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Molecular dynamics of postsynaptic receptors and scaffold proteins

Marianne Renner[1,2], Christian G Specht[1,2] and Antoine Triller[1,2]

The activity of neurotransmitter receptors determines the strength of synaptic transmission. Therefore, the clustering of receptors at synapses is an important mechanism underlying synaptic plasticity. The dynamic exchange of receptors between synaptic and extrasynaptic membranes is dependent on their interaction with synaptic scaffold proteins. Here, we review the recent advances and emerging concepts related to the dynamics of synaptic proteins at inhibitory and excitatory synapses. These include the imaging techniques that enable the study of protein dynamics in cells, the differences and similarities of receptor dynamics at excitatory and inhibitory synapses, the relationship between the exchange of receptor and scaffold proteins, as well as the role of receptor fluxes in the modulation of synaptic strength.

Addresses
[1] Inserm U789, Biologie Cellulaire de la Synapse, ENS, 46 rue d'Ulm, 75005 Paris, France
[2] Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France

Corresponding author: Triller, Antoine (triller@biologie.ens.fr)

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Introduction

The law of *the Dynamic Polarisation of the Neuron* by Santiago Ramón y Cajal states the directed flow of information in neurons from the somato-dendritic to the axonal compartment, where the signal is transmitted to a neuron downstream in the network. The consequence of this directionality is an asymmetric organisation of the neuron, which is as we now know reflected in the molecular compartmentalisation of the plasma membrane at the presynaptic and the postsynaptic side. Indeed, the heterogeneous distribution of membrane molecules like channels and receptors forms the basis of neuronal transmission. Neurons evolved to maintain this precise organisation together with the capacity to change it in response to plasticity events. They succeed to do so despite the irritating and inevitable habit of molecules to move in response to thermal agitation. The Brownian movements that result are translated into diffusion at a macroscopic level, which tends to favour the homogeneous distribution of the molecules. In other words, neurons as well as other cells must deal with molecular diffusion, spending energy and making efforts to maintain molecules in certain cellular locations, which has recently been formulated as the *brake* concept [1].

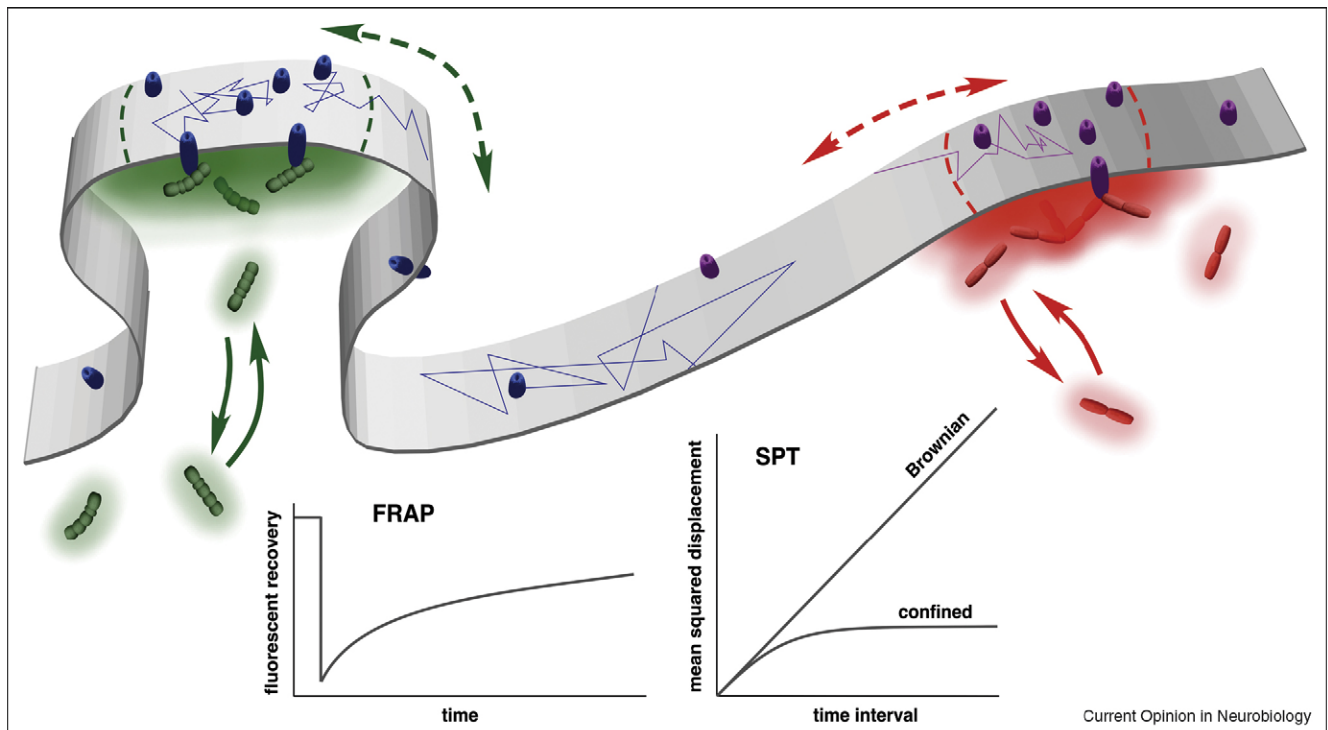
In neuronal synapses receptors for neurotransmitters are stabilised at the postsynaptic membrane by interactions with a subjacent meshwork of scaffolding proteins. Nevertheless, their stabilisation is transient and receptors diffuse in and out of the synaptic membrane at unexpectedly high rates (reviewed in [2]) (Figure 1). The synaptic scaffold proteins are also highly dynamic. Recent data about the dynamics of synaptic components supports the view of the synapse as a steady-state structure with different local equilibrium states, each one corresponding to a given activity level. The concept that emerges from this is that the population of extrasynaptic receptors is in equilibrium with the synaptic one. Therefore, it could be possible to rapidly shift this equilibrium in either direction, merely by modifying the exchange rates. Another important concept is that the extrasynaptic membrane acts as a reserve pool for synaptic receptors.

In addition to this fast exchange because of lateral diffusion, most neurotransmitter receptors cycle between the membrane and the intracellular stores [3–5]. These types of trafficking processes involve endocytosis and exocytosis and are beyond the scope of this review. We will concentrate on recent data concerning the dynamic properties of receptors at the plasma membrane and of scaffolding proteins at inhibitory and excitatory synapses.

A word about diffusion studies

The most frequently used method to study the dynamic exchange of proteins is the fluorescence recovery after photobleaching or FRAP. The molecules of interest are tagged typically with a fluorescent protein such as GFP. A small fluorescent region of the cell is then bleached with a high intensity laser, thus creating optically separate pools of the same protein in separate cellular compartments. These two pools subsequently exchange according to their diffusion properties, and this exchange is detected as fluorescence recovery over time (Figure 1). The technique is easily implemented. However, the interpretation of the obtained data is not straightforward despite its apparent simplicity [6]. FRAP provides a bulk measurement of diffusion and tells about the proportion of a

Figure 1



Dynamic behaviour of synaptic proteins. The diffusion of excitatory (blue) and inhibitory receptor complexes (purple) in the plasma membrane is free (Brownian) at extrasynaptic locations and confined within synapses. These patterns of diffusion can be differentiated by SPT of individual receptor complexes (see right graph). At excitatory (left, green) and inhibitory synapses (right, red) receptors are immobilised through interactions with the synaptic scaffold. Finally, receptors can enter and exit synapses (blue and red dashed arrows). Synaptic scaffold proteins such as PSD-95 (at excitatory synapses, green) and gephyrin (inhibitory, red) also exchange between synaptic and nonsynaptic compartments. Their dynamic exchange is frequently measured by FRAP (see left graph). Dotted lines delineate excitatory (green, left) and inhibitory synaptic regions (red, right).

population of molecules that exchange. It can, in theory, be used to determine the diffusion coefficients and the binding constants of the interactions between receptors and their scaffolding proteins. However, this requires the development of appropriate equations that take into account the particular geometry of neurites, as described in Holcman and Triller [7]. In principle molecules should move with a Brownian (free) form of displacement, but this is not always the case in biological membranes. Indeed, the diffusion behaviours that molecules can adopt (free, anomalous, confined, directed; [1]) reflect the organisation of the membrane. As molecules must encounter in order to interact, their diffusion behaviour may influence the success of chemical reactions, especially when they concern only a few molecules as in the case of receptors (few tens up to 200 molecules, average of about 100 copies [8]). An important disadvantage of the FRAP approach is that it cannot distinguish between different types of diffusion in a given population of molecules and that its accuracy is furthermore limited by the optical point spread function of the experimental set-up and preparation (200–300 nm).

In recent years another approach started to be successfully applied to measure the diffusion of individual receptors in neurons. Single particle tracking (SPT) techniques consist in following the movements of individual molecules to which a particle is bound that in turn can be detected by optical imaging, that is a latex bead, a fluorophore or a quantum dot (QD). This particle can be attached to the molecule covalently or by a specific ligand. SPT is more complicated in comparison to FRAP regarding the data analysis; and the size of the attached particle can influence the capacity of molecules to enter into narrow spaces like the synaptic cleft [9]. Nonetheless, it provides unique information about the heterogeneity of the population, as well as the type of diffusion behaviour of individual molecules (Figure 1). More importantly, it provides much higher spatial resolution than conventional optical imaging [1]. The interpretation of SPT data generally takes several parameters into account such as the diffusion coefficient (area explored per unit of time), the diffusion behaviour (free, confined, etc.) or the dwell time (residency time) [10]. As the absolute numbers obtained strongly depend on the data acquisition parameters, notably on the sampling

frequency [1], comparisons between studies are difficult. Instead, one can compare the relative changes that are reported in each study.

The dynamics at inhibitory synapses

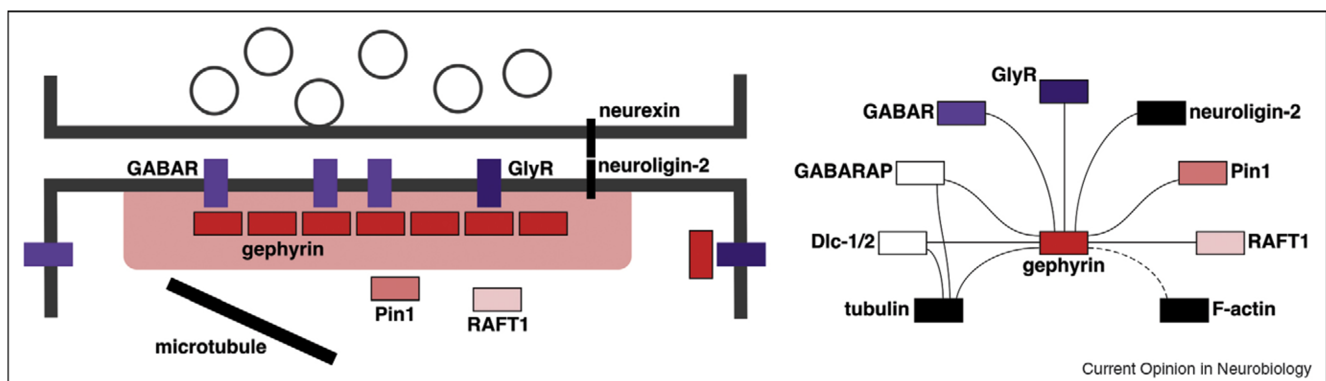
At inhibitory synapses, glycine and GABA receptors are embedded in most postsynaptic membranes in the lower CNS while GABA receptors alone predominate in the rostral parts (reviewed in [11]). Activation of these receptor systems leads to hyperpolarisation of the postsynaptic terminal along with the influx of chloride ions, thus reducing the likelihood of depolarisation of the neuron through excitatory stimuli. The clustering of glycine and GABA receptors at synapses depends on the adaptor protein gephyrin that not only provides binding sites for the immobilisation of inhibitory receptors, but also interacts with microtubules and, indirectly, with the actin cytoskeleton (Figure 2; for review see [12]). The synaptic clustering of gephyrin itself is shaped by the ability of the N-terminal and C-terminal domains of gephyrin to trimerise and to dimerise, respectively. The gephyrin scaffold is therefore believed to be a relatively organised hexagonal lattice that is dynamically regulated through the insertion or through the removal of individual gephyrin monomers [13].

Glycine receptors

Early experiments using latex beads showed that glycine receptors move laterally in the cell membrane of COS7 cells and young spinal cord neurons [14•]. Despite the size of the probe that hindered the tagged receptors to enter into synapses, two distinctive behaviours were recognised: free (Brownian) diffusion outside of synapses and long confinement periods of GlyRs on top of the synaptic gephyrin clusters. In other words, receptors are free to move outside of gephyrin clusters and the change of behaviour on top of the clusters reflects their interaction with gephyrin that puts a brake on the diffusion of the receptors [14•]. The use of QDs, nanometer-sized semiconductor crystals for molecular labelling that provide long-lasting fluorescence emission, was a great improvement for SPT and remains the most commonly used method to study receptor dynamics by SPT to date. The measurements of GlyR diffusion on spinal cord neurons using QDs allowed the first direct observation of the dynamics of receptors at synapses [15••]. Receptors displayed confined diffusion at synapses and were shown to exchange between synaptic and extrasynaptic locations within minutes.

The change in diffusion behaviour at synapses reflects the differences in the structure of the synaptic membrane in comparison to the extrasynaptic one. The mobility of receptors is affected by the stabilisation through scaffolding proteins but diffusion is also altered by the presence of obstacles (immobile or less mobile molecules) or barriers in or next to the membrane (scaffold, adhesion proteins and cytoskeletal structures). The capacity of synapses to retain receptors does not only depend on the interactions with the scaffold but also on the regulation of receptor fluxes into or out of the synapse [15••]. Indeed, the diffusion of GlyRs is regulated by the cytoskeleton. The depolymerisation of F-actin and microtubules was shown to decrease the amount of receptors at synapses by shifting the equilibrium toward the extrasynaptic pool. This is also reflected by a decrease in the dwell time of the receptors at synapses and the concomitant increase in the exchange of receptors between synaptic and extrasynaptic locations [16]. Although the mobility of receptors is greatly reduced at synapses, there

Figure 2



The scaffold at inhibitory synapses. (Left) The scaffold at inhibitory synapses is largely composed of gephyrin (red), which is believed to oligomerise and form a submembranous lattice. Gephyrin offers binding sites for the attachment of the main types of inhibitory receptors, GlyRs and GABARs (purple), at synapses. Gephyrin also interacts with the cytoskeleton and adhesion proteins (black), proteins that are involved in trafficking such as GABARAP and the dynein light chain (Dlc) proteins (white), as well as with synaptic regulatory proteins (light red tones) such as Pin1 and RAFT. Pin1 has been proposed to induce a conformational change of gephyrin and thus control the affinity of the GlyR–gephyrin interaction. (Right) Network of interactions between the constituents of the inhibitory postsynaptic density. Direct and indirect interactions are represented as black and dashed lines, respectively.

remains a large heterogeneity of behaviours, as judged by a large distribution of diffusion coefficients [15••,16,17]. In fact, receptors appear to be retained at synapses by interactions that follow different kinetics. In the case of GlyRs one may distinguish between two synaptic populations of receptors: those that are tightly bound to gephyrin and those that are slowed down at synapses because of steric constraints [17]. Interestingly, the diffusion behaviours of extrasynaptic GlyRs suggest that they diffuse in a gephyrin-bound form [17].

Changes in receptor dynamics are translated into alterations in the number of synaptic receptors, allowing the synapse to rapidly adjust its efficacy to the required levels. For example, the clustering of GlyRs at inhibitory synapses has recently been found to be subject to a rapid homeostatic regulation in response to excitatory activity [18••]. NMDA receptor stimulation reduced the diffusion coefficient of the GlyR, while increasing its clustering at synapses.

GABA receptors

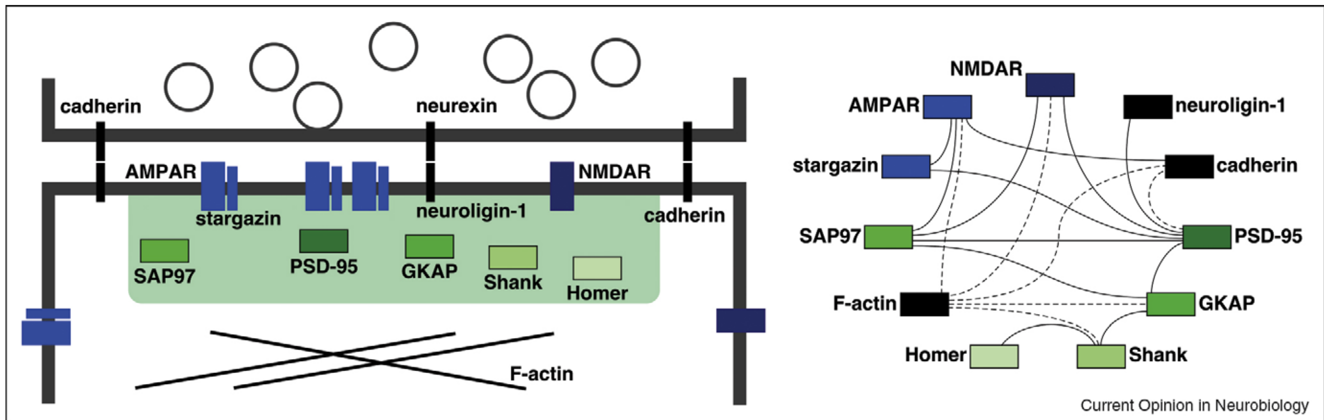
The inhibitory $GABA_A$ receptors also diffuse laterally in the plasma membrane. This was studied by different bulk approaches. Using a pHluorin-tagged $GABA_AR$ β3 subunit the diffusion of surface receptors was addressed by FRAP. It was found that $GABA_ARs$ are slowed down at synapses as compared to extrasynaptic receptors, and that this depended on the presence of the scaffold protein gephyrin [19]. Bogdanov *et al.* [20••] labelled pHluorin-tagged $GABA_ARs$ with fluorescent α-bungarotoxin to study the surface trafficking of receptors. In analogy with what was observed for the GlyR, this revealed that synaptic $GABA_ARs$ are directly recruited from the extrasynaptic pool. Another method to reveal and quantify the exchange of synaptic receptors, named electrophysiological tagging, consists in using a specific and irreversible antagonist to the receptor. The recovery of the activity is an indication of the replacement of blocked receptors with active ones. By this means it was shown that the total surface population of $GABA_ARs$ shows no fast recovery (at least up to 30–40 min) after irreversible inhibition. However, the synaptic population was exchanged within minutes [21]. In conclusion, extrasynaptic receptors represent a reserve pool that can rapidly counteract the modifications of synaptic receptors. This in turn can have profound effects on synaptic plasticity.

The scaffold at inhibitory synapses

The accumulation of proteins in the postsynaptic terminal creates an electron-dense submembranous structure referred to as postsynaptic density or PSD. At inhibitory synapses, the main constituent of the synaptic scaffold is gephyrin that offers binding sites for the immobilisation of glycine and GABA receptors [22,23•] and for other molecular constituents of the PSD [12] (Figure 2). The influence of gephyrin on the dynamics of the receptor is not, however, confined to the synapse. In the extrasynaptic membrane approximately 40% of GlyRs appear to be attached to gephyrin microclusters that reduce their speed of diffusion significantly [17]. Gephyrin is also present in intracellular trafficking complexes for the transport of GlyR-containing vesicles along microtubules [24], together with the dynein light chain proteins Dlc-1 and Dlc-2 in retrograde trafficking [25]. This indicates that gephyrin is involved in most steps of the glycine receptor life cycle from intracellular trafficking to membrane diffusion, synaptic anchoring and retrograde transport. Nonetheless, little is known on the dynamic properties of gephyrin itself. When compared to the fast exchange of GlyRs the replacement of gephyrin molecules at synapses is relatively slow, as judged by FRAP. Only around 30% of the synaptic gephyrin is exchanged within 5 min, followed by an even slower phase of recovery with a time constant of over one hour (Charrier, Calamai and Triller, unpublished data). These observations suggest that as an entity the synaptic gephyrin scaffold is more stable than the receptor pool, offering an excess of binding sites for the immobilisation of diffusing receptors.

The clustering of gephyrin is in turn dependent on the synaptic proteins with which it interacts: receptors, adhesion proteins as well as the cytoskeleton (Figure 2). For example, receptors have the potential to induce the clustering of gephyrin, as has been shown for the $GABA_AR$ [26]. The cytoskeleton also determines the stability of the synaptic scaffold, and its disruption decreases the synaptic clustering of gephyrin [16]. Fast rearrangements of the gephyrin scaffold are dependent on F-actin and may represent the dynamics of individual gephyrin molecules in the lateral morphing of the PSD [27]. On the other hand, the lateral movement of the entire gephyrin clusters along dendrites is increased by the disruption of the microtubules. The lateral movements of gephyrin clusters were also reduced in response to synaptic activity, though it remains unclear how these effects are mediated [27]. Last but not least, the clustering of gephyrin is complicated by the presence of many different splice variants, whose functions are only beginning to be understood [12]. The insertion of an additional exon in the N-terminal domain of gephyrin was found to reduce the ability of gephyrin to oligomerise and to clusterise [13]. Taken together, the regulation of gephyrin clustering provides an ideal mechanism for the plastic regulation of receptor numbers at synapses. Nonetheless, the synaptic clustering of inhibitory receptors may also be controlled by the modulation of the gephyrin–receptor interaction, as has been proposed in the fast homeostatic response of GlyRs to excitatory stimulation [18••]. Indeed, the phosphorylation of gephyrin and a subsequent conformational change by prolyl-isomerisation through Pin1 result in a higher affinity of gephyrin for GlyRs and may thus represent a mechanism to control GlyR clustering and function [28•].

Figure 3



The scaffold at excitatory synapses. A high complexity characterises the synaptic scaffold at excitatory synapses. The simplified model (left) shows only a limited number of synaptic scaffold or adaptor proteins (green shades) that provide binding sites for excitatory receptor types (AMPARs and NMDARs, blue) as well as cytoskeletal, adhesion and adaptor proteins. The postsynaptic density (PSD, green area) at excitatory synapses displays a subsynaptic organisation (see text for detail). While, for example, the scaffold protein PSD-95 is present throughout the entire PSD, the cadherins and SAP97 are located at the periphery of the PSD. (Right) Network of interactions between the constituents of excitatory PSDs. The dynamics of synaptic components are complicated by the complexity of the possible interactions in the PSD at excitatory synapses. Direct and indirect interactions are represented as black and dashed lines, respectively.

The dynamics at excitatory synapses

The PSD at excitatory synapses is thicker and much more complex than that at inhibitory synapses, and brings together receptors, scaffold and adhesion proteins, kinases and phosphatases, as well as cytoskeletal elements [8] (Figure 3). Posttranslational modifications such as the phosphorylation of synaptic proteins also add to the complexity of PSDs [29]. This complexity forms the basis of the outstanding signal processing capabilities of excitatory synapses. Recent studies have begun to dissect the internal structure of the PSD and to identify lateral and laminar patterns of organisation, as well as the presence of microdomains within the PSD. The main ionotropic receptors at excitatory synapses are AMPA-type and NMDA-type glutamatergic receptors. While NMDARs are distributed throughout the entire PSD membrane, AMPARs appear to localise to small subsynaptic domains within the PSD [30]. Among the different synaptic adhesion proteins, the cadherins localise to peripheral regions of the PSD, where they not only represent barriers for diffusion, but also provide binding sites for receptors, for example through their interaction with the extracellular domain of GluR2-containing AMPARs [31]. By contrast, the central scaffold protein PSD-95 is distributed throughout the entire PSD [32], and may be a component of vertical filaments [33]. The related adaptor protein SAP97 on the contrary is located rather at the edge of the PSD, in agreement with its role in the trafficking of AMPARs [32]. Both adaptor proteins can bind to AMPARs, however, PSD-95 binding to GluR1-containing receptors is mediated by the membrane protein stargazin [34]. The adaptor protein Shank3 may also play a key role in the organisation of the PSD through its assembly into helical fibres [35]. It is localised more on the cytoplasmic side of the PSD, where filamentous structures extend to the F-actin cytoskeleton of the synaptic spine [36].

AMPA-type glutamatergic receptors

The mobility of AMPARs at the cell surface was initially demonstrated using latex beads [37]. This was confirmed by labelling GluR1 or GluR2 receptor subunits with single fluorophores or QDs [37–40,41••]. While some synaptic receptors were immobilised to the extent that little or no diffusion was observed in the time range of SPT experiments (seconds to minutes), a large proportion of the receptor population (around 50%) remained much more mobile [38]. The exchange of synaptic AMPARs was confirmed by FRAP experiments using pHluorin-tagged GluR2 [42] or EYFP-GluR1 [43], or by electrophysiological tagging, where synaptic receptors were irreversibly inhibited by the photoreactive receptor antagonist ANQX [44]. The slow-down of receptors at synapses is related to their interaction with the scaffolding molecule PSD-95 via stargazin [45].

Because AMPARs have a preponderant role in the expression of synaptic plasticity, a central question is whether receptor diffusion is regulated by synaptic activity. Indeed, global changes in network activity modify the mobility of AMPARs. The application of glutamate, for example, increases the diffusion of GluR2-containing AMPARs, while blocking inhibitory activity with bicuculline did not exert any effect [38]. Depolarisation of

neurons with high concentrations of KCl or decreasing neuronal activity by tetrodotoxin (TTX) modified AMPAR but not NMDAR mobility, whereas the activation of PKC modified both [39]. Finally, using viral infections to silence synapses it was shown that AMPARs, which were confined to small areas in active synapses, explored much larger areas in inactive ones [40]. An exciting report recently described the effect of lateral diffusion on the recovery from paired-pulse depression [41••]. This fast recovery within tens of milliseconds can be explained in part by the rapid exchange of desensitised AMPARs by naïve ones [41••]. Therefore, the extrasynaptic membrane compartment provides a reserve pool of ready-to-use receptors that plays an important role in the maintenance of synaptic transmission. The exchange by lateral diffusion happens in a time range of seconds [38,40,45], whereas trafficking events depending on endocytosis and exocytosis take place on a time scale of tens of minutes (Refs. in [5]). Altogether, neurons evolved not to completely overcome thermal agitation and lateral diffusion, but instead to utilise diffusion as a fast and energetically advantageous way to modify the synaptic strength.

NMDA receptors

An initial electrophysiological study in autaptic neurons showed that NMDAR currents recover rapidly (10–20 min) after the irreversible blockade of synaptic receptors with the antagonist MK801 [46]. This recovery could only be explained by the replacement of synaptic receptors, confirming that NMDARs also diffuse between synaptic and extrasynaptic membranes. The rate of recovery was about 65% within 7 min, indicating that most NMDARs exchange quickly between the two compartments [46]. In fact, SPT experiments revealed that NMDARs are less mobile than AMPARs [39]. This observation is in agreement with the differences in the turnover of the two types of receptor as determined by FRAP [43]. In addition, NMDARs display different mobility depending on their subunit composition. NR2A-containing receptors are less mobile than NR2B-containing ones [47]. This is in line with the decrease of NMDARs mobility during synaptogenesis, which can be explained by the increased NR2A/NR2B ratio in mature synapses. The stabilisation of receptors at synapses is not limited to their interaction with scaffold molecules. For instance, the dopamine receptor D1 diffuses freely in dendrites but it can be stabilised in spines by interacting with NMDARs [48]. This interaction requires the activation of NMDARs, which change their conformation after ligand binding. The NMDAR–DR1 complex displays a reduced mobility and the overall effect is to stabilise DR1 at dendritic spines [48]. This link between the glutamatergic and the dopaminergic systems may have functional consequences for synaptic plasticity and also play a role in psychiatric diseases such as schizophrenia.

The scaffold at excitatory synapses

The adaptor protein PSD-95 forms the core of the scaffold at excitatory synapses (Figure 3). The maintenance of the synaptic structure requires a constant replacement of proteins lost by degradation with newly synthesised ones. During constitutive replacement, however, the synaptic strength must be preserved, which is particularly important when it relates to the exchange of scaffold proteins, because these offer the necessary binding sites that regulate the receptor number at synapses. In line with these observations, synaptic PSD-95 clusters may be stable for days, yet individual PSD-95 molecules exchange with a half-life of less than one hour [49••]. The presence of a soluble pool of PSD-95 also means that individual adaptor proteins can exchange between nearby synapses. The exchange of PSD-95 was repeatedly measured by FRAP in primary neuron cultures. Despite the differences in the precise methodology employed in these studies, the exchange rates of PSD-95 are remarkably reproducible, ranging from 25% to 35% of recovery within 5 min, followed by a slow component of recovery [43,50•,51]. By contrast, the related protein SAP97 has a faster exchange rate with over 50% of recovery within 5 min [52]. The different exchange rates of these two proteins may be related to their respective functions, PSD-95 being involved in the anchoring of AMPARs in the PSD and SAP97 playing a role in the trafficking of AMPARs. At the same time, the differences in the exchange rates may reflect the subsynaptic localisations of PSD-95 and SAP97, the latter being restricted to the edges of the PSD [32]. The exchange rates of PSD-95 and SAP97 appear to be influenced also by the presence of alternatively spliced N-termini. While the main variant of SAP97 (βSAP97) contains an L27 protein interaction domain and displays a relatively high speed of recovery, the αSAP97 variant with its palmitoylated N-terminus has a much slower exchange rate that resembles that of palmitoylated PSD-95 (Waites, Specht and Garner, unpublished data). Furthermore, both PSD-95 and αSAP97 enhance the synaptic AMPAR currents, implying a certain degree of functional redundancy between the palmitoylated isoforms [53].

Comparable to the situation at inhibitory synapses, the scaffold at excitatory synapses offers binding sites for its respective receptor types (Figure 3). Yet the number of PSD-95 molecules exceeds the number of synaptic receptors by about threefold ($\sim$300 copies of PSD-95) [8]. Taking into account the presence of other synaptic adaptor proteins such as the GKAP, Shank and Homer families, the ratio between receptors and adaptors differs by at least one order of magnitude [8]. The sequential order in which these adaptor proteins are associated with the PSD-95 scaffold is paralleled by their exchange kinetics. In other words, the slowest fluorescence recovery was observed for PSD-95 itself, followed by GKAP, Shank2 and Homer 1c in ascending order [50•]. Another

interesting feature of the different scaffold proteins is that their kinetic properties are differentially regulated by synaptic activity. Synaptic stimulation protocols using bicuculline and 4-AP did not alter the steady-state levels of PSD-95, increased the synaptic localisation of GKAP and caused the dispersal of Shank2 and Homer 1c [50•]. An acceleration of the exchange rate of Shank3 was also observed in response to electric stimulation during the recovery phase, though this was not reflected in changes in the steady-state level of the protein [54••].

In summary, these findings show the strength of FRAP and related methods to measure the exchange of protein populations, though the exact interpretation of FRAP results remains controversial [6]. The results also confirm that a discrepancy exists between the fast exchange of receptors through lateral diffusion on the time scale of minutes, and the slower exchange of adaptor proteins that may take hours.

Is all this true *in vivo*?

While our knowledge about lateral diffusion in cultured neurons is rapidly growing, technical limitations complicate studies *in vivo* and even in organotypic cultures. SPT techniques are currently applicable only to cultured neurons because the membranes of these cells are easily labelled with fluorescent ligands and can be efficiently rinsed after labelling. By contrast, the penetration of SPT probes such as QDs in tissues is problematic. Nevertheless, this is a very active field of research and the first report on the *in vivo* dynamics of PSD-95 has recently been published. Using two-photon microscopy, Gray *et al.* [49••] could show that PSD-95 molecules tagged with photoactivatable GFP have a rapid turnover (20–60 min) in cortical neurons, while the PSD-95 clusters themselves that represent synapses are stable for days. Moreover, the retention times obtained are in good agreement with measurements done in cultured cells. Whether the dynamic properties of proteins in cultured cells more generally match those obtained by *in vivo* studies remains, however, to be seen.

Functional implications

The continuous insertion of receptor proteins into the plasma membrane is balanced by their internalisation. The characteristic time of this basal exchange between intracellular and cell surface populations of receptors is probably in the order of hours (e.g. 16 hours for AMPARs, [44]). Within the plane of the membrane, however, there is a must faster exchange between synaptic and extrasynaptic compartments, as discussed above. Extrasynaptic receptors are not easily detected by immunocytochemistry experiments, because of the limitations of this technique for the detection of antigens at low concentration (Refs. in [2]). However, despite the fact that they are 10–100 times less concentrated than at synapses, extrasynaptic receptors are actually much more numerous. This is due to the fact that the extrasynaptic membrane has a much larger surface area than the synaptic one. Therefore, the extrasynaptic receptors constitute a reserve pool that can be rapidly mobilised into the synapse in response to activity, allowing fast regulation of synaptic strength. This has recently been demonstrated for the homeostatic regulation of the inhibitory glycinergic response mediated by the lateral diffusion of glycine receptors in response to NMDA receptor activation [18••]. Furthermore the peri-synaptic receptors, wandering in quest of a synaptic identity, are likely to be the ones activated by spill-over of neurotransmitters following intense release (Refs. in [2]). Lateral diffusion of receptor is also likely to play a key role in synaptic reliability. Indeed, molecules that have been locally modified (desensitisation of receptors, phosphorylation, etc.) can be rapidly replaced by naïve ones. In excitatory synapses the recovery of AMPARs from desensitisation has been proposed to depend in part on the fast exchange of the synaptic receptor pool [41••].

The mobility observed for both receptors (that diffuse in the plane of the membrane) and scaffold proteins (that diffuse in the cytoplasm) suggests that they can visit different synapses over time. This raises the interesting possibility of a competition between synapses to gather their components [49••,54••]. A future challenge will be to measure the characteristic times of synaptic localisation or stabilisation for each molecule at various states of activity, which will further our understanding on how the synapse is constructed, maintained and modified by plasticity. Interestingly, the receptor–scaffold interactions appear to be rapidly reversible. Together with the high turnover of scaffold proteins this hints to reciprocity of stabilisation between receptors and the synaptic scaffold.

Conclusions

Probably the most important feature of synapses is their capacity to remain stable for long periods of time without loosing their ability to remodel. In addition, we are now aware that receptors enter and leave the synaptic region at rates varying between seconds and tens of minutes. Thanks to the improvement of single molecule tracking techniques we can now determine the characteristic times of the interactions established by individual proteins at synapses to understand the structural plasticity of synapses at a nanoscopic level. The complexity of the molecular composition at synapses together with the continuous mobility of synaptic proteins makes us think that the synaptic structure is the result of a synergy between numerous weak interactions. In other words, the synapse exists thanks to the continuous exchange of its components together with the multiplicity of weak molecular interactions. Therefore, both stability and plasticity could be achieved by modulating the fluxes of molecules that enter and exit the synaptic area. Although this can be intuitively understood, it will be important to characterise the dynamics of molecular

interactions that enable the transition from one set point of the equilibrium with a given number of synaptic receptors to another state, without the disruption of the synaptic organisation. A future challenge will be to unravel at the mechanistic level the conditions of quasi-equilibrium within the PSD that allow stability despite plasticity and plasticity despite stability. Finally, a next frontier will be to understand how these diffusion-reaction processes impact on the computational properties of neurons within networks *in vivo*, and how dysfunction or interferences in this process may be at the origin of symptoms in various synaptopathies.

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