REVIEW SUMMARY

NEUROSCIENCE

# Linking glutamate receptor movements and synapse function

Laurent Groc* and Daniel Choquet*

**BACKGROUND:** Since it was established that the cognitive brain is formed mostly by an interconnected network of neurons that communicate at contact sites termed synapses, intense research has aimed at identifying their molecular composition and physiological roles. The discovery that the efficacy of synaptic transmission can be modified by neuronal activity has undoubtedly been a major step in understanding brain function. The various forms of activity-dependent synaptic plasticity were early on proposed to play central roles in brain adaptation, learning, and memory. This motivated neurophysiologists to understand the mechanisms of synaptic plasticity, initially within the sole framework of the quantal properties of transmitter release, largely ignoring the cell biology revolution that was occurring in parallel. In the 1970s, at the same time that synaptic plasticity was discovered, the fluidity of cell membranes was established. Surprisingly, these contemporary findings seldom crossed paths. As cell biologists established the major roles of receptor trafficking in cell function, neurophysiologists still largely viewed synapse function as based on unitary receptor properties and control of transmitter release. It has been only about 20 years since the two fields cross-fertilized and the regulation of receptor movements into and out of synapses emerged as a fundamental mechanism for synaptic plasticity.

**ADVANCES:** Largely based on the development of imaging approaches, including single-molecule tracking, receptors have been demonstrated to undergo a variety of movements, from long-range rapid motor-based intracellular transport, to short-range Brownian surface diffusion, and intercompartment exchange by membrane trafficking. For efficient synaptic transmission, receptors must accumulate in front of neurotransmitter release sites. This is accomplished through a set of interactions with intracellular scaffold proteins, transmembrane auxiliary subunits, or adhesion proteins and other extracellular elements. This duality of receptor movements and stabilization has led to the important concept that the number of functionally responsive receptors at synapses results from the interplay between reversible receptor stabilization and dynamic equilibrium between pools of receptors in the synaptic, extrasynaptic, and intracellular compartments. Coarse receptor distribution along dendrites is largely achieved by intracellular transport. Because exchange of receptors between surface and intracellular compartments seems to occur largely at extrasynaptic sites, reversible surface receptor diffusion trapping at synapses has emerged as a central mechanism to control their availability for synaptic activation. Receptor stabilization and movements are all profoundly regulated by short- and long-term neuronal activity patterns. Reciprocally, evidence has accumulated that receptor movements participate in many forms of synaptic plasticity. Notably, altered receptor movements are observed in many neurodevelopmental, psychiatric, or neurodegenerative pathological models as indicated in the figure [the + and – signs indicate the reported positive and negative modulation of the indicated trafficking and stabilization processes during either normal (blue) or pathological (red) synaptic function]. Whether altered receptor trafficking represents the primum movens of some neurological diseases remains to be established, but is certainly an attractive hypothesis.

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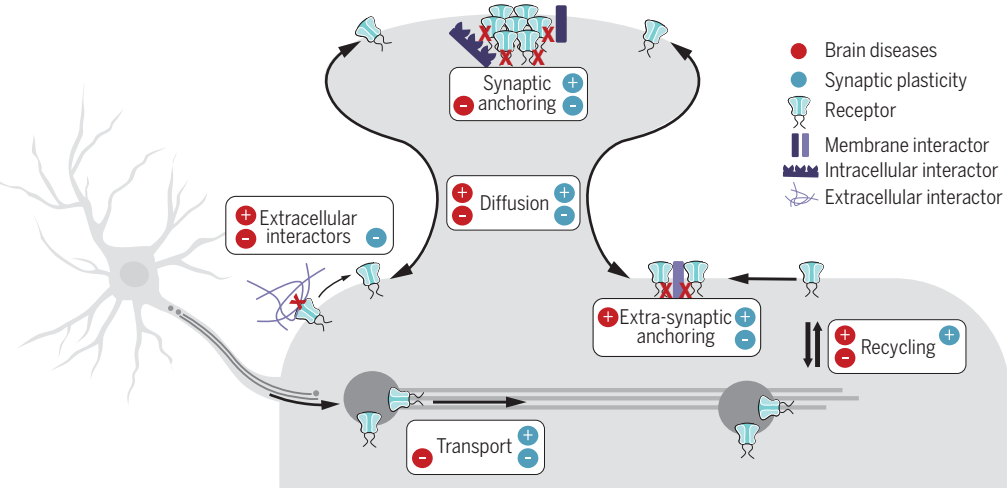
**OUTLOOK:** Most receptor trafficking studies have been performed in reduced experimental systems such as neuronal cultures. This has limited our understanding of the physiological impact of these processes. The development of brighter and smaller probes together with new imaging modalities are on the verge of allowing routine measurement of receptor movements in more physiological settings such as brain slices and in vivo. There is little doubt that qualitatively comparable trafficking modalities will be identified. Reciprocally, tools are being developed to control the various types of receptor movements, from blocking surface diffusion by receptor cross-linking to stopping receptor exocytosis with light-activated toxins. Often, these trafficking tools do not impair basic synaptic function, because resilience of the synapse to trafficking alterations is high owing to the amount of available receptors, as well as the trapping capacities and nanoscale organization of the synapse. Combining measurement and control of receptor movements will not only allow better understanding of their contribution to synaptic and neuronal function but also provide valuable tools for identifying the role of synaptic plasticity in higher brain functions. Controlling receptor movements or stabilization may eventually represent an alternative therapeutic strategy to receptor activity modulation approaches in a variety of synaptic and network-based brain diseases. ■




**Neurotransmitter receptors undergo a variety of large- and small-scale movements.** Movements of large amplitude constantly reshuffle the receptor distribution (e.g., surface diffusion and intracellular transport). Movements at interfaces (e.g., between synaptic and extrasynaptic sites, between intracellular and surface compartments) are of small amplitude but have huge functional impacts. Each of these movements is highly regulated and finely tuned in physiological and pathological conditions.

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# Linking glutamate receptor movements and synapse function

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Regulation of neurotransmitter receptor content at synapses is achieved through a dynamic equilibrium between biogenesis and degradation pathways, receptor stabilization at synaptic sites, and receptor trafficking in and out synapses. In the past 20 years, the movements of receptors to and from synapses have emerged as a series of highly regulated processes that mediate postsynaptic plasticity. Our understanding of the properties and roles of receptor movements has benefited from technological advances in receptor labeling and tracking capacities, as well as from new methods to interfere with their movements. Focusing on two key glutamatergic receptors, we review here our latest understanding of the characteristics of receptor movements and their role in tuning the efficacy of synaptic transmission in health and brain disease.

Activity-dependent modulations of the efficacy of synaptic transmission between neurons, commonly termed synaptic plasticity, are key for brain development and functions, among which are primarily learning and memory (*1*). Alterations in synapse function are believed to be at the origin of brain dysfunction in many diseases. Despite having been studied for decades, the mechanisms of activity-dependent forms of synaptic plasticity remain largely unknown. We believe that this dearth of knowledge originates from our current inability to incorporate the diverse and dynamic biochemical properties of the molecular components of synapses at the nanoscale level into a comprehensive model. This understanding is key as synapses are the elementary components of neural network function underlying behavior.

The efficacy of synaptic transmission has been classically determined by presynaptic transmitter release properties, and postsynaptic receptor numbers and properties. In addition, the presynaptic and postsynaptic compartments have until recently been considered to be relatively independent functional entities. The efficacy of synaptic transmission between two neurons is traditionally viewed as the product of the number of release sites $n$, their release probability $p$, and the elementary postsynaptic response to a release event $q$ (*2*). The total postsynaptic response $R$ following an action potential is thus given by the simple equation

$$R = npq$$

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Although $q$ was initially thought to rely on individual receptor properties and numbers, recent investigations on the nanoscale organization and dynamics of receptors have highlighted the additional level of regulation provided by the precise positioning of receptors. Nanoscale changes in receptor organization within the postsynaptic density (PSD) may control synaptic efficacy without the need for changes in absolute receptor numbers or biophysical properties (*3*–*5*). This is particularly important for receptors that have a low affinity for their ligand, such as AMPA receptors (AMPARs) [median effective concentration ($EC_{50}$) ~100 to 1000 μM], as their probability of activation drops rapidly with distance—tens of nanometers—from the site of transmitter release. This is likely less crucial for higher-affinity receptors such as *N*-methyl-D-aspartate (NMDA) ($EC_{50}$ ~0.1 to 5 μM) or metabotropic glutamate ($EC_{50}$ ~tens of μM) receptors.

The regulation of the efficacy of synaptic transmission through the control of $q$ has emerged as a major postsynaptic mechanism that complements the presynaptic control of transmitter release by $n$ and $p$. Initially, the regulation of $q$ had been attributed to changes in individual receptor properties, such as conductance or permeability through posttranslational modifications (*6*), binding and unbinding of endogenous ligands (*7*), or endogenous pore blockers (*8*). A major paradigm shift occurred when work from many laboratories indicated that neurotransmitter receptors could enter and leave the PSD at rates compatible with the onset of synaptic plasticity—i.e., seconds to minutes (*9*, *10*). This key notion—that neurotransmitter receptors could move in and out of the PSD—laid the groundwork for the concept that $q$ could be controlled through the regulation of the type and number of receptors at the PSD on short time scales, compatible with the early expression of synaptic plasticity after an induction stimulus (*9*). Receptor entry and exit to and from the PSD, first thought to be limited to endocytic and exocytic membrane trafficking between intracellular pools and the neuronal surface (*11*), were rapidly complemented by lateral diffusion in the plane of the membrane (*12*–*14*). Indeed, we and others have proposed that the main pathway for receptors to enter and leave the PSD was Brownian movement of receptors in the plasma membrane powered by thermal agitation (*9*, *15*). Determining the respective roles of changes in receptor nanoscale positioning, absolute numbers, and posttranslational modifications in the expression of synaptic plasticity represents a major challenge.

Receptors are concentrated in the PSD through interactions with a variety of intracellular scaffold, transmembrane, and extracellular proteins. These interactions are often transient and of relatively low affinity, so that the actual number of receptors present in front of neurotransmitter release sites results from an interplay between their movements and their stabilization (*9*). This has led to the concept of reversible diffusion trapping of receptors and more generally to the notion that the number of receptors in the PSD, which largely sets $q$, results from a dynamic equilibrium between receptors in various subcellular compartments. Understanding the regulation of receptor number at synapses can only be achieved by taking into account this duality between receptor movements and stabilization. In this review, we will analyze and comment on our current state of knowledge of the various types of receptor movements—and the entangled stabilization processes, the latest techniques developed to measure and interfere with them, and most notably, their various functions in the normal and pathological synapse.

## Types of receptor movements and methods for their study

Neurons exploit a wide range of categories of subcellular movements at different spatial and temporal scales to cope with their morphological complexity. The extensive distances between the cell body and distal synapses require the recruitment of specific strategies to deliver molecular components to their appropriate sites of action over long ranges but with exquisite precision. This is helped in part by the distribution of the biosynthetic machinery, including the endoplasmic reticulum (ER) and ER-Golgi intermediate compartment (ERGIC), throughout the dendrite (*16*, *17*). Receptor movements can roughly be divided in two categories: (i) the movements associated with the trafficking of vesicles in which the receptors are trapped (vesicles formed during endocytosis, exocytosis, endosomal recycling, intracellular transport, exchange between endoplasmic reticulum, ERGIC,

and Golgi compartments); and (ii) Brownian diffusion in membrane planes of sufficient dimension to allow measurable movements (plasma membrane, ER, Golgi) and solely powered by thermal agitation, but limited by protein-protein and protein-lipid interactions.

### *Methods for measuring receptor movements*

Measuring receptor movements is obviously best performed in live cells, although valuable indirect information can be obtained through time-lapse snapshots in fixed cells or even more crudely through biochemical means. In live neurons, the oldest and still the most popular approach for measuring receptor movements is fluorescence recovery after photobleaching (FRAP), in which fluorescently labeled receptors (Fig. 1A) are locally photobleached through a focused laser beam, and the recovery of fluorescence in the bleached zone measures the rate of receptor mobility in the surrounding membrane environment (*18*). The asymptotic level of recovery tentatively measures the fraction of immobile receptors. It is hard to interpret precisely the mechanistic origin of this value, which mixes the availability of receptors outside the bleached spot and the fraction of receptor trapping sites inside the bleached spot, among other parameters. For FRAP, receptors are most often tagged with fluorescent proteins that allow specific imaging of cell surface receptors (*19*). An interesting alternative that allows labeling of endogenous receptors is the use of monovalent fluorescent ligands. FRAP is a versatile method, easy to implement on commercial microscopes, that can measure receptor movements in neuronal cultures, in brain slices, or even in vivo, and is thus very popular (*13*, *20*). However, it has several severe drawbacks, remaining a bulk imaging approach, lacking spatial resolution (which is limited by diffraction to a couple of hundred nanometers at best; hence it lacks subsynaptic resolution or information on the directionality of movements), and often requiring receptor overexpression. The last point is a serious issue, as receptor overexpression strongly biases mobility measurements—through the saturation of trapping sites, for example. This latter point will be largely overcome by the recent development of CRISPR-mediated tagging of endogenous receptors (*21*), although the signal from tagged endogenous receptors will likely be disappointingly low with respect to that of overexpressed receptors. An interesting electrophysiological alternative uses high-affinity open channel blockers to measure the mobility of synaptically activated receptors (Fig. 1B). Upon transmitter release, activated open receptors are irreversibly blocked. Recovery of synaptic responses over time is then a measure of receptor exchange (*22*).

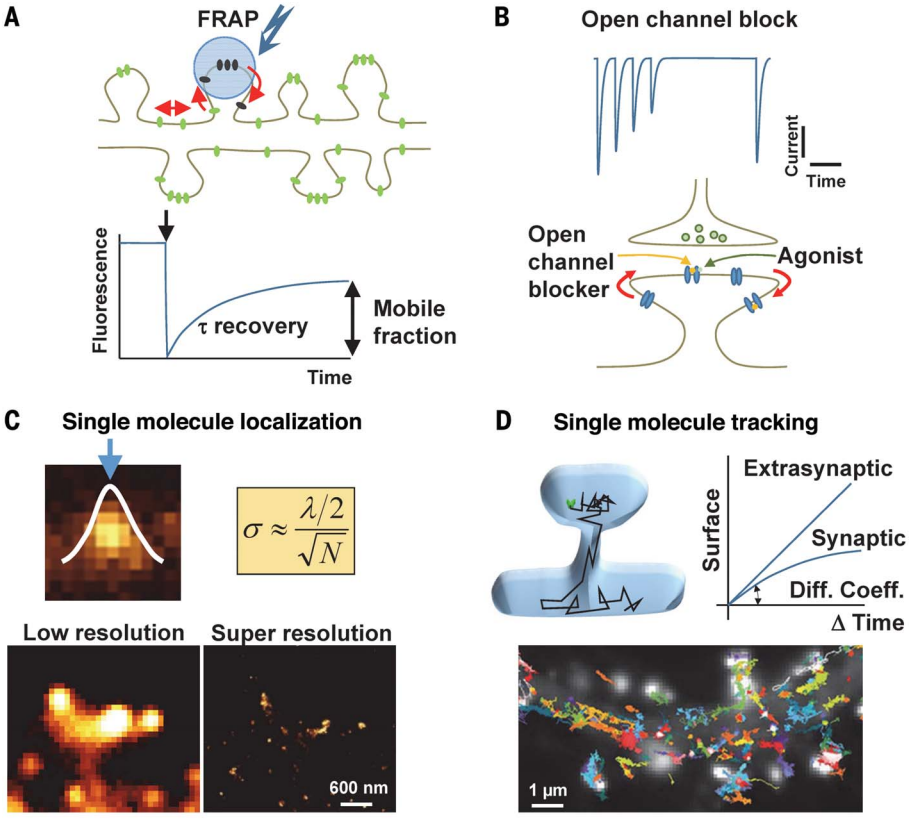


**Fig. 1. Methods for studying receptor movements.** (**A**) In fluorescence recovery after photobleaching (FRAP), fluorescently labeled receptors are locally photobleached and the recovery of fluorescence levels in the bleached area measures both the rate of movement of the receptors and the fraction of immobile receptors. (**B**) Irreversible open channel blockers can be used to block specifically synaptic receptors opened upon transmitter release. The rate of recovery of synaptic responses upon washout of the blocker measures the exchange rate of synaptic receptors. (**C**) In single-molecule localization microscopy, the diffraction-limited fluorescence spot emitted by a single dye can be fitted by a Gaussian curve whose centroid localizes the dye with a precision proportional to the square root of the number of emitted photons. Sequential localization of thousands of dyes allows reconstitution of a super-resolved image. (**D**) Localizing single molecules in live cells in time-lapse imaging allows tracking of individual receptors. The surface explored by the receptors over time allows measurement of their types of movements and diffusion rates. Extrasynaptic receptors typically display free Brownian movements (linear curve), whereas synaptic receptors display confined movements.

The gold standard for measuring receptor movements is single-particle or single-molecule tracking. It has evolved from a relatively coarse approach using receptor-bound nanogold or latex particle tracking to a sophisticated method that can track with high speed (up to kilohertz) and high resolution (in the 10-nm range) single fluorochromes attached to receptors (*23*–*25*) (Figs. 1C and 2). Because of their intrinsic single-molecule sensitivity, these approaches are ideally suited to tracking endogenous receptors that are often expressed at low copy numbers. The two key features needed to achieve single-molecule localization microscopy (SMLM) of endogenous receptors are (i) use of a high-affinity specific labeling method (Table 1 and Fig. 2) and (ii) a sparse (<1 molecule per $\mu m^2$) distribution of emitted fluorescence spots at any given time point to detect individual molecules. Then, each fluorescent spot (i.e., receptor) can be detected with a precision proportional to the square root of the number of emitted photons, to a resolution of 10 nm. Because of the need for efficient photon collection, SMLM has been mostly used in two-dimensional (2D) cultured cells but has also been successfully implemented to track the movement of receptors in brain slices (*26*). SMLM-based super-resolution imaging approaches now enable multicolor maps of receptor distribution to be obtained in live or fixed neurons with 10-nm resolution (Fig. 1D) (*23*, *27*). Single-particle tracking approaches have also been successfully implemented to track the movement of receptors transported into intracellular vesicles (*28*).

### *Intracellular movements*

The intracellular environment is relatively viscous; hence, intracellular membranous compartments can only move slowly unless displaced by active motors. To our knowledge, the

intracellular diffusion rate of vesicles containing receptors in the postsynaptic or dendritic compartment has not been explicitly measured. However, by analogy to the diffusion of presynaptic vesicles that have been amply studied, postsynaptic vesicles may reach diffusion rates on the order of 0.01 $\mu m^2/s$ and could be highly confined (*29*). Efficient vesicle movement through the cytoplasm requires energy. Actin dynamics generate forces that manipulate membranes in the process of vesicle biogenesis, for propelling vesicles through short distances in the cytoplasm to reach their destination (*30*). The actin dynamics–based movements of receptors associated with the exocytosis and endocytosis of vesicles are of critical importance, as they allow such receptors to exchange between intracellular compartments and the neuronal surface, although this represents a distance of only a few nanometers. Our view is that these receptor recycling events are centrally involved in controlling total surface receptor content but only indirectly regulate synaptic receptor numbers.

Longer-distance and more efficient intracellular transport systems involve adenosine 5′-triphosphate (ATP)–driven molecular motors. Actin-based molecular processes have been involved in both endocytic and exocytic processes (*31*). The actin-based motor myosin VI is directly required for activity-induced, clathrin-mediated endocytosis of AMPA receptors for synaptic long-term depression in hippocampal neurons (*32*) and at parallel fiber–Purkinje cell synapses (*33*). Dendritic spines are highly enriched in actin and mostly devoid of microtubules and are thus likely the primary site where myosin-based movements are at play. During LTP induction, myosin Vb or Va interacts with GluA1-containing recycling endosomes in the dendritic shaft to drive their delivery into spines. There, the recycling endosomes fuse with the plasma membrane, leading to both the surface insertion of GluA1 AMPAR subunits and the spine surface growth that

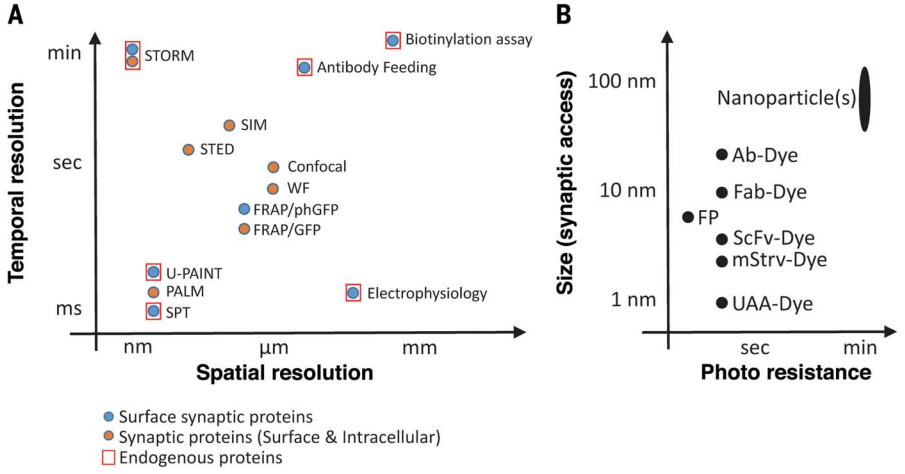


**Fig. 2. Temporal and spatial resolution, as well as synaptic access, achieved by various methods to follow receptor dynamics.** (**A**) Temporal versus spatial resolution determined by various methods commonly used to measure receptor dynamics. Note that spatial resolution spans orders of magnitude. The approaches are differentially suited to measuring the dynamics of surface proteins (blue) versus generic synaptic proteins (orange). Some are particularly suited to tracking endogenous receptors (boxed). STORM: stochastic optical reconstruction microscopy; SIM: structured illumination microscopy; STED: stimulation emission depletion; WF: wide field; FRAP: fluorescence recovery after photobleaching; phGFP: phluorin GFP (green fluorescent protein); U-PAINT: universal point accumulation imaging in nanoscale topography; SPT: single-particle tracking. (**B**) Respective sizes of various reporter-ligand complexes. The smaller the complex, the better is the access to receptors in the synaptic cleft. See Table 1 for abbreviations.

**Table 1. Different labeling strategies for imaging receptor dynamics.** Generally, a reporter must be bound to the target receptor through a ligand. Three broad categories of reporters can be used: fluorescent proteins (FP) genetically fused to the receptors, organic dyes, or nanoparticles (such as quantum dots, gold particles, carbon nanotubes, etc.). The latter two must be attached to the target receptor through a ligand. There is a broad variety of ligands, from antibodies and their derivatives (single-chain ScFv or Fab fragments) to the more recent monomeric avidin, fibronectin domains (FN3), or intrabodies. These ligands can recognize either endogenous receptors or epitope tags genetically fused to the receptors. A promising method for labeling the smallest ligands lies in the use of unnatural amino acids (UAA) that can be labeled by organic dyes. These combinations of labels-ligands-reporters can then be imaged by various methods, including diffraction-limited FRAP and wide-field (WF) or scanning methods (including confocal, spinning disk, and STED microscopy).

| Labeling methods | Label family | Label | Ligand | Reporter | Size | Imaging method |
|---|---|---|---|---|---|---|
| Genetic fusion or modification | Fluorescent protein | FP | - | FP | 4 nm | FRAP, WF, Scanning |
| | | pH sensitive FP | - | FP | 4 nm | FRAP, WF, Scanning |
| | | Photoswitchable FP | - | FP | 4 nm | PALM |
| | Epitope tag | FP, HA, myc, V5, ... | Antibody | Organic dye | 10 nm | SPT, U-PAINT, AF, FRAP |
| | | | ScFv, Fab | Nanoparticles | 20-50 nm | SPT |
| | | Biotinylation acceptor | Monomeric avidin | | 3 nm | SPT, U-PAINT, AF, FRAP |
| | UAA | UAA | Clickable ligand | Organic dye | 1 nm | SPT, AF, FRAP |
| Ligand to endogenous protein | | | Antibody | Organic dye | 10 nm | SPT, U-PAINT, AF |
| | | | ScFv, Fab, FN3 domain | Nanoparticles | 20-50 nm | SPT |
| | | | Intrabodies | FP, organic dye | 4 nm | SPT, FRAP, WF, Scanning |
| | | | Peptides, aptamers | Dye | 2-4 nm | SPT, FRAP, WF, Scanning |
| | | | | Nanoparticles | 20-50 nm | SPT |

accompanies LTP (*34*). It is currently unclear whether actin-based transport is also at play for longer movements in dendrites. The preferred active transport pathway for long-range intracellular movements, either between the ER, ERGIC, and Golgi or for post-Golgi trafficking, is microtubule-based transport, allowing directional movements at speeds up to 2 to 5 μm/s.

Few studies have yet directly studied glutamate receptor intracellular transport. This is mostly due to imaging limitations that arise from the use of fluorescent protein–tagged receptors. A first comprehensive report of GLR-1 intracellular transport was achieved in *Caenorhabditis elegans* (*35*). In cultured hippocampal neurons, several studies recently reported the microtubule-based rapid transport of AMPAR (*28*) or KAR subunits (*36*). Glutamate receptor intracellular transport is fully bidirectional, suggesting that it is primarily used by the neuron as a means to rapidly disperse receptors over the whole dendrite rather than as a point-to-point transport system. This likely eases the availability of receptors over the whole dendrite for rapid on-demand delivery or synaptic capture during activity-dependent plasticity rather than for directed processes. Selective imaging of transport of neo-synthetized GluA1 revealed that synaptic activity and rapid increases in intracellular calcium concentrations stop vesicle transport presumably priming them for surface delivery in response to synaptic activity. During later phases of neuronal stimulation, intracellular AMPAR transport is largely increased, probably to replenish the intracellular content in the dendrite (*28*). Altogether, the high level of activity-dependent regulation of glutamate receptor intracellular transport (*28*, *36*) strongly suggests that it plays a far more important regulatory role in controlling receptor availability during synaptic plasticity than expected and thus deserves better scrutiny. An important unsettled question at present is whether the mechanisms whereby activity regulates trafficking of AMPA glutamate receptors are specific to these proteins or general to neurotransmitter receptors or even all dendritic recycling cargo.

### *Surface movements*

The plasma membrane is a viscous compartment, known to be highly dynamic but compartmentalized (*37*). As such, all neurotransmitter receptors move through Brownian diffusion when not stabilized by interactions with stable elements such as cytoskeletal or scaffold proteins. Tracking the surface movements of endogenous single receptors in neurons has revealed these expected features as well as unexpected ones. This is particularly true for receptors in the extrasynaptic compartment that usually diffuse freely at rates up to 1 $\mu m^2/s$ (*13*, *38*–*40*). Similarly, receptors are highly mobile in intracellular membranes such as the ER (*41*). Brownian diffusion is solely powered by thermal agitation and as such represents a "free" movement that is energetically neutral for the cell. The distance traveled by diffusion is proportional to the square root of time; hence, it is an efficient system for short- but not long-range displacement. On average, at 0.2 $\mu m^2/s$, a receptor travels close to a micrometer in 1 s but only 20 μm in 100 s. By comparison, intracellular motor-driven transport allows a receptor to travel ~200 μm in 100 s and is thus much more efficient for long-range displacement than diffusion, despite requiring ATP to supply energy. To date, there are almost no reports of directed receptor movement on the neuronal surface [see, however, (*42*)].

Brownian diffusion is random in direction and would thus be inefficient as a specific spatial targeting method. However, most receptors harbor binding partners that allow their local stabilization. AMPA and NMDA receptors are concentrated at synaptic sites through binding with specific scaffolds that can be either intracellular, transmembrane, or extracellular (*10*). These interactions are usually of relatively low affinity, allowing receptor binding and unbinding within seconds. This has led to the key concept of reversible diffusion trapping that sets the interplay between receptor movements and stabilization (*9*). This concept applies to almost all membrane proteins in all cell types but bears a particular interest for synaptic receptors whose function critically depends on their localization in front of release sites. Because this is a dynamic equilibrium, the number of receptors concentrated at synapses and the ratio between synaptic and extrasynaptic receptors at a given time result from a combination of their diffusion rate inside and outside synapses, the number and affinity of their anchoring sites (or slots), and the total number of surface receptors. The latter is largely set by the rates of biogenesis and exchange with intracellular compartments through membrane trafficking. The reversibility of diffusion trapping at synapses demonstrated for all ionotropic glutamate receptors (*14*, *43*, *44*) is correlated to the existence of a reserve pool of extrasynaptic receptors. This is particularly true for AMPARs and NMDARs where the existence of a large pool of extrasynaptic receptors has been confirmed by local electrophysiological experiments (*45*). Although a large fraction of receptors can usually exchange between synaptic and extrasynaptic sites [e.g., typically ~30% for AMPARs (*9*)], most glutamate receptors are concentrated at synapses, with a ratio of synaptic to extrasynaptic receptor >10 (*45*). This originates from the existence of a large pool of stabilized receptors in the PSD—for example, in the form of nanodomains (*3*, *46*, *47*). The existence of both mobile and immobile receptors at synapses together with a high concentration of receptors at the PSD might be best explained by the combined concepts of highly cooperative receptor–scaffold interactions (*48*, *49*) and the phase separation created by scaffold-scaffold (*50*) and scaffold-receptor interactions (*51*) that result in the formation of well-defined postsynaptic domains.

Because a fraction of receptors enter and leave synaptic sites, they have to move inside synapses. The PSD provides many obstacles to free diffusion in addition to specific binding sites. Accordingly, SMLM studies have all reported confined movement for proteins and lipids within the PSD (*52*, *53*). Nonetheless, most studies using FRAP or SMLM have reported a high fraction of mobile synaptic AMPARs, from 30 to 60% (*13*, *39*, *54*) to as much as 80 to 90%, and from 25 to 50% for NMDARs (*22*, *55*). Whereas FRAP can only measure exchange rates between synaptic and extrasynaptic sites (because of the diffraction limit), SMLM provides direct access to intra-PSD movements. The combination of various super-resolution imaging modalities has recently allowed measurement of AMPAR diffusion strictly in the PSD (*40*) and indicated that ~25% of AMPARs move at rates >0.01 $\mu m^2/s$ inside the PSD—that is, >60 nm per 100 ms. The high fraction of exchange between synaptic and extrasynaptic pools of receptors, as well as the existence of these intrasynaptic movements, has far-reaching functional consequences, particularly given their exquisite regulation by neuronal activity and the recently characterized nanoscale subsynaptic organization of receptors with respect to transmitter release sites (*4*, *5*).

## Role of receptor dynamics in synapse function and plasticity

### *Receptor movements and nanoscale organization: Short-term plasticity*

The subsynaptic localization of receptors with respect to neurotransmitter release sites has a major impact on the probability of activation for receptors that have relatively low affinity for glutamate (e.g., AMPARs with a glutamate affinity in the hundreds of micromolar range) (*54*, *56*, *57*). This is because presynaptic vesicles contain only ~2000 glutamate molecules, and their release creates a steep glutamate gradient. This spatial sensitivity is expected to be less prominent for receptors with higher affinity, such as the NMDARs or mGluRs. Modeling predicts that displacing AMPARs 100 nm away from the glutamate release site could decrease their probability of activation by half, depending on the number of glutamate molecules per vesicle (*4*). Accordingly, recent studies have suggested that about half of synaptic AMPARs are organized in nanoclusters (*3*, *47*) that are aligned with presynaptic transmitter release sites (*4*, *5*, *58*), supporting the concept

of functional nanocolumns to increase the fidelity of fast excitatory transmission. This peculiar organization might also support the proposal that we made 10 years ago that fast surface diffusion of AMPARs tunes frequency-dependent short-term plasticity (FD-STP) by allowing the fast replacement of desensitized receptors by naïve ones (*54*) (Fig. 3A). This process is favored by the increased diffusion of desensitized AMPARs as compared to naïve ones (*39*). Promotion of AMPAR diffusion by removal of the extracellular matrix facilitates FD-STP (*59*), whereas calcium/calmodulin-dependent protein kinase II (CaMKII)–mediated decrease in AMPAR mobility depresses FD-STP (*38*). This process might explain the decrease in FD-STP observed during LTP and CaMKII activation (*60*). Along the same lines, the secreted glycoprotein Noelin1 can limit AMPAR diffusion and modulates FD-STP (*61*). Altogether, converging elements indicate that, contrary to common belief, modulation of FD-STP does not strictly depend on changes in neurotransmitter release but rather also depends upon postsynaptic AMPAR surface diffusion and fast reorganization. This process would obviously be of prime importance at synapses with high release probability, such as some synapses onto CA1 pyramidal neurons (*62*), and would be favored by high-frequency sequential release at the same location as could occur as a result of the nanocolumnar organization of release sites with AMPAR nanodomains (*4*, *5*). Caution should thus be used in assigning changes in paired-pulse ratio of synaptic responses solely to presynaptic mechanisms.

### *Receptor movements and nanoscale organization: Long-term plasticity*

Over the past three decades, the hypothesis that activity-dependent changes in synaptic strength, such as the canonical NMDAR-dependent LTP or LTD, arise from rapid changes in postsynaptic responsiveness to glutamate through increase or decrease in AMPAR numbers has gained enormous support (*63*–*66*) (Fig. 4). A wealth of studies unveiled the trafficking pathways and molecular mechanisms underlying these activity-dependent rapid and enduring changes in receptor numbers (*9*, *10*). Several points are worth mentioning. Initially, it was suggested that AMPARs are directly delivered to, or removed from, synapses by exocytosis or endocytosis, respectively (*66*, *67*). However, the lack of direct visualization of these membrane trafficking events at PSDs and their extensive occurrence at nonsynaptic sites in the dendritic shaft suggested rather that AMPARs could reach and leave synapses by lateral diffusion, whereas membrane trafficking events critically regulate their total amount at the cell surface (*15*, *68*, *69*). Because we observed that local increases in intracellular calcium immobilize AMPARs in a CaMKII-dependent manner, the hypothesis that diffusion trapping of AMPARs at synapses underlies the increase in receptor number during LTP emerged (*12*, *38*). Surface receptor cross-linking confirmed that AMPAR surface diffusion is mandatory both for the initial phase of postsynaptic potentiation and for further diffusion of exocytosed receptors to the synapse (*70*). These and complementary (*15*) data thus support a model in which the initial phase of synaptic potentiation is primarily due to diffusion trapping of surface AMPARs. This anchoring could be dependent on the binding of AMPAR complexes to synaptic slots mediated by the major synaptic scaffold protein PSD-95, through phosphorylation of the γ2 TARP auxiliary subunit (*71*, *72*). Phosphorylation of a stretch of serine residues in γ2 would trigger unbinding of the C terminus from the membrane and its increased binding to deep PSD-95 PDZ domains, a process facilitated by the N-terminal anchoring of palmitoylated PSD95 to the plasma membrane (*71*, *73*).

This model likely needs to be revisited as the major TARP at hippocampal synapses—γ8 (*74*)—seems to behave differently from γ2, and its phosphorylation may decrease binding to PSD95 (*51*). There might thus be different rules for plasticity at different synapses or regions of the brain because of differences in the molecular players. Furthermore, it remains to be demonstrated whether exocytosis of AMPAR per se is necessary for LTP. Given the relatively large amount of extrasynaptic AMPARs already present in basal conditions, there should, in theory, be no need for the addition of a few more extrasynaptic receptors by exocytosis to allow synaptic potentiation. Exocytosis may be required to traffic to the neuronal surface an unidentified factor(s) that would maintain accumulated synaptic receptors and stabilize spine growth (*15*). The specific molecular players and mechanisms responsible for the increased AMPAR responsiveness, as well as the respective roles of AMPAR diffusion trapping and exocytosis, have not yet been identified. The same molecular uncertainty holds true for LTD that combines

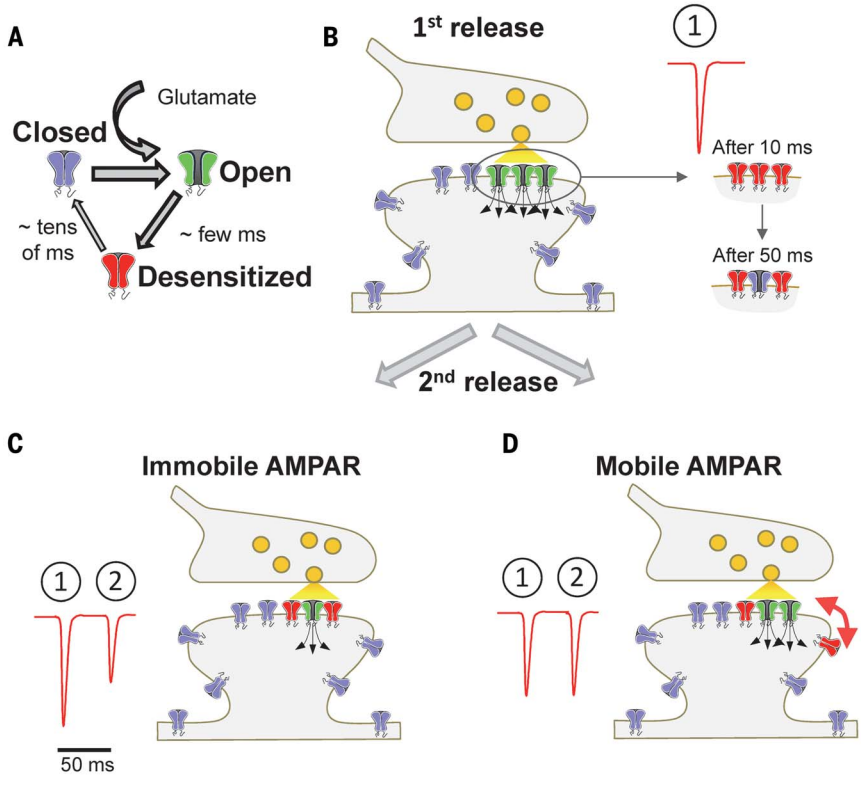


**Fig. 3. AMPAR surface diffusion tunes short-term plasticity.** (**A**) Simplified scheme of AMPAR switching from closed (blue) to open (green) and desensitized (red) upon glutamate binding. (**B**) The first action potential triggers release of a glutamate vesicle and opening of the AMPAR facing the release site. Within a few milliseconds, receptors become desensitized and recover slowly from desensitization. (**C**) When a second action potential arrives at the terminal 50 ms after the first one, triggering a second release of glutamate at a similar location, few receptors are available for activation if they are immobile, as long as the other ones remain desensitized. (**D**) If receptors are mobile, desensitized receptors are exchanged for naïve ones within the interstimulus interval, allowing more receptors to be available for activation and a faster recovery from synaptic depression mediated by receptor desensitization.

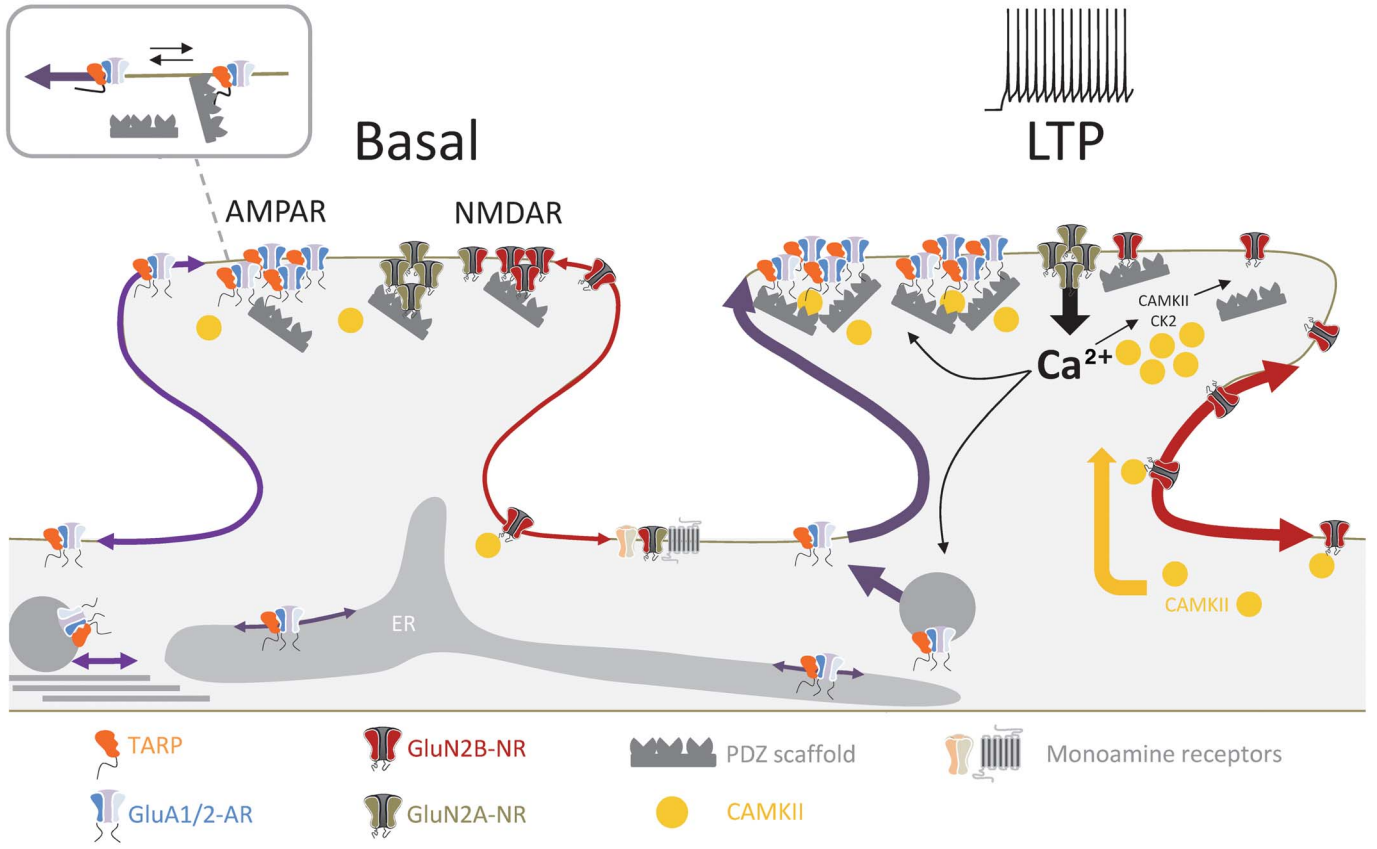


**Fig. 4. AMPAR and NMDAR surface trafficking during long-term potentiation of glutamate synapses.** Left: In the basal state, AMPARs and NMDARs diffuse at the surface of hippocampal neurons, alternating between a confined state in the PSD and a free diffusion state in the extrasynaptic compartment. In the latter compartment, NMDARs can interact with other neurotransmitter receptors such as those for dopamine. Right: During LTP, both AMPARs and NMDARs alter their surface dynamics and nanoscale organization. The activation of NMDARs and calcium influx trigger signaling cascades that laterally relocate GluN2B-NMDARs to the perisynaptic compartment to favor CAMKII recruitment to the spine head and activation of casein kinase 2 (CK2). In parallel, AMPARs are likely exocytosed to the plasma membrane and accumulate within the PSD area.

AMPAR escape from the PSD by diffusion and endocytosis. A clue could come from the existence of small nanoscale reorganizations of receptors within the PSD, allowing activity-dependent changes in AMPAR alignment to release sites without requiring a change in net receptor numbers.

Similarly, the fine processes underpinning NMDAR regulation during synaptic long-term plasticity remain a subject of debate. It was initially proposed that the main, if not sole, contribution of NMDARs in LTP is to flux calcium and activate protein kinases and associated signaling pathways. This is because the NMDAR pool is stable during LTP, and NMDARs are overall more stable within synapses than are AMPARs (*14*, *75*). Yet, there is emerging evidence that changes in NMDAR membrane trafficking and subtype composition are required for long-term synaptic plasticity. First, stable and specific changes in NMDAR transmission, so-called NMDAR LTP, occur in some hippocampal synapses after certain stimulation protocols (*76*, *77*). In the canonical AMPAR LTP, NMDAR signaling changes slowly, over a period of hours, after LTP (*78*), possibly contributing to synaptic homeostasis and metaplastic processes. Yet, shortly after the induction of LTP at CA3-CA1 synapses of young mice, the contribution of GluN2B-NMDAR–mediated current decreases (*79*), suggesting a rapid change in NMDAR subtypes within the synapse. During LTP induction at immature synapses, GluN2B-NMDARs specifically escape the synapse and actively traffic within the perisynaptic area, in a CAMKII- and casein kinase 2–dependent process (*80*). This activity-dependent redistribution of surface GluN2B-NMDARs is necessary for the establishment of in vitro, ex vivo, and in vivo LTP at hippocampal synapses, as well as for associative memory (*80*, *81*). This finding provided the first evidence that membrane diffusion of glutamate receptors is required for LTP.

How could GluN2B-NMDAR synaptic escape and trafficking in the perisynaptic area contribute to the establishment of LTP? We showed that the increase in GluN2B-NMDAR surface dynamics after stimuli that induce LTP directly contributes to the accumulation of α-CAMKII within the spine head (*80*). The highly diffusive GluN2B-NMDAR could "shuttle" α-CAMKII to the PSD through their direct binding, consistent with the well-defined and key role of the interaction between NMDAR and α-CAMKII into LTP and learning processes (*82*). Thus, NMDAR surface dynamics, and especially that of GluN2B-NMDAR, is tuned by neuronal activity and is necessary for the reorganization of NMDARs and associated postsynaptic proteins during long-term synaptic plasticity. Most of the evidence supports a twofold model of the role of NMDARs in LTP: (i) Strongly anchored NMDARs (e.g., GluN2A-NMDAR) serve as ionotropic calcium providers to trigger essential protein kinase and signaling cascades; and (ii) labile NMDARs (e.g., GluN2B-NMDAR) shuttle and redistribute some key intracellular actors after calcium influx (*80*, *83*). After LTP, the potentiated hippocampal synapses thus exhibit an increased GluN2A/2B synaptic ratio that likely tunes their plastic range by limiting further potentiation and even favoring depotentiation (*84*). Surface NMDARs thus have classical ionotropic but also non-ionotropic functions related to their movements, as described above for LTP and that could relate to other synaptic paradigms (*85*, *86*).

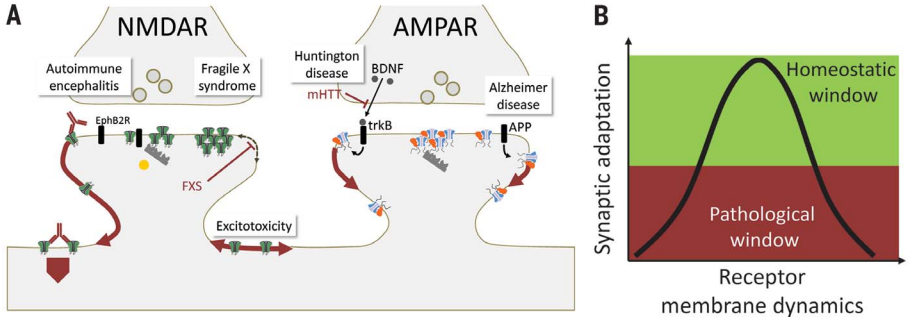


**Fig. 5. Altered receptor surface diffusion homeostasis in pathological synapse dysfunction.** (**A**) Alterations in NMDAR and AMPAR surface dynamics have been reported in various models of neurological and psychiatric disorders. In particular, the deficit in NMDAR trafficking in the presence of autoantibodies directed against the receptor provided the first evidence of a direct link between receptor surface dynamics and human brain pathology. (**B**) A hypothetical inverted-U curve shows that glutamate receptors must be dynamic within a certain range to promote synaptic adaptation. If the receptor dynamics fall above or below this range, the homeostatic range of the glutamate synapse is corrupted, favoring the development of pathological conditions.

Induction and expression of long-term synaptic plasticity thus involve a redistribution of both AMPARs and NMDARs through lateral diffusion to modulate their number and nanoscale organization. This mechanism is likely a general process for other forms of plasticity that involve neurotransmitter receptor reorganization—for example, at inhibitory synapses (*87*, *88*).

### Receptor dynamics and brain diseases

Dysfunctions of the glutamatergic and γ-aminobutyric acid (GABA)–mediated synaptic transmissions, and particularly their lack of adaptation, have been increasingly associated with the etiology of major neurological and psychiatric disorders. We and others investigated whether the membrane organization and dynamics of receptors represent some of the primary elements that are corrupted in major brain diseases. Defining the molecular and cellular processes underpinning synaptic dysfunction in neuropsychiatric conditions and animal models has, however, been difficult because several confounding factors likely contribute to the etiology of most brain illnesses. For instance, distinct proteins of glutamatergic synapses (e.g., receptor, scaffold, and signaling proteins) are implicated in psychiatric diseases such as schizophrenia and autism spectrum disorder (*89*). Glutamate receptors per se are not usually genetically altered, contrary to the synaptic environment that is essential for their proper trafficking and organization, fueling the hypothesis that receptor trafficking rather than biophysical properties has a decisive etiological role in brain diseases (Fig. 5A). Support for this proposition recently came from an autoimmune disorder, NMDAR encephalitis. Patients with NMDAR encephalitis have prominent psychiatric and neurological symptoms that directly correlate with autoantibodies targeting GluN1 subunit extracellular domain(s) (NMDAR-Ab) (*90*). The symptoms vanish once NMDAR-Ab are clinically removed. The NMDAR-Ab induce a massive loss of NMDAR membrane content in limbic structures. SMLM demonstrated that NMDAR-Ab induce a rapid dispersal of synaptic NMDAR toward the extrasynaptic membrane compartment, in which they are efficiently cross-linked and eventually internalized. The lateral escape of NMDARs from synapses decreases NMDAR-mediated transmission, abolishes NMDAR-dependent synaptic plasticity, alters neuronal network activity, and induces major behavioral alterations (*80*, *91*–*93*).

The hypofunction of synaptic NMDARs (namely, the reduced number of synaptic NMDARs) in the presence of autoantibodies is solely due to trafficking alterations, as their ionotropic function remains unaffected (*91*). The internalization of extrasynaptic NMDARs in the presence of autoantibodies is also observed in the presence of an NMDAR antagonist (*93*). Thus, NMDAR-Ab from patients with autoimmune encephalitis, as well as patients with mainly psychotic symptoms (*94*), impair the membrane dynamics and organization of NMDARs, shedding new light on the unexpected molecular mechanisms underpinning NMDAR dysfunction in major psychotic disorders such as schizophrenia. The detection of autoantibodies directed against other glutamatergic and GABAergic receptors in patients with various neurological and psychiatric conditions raises the possibility that autoantibody-mediated neuropsychiatric symptoms are more widely mediated through disturbed membrane organization and dynamics of neurotransmitter receptors. For example, in autoimmune encephalitis, antibodies against GluA2 induce receptor internalization and a reduction in the number of synaptic GluA2-containing AMPARs followed by compensatory incorporation of synaptic GluA2-lacking AMPARs (*95*). Alterations in NMDAR surface trafficking have also been reported in various models of neurological and psychiatric disorders, such as fragile X syndrome (*96*), Huntington's disease (*97*), Alzheimer's disease (*98*), and addiction (*99*). In addition, mutations of the receptor itself associated with autism or neurodevelopmental encephalopathy can also strongly affect receptor membrane trafficking (*100*, *101*). Similar alterations in membrane diffusion and organization have been reported for AMPARs in models of Alzheimer's (*102*) and Huntington's diseases (*103*), and for $GABA_A$ receptors in Parkinson's disease (*104*), supporting the idea that a corrupted neurotransmitter receptor dynamics contributes to the deficits in synaptic transmission in neurodegenerative disorders (*105*).

### Outlook

Studies from the past 20 years have firmly established that glutamate receptor trafficking is a key factor in short and long-term synaptic plasticity. Although most studies of receptor movements have been carried out in reduced experimental systems, there is no reason to believe that receptors should move differently in more physiological settings. While we remember the statement of a prominent neuroscientist that "maybe receptors move in culture, but not in my head," substantial evidence does indicate that glutamate receptors diffuse at the surface of neurons in brain slices (*26*, *54*). Measuring receptor dynamics in vivo is still to be achieved, but it is already known that the AMPAR synaptic content is dynamic in live animals (*106*, *107*). New technological developments—for example, the use of gene editing to incorporate small tags or unnatural amino acids in receptors together with engineering of brighter probes—should enable routine and faithful tracking of endogenous receptors in brain slices and even in vivo, making it possible to study modulations of receptor movement during physiological plasticity and adaptive behaviors. This advancement will help to define the role of receptor subunit composition and regulation by scaffolding proteins and signaling cascades during various types of physiologically relevant experimental paradigms of plasticity. It will also enable the respective contributions of the various modalities of receptor movements to the different forms of synaptic plasticity to be determined. Besides visualizing receptor movements, new approaches are emerging to artificially control these movements (*70*, *81*)—for example, through surface cross-linking. Further refining these tools with better spatiotemporal resolution will allow a full picture of the roles of receptor movements in brain functions to be obtained.

The dynamics and organization of glutamatergic receptors, and likely other neurotransmitter families, are altered in various neurological and psychiatric conditions. Elucidating the mechanisms underpinning these trafficking abnormalities will open new avenues for innovation in therapeutic strategies, an unmet and urgent need. For instance, most of the past efforts to treat neuropsychiatric disorders associated with glutamatergic receptors, such as NMDAR activity in schizophrenia and excitotoxic conditions, have focused on modulating the ionotropic activity of receptors. The poor outcome, so far, of these programs should prompt us to explore other ways of manipulating glutamate receptor–mediated transmission. Models of brain diseases have consistently been associated with either a down- or up-regulation of glutamate receptor surface diffusion, suggesting that either modification can lead to the pathological loss of synaptic adaptation and neuronal integration. We hypothesize that normal synaptic transmission and plasticity are associated with a homeostatic range of membrane receptor dynamics, simultaneously ensuring sufficient receptor flux and stabilization in signaling domains (Fig. 5B). In pathological conditions, membrane receptor dynamics would escape this homeostatic range, being either too low (e.g., reduced diffusion) or too high (e.g., reduced anchoring). The development of therapeutic strategies to finely and specifically tune membrane receptor dynamics and organization in brain cells thus offers a new and unexplored opportunity to restore balance in the glutamatergic drive of the diseased brain.

## REFERENCES AND NOTES


1. T. Takeuchi, A. J. Duszkiewicz, R. G. Morris, The synaptic plasticity and memory hypothesis: Encoding, storage and persistence. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20130288 (2013). doi: 10.1098/rstb.2013.0288; pmid: 24298167
2. J. del Castillo, B. Katz, Quantal components of the end-plate potential. *J. Physiol.* **124**, 560–573 (1954). doi: 10.1113/jphysiol.1954.sp005129; pmid: 13175199
3. D. Nair *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). doi: 10.1523/JNEUROSCI.2381-12.2013; pmid: 23926273
4. K. T. Haas *et al.*, Pre-post synaptic alignment through neuroligin-1 tunes synaptic transmission efficiency. *eLife* **7**, e31755 (2018). doi: 10.7554/eLife.31755; pmid: 30044218
5. A. H. Tang *et al.*, A trans-synaptic nanocolumn aligns neurotransmitter release to receptors. *Nature* **536**, 210–214 (2016). doi: 10.1038/nature19058; pmid: 27462810
6. A. S. Kristensen *et al.*, Mechanism of Ca2+/calmodulin-dependent kinase II regulation of AMPA receptor gating. *Nat. Neurosci.* **14**, 727–735 (2011). doi: 10.1038/nn.2804; pmid: 21516102
7. A. Panatier *et al.*, Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* **125**, 775–784 (2006). doi: 10.1016/j.cell.2006.02.051; pmid: 16713567
8. D. Bowie, Polyamine-mediated channel block of ionotropic glutamate receptors and its regulation by auxiliary proteins. *J. Biol. Chem.* **293**, 18789–18802 (2018). doi: 10.1074/jbc.TM118.003794; pmid: 30333231
9. D. Choquet, Linking Nanoscale Dynamics of AMPA Receptor Organization to Plasticity of Excitatory Synapses and Learning, Linking Nanoscale Dynamics of AMPA Receptor Organization to Plasticity of Excitatory Synapses and Learning. *J. Neurosci.* **38**, 9318–9329 (2018). doi: 10.1523/JNEUROSCI.2119-18.2018; pmid: 30381423
10. B. E. Herring, R. A. Nicoll, Long-Term Potentiation: From CaMKII to AMPA Receptor Trafficking. *Annu. Rev. Physiol.* **78**, 351–365 (2016). doi: 10.1146/annurev-physiol-021014-071753; pmid: 26863325
11. R. C. Carroll, E. C. Beattie, M. von Zastrow, R. C. Malenka, Role of AMPA receptor endocytosis in synaptic plasticity. *Nat. Rev. Neurosci.* **2**, 315–324 (2001). doi: 10.1038/35072500; pmid: 11331915
12. A. J. Borgdorff, D. Choquet, Regulation of AMPA receptor lateral movements. *Nature* **417**, 649–653 (2002). doi: 10.1038/nature00780; pmid: 12050666
13. M. C. Ashby, S. R. Maier, A. Nishimune, J. M. Henley, Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology. *J. Neurosci.* **26**, 7046–7055 (2006). doi: 10.1523/JNEUROSCI.1235-06.2006; pmid: 16807334
14. L. Groc *et al.*, Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors. *Nat. Neurosci.* **7**, 695–696 (2004). doi: 10.1038/nn1270; pmid: 15208630
15. B. G. Hiester, M. I. Becker, A. B. Bowen, S. L. Schwartz, M. J. Kennedy, Mechanisms and Role of Dendritic Membrane Trafficking for Long-Term Potentiation. *Front. Cell. Neurosci.* **12**, 391 (2018). doi: 10.3389/fncel.2018.00391; pmid: 30425622
16. C. Hanus, M. D. Ehlers, Specialization of biosynthetic membrane trafficking for neuronal form and function. *Curr. Opin. Neurobiol.* **39**, 8–16 (2016). doi: 10.1016/j.conb.2016.03.004; pmid: 27010827
17. M. J. Kennedy, C. Hanus, Architecture and Dynamics of the Neuronal Secretory Network, Architecture and Dynamics of the Neuronal Secretory Network. *Annu. Rev. Cell Dev. Biol.* **35**, 543–566 (2019). doi: 10.1146/annurev-cellbio-100818-125418; pmid: 31283381
18. D. Axelrod, D. E. Koppel, J. Schlessinger, E. Elson, W. W. Webb, Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophys. J.* **16**, 1055–1069 (1976). doi: 10.1016/S0006-3495(76)85755-4; pmid: 786399
19. M. C. Ashby, K. Ibaraki, J. M. Henley, It's green outside: Tracking cell surface proteins with pH-sensitive GFP. *Trends Neurosci.* **27**, 257–261 (2004). doi: 10.1016/j.tins.2004.03.010; pmid: 15111007
20. O. Thoumine *et al.*, Probing the dynamics of protein-protein interactions at neuronal contacts by optical imaging. *Chem. Rev.* **108**, 1565–1587 (2008). doi: 10.1021/cr078204m; pmid: 18447398
21. Y. Gao *et al.*, Plug-and-Play Protein Modification Using Homology-Independent Universal Genome Engineering. *Neuron* **103**, 583–597.e8 (2019). doi: 10.1016/j.neuron.2019.05.047; pmid: 31272828
22. K. R. Tovar, G. L. Westbrook, Mobile NMDA receptors at hippocampal synapses. *Neuron* **34**, 255–264 (2002). doi: 10.1016/S0896-6273(02)00658-X; pmid: 11970867
23. Z. Liu, L. D. Lavis, E. Betzig, Imaging live-cell dynamics and structure at the single-molecule level. *Mol. Cell* **58**, 644–659 (2015). doi: 10.1016/j.molcel.2015.02.033; pmid: 26000849
24. J. P. Dupuis, L. Groc, Surface trafficking of neurotransmitter receptors: From cultured neurons to intact brain preparations. *Neuropharmacology* **169**, 107642 (2020). doi: 10.1016/j.neuropharm.2019.05.019; pmid: 31108111
25. L. Cognet, B. Lounis, D. Choquet, Tracking receptors using individual fluorescent and nonfluorescent nanolabels. *Cold Spring Harb. Protoc.* **2014**, 207–213 (2014). doi: 10.1101/pdb.prot080416; pmid: 24492778
26. J. A. Varela *et al.*, Targeting neurotransmitter receptors with nanoparticles in vivo allows single-molecule tracking in acute brain slices. *Nat. Commun.* **7**, 10947 (2016). doi: 10.1038/ncomms10947; pmid: 26971573
27. Y. M. Sigal, R. Zhou, X. Zhuang, Visualizing and discovering cellular structures with super-resolution microscopy. *Science* **361**, 880–887 (2018). doi: 10.1126/science.aau1044; pmid: 30166485
28. E. Hangen, F. P. Cordelières, J. D. Petersen, D. Choquet, F. Coussen, Neuronal Activity and Intracellular Calcium Levels Regulate Intracellular Transport of Newly Synthesized AMPAR, Neuronal Activity and Intracellular Calcium Levels Regulate Intracellular Transport of Newly Synthesized AMPAR. *Cell Rep.* **24**, 1001–1012.e3 (2018). doi: 10.1016/j.celrep.2018.06.095; pmid: 30044968
29. M. Joensuu *et al.*, Subdiffractional tracking of internalized molecules reveals heterogeneous motion states of synaptic vesicles. *J. Cell Biol.* **215**, 277–292 (2016). doi: 10.1083/jcb.201604001; pmid: 27810917
30. J. G. Hanley, Actin-dependent mechanisms in AMPA receptor trafficking. *Front. Cell. Neurosci.* **8**, 381 (2014). doi: 10.3389/fncel.2014.00381; pmid: 25429259
31. M. Kneussel, W. Wagner, Myosin motors at neuronal synapses: Drivers of membrane transport and actin dynamics. *Nat. Rev. Neurosci.* **14**, 233–247 (2013). doi: 10.1038/nrn3445; pmid: 23481482
32. E. Osterweil, D. G. Wells, M. S. Mooseker, A role for myosin VI in postsynaptic structure and glutamate receptor endocytosis. *J. Cell Biol.* **168**, 329–338 (2005). doi: 10.1083/jcb.200410091; pmid: 15657400
33. W. Wagner *et al.*, Myosin VI Drives Clathrin-Mediated AMPA Receptor Endocytosis to Facilitate Cerebellar Long-Term Depression. *Cell Rep.* **28**, 11–20.e9 (2019). doi: 10.1016/j.celrep.2019.06.005; pmid: 31269433
34. Z. Wang *et al.*, Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell* **135**, 535–548 (2008). doi: 10.1016/j.cell.2008.09.057; pmid: 18984164
35. F. J. Hoerndli *et al.*, Kinesin-1 regulates synaptic strength by mediating the delivery, removal, and redistribution of AMPA receptors. *Neuron* **80**, 1421–1437 (2013). doi: 10.1016/j.neuron.2013.10.050; pmid: 24360545
36. A. J. Evans, S. Gurung, K. A. Wilkinson, D. J. Stephens, J. M. Henley, Assembly, Secretory Pathway Trafficking, and Surface Delivery of Kainate Receptors Is Regulated by Neuronal Activity. *Cell Rep.* **19**, 2613–2626 (2017). doi: 10.1016/j.celrep.2017.06.001; pmid: 28636947
37. S. J. Singer, G. L. Nicolson, The fluid mosaic model of the structure of cell membranes. *Science* **175**, 720–731 (1972). doi: 10.1126/science.175.4023.720; pmid: 4333397
38. P. Opazo *et al.*, CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. *Neuron* **67**, 239–252 (2010). doi: 10.1016/j.neuron.2010.06.007; pmid: 20670832
39. A. Constals *et al.*, Glutamate-induced AMPA receptor desensitization increases their mobility and modulates short-term plasticity through unbinding from Stargazin. *Neuron* **85**, 787–803 (2015). doi: 10.1016/j.neuron.2015.01.012; pmid: 25661182
40. V. V. G. K. Inavalli *et al.*, A super-resolution platform for correlative live single-molecule imaging and STED microscopy. *Nat. Methods* **16**, 1263–1268 (2019). doi: 10.1038/s41592-019-0611-8; pmid: 31636458
41. T. Cui-Wang *et al.*, Local zones of endoplasmic reticulum complexity confine cargo in neuronal dendrites. *Cell* **148**, 309–321 (2012). doi: 10.1016/j.cell.2011.11.056; pmid: 22265418
42. R. D. Taylor, M. Heine, N. J. Emptage, L. C. Andreae, Neuronal Receptors Display Cytoskeleton-Independent Directed Motion on the Plasma Membrane. *Iscience* **10**, 234–244 (2018). doi: 10.1016/j.isci.2018.12.001; pmid: 30557785
43. C. Bats, L. Groc, D. Choquet, The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron* **53**, 719–734 (2007). doi: 10.1016/j.neuron.2007.01.030; pmid: 17329211
44. M. Carta *et al.*, CaMKII-dependent phosphorylation of GluK5 mediates plasticity of kainate receptors. *EMBO J.* **32**, 496–510 (2013). doi: 10.1038/emboj.2012.334; pmid: 23288040
45. M. Matsuzaki *et al.*, Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat. Neurosci.* **4**, 1086–1092 (2001). doi: 10.1038/nn736; pmid: 11687814
46. B. Kellermayer *et al.*, Differential Nanoscale Topography and Functional Role of GluN2-NMDA Receptor Subtypes at Glutamatergic Synapses. *Neuron* **100**, 106–119.e7 (2018). doi: 10.1016/j.neuron.2018.09.012; pmid: 30269991
47. H. D. MacGillavry, Y. Song, S. Raghavachari, T. A. Blanpied, Nanoscale scaffolding domains within the postsynaptic density concentrate synaptic AMPA receptors. *Neuron* **78**, 615–622 (2013). doi: 10.1016/j.neuron.2013.03.009; pmid: 23719161
48. M. Sainlos *et al.*, Biomimetic divalent ligands for the acute disruption of synaptic AMPAR stabilization. *Nat. Chem. Biol.* **7**, 81–91 (2011). doi: 10.1038/nchembio.498; pmid: 21186349
49. K. Sekimoto, A. Triller, Compatibility between itinerant synaptic receptors and stable postsynaptic structure. *Phys. Rev. E* **79**, 031905 (2009). pmid: 19391969
50. M. Zeng *et al.*, Phase Transition in Postsynaptic Densities Underlies Formation of Synaptic Complexes and Synaptic

Plasticity. *Cell* **166**, 1163–1175.e12 (2016). doi: 10.1016/j.cell.2016.07.008; pmid: 27565345
51. M. Zeng *et al*., Phase Separation-Mediated TARP/MAGUK Complex Condensation and AMPA Receptor Synaptic Transmission. *Neuron* **104**, 529–543.e6 (2019). doi: 10.1016/j.neuron.2019.08.001; pmid: 31492534
52. M. Renner, D. Choquet, A. Triller, Control of the postsynaptic membrane viscosity. *J. Neurosci.* **29**, 2926–2937 (2009). doi: 10.1523/JNEUROSCI.4445-08.2009; pmid: 19261888
53. C. Tardin, L. Cognet, C. Bats, B. Lounis, D. Choquet, Direct imaging of lateral movements of AMPA receptors inside synapses. *EMBO J.* **22**, 4656–4665 (2003). doi: 10.1093/emboj/cdg463; pmid: 12970178
54. M. Heine *et al*., Surface mobility of postsynaptic AMPARs tunes synaptic transmission. *Science* **320**, 201–205 (2008). doi: 10.1126/science.1152089; pmid: 18403705
55. L. Groc *et al*., NMDA receptor surface trafficking and synaptic subunit composition are developmentally regulated by the extracellular matrix protein Reelin. *J. Neurosci.* **27**, 10165–10175 (2007). doi: 10.1523/JNEUROSCI.1772-07.2007; pmid: 17881522
56. H. D. MacGillavry, J. M. Kerr, T. A. Blanpied, Lateral organization of the postsynaptic density. *Mol. Cell. Neurosci.* **48**, 321–331 (2011). doi: 10.1016/j.mcn.2011.09.001; pmid: 21920440
57. B. L. Sinnen *et al*., Optogenetic Control of Synaptic Composition and Function. *Neuron* **93**, 646–660.e5 (2017). doi: 10.1016/j.neuron.2016.12.037; pmid: 28132827
58. M. Hruska, N. Henderson, S. J. Le Marchand, H. Jafri, M. B. Dalva, Synaptic nanomodules underlie the organization and plasticity of spine synapses. *Nat. Neurosci.* **21**, 671–682 (2018). doi: 10.1038/s41593-018-0138-9; pmid: 29686261
59. R. Frischknecht *et al*., The brain extracellular matrix limits lateral diffusion of AMPA receptors and modulates short-term synaptic plasticity. *Nat. Neurosci.* **12**, 897 (2009). doi: 10.1038/nn.2338; pmid: 19483686
60. J. H. Wang, P. T. Kelly, Attenuation of paired-pulse facilitation associated with synaptic potentiation mediated by postsynaptic mechanisms. *J. Neurophysiol.* **78**, 2707–2716 (1997). doi: 10.1152/jn.1997.78.5.2707; pmid: 9356420
61. N. J. Pandya *et al*., Noelin1 Affects Lateral Mobility of Synaptic AMPA Receptors. *Cell Rep.* **24**, 1218–1230 (2018). doi: 10.1016/j.celrep.2018.06.102; pmid: 30067977
62. N. Helassa *et al*., Ultrafast glutamate sensors resolve high-frequency release at Schaffer collateral synapses. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 5594–5599 (2018). doi: 10.1073/pnas.1720648115; pmid: 29735711
63. J. T. Isaac, R. A. Nicoll, R. C. Malenka, Evidence for silent synapses: Implications for the expression of LTP. *Neuron* **15**, 427–434 (1995). doi: 10.1016/0896-6273(95)90046-2; pmid: 7646894
64. H. Y. Man *et al*., Regulation of AMPA receptor-mediated synaptic transmission by clathrin-dependent receptor internalization. *Neuron* **25**, 649–662 (2000). doi: 10.1016/S0896-6273(00)81067-3; pmid: 10774732
65. S. H. Shi *et al*., Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* **284**, 1811–1816 (1999). doi: 10.1126/science.284.5421.1811; pmid: 10364548
66. R. C. Carroll, D. V. Lissin, M. von Zastrow, R. A. Nicoll, R. C. Malenka, Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nat. Neurosci.* **2**, 454–460 (1999). doi: 10.1038/8123; pmid: 10321250
67. P. M. Lledo, X. Zhang, T. C. Südhof, R. C. Malenka, R. A. Nicoll, Postsynaptic membrane fusion and long-term potentiation. *Science* **279**, 399–403 (1998). doi: 10.1126/science.279.5349.399; pmid: 9430593
68. G. A. Yudowski *et al*., Real-time imaging of discrete exocytic events mediating surface delivery of AMPA receptors. *J. Neurosci.* **27**, 11112–11121 (2007). doi: 10.1523/JNEUROSCI.2465-07.2007; pmid: 17928453
69. D. T. Lin *et al*., Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation. *Nat. Neurosci.* **12**, 879–887 (2009). doi: 10.1038/nn.2351; pmid: 19503082
70. A. C. Penn *et al*., Hippocampal LTP and contextual learning require surface diffusion of AMPA receptors. *Nature* **549**, 384–388 (2017). doi: 10.1038/nature23658; pmid: 28902836
71. A. S. Hafner *et al*., Lengthening of the Stargazin Cytoplasmic Tail Increases Synaptic Transmission by Promoting Interaction to Deeper Domains of PSD-95. *Neuron* **86**, 475–489 (2015). doi: 10.1016/j.neuron.2015.03.013; pmid: 25843401
72. S. Tomita, V. Stein, T. J. Stocker, R. A. Nicoll, D. S. Bredt, Bidirectional synaptic plasticity regulated by phosphorylation of stargazin-like TARPs. *Neuron* **45**, 269–277 (2005). doi: 10.1016/j.neuron.2005.01.009; pmid: 15664178
73. A. Sumioka, D. Yan, S. Tomita, TARP phosphorylation regulates synaptic AMPA receptors through lipid bilayers. *Neuron* **66**, 755–767 (2010). doi: 10.1016/j.neuron.2010.04.035; pmid: 20547132
74. J. Park *et al*., CaMKII Phosphorylation of TARPγ-8 Is a Mediator of LTP and Learning and Memory. *Neuron* **92**, 75–83 (2016). doi: 10.1016/j.neuron.2016.09.002; pmid: 27667007
75. R. A. Nicoll, Expression mechanisms underlying long-term potentiation: A postsynaptic view. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **358**, 721–726 (2003). doi: 10.1098/rstb.2002.1228; pmid: 12740118
76. H. B. Kwon, P. E. Castillo, Long-term potentiation selectively expressed by NMDA receptors at hippocampal mossy fiber synapses. *Neuron* **57**, 108–120 (2008). doi: 10.1016/j.neuron.2007.11.024; pmid: 18184568
77. M. C. Lee, R. Yasuda, M. D. Ehlers, Metaplasticity at single glutamatergic synapses. *Neuron* **66**, 859–870 (2010). doi: 10.1016/j.neuron.2010.05.015; pmid: 20620872
78. D. L. Hunt, P. E. Castillo, Synaptic plasticity of NMDA receptors: Mechanisms and functional implications. *Curr. Opin. Neurobiol.* **22**, 496–508 (2012). doi: 10.1016/j.conb.2012.01.007; pmid: 22325859
79. C. Bellone, R. A. Nicoll, Rapid bidirectional switching of synaptic NMDA receptors. *Neuron* **55**, 779–785 (2007). doi: 10.1016/j.neuron.2007.07.035; pmid: 17785184
80. J. P. Dupuis *et al*., Surface dynamics of GluN2B-NMDA receptors controls plasticity of maturing glutamate synapses. *EMBO J.* **33**, 842–861 (2014). doi: 10.1002/embj.201386356; pmid: 24591565
81. M. Potier *et al*., Temporal Memory and Its Enhancement by Estradiol Requires Surface Dynamics of Hippocampal CA1 N-Methyl-D-Aspartate Receptors. *Biol. Psychiatry* **79**, 735–745 (2016). doi: 10.1016/j.biopsych.2015.07.017; pmid: 26321020
82. K. U. Bayer, H. Schulman, CaM Kinase: Still Inspiring at 40. *Neuron* **103**, 380–394 (2019). doi: 10.1016/j.neuron.2019.05.033; pmid: 31394063
83. A. Sanz-Clemente, J. A. Gray, K. A. Ogilvie, R. A. Nicoll, K. W. Roche, Activated CaMKII couples GluN2B and casein kinase 2 to control synaptic NMDA receptors. *Cell Rep.* **3**, 607–614 (2013). doi: 10.1016/j.celrep.2013.02.011; pmid: 23478024
84. O. A. Shipton, O. Paulsen, GluN2A and GluN2B subunit-containing NMDA receptors in hippocampal plasticity. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **369**, 20130163 (2013). doi: 10.1098/rstb.2013.0163; pmid: 24298164
85. H. W. Kessels, S. Nabavi, R. Malinow, Metabotropic NMDA receptor function is required for β-amyloid-induced synaptic depression. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 4033–4038 (2013). doi: 10.1073/pnas.1219605110; pmid: 23431156
86. B. C. Carter, C. E. Jahr, Postsynaptic, not presynaptic NMDA receptors are required for spike-timing-dependent LTD induction. *Nat. Neurosci.* **19**, 1218–1224 (2016). doi: 10.1038/nn.4343; pmid: 27399842
87. C. A. Haselwandter, M. Calamai, M. Kardar, A. Triller, R. Azeredo da Silveira, Formation and stability of synaptic receptor domains. *Phys. Rev. Lett.* **106**, 238104 (2011). doi: 10.1103/PhysRevLett.106.238104; pmid: 21770547
88. A. Triller, D. Choquet, Surface trafficking of receptors between synaptic and extrasynaptic membranes: And yet they do move! *Trends Neurosci.* **28**, 133–139 (2005). doi: 10.1016/j.tins.2005.01.001; pmid: 15749166
89. L. Volk, S. L. Chiu, K. Sharma, R. L. Huganir, Glutamate synapses in human cognitive disorders. *Annu. Rev. Neurosci.* **38**, 127–149 (2015). doi: 10.1146/annurev-neuro-071714-033821; pmid: 25897873
90. J. Dalmau, F. Graus, Antibody-Mediated Encephalitis. *N. Engl. J. Med.* **378**, 840–851 (2018). doi: 10.1056/NEJMra1708712; pmid: 29490181
91. L. Mikasova *et al*., Disrupted surface cross-talk between NMDA and Ephrin-B2 receptors in anti-NMDA encephalitis. *Brain* **135**, 1606–1621 (2012). doi: 10.1093/brain/aws092; pmid: 22544902
92. J. Planagumà *et al*., Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain* **138**, 94–109 (2015). doi: 10.1093/brain/awu310; pmid: 25392198
93. E. G. Hughes *et al*., Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J. Neurosci.* **30**, 5866–5875 (2010). doi: 10.1523/JNEUROSCI.0167-10.2010; pmid: 20427647
94. J. Jézéquel, E. M. Johansson, M. Leboyer, L. Groc, Pathogenicity of Antibodies against NMDA Receptor: Molecular Insights into Autoimmune Psychosis. *Trends Neurosci.* **41**, 502–511 (2018). doi: 10.1016/j.tins.2018.05.002; pmid: 29807730
95. H. Haselmann *et al*., Human Autoantibodies against the AMPA Receptor Subunit GluA2 Induce Receptor Reorganization and Memory Dysfunction. *Neuron* **100**, 91–105.e9 (2018). doi: 10.1016/j.neuron.2018.07.048; pmid: 30146304
96. E. Aloisi *et al*., Altered surface mGluR5 dynamics provoke synaptic NMDAR dysfunction and cognitive defects in Fmr1 knockout mice. *Nat. Commun.* **8**, 1103 (2017). doi: 10.1038/s41467-017-01191-2; pmid: 29062097
97. M. M. Fan, L. A. Raymond, N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog. Neurobiol.* **81**, 272–293 (2007). doi: 10.1016/j.pneurobio.2006.11.003; pmid: 17188796
98. E. M. Snyder *et al*., Regulation of NMDA receptor trafficking by amyloid-beta. *Nat. Neurosci.* **8**, 1051–1058 (2005). doi: 10.1038/nn1503; pmid: 16025111
99. L. M. Mao *et al*., Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. *Nat. Neurosci.* **12**, 602–610 (2009). doi: 10.1038/nn.2300; pmid: 19349975
100. S. Liu *et al*., A Rare Variant Identified Within the GluN2B C-Terminus in a Patient with Autism Affects NMDA Receptor Surface Expression and Spine Density. *J. Neurosci.* **37**, 4093–4102 (2017). doi: 10.1523/JNEUROSCI.0827-16.2017; pmid: 28283559
101. D. Soto *et al*., L-Serine dietary supplementation is associated with clinical improvement of loss-of-function *GRIN2B*-related pediatric encephalopathy. *Sci. Signal.* **12**, eaaw0936 (2019). doi: 10.1126/scisignal.aaw0936; pmid: 31213567
102. P. Opazo *et al*., CaMKII Metaplasticity Drives Aβ Oligomer-Mediated Synaptotoxicity. *Cell Rep.* **23**, 3137–3145 (2018). doi: 10.1016/j.celrep.2018.05.036; pmid: 29898386
103. H. Zhang *et al*., Modulation of AMPA receptor surface diffusion restores hippocampal plasticity and memory in Huntington's disease models. *Nat. Commun.* **9**, 4272 (2018). doi: 10.1038/s41467-018-06675-3; pmid: 30323233
104. A. N. Shrivastava *et al*., α-synuclein assemblies sequester neuronal α3-Na+/K+-ATPase and impair Na+ gradient. *EMBO J.* **34**, 2408–2423 (2015). doi: 10.15252/embj.201591397; pmid: 26323479
105. A. N. Shrivastava, A. Aperia, R. Melki, A. Triller, Physico-Pathologic Mechanisms Involved in Neurodegeneration: Misfolded Protein-Plasma Membrane Interactions. *Neuron* **95**, 33–50 (2017). doi: 10.1016/j.neuron.2017.05.026; pmid: 28683268
106. Y. Zhang, R. H. Cudmore, D. T. Lin, D. J. Linden, R. L. Huganir, Visualization of NMDA receptor-dependent AMPA receptor synaptic plasticity in vivo. *Nat. Neurosci.* **18**, 402–407 (2015). doi: 10.1038/nn.3936; pmid: 25643295
107. R. H. Roth *et al*., Cortical Synaptic AMPA Receptor Plasticity during Motor Learning. *Neuron* **105**, 895–908.e5 (2020). doi: 10.1016/j.neuron.2019.12.005 pmid: 31901303

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# Linking glutamate receptor movements and synapse function

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**Receptors moving in and out of the synapse**

The number of neurotransmitter receptors and their spatial organization on the postsynaptic site is a central determinant of synaptic efficacy. Sophisticated techniques to visualize and track the movement of single molecules have provided us with profound new insights into these dynamics. We now know that neurotransmitter receptors undergo movements on different scales. Groc and Choquet review our present understanding of the mechanisms that regulate glutamate receptor localization and clustering. Receptor movements are fundamental to basic synaptic function and participate in many forms of synaptic plasticity.

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