PERSPECTIVE | FOCUS
https://doi.org/10.1038/s41593-019-0480-6
nature neuroscience

The next generation of approaches to investigate the link between synaptic plasticity and learning

Yann Humeau[1,2] and Daniel Choquet[1,2,3]

Activity-dependent synaptic plasticity has since long been proposed to represent the subcellular substrate of learning and memory, one of the most important behavioral processes through which we adapt to our environment. Despite the undisputed importance of synaptic plasticity for brain function, its exact contribution to learning processes in the context of cellular and connectivity modifications remains obscure. Causally bridging synaptic and behavioral modifications indeed remains limited by the available tools to measure and control synaptic strength and plasticity in vivo under behaviorally relevant conditions. After a brief summary of the current state of knowledge of the links between synaptic plasticity and learning, we will review and discuss the available and desired tools to progress in this endeavor.

Long-term forms of synaptic plasticity (SP) were discovered in the 1970s, and have since been extensively studied at various synapse subtypes, with the objective of understanding whether and how they mediate the modifications in brain activity that underlie behavioral adaptation. Ex vivo, very diverse SP mechanisms have been identified, including presynaptic and/or postsynaptic induction and expression mechanisms elicited in response to a variety of neuronal activity patterns. In the 1980s and 90s, the global concept that long-term SP is essential for learning and memory emerged: the 'synaptic plasticity and memory' theory[1,2]. This was mainly supported by the observation that in vivo pharmacological targeting of key players of SP, such as NMDA receptors (NMDARs) or downstream signaling pathways, induced memory impairments in behavioral tests[3,4].

Learning and memory have since long been proposed to be multistep phenomena comprising, at least, an encoding and a consolidation phase, respectively occurring during wake and sleep states. However, the causal link between given specific forms of SP and given phases of learning or memory remains poorly established experimentally. In our view, this results from (i) the paucity of available tools that can efficiently and specifically block SP without perturbing basal neurotransmission, (ii) the complexity of studying functional and structural synaptic changes in the behaving animal, (iii) the difficulty in observing the spatial and temporal dynamics of the memory engrams eventually generated by behavioral protocols that mix encoding and consolidation memory phases, and (iv) the discrepancies in stimulus–response time scales between ex vivo and in vivo studies.

Thus, a main challenge is to determine the rules that enable the formation of a coherent memory engram from seemingly random independent synaptic contacts. It will require understanding how and when specific neuronal populations are activated during different memory phases, how they engage in region-specific brain oscillations, and their functional consequences on both local and remote synaptic populations.

To achieve this goal, we urgently need to develop new tools that enable SP changes to be detected at the single contact level, but sampled in large populations. Based on the accumulating knowledge of the molecular and physiological mechanisms allowing bidirectional SP, we must also invent new strategies to manipulate SP in a specific manner. With these tools in hand, we should be in a position to test directly the necessity of SP for learning and memory and to validate or invalidate the numerous theories describing learning at the synaptic, cellular and network levels.

A framework for linking SP to memory

The vast majority of our knowledge about learning and memory at the synaptic and cellular levels results from the seminal discovery of maintained forms of synaptic potentiation (long term potentiation or LTP) in the rabbit hippocampus following repeated stimulations of the perforant path[5]. The repeated identification of contingencies between memory formation and SP mechanisms, such as the involvement of numerous signaling cascades in SP (for example, CaMKII or PKM kinase, or rho and rac GTPase activations), and the decreased behavioral performance of mice when these are inactivated[6,7], have reinforced this link. A key enduring question has been whether the molecular and structural synaptic changes that occur during SP constitute the memory trace itself, or whether they generate the conditions allowing information storage that accompanies memory formation[2]. Indeed, while we would all agree that behavioral adaptation must derive from a combination of synaptic, cellular and network modifications, their respective contributions remain to be disentangled.

Validating a link between SP and memory requires three types of tests: necessity (i.e., requirement of SP for a given memory), saturation or occlusion (i.e., suppression of SP capacity after acquisition of a memory and vice a versa) and erasure (i.e., a transiently applied agent that attacks a putative SP leads to persistent erasure of previously induced memory). This mimics the proposal by Lisman[8] to validate the involvement of given molecules in both LTP and learning. For example, a notable occlusion experiment in the hippocampus showed that one-trial inhibitory avoidance learning in rats produced the same changes in glutamate receptors as induction of LTP with high-frequency stimulation and caused a spatially restricted increase in the amplitude of evoked synaptic transmission in CA1 in vivo[9]. However, of note, some erasure experiments do not establish the need for LTP in memory[10].

To date, the strongest evidence for the role of LTP and long term depression (LTD) in mammalian associative learning expression likely comes from experiments using cued fear-conditioning paradigms. Cued fear conditioning is an implicit associative memory that is easily generated within minutes by the temporal association

[1]Interdisciplinary Institute for Neuroscience, UMR 5297, University of Bordeaux, Bordeaux, France. [2]Interdisciplinary Institute for Neuroscience, UMR 5297, Centre National de la Recherche Scientifique, Bordeaux, France. [3]Bordeaux Imaging Center, UMS 3420 CNRS - University of Bordeaux, US4 INSERM, Bordeaux, France. e-mail: yann.humeau@u-bordeaux.fr; daniel.choquet@u-bordeaux.fr

between an unconditioned stimulus, such as a foot shock, and a conditioned stimulus (a sensory stimulus, often a sound)[11]. Subsequent presentation of the conditioned stimulus alone then induces a conditioned fear response. It has been shown that optogenetic stimulation of auditory inputs targeting the amygdala that induces LTD and LTP inactivated or reactivated the memory[12]. These experiments fulfill both the criteria of erasure and necessity. However, this simple form of memory does not recapitulate the sequence of events associated with explicit memories that require multiple learning sessions to apprehend the complex rules that govern animal choices toward goals or to avoid danger. In the rest of this review we will concentrate on these more complex memory processes, focusing in particular on NMDAR-dependent SP in hippocampal and cortical pyramidal neurons.

Memory encoding from synapses to networks. The encoding process aims at generating a memory engram, here defined as a neuronal ensemble whose reactivation will be sufficient to express the learned memory[13–16]. SP and especially spike-timing dependent plasticity (STDP) may be important for encoding[17,18]. In vitro, STDP can be induced by introducing millisecond delays between pre- and postsynaptic activations. This leads to the stable decrease (LTD) or increase (LTP) of postsynaptic responses. The direction of STDP plasticity can be well predicted by the level of intracellular Ca^{2+} that is generated in the postsynaptic compartment upon neuronal activation[19] and mediated predominantly by NMDARs[20]. Downstream of Ca^{2+} transients, a number of molecular cascades are activated, mediating early functional and morphological synaptic changes (Fig. 1 and see ref. [20]). These rapid, local, but labile modifications are proposed to also generate a synaptic tagging[21] that allows later consolidation—synaptic capture—by subsequent neuronal activations[21]. In reward-based learning, synaptic tagging depends on dopamine, which is proposed to regulate the gain of NMDAR-dependent Hebbian plasticity via CaMKII activity, forming an eligibility trace that determines the time window for reward action[22–24].

STDP could be translated in vivo through network activity. In the hippocampus, spatial memory encoding is associated with brain activities of moderate frequencies (theta, 8–12 Hz, and the low gamma range, 15–40 Hz)[25,26]. Importantly, these oscillations allow repeated, phase-locked activations of neuronal populations within the network, a mechanism that is efficient in producing SP in vitro[27], and they potentially add a pre- or postsynaptic millisecond delay that could enable the orientation of SP toward LTP or LTD. Spatial learning processes also depend on theta-locked hippocampal sharp wave ripples (SPW-Rs): at rest and during wait periods, forward or backward place cell reactivation sequences are initiated within SPW-Rs to code the next trajectory or recall previous trajectories, respectively[28]. Interfering with awake SPW-Rs delays working memory-based spatial rules[29]. Furthermore, upon learning, there is a trial-by-trial dynamic remodeling of hippocampal place cells and cortical grid cells toward the salient rewarded places. This phenomenon seems temporally uncoupled from animal behavior adaptation[30], but is essential[31]. A likely hypothesis is thus that SP is at play to allow generating and organizing these specific network reactivations. However, SP forms involved in the encoding of spatial explicit memories remain to be fully identified, and they may not be restricted to canonical bidirectional plasticity[24,32,33].

Overall, although the concept of an involvement of activity-dependent associative SP in memory encoding and consolidation is strongly anchored in the field and supported by computational models, it is still poorly demonstrated experimentally. In our view, two key steps in linking SP to learning and memory must be developed: (i) the ability to measure synaptic strength at individual, specified synapses in experimental conditions of learning; and (ii) the ability to control and/or perturb SP to measure its specific impact during identified learning steps. In the next section, we will discuss the advantages and limitations of the state of the art strategies in the development of these tools.

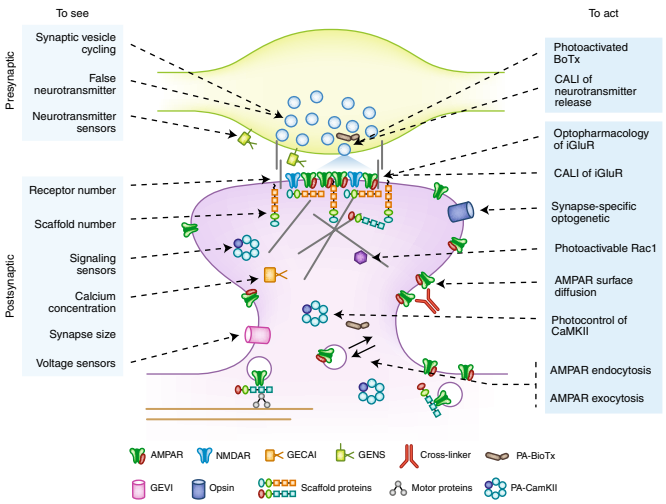

Fig. 1 | Scheme of the synapse, illustrating key functional and molecular steps that allow observing and acting on SP. Left: a list of synaptic elements and means used to evaluate the status of a given synaptic contact. At the presynaptic level, neurotransmitter release can be assessed by detecting synaptic vesicle fusion events and the presence of extracellular neurotransmitters. Postsynaptic proxies of synaptic strength include synapse size and the amount of scaffold proteins or neurotransmitter receptor numbers. Postsynaptic activity can be visualized by calcium dynamics and local membrane potential; plasticity induction can be visualized by the activation of signaling cascades such as CaMKII or Rac. Right: a list of some tools presently used to manipulate these key elements. BoTx, botulinum neurotoxin B; CALI, chromophore-assisted light inactivation; PA, photoactivated; GECAI, genetically encoded calcium indicator; GENS, genetically encoded neurotransmitter sensor; GEVI, genetically encoded voltage indicator.

Visualizing synaptic strength at the single-synapse level in vivo

Electrophysiological recordings of synaptic currents have since long been the gold standard for measuring the efficacy of synaptic transmission. However, despite their proven power to unravel the molecular and physiological properties of synapses, they lack spatial resolution and high-throughput capacity. Other than at well-organized inputs such as the Schaeffer collateral to CA1 pyramidal neurons in the hippocampus, measuring synaptic strength in vivo using extracellular techniques is nearly impossible. Alternatively, intracellular and patch-clamp approaches are limited to recording only a handful of cells simultaneously, very far from the hundreds to thousands of neurons involved in any given learning event[13–16]. The advent of single-unit recordings and other silicon probes has greatly enhanced the multiplexing recording capacity of neuronal activity, but these extracellular approaches do not yet achieve clear synaptic resolution (but see ref. [34] for a state-of-the-art analysis of single-connectivity analysis in vivo). The same applies for most conventional calcium-based in vivo imaging of somatic neuronal activities. Only high-resolution optical imaging has the potential to measure synaptic activity with high enough precision and throughput capacity to envision a direct correlation between synaptic strength and a learning event.

Structural readouts: synapse size as an index for SP. Since their early characterization by Ramon y Cajal[35], dendritic spines have

been demonstrated to undergo changes in size, density and shape, and they are postulated to underlie the anatomical locus of plasticity[36,37]. The size of a dendritic spine has long been considered a proxy for synaptic strength, as it is proportional to the size of its postsynaptic density (PSD) and number of glutamate receptors that, in large part, define the efficacy of synaptic transmission[38–40]. Indeed, large PSDs and spines have been observed to contain more AMPA and NMDA receptors than small ones[41,42].

A major advance for the in vivo visualization of spines has been the development of two-photon imaging[43] and the use of Thy1 transgenic mice expressing fluorescent proteins in a sparse subset of neurons[44]. A breadth of studies reported spine turnover and experience-dependent structural plasticity of spine size and shape in the adult brain[37,45–47]. This has led to the conclusion that sensory experience drives the formation and elimination of synapses and that these changes might underlie adaptive remodeling of neural circuits.

Further refinement of spine morphological imaging has been achieved through stimulated emission depletion microscopy in living brain slices[48] and in vivo[49]. The visual and motor cortices have been extensively used to study sensory-related structural plasticity due to the ease of access to two-photon imaging and relatively straightforward stimulus–response coupling. For example, monocular deprivation episodes double the rate of spine formation, thereby increasing spine density. This establishes a link between functional plasticity and specific synaptic rearrangements, revealing a mechanism for how prior experiences could be stored in cortical circuits[50,51]. Similarly, structural spine plasticity occurs within the barrel cortex during the initial phases of whisker-dependent learning[52].

Structural plasticity in the hippocampus, where SP is proposed to mediate explicit spatial memory (see above), has been harder to access due to its depth. Removing the overlaying cortex and using two-photon stimulated emission depletion microscopy allowed measuring up to 40% spine turnover in the dorsal hippocampus within 4 days[49]. New avenues will be opened with the recent developments of two-photon endoscopes that achieve single spine resolution in live behaving mice[53,54].

Most reports of structural plasticity in vivo refer to relatively slow stimulus–response pairs, occurring over the course of tens of minutes to hours or days. This time scale is quite at variance with the ex vivo studies of structural plasticity observed during high frequency stimulation leading to LTP or its postsynaptic proxy using glutamate uncaging[55]. Although this protocol has been a gold mine for progress in our understanding of the molecular mechanisms of activity-dependent SP at identified spines[56], it is still relatively unclear when and how changes in spine morphology contribute to in vivo experience-dependent SP, especially on the short-term time scales related to memory encoding. In this context, it is particularly important to develop more acute, specific and faithful alternative readouts of synaptic efficacy.

Structural readouts: scaffold and receptor numbers. A more representative readout of synaptic strength than mere spine size is the number of AMPA receptors (AMPARs) in the postsynapse, which directly scales with the amplitude of postsynaptic currents (Fig. 1). Early work has demonstrated that synaptic potentiation is associated with increased AMPAR numbers at synapses[57,58]. Measurement of this parameter in vivo by imaging AMPAR subunits tagged with pHluorin (a pH-sensitive GFP variant that fluoresces only on the cell surface) has allowed the visualization of NMDAR-dependent AMPAR SP in vivo in the mouse somatosensory barrel cortex[59] (Fig. 2). Acute whisker stimulation led to a significant increase in the intensity of surface AMPAR GluA1 subunits (sGluA1) in both spines and dendritic shafts, but only a small increase in spine size relative to pre-stimulation values[59,60]. This further indicates a potential disconnect between functional and structural plasticity

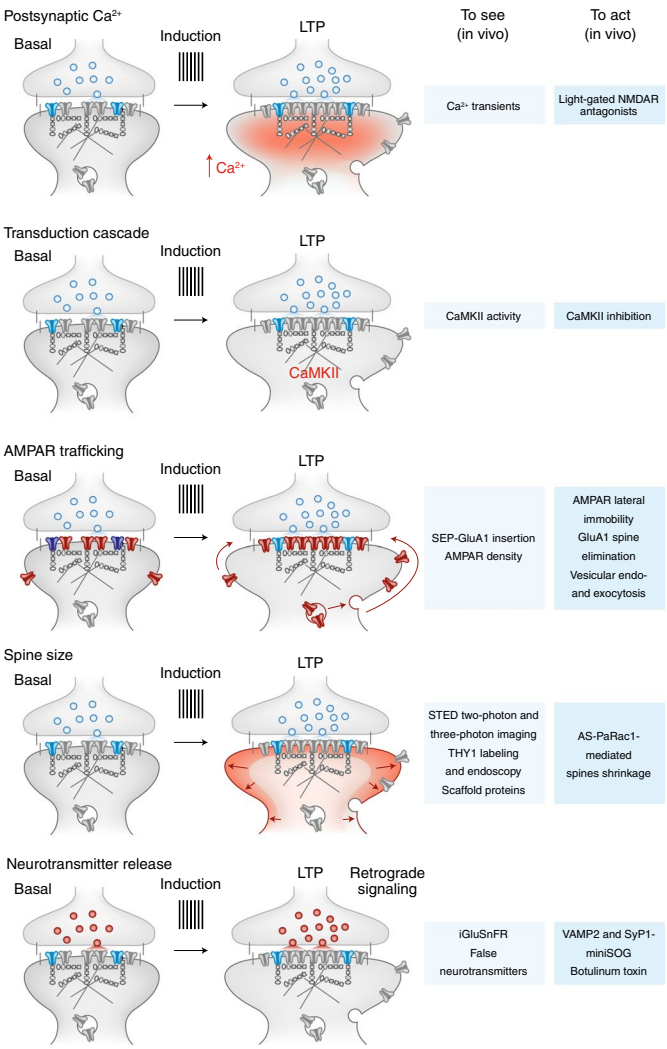

Fig. 2 | Schematic showing state-of-the-art strategies allowing specific and acute control of synaptic strength in vivo. From top to bottom: SP induction is classically dependent on NMDAR opening triggering iCa^{2+} increase. Light-gated NMDAR antagonists may help precisely control LTP induction in vivo. Downstream of Ca^{2+} entry, activation of transduction cascades is exemplified by CaMKII, which translocates to the activated synapses. AMPAR quantity and quality are good proxies of LTP occurrence, and they can be visualized in vivo. Some ways of acting on AMPAR insertion include surface-receptor cross-linking, exocytosis blockade and elimination of GluA1 homomers. Activity-dependent changes in synaptic size were identified in vitro in the late 90s using conventional fluorescent imaging technics. Refined versions are now commonly used in vivo. A recent strategy for eliminating activated spines has been developed based on Rac1 activation. Finally, even if they do not exist at all synapses, presynaptic expression mechanisms, often activated by retrograde signaling molecules, can be detected as changes in release probability using neurotransmitter sniffers or false fluorescent neurotransmitters. Synaptic vesicle fusion processes can be affected by light-gated bacterial toxins or CALI strategies targeting vesicular and SNARE proteins. SEP, super ecliptic phluorin; miniSOG, mini-singlet oxygen generator.

and suggests that spine size may not always be a reliable proxy for SP. Chemical labeling for visualizing native AMPARs in live neurons based on a covalent chemical graft of small fluorophores to AMPARs[61] also has the potential to longitudinally track the amount of synaptic receptors. A more indirect molecular correlate to synaptic strength is the number of PSD-95 molecules, which play a key

role in anchoring AMPARs at synapses[62,63]. Although PSD95 levels seem indeed central to set synaptic strength, and rapid changes in synaptic PSD95 content have been reported in vivo[64,65], a direct correlation between PSD95 levels at identified synapses and learning is still missing. Of note, the AMPAR—and, even less so, scaffold protein—content of a synapse is only an approximation of synapse strength and does not take into account other variables such as changes in transmitter release or local nanoscale pre- and/or postsynaptic organization[66–68]. The latter are unlikely to be observable in the brains of behaving mice in the near future due to current technical limitations of super-resolution approaches.

Functional postsynaptic readouts. There is still, at present, a serious lack of adequate readouts of functional synaptic strength in vivo with individual spine resolution (see review in ref. [69]). The current gold standard high-throughput functional readout of cellular activity in vivo is arguably intracellular calcium levels measured by genetically encoded calcium sensors. These are now extensively used to image cellular activity in wide-fields and volumes in vivo, in concert with various behavioral tasks. Spine calcium levels can also be measured in vivo using calcium indicators[70,71]. However, basal transmission does not always trigger detectable changes in spine calcium levels, and increases in spine calcium are dependent on the opening of NMDARs or voltage-dependent calcium channels. Hence, this readout is of relatively limited use when trying to measure fine changes in synaptic strength that could occur as a substrate of learning. Ideally, one would like to directly image voltage changes with spine resolution. Recent improved near-infrared voltage indicators and targeted gene expression schemes enable simultaneous in vivo recordings of supra- and subthreshold voltage dynamics in multiple neurons in the hippocampus of behaving mice[72]. Tremendous progress has been made in imaging SP-related signaling cascades, from CaMKII activation[73] to a whole biochemical cascade during SP[56], but these are nevertheless indirect correlates to synaptic strength.

An alternative strategy is to measure the occurrence of SP in ex vivo acute slices prepared from animals that have undergone behavioral tasks. There, the whole breadth of approaches that allow ex vivo SP measurements becomes available (Fig. 2), such as measuring NMDAR/AMPAR ratios[12,74,75] or incorporation of GluA1 AMPAR subunits at dedicated synapses. This strategy has, for example, been used at thalamic excitatory projections to the lateral amygdala after fear conditioning[76]. These complement other strategies to explore the level of AMPAR function at synapses such as LTP occlusion, miniature analysis or input–output curves[77,78]. In any case, these physiological measurements in ex vivo preparations all share the same limitations of being temporally decoupled from behavioral learning. However, their combination with engram cellular labeling methods—such as CaMPARI[79]—may still be useful to validate the existence of learning-associated SP at specified synapses in the future.

Functional presynaptic readouts. Measuring vesicular release has long represented a way to track the activity of specific neuronal outputs independent of postsynaptic processing and as a proxy to measure synaptic strength (Fig. 1). Some of these approaches are now maturing to reach synapse resolution in vivo. In cultured neurons, imaging pHluorin-tagged members of the release machinery or transmitter transporters has been the technique of choice to measure vesicular release[80]. However, to our knowledge, the poor signal-to-noise ratio of pHluorin has precluded its use in vivo. Vesicle cycling in vivo can be assessed using SynaptoZip, a genetically encoded bait moiety deployed in the vesicular lumen designed to capture a labeled alien peptide, Synbond, upon exocytosis[81] (Fig. 2).

Fluorescent false neurotransmitters provide a first means for optical imaging of synaptic vesicular content release, for example at individual dopaminergic presynaptic boutons, and an approach for studying SP in brain slices[82] and in vivo[83]. This approach has been extended to several other transmitters, including norepinephrine, in vivo[84]. An important step forward in measuring endogenous transmitter release has been achieved through the development of genetically encoded fluorescent sensors. The development of the glutamate sensor iGluSnFR[85,86] has opened the way to measuring synaptic transmission in vivo at the single-synapse level with a high signal-to-noise ratio. A similar strategy has been used for developing sensors for dopamine[87,88] and acetylcholine[89]. These sensors could be employed to access the roles of neuromodulatory circuits, or other co-transmitters, in shaping glutamatergic SP and learning, and possibly also in linking them with memory deficits like Alzheimer's disease, especially with an acetylcholine sensor. Ideally, a combination of sensors simultaneously measuring AMPAR content and glutamate release levels would allow a perfect monitoring of presynaptic release and synapse strength.

Manipulating SP in vivo

To investigate the relationship between SP and learning requires that techniques to manipulate synaptic transmission on demand are used in complement to those assessing synaptic strength (Figs. 1 and 2). Ideally, these should be specific for given forms of plasticity and cell type, would not affect basal transmission, and should allow precise spatiotemporal control. To date, two broad categories of tools have been used: pharmacological and genetically engineered.

Optopharmacology. A recent refinement in the pharmacological approach to spatiotemporal control of synaptic activity lies in the manipulation of receptor activity with light-sensitive receptor switches (optopharmacology). All three of the iGluR subtypes have been targeted with photoswitchable agonists or antagonists (references in ref. [90]). Optogenetic control of SP by controlling NMDARs would be more useful for studying the link with memory, as basal transmission is not affected. A strategy combining unnatural amino acids and azobenzene photochemistry was recently developed to reversibly control the potential of a set of synapses to undergo NMDAR-dependent plasticity[91]. Other more drastic methods have been developed to specifically and irreversibly inactivate homomeric GluA1 AMPARs in vivo using chromophore-assisted light inactivation[92], suggesting a direct link between the incorporation of GluA1 homomers at potentiated synapses and fear memory. If refined to target specific AMPAR complexes or release processes[93], these approaches may prove powerful to study the link between SP and learning.

Preventing synapse activity. A radical approach has been developed to destroy potentiated synapses with a synaptic optoprobe, AS-PaRac1 (activated synapse-targeting photoactivatable Rac1) that labels generated or potentiated spines and induces their selective shrinkage upon illumination[94]. In vivo imaging of AS-PaRac1 revealed that a motor-learning task induced substantial synaptic remodeling in a small subset of neurons. The acquired motor learning was disrupted by the optical shrinkage of the potentiated spines. On the presynaptic side, botulinum neurotoxin B has been engineered to be activated with blue light[95] and could optically disrupt excitatory neurotransmission (Fig. 2). These approaches may be useful to precisely localize the 'synaptic engram' of a given learning task. A more specific and reversible control of spine activity could be achieved through manipulations of their electrical potential by activity-dependent expression of channel rhodopsin using a hybrid RNA–protein approach[96]. This enables selective tagging of potentiated spines following the encoding of a novel context in the hippocampus. This approach, if generalized to express depolarizing

and hyperpolarizing opsins at the postsynaptic site, would provide a lever to manipulate STDP threshold in situ during identified memory processes.

Manipulating signaling. SP depends on a set of complex signaling cascades that eventually lead to changes in synaptic receptor content and type or to variations in transmitter release properties. A major effort is devoted to developing light-sensitive probes to measure and control these signaling cascades to establish the link between SP and learning. Initial work used plain dominant–negative or dominant–positive variants of a variety of kinases. Knock-out of CaMKII-α, the central kinase to SP[6], or knock-in of a mutant form that cannot autophosphorylate (T286A)[97] or is catalytically dead (K42 M)[98] greatly reduces LTP and memory, reinforcing the link between plasticity and memory. Memory erasure experiments have similarly confirmed a critical role of CaMKII in memory storage[99]. Light-sensitive inhibitors of CaMKII have been recently developed[100]. These tools could prove invaluable to block NMDA-dependent SP at identified synapses and at defined memory steps. The differential and time gated use of other photoswitchable kinases[101] is likely to enlighten our understanding of the link between the different phases of SP and memory, respectively.

On another side, a breadth of studies has identified various post-translational modifications of AMPAR subunits[102] or associated auxiliary proteins[103] that have regularly been correlated to phases of learning. For example, calcium-permeable AMPAR dynamics have been proposed to mediate fear-memory erasure[104], while LTP and memory formation, but not basal transmission, are substantially impaired in mice lacking CaMKII phosphorylation sites of TARP γ-8[105]. Developing tools to specifically image or modify these post-translational events at identified synapses would be invaluable to link SP to learning.

Receptor trafficking. A key paradigm shift in understanding the molecular basis of SP emerged at the end of the 90s through the discovery that AMPARs are not stable in the PSD, but are constantly exchanged through trafficking between the PSD, extrasynaptic membrane and intracellular compartments[106]. These trafficking pathways involve lateral diffusion in the plane of the plasma membrane and vesicular trafficking between intracellular compartments and the neuronal surface. Synaptic strength, as set by the number and organization of AMPARs in the PSD, thus results from a dynamic equilibrium of AMPARs between sub-compartments and their reversible stabilization at the PSD through interactions with scaffold elements. Importantly, most processes of SP at the postsynapse seem to involve either regulation of these trafficking pathways[102,106–108] or of one of the AMPAR stabilization mechanisms[103], while there seems to exist a clear separation between the molecular pathways involved in the control of basal transmission and those engaged for SP. This creates opportunities for specific exogenous control of plasticity.

Three main trafficking pathways have been targeted to modulate SP. AMPAR exocytosis was first identified as playing an important function in increasing AMPAR numbers at synapses for LTP[109], thanks to the use of the SNARE-targeting botulinum toxin. Twenty years later, the first light-sensitive botulinum toxin was designed[95] to block transmitter release. This type of tool will be of great interest to those studying the role of AMPAR exocytosis in the control of synaptic potentiation and its link to memory processes. Identifying the molecular machinery involved in regulated AMPAR exocytosis[110,111] will allow development of tools to refine the link between plasticity and learning.

Conversely, AMPAR endocytosis has been extensively involved in LTD[112], and recent work has correlated the endocytic pathway involved in LTD with forgetting[113]. Manipulating endocytosis could thus prove as valuable as exocytosis in studying the links between plasticity and learning. Recent work using a new optogenetic tool, PhotonSABER, which acutely inhibits AMPAR endocytosis during LTD, shows that it plays a direct role in motor learning at parallel fiber–Purkinje cell synapses in the cerebellar flocculus in vivo[114].

Finally, the now widely accepted model of AMPAR dynamic equilibrium posits that endo- and exocytosis processes occur mostly at extra-PSD sites[107]. The corollary is that receptors enter and exit the PSD through lateral diffusion in the plane of the membrane. As synaptic stabilization of receptors encompasses regulated interactions with intracellular, extracellular and transmembrane targets, the concept of reversible and regulated diffusion trapping is becoming center stage for the control of receptor numbers at the PSD[106,115]. We proposed that a straightforward and powerful way to prevent receptor addition or loss to and from the PSD during plasticity, respectively, would be to prevent their surface movement. This is a priori easier said than done, as surface movement depends strictly on Brownian forces that can only be controlled by temperature and viscosity of the plasma membrane, so we developed approaches to prevent receptor surface diffusion through addition of cross-linking elements (antibodies, avidin, etc.). Because around 50% of receptors are naturally immobile—bound to scaffold or extracellular or trans-synaptic partners—upon addition of an exogenous cross-linker, mobile receptors become linked to the most proximal endogenously immobile receptors. The important consequence is that upon addition of a cross-linker, the spatial distribution of receptors will not be sizably affected. We demonstrated that acute cross-linking of surface AMPARs or NMDARs does not noticeably affect their distribution or basal transmission[115–117]. Of note, however, longer exposure (i.e., hours to days) to cross-linking antibodies, in particular pathological ones generated in autoimmune diseases, does modify receptor function, distribution and basal transmission[118,119]. With shorter exposures within the window of innocuous effect of cross-linkers on basal transmission, we showed that AMPAR immobilization completely blocks LTP up to 1 h after induction, likely due to the inability of extrasynaptic receptors to accumulate at synapses tagged for potentiation[115]. In parallel experiments, we showed that application of the same cross-linker in vivo to the dorsal hippocampus drastically decreases fear conditioning, suggesting that AMPAR-mobility-dependent SP is required for this learning paradigm.

This cross-linking tool thus appears to have great potential to allow specific blockade of activity-induced changes in synaptic strength without modifying basal transmission. Refining this tool, making it cell-specific and light-sensitive, thus holds great promise for studying the implication of given modifications in synaptic strength at identified synaptic contacts in diverse networks and behavioral paradigms. At present, one drawback of this approach is that it cannot differentiate between synaptic potentiation and depression, as it likely 'freezes' receptor exchange in both directions. Future developments using extrasynaptic or synapse-specific cross-linkers may overcome this limitation.

Outlook

The causal link between the various forms of SP (pre- or postsynaptic, potentiating or depressing, short-term or long-term, etc.) and learning and memory is still hard to establish. This results from several factors, including that the description of brain activities and oscillations generated in particular brain regions in a given animal state are still patchy and on-going, and that such activities and oscillations are not yet aligned with the more comprehensively described repertoire of SP in ex vivo systems. A new generation of techniques and methods combining genetically driven expression of molecular tools aimed at visualizing and manipulating synaptic strength in vivo is needed and emerging.

The detection of memory-related synaptic changes requires measures of morphological, functional and biochemical characteristics of synaptic contacts without a priori issues. Most actual

proxies for synaptic function and plasticity in vivo are linked to fluorescent reporters. Actual limitations in synaptic studies in behaving animals reside in (i) emitted photon detection due to tissue scattering and detector sensitivity, (ii) recording stability in spatial and temporal dimensions, and (iii) the size and weight of 'onboard' imaging devices, especially in small rodents. The progressive development of transgenic rats, CRISPR–CAS9 gene editing, and miniaturization of recording devices will allow progress. However, we do urgently need technical breakthroughs in reporters and detectors to increase their quantum efficiency and signal-to-noise ratios. This will improve the resolution and decrease phototoxicity for efficient longitudinal, high-resolution and exhaustive synaptic recordings. In addition to technical developments, new generation probes have to go further in the infrared to allow deeper imaging. Development of a direct fluorescent reporter of AMPAR activation would allow a direct readout of synaptic strength, as there should be more (or less, respectively) AMPAR activation following synaptic potentiation (or depression), regardless of the mechanism. More generally, new reporters of surface receptor density to replace the somewhat outdated super-ecliptic pHluorin are urgently needed. In that direction, the development of unnatural amino acid incorporation in receptors seems particularly attractive to add on fluorophores on demand[120].

It is also important to continue identifying the molecules involved in SP and their time courses, particularly as these signals could also potentially be used to mark synapses that have undergone or are available to undergo plasticity. Many current reporters use Förster resonnance energy transfer (FRET), which is difficult to use in vivo, and hence development of direct fluorescent reporters of signaling pathways, such as the ones based on circularly permutated GFP, would be useful. In another line of exploration, there is mounting evidence[66] for a coherent and reciprocal dialog between pre- and postsynaptic compartments based on changes in trans-synaptic protein–protein interactions during SP. The design of probes reporting for pre- and postsynaptic protein interactions could therefore be of interest to test for the potentiation or depression status of a given synapse. It will then remain to apply these novel tools in behavioral tests that allow different memory phases to be clearly separated.

Finally, a key milestone will be reached when the spatial and temporal resolution of these tools is sufficient to operate at the time scale of associative LTP and LTD induction, in a cell-specific manner and with minimal deleterious effects on basal synaptic transmission and globally on brain homeostasis and physiology.

Received: 17 April 2019; Accepted: 26 July 2019;
Published online: 2 September 2019

References

1. Takeuchi, T., Duszkiewicz, A. J. & Morris, R. G. The synaptic plasticity and memory hypothesis: encoding, storage and persistence. *Philos. Trans. R. Soc. Lond. B* **369**, 20130288 (2013).
2. Martin, S. J. & Morris, R. G. New life in an old idea: the synaptic plasticity and memory hypothesis revisited. *Hippocampus* **12**, 609–636 (2002).
3. Collingridge, G. L., Kehl, S. J. & McLennan, H. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol. (Lond.)* **334**, 33–46 (1983).
4. Morris, R. G., Anderson, E., Lynch, G. S. & Baudry, M. Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-d-aspartate receptor antagonist, AP5. *Nature* **319**, 774–776 (1986).
5. Bliss, T. V. & Lomo, T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol. (Lond.)* **232**, 331–356 (1973).
6. Silva, A. J., Paylor, R., Wehner, J. M. & Tonegawa, S. Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science* **257**, 206–211 (1992).
7. Silva, A. J., Stevens, C. F., Tonegawa, S. & Wang, Y. Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. *Science* **257**, 201–206 (1992).
8. Lisman, J. Criteria for identifying the molecular basis of the engram (CaMKII, PKMzeta). *Mol. Brain* **10**, 55 (2017).
9. Whitlock, J. R., Heynen, A. J., Shuler, M. G. & Bear, M. F. Learning induces long-term potentiation in the hippocampus. *Science* **313**, 1093–1097 (2006).
10. Ryan, T. J., Roy, D. S., Pignatelli, M., Arons, A. & Tonegawa, S. Memory. Engram cells retain memory under retrograde amnesia. *Science* **348**, 1007–1013 (2015).
11. LeDoux, J. E. Emotion circuits in the brain. *Annu. Rev. Neurosci.* **23**, 155–184 (2000).
12. Nabavi, S. et al. Engineering a memory with LTD and LTP. *Nature* **511**, 348–352 (2014).
13. Tonegawa, S., Morrissey, M. D. & Kitamura, T. The role of engram cells in the systems consolidation of memory. *Nat. Rev. Neurosci.* **19**, 485–498 (2018).
14. Rao-Ruiz, P., Yu, J., Kushner, S. A. & Josselyn, S. A. Neuronal competition: microcircuit mechanisms define the sparsity of the engram. *Curr. Opin. Neurobiol.* **54**, 163–170 (2019).
15. Liu, X. et al. Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* **484**, 381–385 (2012).
16. Han, J. H. et al. Selective erasure of a fear memory. *Science* **323**, 1492–1496 (2009).
17. Bi, G. Q. & Poo, M. M. Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J. Neurosci.* **18**, 10464–10472 (1998).
18. Markram, H., Lübke, J., Frotscher, M. & Sakmann, B. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**, 213–215 (1997).
19. Bienenstock, E. L., Cooper, L. N. & Munro, P. W. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J. Neurosci.* **2**, 32–48 (1982).
20. Lüscher, C. & Malenka, R. C. NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb. Perspect. Biol.* **4**, a005710 (2012).
21. Seibt, J. & Frank, M. G. Primed to sleep: the dynamics of synaptic plasticity across brain states. *Front. Syst. Neurosci.* **13**, 2 (2019).
22. Yagishita, S. et al. A critical time window for dopamine actions on the structural plasticity of dendritic spines. *Science* **345**, 1616–1620 (2014).
23. He, K. et al. Distinct eligibility traces for LTP and LTD in cortical synapses. *Neuron* **88**, 528–538 (2015).
24. Bittner, K. C., Milstein, A. D., Grienberger, C., Romani, S. & Magee, J. C. Behavioral time scale synaptic plasticity underlies CA1 place fields. *Science* **357**, 1033–1036 (2017).
25. Zarnadze, S. et al. Cell-specific synaptic plasticity induced by network oscillations. *eLife* **5**, e14912 (2016).
26. Trimper, J. B., Galloway, C. R., Jones, A. C., Mandi, K. & Manns, J. R. Gamma oscillations in rat hippocampal subregions dentate gyrus, CA3, CA1, and subiculum underlie associative memory encoding. *Cell Rep.* **21**, 2419–2432 (2017).
27. Larson, J., Munkácsy, E. & Theta-burst, L. T. P. *Brain Res.* **1621**, 38–50 (2015).
28. Xu, H., Baracskay, P., O'Neill, J. & Csicsvari, J. Assembly responses of hippocampal CA1 place cells predict learned behavior in goal-directed spatial tasks on the radial eight-arm maze. *Neuron* **101**, 119–132.e4 (2019).
29. Jadhav, S. P., Kemere, C., German, P. W. & Frank, L. M. Awake hippocampal sharp-wave ripples support spatial memory. *Science* **336**, 1454–1458 (2012).
30. Dupret, D., O'Neill, J., Pleydell-Bouverie, B. & Csicsvari, J. The reorganization and reactivation of hippocampal maps predict spatial memory performance. *Nat. Neurosci.* **13**, 995–1002 (2010).
31. Turi, G. F. et al. Vasoactive intestinal polypeptide-expressing interneurons in the hippocampus support goal-oriented spatial learning. *Neuron* **101**, 1150–1165.e8 (2019).
32. Bittner, K. C. et al. Conjunctive input processing drives feature selectivity in hippocampal CA1 neurons. *Nat. Neurosci.* **18**, 1133–1142 (2015).
33. Hollup, S. A., Molden, S., Donnett, J. G., Moser, M. B. & Moser, E. I. Accumulation of hippocampal place fields at the goal location in an annular watermaze task. *J. Neurosci.* **21**, 1635–1644 (2001).
34. Dupret, D., O'Neill, J. & Csicsvari, J. Dynamic reconfiguration of hippocampal interneuron circuits during spatial learning. *Neuron* **78**, 166–180 (2013).
35. Ramon y Cajal, S. *Textura del Sistema Nervioso del Hombre y de los Vertebrados: Estudios Sobre el Plan Estructural y Composición Histológica de los Centros Nerviosos Adicionados de Consideraciones Fisiológicas Fundadas en los Nuevos Descubrimentos* (Moya, 1899).
36. Segal, M. Dendritic spines: morphological building blocks of memory. *Neurobiol. Learn. Mem.* **138**, 3–9 (2017).
37. Holtmaat, A. & Svoboda, K. Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat. Rev. Neurosci.* **10**, 647–658 (2009).

38. Harris, K. M. & Stevens, J. K. Dendritic spines of CA 1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *J. Neurosci.* **9**, 2982–2997 (1989).
39. Bourne, J. N. & Harris, K. M. Balancing structure and function at hippocampal dendritic spines. *Annu. Rev. Neurosci.* **31**, 47–67 (2008).
40. Nimchinsky, E. A., Sabatini, B. L. & Svoboda, K. Structure and function of dendritic spines. *Annu. Rev. Physiol.* **64**, 313–353 (2002).
41. Noguchi, J., Matsuzaki, M., Ellis-Davies, G. C. & Kasai, H. Spine-neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites. *Neuron* **46**, 609–622 (2005).
42. Nusser, Z. et al. Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* **21**, 545–559 (1998).
43. Denk, W., Strickler, J. H. & Webb, W. W. Two-photon laser scanning fluorescence microscopy. *Science* **248**, 73–76 (1990).
44. Feng, G. et al. Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* **28**, 41–51 (2000).
45. Trachtenberg, J. T. et al. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* **420**, 788–794 (2002).
46. Maletic-Savatic, M., Malinow, R. & Svoboda, K. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* **283**, 1923–1927 (1999).
47. Engert, F. & Bonhoeffer, T. Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**, 66–70 (1999).
48. Nägerl, U. V., Willig, K. I., Hein, B., Hell, S. W. & Bonhoeffer, T. Live-cell imaging of dendritic spines by STED microscopy. *Proc. Natl Acad. Sci. USA* **105**, 18982–18987 (2008).
49. Pfeiffer, T. et al. Chronic 2P-STED imaging reveals high turnover of dendritic spines in the hippocampus in vivo. *eLife* **7**, e34700 (2018).
50. Hofer, S. B., Mrsic-Flogel, T. D., Bonhoeffer, T. & Hübener, M. Experience leaves a lasting structural trace in cortical circuits. *Nature* **457**, 313–317 (2009).
51. El-Boustani, S. et al. Locally coordinated synaptic plasticity of visual cortex neurons in vivo. *Science* **360**, 1349–1354 (2018).
52. Kuhlman, S. J., O'Connor, D. H., Fox, K. & Svoboda, K. Structural plasticity within the barrel cortex during initial phases of whisker-dependent learning. *J. Neurosci.* **34**, 6078–6083 (2014).
53. Zong, W. et al. Fast high-resolution miniature two-photon microscopy for brain imaging in freely behaving mice. *Nat. Methods* **14**, 713–719 (2017).
54. Meng, G. et al. High-throughput synapse-resolving two-photon fluorescence microendoscopy for deep-brain volumetric imaging in vivo. *eLife* **8**, e40805 (2019).
55. Matsuzaki, M., Honkura, N., Ellis-Davies, G. C. & Kasai, H. Structural basis of long-term potentiation in single dendritic spines. *Nature* **429**, 761–766 (2004).
56. Nakahata, Y. & Yasuda, R. Plasticity of spine structure: local signaling, translation and cytoskeletal reorganization. *Front. Synaptic Neurosci.* **10**, 29 (2018).
57. Shi, S. H. et al. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* **284**, 1811–1816 (1999).
58. Hayashi, Y. et al. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287**, 2262–2267 (2000).
59. Zhang, Y., Cudmore, R. H., Lin, D. T., Linden, D. J. & Huganir, R. L. Visualization of NMDA receptor-dependent AMPA receptor synaptic plasticity in vivo. *Nat. Neurosci.* **18**, 402–407 (2015).
60. Roth, R. H., Zhang, Y. & Huganir, R. L. Dynamic imaging of AMPA receptor trafficking in vitro and in vivo. *Curr. Opin. Neurobiol.* **45**, 51–58 (2017).
61. Wakayama, S. et al. Chemical labelling for visualizing native AMPA receptors in live neurons. *Nat. Commun.* **8**, 14850 (2017).
62. Schnell, E. et al. Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proc. Natl Acad. Sci. USA* **99**, 13902–13907 (2002).
63. Bats, C., Groc, L. & Choquet, D. The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron* **53**, 719–734 (2007).
64. Gray, N. W., Weimer, R. M., Bureau, I. & Svoboda, K. Rapid redistribution of synaptic PSD-95 in the neocortex in vivo. *PLoS Biol.* **4**, e370 (2006).
65. Wegner, W., Mott, A. C., Grant, S. G. N., Steffens, H. & Willig, K. I. In vivo STED microscopy visualizes PSD95 sub-structures and morphological changes over several hours in the mouse visual cortex. *Sci. Rep.* **8**, 219 (2018).
66. Tang, A. H. et al. A trans-synaptic nanocolumn aligns neurotransmitter release to receptors. *Nature* **536**, 210–214 (2016).
67. Haas, K. T. et al. Pre-post synaptic alignment through neuroligin-1 tunes synaptic transmission efficiency. *eLife* **7**, e31755 (2018).
68. Nair, D. et al. Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. *J. Neurosci.* **33**, 13204–13224 (2013).
69. Lin, M. Z. & Schnitzer, M. J. Genetically encoded indicators of neuronal activity. *Nat. Neurosci.* **19**, 1142–1153 (2016).
70. Chen, X., Leischner, U., Rochefort, N. L., Nelken, I. & Konnerth, A. Functional mapping of single spines in cortical neurons in vivo. *Nature* **475**, 501–505 (2011).
71. Chen, T. W. et al. Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* **499**, 295–300 (2013).
72. Adam, Y. et al. Voltage imaging and optogenetics reveal behaviour-dependent changes in hippocampal dynamics. *Nature* **569**, 413–417 (2019).
73. Lee, S. J., Escobedo-Lozoya, Y., Szatmari, E. M. & Yasuda, R. Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* **458**, 299–304 (2009).
74. Kourrich, S., Rothwell, P. E., Klug, J. R. & Thomas, M. J. Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *J. Neurosci.* **27**, 7921–7928 (2007).
75. Sepulveda-Orengo, M. T., Lopez, A. V., Soler-Cedeño, O. & Porter, J. T. Fear extinction induces mGluR5-mediated synaptic and intrinsic plasticity in infralimbic neurons. *J. Neurosci.* **33**, 7184–7193 (2013).
76. Rumpel, S., LeDoux, J., Zador, A. & Malinow, R. Postsynaptic receptor trafficking underlying a form of associative learning. *Science* **308**, 83–88 (2005).
77. Humeau, Y. et al. A pathway-specific function for different AMPA receptor subunits in amygdala long-term potentiation and fear conditioning. *J. Neurosci.* **27**, 10947–10956 (2007).
78. Houbaert, X. et al. Target-specific vulnerability of excitatory synapses leads to deficits in associative memory in a model of intellectual disorder. *J. Neurosci.* **33**, 13805–13819 (2013).
79. Fosque, B. F. et al. Neural circuits. Labeling of active neural circuits in vivo with designed calcium integrators. *Science* **347**, 755–760 (2015).
80. Dittman, J. & Ryan, T. A. Molecular circuitry of endocytosis at nerve terminals. *Annu. Rev. Cell Dev. Biol.* **25**, 133–160 (2009).
81. Ferro, M. et al. Functional mapping of brain synapses by the enriching activity-marker SynaptoZip. *Nat. Commun.* **8**, 1229 (2017).
82. Gubernator, N. G. et al. Fluorescent false neurotransmitters visualize dopamine release from individual presynaptic terminals. *Science* **324**, 1441–1444 (2009).
83. Rodriguez, P. C. et al. Fluorescent dopamine tracer resolves individual dopaminergic synapses and their activity in the brain. *Proc. Natl Acad. Sci. USA* **110**, 870–875 (2013).
84. Dunn, M. et al. Designing a norepinephrine optical tracer for imaging individual noradrenergic synapses and their activity in vivo. *Nat. Commun.* **9**, 2838 (2018).
85. Marvin, J. S. et al. An optimized fluorescent probe for visualizing glutamate neurotransmission. *Nat. Methods* **10**, 162–170 (2013).
86. Marvin, J. S. et al. Stability, affinity, and chromatic variants of the glutamate sensor iGluSnFR. *Nat. Methods* **15**, 936–939 (2018).
87. Patriarchi, T. et al. Ultrafast neuronal imaging of dopamine dynamics with designed genetically encoded sensors. *Science* **360**, eaat4422 (2018).
88. Sun, F. et al. A genetically encoded fluorescent sensor enables rapid and specific detection of dopamine in flies, fish, and mice. *Cell* **174**, 481–496. e19 (2018).
89. Jing, M. et al. A genetically encoded fluorescent acetylcholine indicator for in vitro and in vivo studies. *Nat. Biotechnol.* **36**, 726–737 (2018).
90. Barber, D. M. et al. Optical control of AMPA receptors using a photoswitchable quinoxaline-2,3-dione antagonist. *Chem. Sci.* **8**, 611–615 (2017).
91. Klippenstein, V., Hoppmann, C., Ye, S., Wang, L. & Paoletti, P. Optocontrol of glutamate receptor activity by single side-chain photoisomerization. *eLife* **6**, e25808 (2017).
92. Takemoto, K. et al. Optical inactivation of synaptic AMPA receptors erases fear memory. *Nat. Biotechnol.* **35**, 38–47 (2017).
93. Lin, J. Y. et al. Optogenetic inhibition of synaptic release with chromophore-assisted light inactivation (CALI). *Neuron* **79**, 241–253 (2013).
94. Hayashi-Takagi, A. et al. Labelling and optical erasure of synaptic memory traces in the motor cortex. *Nature* **525**, 333–338 (2015).
95. Liu, Q. et al. A photoactivatable botulinum neurotoxin for inducible control of neurotransmission. *Neuron* **101**, 863–875.e6 (2019).
96. Gobbo, F. et al. Activity-dependent expression of Channelrhodopsin at neuronal synapses. *Nat. Commun.* **8**, 1629 (2017).
97. Giese, K. P., Fedorov, N. B., Filipkowski, R. K. & Silva, A. J. Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science* **279**, 870–873 (1998).
98. Yamagata, Y. et al. Kinase-dead knock-in mouse reveals an essential role of kinase activity of Ca^{2+}/calmodulin-dependent protein kinase IIalpha in dendritic spine enlargement, long-term potentiation, and learning. *J. Neurosci.* **29**, 7607–7618 (2009).
99. Rossetti, T. et al. Memory erasure experiments indicate a critical role of CaMKII in memory storage. *Neuron* **96**, 207–216.e2 (2017).
100. Murakoshi, H. et al. Kinetics of endogenous CaMKII required for synaptic plasticity revealed by optogenetic kinase inhibitor. *Neuron* **94**, 690 (2017).

101. Kelleher, R. J. III, 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).
102. Diering, G. H. & Huganir, R. L. The AMPA receptor code of synaptic plasticity. *Neuron* **100**, 314–329 (2018).
103. Nicoll, R. A. A brief history of long-term potentiation. *Neuron* **93**, 281–290 (2017).
104. Clem, R. L. & Huganir, R. L. Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science* **330**, 1108–1112 (2010).
105. Park, J. et al. CaMKII phosphorylation of TARPγ-8 is a mediator of LTP and learning and memory. *Neuron* **92**, 75–83 (2016).
106. Choquet, D. Linking nanoscale dynamics of AMPA receptor organization to plasticity of excitatory synapses and learning. *J. Neurosci.* **38**, 9318–9329 (2018).
107. Choquet, D. & Triller, A. The dynamic synapse. *Neuron* **80**, 691–703 (2013).
108. Kennedy, M. J. & Ehlers, M. D. Organelles and trafficking machinery for postsynaptic plasticity. *Annu. Rev. Neurosci.* **29**, 325–362 (2006).
109. Lledo, P. M., Zhang, X., Südhof, T. C., Malenka, R. C. & Nicoll, R. A. Postsynaptic membrane fusion and long-term potentiation. *Science* **279**, 399–403 (1998).
110. Jurado, S. et al. LTP requires a unique postsynaptic SNARE fusion machinery. *Neuron* **77**, 542–558 (2013).
111. Wu, D. et al. Postsynaptic synaptotagmins mediate AMPA receptor exocytosis during LTP. *Nature* **544**, 316–321 (2017).
112. Collingridge, G. L., Peineau, S., Howland, J. G. & Wang, Y. T. Long-term depression in the CNS. *Nat. Rev. Neurosci.* **11**, 459–473 (2010).
113. Awasthi, A. et al. Synaptotagmin-3 drives AMPA receptor endocytosis, depression of synapse strength, and forgetting. *Science* **363**, eaav1483 (2019).
114. Kakegawa, W. et al. Optogenetic control of synaptic AMPA receptor endocytosis reveals roles of LTD in motor learning. *Neuron* **99**, 985–998.e6 (2018).
115. Penn, A. C. et al. Hippocampal LTP and contextual learning require surface diffusion of AMPA receptors. *Nature* **549**, 384–388 (2017).
116. Heine, M. et al. Surface mobility of postsynaptic AMPARs tunes synaptic transmission. *Science* **320**, 201–205 (2008).
117. Dupuis, J. P. et al. Surface dynamics of GluN2B-NMDA receptors controls plasticity of maturing glutamate synapses. *EMBO J.* **33**, 842–861 (2014).
118. Peng, X. et al. Cellular plasticity induced by anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor encephalitis antibodies. *Ann. Neurol.* **77**, 381–398 (2015).
119. Haselmann, H. et al. Human autoantibodies against the AMPA receptor subunit GluA2 induce receptor reorganization and memory dysfunction. *Neuron* **100**, 91–105.e9 (2018).
120. Neubert, F. et al. Bioorthogonal click chemistry enables site-specific fluorescence labeling of functional NMDA receptors for super-resolution imaging. *Angew. Chem. Int. Edn Engl.* **57**, 16364–16369 (2018).

Acknowledgements

We acknowledge the critical suggestions of A. Getz, F. Lanore and T. Bienvenu on this manuscript. We express our warmest thanks to the many outstanding members of our teams and collaborators that participated in the elaboration of these concepts. This work is currently supported by funding from the Ministère de l'Enseignement Supérieur et de la Recherche, Centre National de la Recherche Scientifique, ERC Grant #787340 Dyn-Syn-Mem, FRM Grant # DEQ20180339189 AMPA-MO-CO and the Conseil Régional de Nouvelle Aquitaine.

Author contributions

D.C. and Y.H. generated the concepts, analyzed the bibliography and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence should be addressed to Y.H. or D.C.

Peer review information: *Nature Neuroscience* thanks Denise Cai, Ryohei Yasuda and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.