition of AMPA receptors to synapses[100]. Increased AMPA-receptor incorporation in specific spine populations has been observed in memory and sensory-experience models, suggesting that synapse-specific LTP-like processes are a general feature of neuronal networks that are adjusting their response properties[101,102]. Blocking AMPA-receptor trafficking to synapses during learning impaired memory formation[103,104]. Causal roles for LTP-like processes in learning were recently established by experiments in which animals could be conditioned by a direct optogenetic pairing of auditory thalamo-amygdala afferent activity with foot shocks. A subsequent synaptic depression protocol applied to these afferents impaired the conditioned response, whereas a follow-up LTP protocol could reactivate it again[105]. By selectively activating thalamo-amygdala afferents, this study had direct control over the synapses that were providing the cue, thereby providing evidence that the associative memory in the amygdala likely depended on LTP-like processes in those synapses.

Hebbian spike-time-dependent plasticity, which requires near-coincidental activity of the pre- and postsynaptic neuron, would seem a particularly effective mechanism for the establishment of neuronal assemblies[106,107]. It could selectively strengthen those synapses that are causing the assembly to fire and at the same time drive competition between different neuronal inputs, generating flexibility in assembly composition. However, this would require that those inputs shaping an assembly effectively induce spiking in all of its neurons, a prerequisite likely not fulfilled in randomly and sparsely connected networks. Instead, several studies have suggested that postsynaptic spikes are not strictly necessary for inducing LTP[108–110]. This implies that in diffusely connected and sparsely spiking networks, locally coordinated synaptic activity may recruit weakly connected neurons to an assembly without a need for inputs to first elicit a sufficient number of action potentials in all neurons of the assembly. Synaptic connections within an assembly might then progressively strengthen and ultimately cause inputs to reach spiking threshold[111]. Furthermore, by promoting strong local dendritic depolarizations, long-range cortical feedback projections in layer (L) 1 might influence synaptic plasticity of intracortical networks and thus gain control over local cortical assembly formation[89,110,112]. Taken together, these findings suggest a scenario in which assembly formation may initially involve the recruitment of nonspiking neurons through local dendritic associative forms of plasticity and in which assembly reinforcement is driven by further activity-dependent strengthening of these inputs.

Structural synaptic plasticity for learning and memory

In addition to modifications in the strength of pre-existing synapses, synaptic plasticity involved in assembly formation includes formation of new synapses and elimination of existing synapses. The formation of functional assemblies is constrained by neuroanatomical features and the geometry of neuronal networks[107,113]. In cortex and in many subcortical areas, axons and dendrites of neighboring neurons are heavily intertwined, but only a fraction of them forms actual synapses[113,114]. Accordingly, two randomly selected neurons in cortex are unlikely to be synaptically connected with one another. Nevertheless, and against the odds, intra-assembly synaptic connectivity is high, for example, among neighboring neurons that share receptive field properties[115]. The underlying mechanism might involve the formation of synapses at sites where axons and dendrites are close enough to be connected to one another through the outgrowth of a postsynaptic spine or a presynaptic terminal bouton[113,114,116]. These sites are termed 'potential synapses'. Usually, only a small fraction of them is occupied by an actual synapse. Thus, specific neuronal pairs within an assembly may form a higher-than-average number of synapses at shared potential synaptic sites[113,114,116]. LTP-like processes may assist in this process, as they can rapidly stabilize new synapses on spines[117]. Consistent with the notion of 'potential synaptic connectivity', imaging studies *in vivo* have revealed extensive synaptic turnover, even in the mature and virtually naive rodent brain (**Fig. 5**)[118–126]. The consensus of these studies is that although the majority of cortical dendritic spines and axonal boutons are long-lived, a substantial fraction turns over[127].

Enhanced spine dynamics, consisting of increased formation and/or removal of synapses, correlate with experience and learning in a vast number of diverse models. Long-lasting changes in sensory input promote stabilization of newly formed dendritic spines on mouse L5 cell apical dendrites in sensory cortices[128–132] as well as on mouse L2/3 cells[133,134] and sensorimotor neurons in songbirds[135]. Likewise, mice that engage in motor learning rapidly exhibit increased spine formation on L5 and L2/3 cells in the motor cortex after the onset of learning (**Fig. 5**)[91,132,136–139]. Similar phenomena are seen after perceptual learning in mice[140] and on sensorimotor neurons in songbirds[141]. Upon further training, a small fraction of the newly generated spines is usually stabilized, while the others are pruned. Spine elimination might involve depression of weakly integrated synapses[142,143]. Specific learning-related spine dynamics have also been reported upon Pavlovian conditioning and its extinction[144–147], where the spine dynamics parallel expression of c-Fos and Arc (ref. 145).

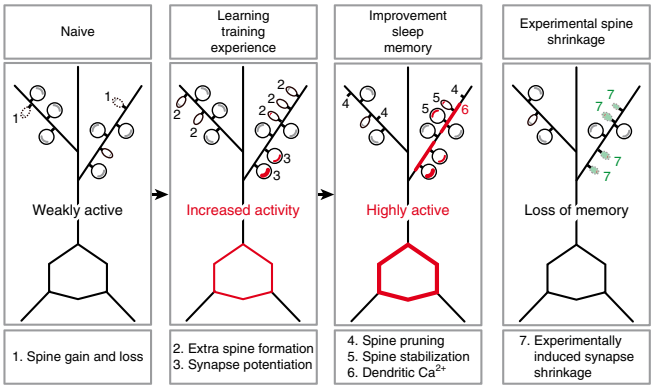

Figure 5 Learning-related spine dynamics and their potential effects on neuronal activity and memory. (1) In naive animals, small dendritic spines are gained and lost at moderate rates. During learning (red), (2) new spines are generated with or without synapses and (3) some pre-existing spines grow in size (representing synapse potentiation), which may occur in a clustered fashion. (4) The majority of the new spines are short lived (transient), but (5) a subset is stabilized, which may occur in a clustered fashion. Plasticity processes may influence stability of neighboring spines (3 and 5), a process that could depend on (6) local dendritic Ca^{2+} events. In turn, clustered activity of spines may cause local Ca^{2+} events, further sculpting spine plasticity. Continuous spine formation and stabilization during learning, sleep and memory formation may increase a neuron's evoked activity and participation in an assembly (red polygons). (7) Targeted shrinkage of spines and synapses that were formed or potentiated during the learning process using photoactivatable Rac1 abolishes the learned behavior, indicating that synapse potentiation is critically important for memory formation. This may have been accompanied by the removal of the neuron from the assembly.

A key question in all these studies is whether spine formation is critically important for changes in network function and behavior. In various learning models, the extent of spine formation and stabilization correlated with the animal's performance at the end of training or conditioning[132,136–141,144,146]. Furthermore, in motor learning, each task induced a new set of spines[136,137,139], suggesting that each memory involves the formation of a specific set of synapses, each probably with a specific set of axons. Conclusive evidence causally relating new spine formation to memory is still lacking, but a recent study showed that specifically compromising those spines that grew upon learning impairs memory[139]. This study involved the design of a construct that allowed transient tagging of newly formed and potentiated synapses upon learning, followed by a photoactivatable Rac1-mediated shrinkage of those tagged synapses. Mice that had successfully learned a motor task showed impaired performance when the new and potentiated synapses were compromised by photoactivation of Rac1 (**Fig. 5**).

From synapse turnover to assembly consolidation

A further set of mechanisms through which synaptic plasticity could mediate memory assembly formation involves rearrangements of local synaptic networks. A feature shared among the learning models that have been used for studying spine dynamics *in vivo* is the increased formation of transient spines, i.e., spines that are present for a day or two and then disappear. A boost in transient spine formation upon learning might provide Hebbian-like plasticity with a window of opportunity to select appropriate connections for memory consolidation[57,116–119,143,148]. In line with this idea, the initial increase and subsequent refinement of cortical assembly activity during motor learning coincides with an increase and selection of transient spines, respectively[75]. In such a scenario, enhanced excitability of assembly neurons might promote both spine formation and stabilization.

While structural plasticity of synapses is likely to be an important factor in the consolidation of neuronal assemblies upon learning, *in vivo* imaging studies have not yet provided detailed insights into how learning-induced spine formation and rearrangements affect local wiring diagrams. Consistent with the notion of 'random outgrowth and selective stabilization', several studies have found that although new spines tend to sprout at many places on dendrites, they stabilize and potentiate in a location-specific manner. This can be specific to cortical domains or cell types[128,130,149], to dendritic domains[91], dendritic branches[90,138,144] or to groups of spines along dendrites[101,137]. Notably, in experiments that likely reflect the refining of neuronal memory assemblies, repetitive learning-related spine stabilization was locally clustered in a motor-task-specific way (**Fig. 5**)[137]. Such clustered spine formation might involve shared instructive signals, for example, as derived from a single axon[137]. Overall, locally cooperative and competitive processes in synaptic plasticity likely provide driving forces for the emergence of small dendritic domains that are dedicated to particular neuronal assemblies (**Fig. 5**). The extent to which the formation of local dendritic domains might be characteristic of assembly consolidation and how it might contribute to specificity and robustness in assembly recruitment remains, however, to be determined.

Conclusions and outlook

Gene technologies, based on the expression of immediate early genes such as *cFos* or *Arc* and opto- or chemogenetics, have allowed remote access to neuronal memory assemblies. This has had a major impact on recent studies of learning and memory. In this review we discussed and illustrated how a conceptual framework based on the notion that learning and memory reflect the formation and consolidation of neuronal cell assemblies can inform mechanistic studies of learning and memory and has prompted hypotheses as to which molecular, synaptic and cellular mechanisms might ultimately relate to behavioral output in learning. Investigations of synaptic plasticity mechanisms should reveal principles through which the recruitment of functional, weak and potential synapses could be orchestrated to gradually sculpt and strengthen initial assemblies, allowing effective and specific retrieval at recall. Related principles might underlie the elaboration and dynamics of neuronal assemblies throughout incremental learning. Research on cellular, subcellular and synaptic mechanisms might further reveal how to strengthen or weaken memories by altering thresholds for retrieval upon recall, which may be of clinical relevance. Furthermore, detailed studies of local network structure should lead to a better and more mechanistic understanding of how cell assemblies emerge in different brain systems and how they are selected during learning. That might include a focus on how local and system-wide coordinated network activities shaped by GABAergic neurons might orchestrate assembly formation and recruitment.

In addition to providing a conceptual framework to productively refocus mechanistic studies of learning and memory, the recent advances highlight challenges for future research. One of them is to elucidate the mechanisms and functional logic of consolidation processes, from the time of acquisition to long-term consolidation of memories 12–14 h after acquisition. A second challenge is to unravel principles that relate intrinsic structural and functional architecture of local networks to assembly formation and consolidation. A further challenge is to the dissect network and synaptic mechanisms that drive the formation of specific assemblies and the associative processes

that underlie learning and memory formation. To this end it will be crucial to obtain a better understanding of how structure relates to function in the brain.

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