Chapter 9
Surface Traffic in Synaptic Membranes

Martin Heine

Abstract The precision of signal transmission in chemical synapses is highly dependent on the structural alignment between pre- and postsynaptic components. The thermal agitation of transmembrane signaling molecules by surrounding lipid molecules and activity-driven changes in the local protein interaction affinities indicate a dynamic molecular traffic of molecules within synapses. The observation of local protein surface dynamics starts to be a useful tool to determine the contribution of intracellular and extracellular structures in organizing a plastic synapse. Local rearrangements by lateral diffusion in the synaptic and perisynaptic membrane induce fast density changes of signaling molecules and enable the synapse to change efficacy in short time scales. The degree of lateral mobility is restricted by many passive and active interactions inside and outside the membrane. AMPAR at the glutamatergic synapse are the best explored receptors in this respect and reviewed here as an example molecule. In addition, transsynaptic adhesion molecule complexes also appear highly dynamically in the synapse and do further support the importance of local surface traffic in subcellular compartments like synapses.

Keywords AMPA-receptors • Endocytosis • Exocytosis • Lateral diffusion • Quantum dots

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9.1 Introduction

The local number, composition, and density of signaling molecules at synapses are important determinants of synaptic plasticity. Changes in synaptic protein content were identified to be basic features of synaptic plasticity and memory formation (Kessels and Malinow 2009). Mechanisms like the endo-exocytotic cycle of synaptic membrane proteins, intracellular transport, and local synthesis of new molecules were shown to change local number, density, and composition of proteins within a time window of minutes to hours (Newpher and Ehlers 2008). Initialization of short- and long-term changes in synaptic plasticity takes place in a few milliseconds and is known to depend on interplay between pre- and postsynaptic mechanisms. Kinetic properties of pre- and postsynaptic molecules are identified to play a major role within this very first moment of activity changes, including calcium-induced facilitation or depression of presynaptic release properties (Catterall and Few 2008; Neher and Sakaba 2008) or postsynaptic receptor saturation and desensitization (Trussell et al. 1993; Xu-Friedman and Regehr 2003). In order to weaken or strengthen synaptic transmission, the alignment between pre- and postsynaptic elements has been shown to be crucial (Franks et al. 2003; Raghavachari and Lisman 2004; Shouval 2005; Xie et al. 1997). In this respect, the view of the synapse as a highly ordered compartment of signaling molecules has to be combined with a high molecular dynamic. The self-agitation of lipids and transmembrane molecules in biological membranes predicts that the position and local density of signaling molecules might be highly variable (Fig. 9.1). Within the membrane, all components can undergo rotational, translational, and lateral motions (Edidin 2003; Saxton and Jacobson 1997). These provoked questions as how is the synaptic molecular structure and density established and maintained? Is lateral mobility controlled or even used as a mechanism for synaptic organization and plasticity? Which quantities of molecules are involved? What is the turnover of crucial molecules?

Here, I will focus on functional implications and consequences of the surface traffic of proteins within and around pre- and postsynaptic membranes. Several mechanisms of dynamic protein stabilization were proposed (Poo 1985, Fig. 1) and recently revisited:

- Diffusion-Mediated trapping by intracellular anchors (Triller, Choquet 2008)
- Interactions with the extracellular matrix (Dityatev et al. 2010; Gundelfinger et al. 2010)
- Changes of membrane viscosity by different lipid composition (Renner et al. 2009a)
- Iontophoretic forces (Fromherz 1988; Savtchenko et al. 2000; Sylantyev et al. 2008).

Plasticity concepts based on a dynamic surface organization within both membrane compartments have been proposed (Franks et al. 2003; Raghavachari and Lisman 2004; Shouval 2005; Xie et al. 1997) but have not been experimentally

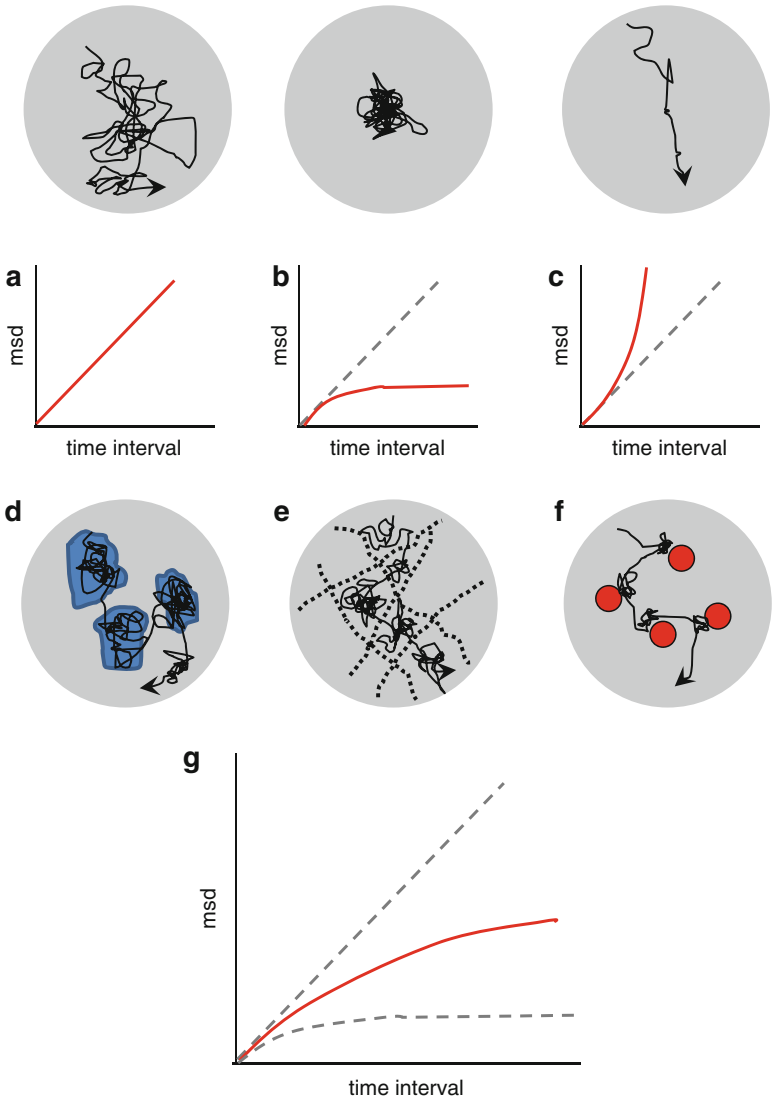

Fig. 9.1 The pictograms should demonstrate the different modes of diffusive behavior in the membrane and possible changes by structural elements within or attached to the membrane. The mean square displacement (msd) over time interval is the basal function for distinct diffusive properties as demonstrated for the extreme cases in **a**–**c**. (**a**) Free Brownian diffusion within the membrane is characterized by a linear msd versus time interval plot. The slope of this relationship determines the diffusion coefficient. (**b**) Confined motions within a defined area are reflected in the curved msd plot. The slope of the first points reflects the diffusion coefficient within the area. (**c**) The combination of diffusion and directed motion will result in a parabolic msd plot and can be separated from purely diffusive behavior. **d**–**f** are examples for anomalous diffusion, which represents the most abandon case of diffusion behavior in neuronal synapses and is an intermediate situation between free and confined diffusion. Several possible structural elements

addressed in much detail. The development and application of imaging techniques as FRAP (Axelrod et al. 1976), single particle tracking (SPT) (Saxton and Jacobson 1997), and STED microscopy (Dyba et al. 2003; Toomre and Bewersdorf 2010) to synaptic structures initiated the visualization of the molecular organization inside and around synapses. Recent developments in the field of microscopy to probe membrane dynamics have been reviewed extensively and will be not introduced here in detail (Marguet et al. 2006; Thoumine et al. 2008).

Application of these techniques has evaluated theoretical concepts of synaptic surface organization and there dynamics in such crowded and tiny compartments (diameter of 500 nm for a cortical synapse). In the past years, many experimental data support the view that the molecular noise induced by the thermal self-agitation of molecules, membrane potential changes, and intermolecular repulsive charges in the neuronal membrane are important variables to describe synaptic function as a dynamic network of signaling molecules. In the following sections, I will review the dynamics of molecules that are examples for the dynamic organization of the synaptic signaling apparatus.

9.2 Diffusion-Mediated Trapping of Postsynaptic Receptors

The first report about surface mobility of synaptic proteins came from FRAP experiments of acetylcholine receptors in the muscular membrane (Edidin and Fambrough 1973). Such experiments gave indications about the proportion of mobile and immobile acetylcholine receptors. With the application of SPT to neuronal cells (Borgdorff and Choquet 2002; Dahan et al. 2003; Meier et al. 2001), many more synaptic molecules were described to be mobile in the plasma membrane (see Table 9.1). The use of SPT provides us with detailed information about the location (accuracy of 10–50 nm), the temporal diffusion properties, relative dwell times, and relative population size of exchanging molecules within small membrane compartments. Diffusive properties can be quantified by the mean square displacement over time as an indication of the explored surface and give a quantitative measure for the apparent viscosity around the molecule of interest (for review, see Kusumi et al. 2005; Saxton and Jacobson 1997).

Fig. 9.1 (continued) are discussed in the text and illustrated here. (**d**) Changes in the membrane viscosity (*blue patches*) can introduce a confinement to free diffusive molecules in the membrane. (**e**) Intracellular as well as extracellular meshwork of structural elements (*dotted lines*) of the cytoskeleton or the extracellular matrix, respectively, can confine free diffusive molecules. (**f**) Transmembrane obstacles (*red dots*) can induce a transient confinement of diffusive molecules. (**g**) The curved msd plot is an often observed phenomenon of diffusive molecules in the membrane. Correlation of the trajectory with structural elements, for example, postsynaptic density markers, can be used to segregate the diffusive behavior in different membrane domains of the cell that are defined in time and space

Table 9.1 Median of diffusion coefficients from FRAP and SPT experiments

Molecule	$D_{synaptic}$ (μm/s)	$D_{extrasynaptic}$ (μm/s)	Mobile fraction synaptic (%)	Cell type	References
Ionotropic neurotransmitter receptor subunits					
GluA1	0.05	0.1	50–60 (D > 0.0075 μm/s)	Hippocampal neurons 14–21 DIV	Ehlers et al. (2007), Heine et al. (2008a)
GluA2	0.01	0.087–0.11	40–50 (D > 0.0075 μm/s)	Hippocampal neurons 10–14 DIV	Groc et al. (2004), Tardin et al. (2003)
TARP (γ2)	~0.01	0.075	25 (D > 0.0075 μm/s)	Hippocampal neurons culture (7–10 DIV)	Bats et al. (2007)
GluN1	0.02	0.037	70 (D > 0.0075 μm/s)	Hippocampal neurons 10–12 DIV	Groc et al. (2004)
GluN2A	0.0002	0.00075	25 (D > 0.0075 μm/s)	Hippocampal neurons 10–14 DIV	Groc et al. (2006)
GluN2B	0.05	0.025	71 (D > 0.0075 μm/s)	Hippocampal neurons 10–14 DIV	Groc et al. (2006)
GABAAR (γ2)	0.024	0.012	–	Hippocampal neurons	Bannai et al. (2009)
GlycinR	~0.005	~0.07	–	DRG neurons	Charrier et al. (2006)
nAchR (α3)	0.07	0.18	34 (total population)	Chick sympathetic ganglion neurons	Fernandes et al. (2010)
nAchR (α7)	0.067	0.18	61 (total population)	Chick sympathetic ganglion neurons	Fernandes et al. (2010)
Metabotropic neurotransmitter receptors					
mGluR5	0.014	0.14	>95 (D > 0.0001 μm/s)	Hippocampal neurons culture 21–27 DIV	Renner et al. (2010)
GABABR	–	–	50 (FRAP)	Hippocampal neurons culture 7 DIV	Pooler and McIlhinney (2007)
Dopamine receptor D1	–	0.7 (FRAP)	65 (FRAP)	Striatal organotypic culture	Scott et al. (2006)
Adhesion molecules					
α-Neurexin	–	–	100 (FRAP)	Parvalbumin-positive cells(organotypic slice)	Fu and Huang (2010)
β-Neurexin	–	~0.07 axon	100 (FRAP)	Parvalbumin-positive cells(organotypic slice) hippocampal neurons culture (6–8 DIV)	Fu and Huang (2010), Saint-Michel et al. (2009)
NCam	–	~0.13	–	Hippocampal neurons culture 10–14 DIV	Bard et al. (2008), Opazo et al. (2010)
SynCam	–	–	–	Hippocampal neurons	Breillat et al. (2007)

(continued)

Table 9.1 (continued)

Molecule	$D_{synaptic}$ (μm/s)	$D_{extrasynaptic}$ (μm/s)	Mobile fraction synaptic (%)	Cell type	References
N-cadherin	–	0.08 growth cone	n.d.	Hippocampal neurons culture (1–2 DIV)	Bard et al. (2008)
L1	–	0.25 axon	70 (D > 0.05 μm/s)	Hippocampal neurons culture (4–5 DIV)	Dequidt et al. (2007)
Membrane lipids					
DOPI	~0.06	–	42	Hippocampal neurons culture (14–20 DIV)	Renner et al. (2009b)
GPI	0.023[a] 0.046[b]	0.17	>99 (D > 0.0001 μm/s)	Hippocampal neurons culture (14–20 DIV)	Renner et al. (2009a)
Cholera toxin (G_{M1})	0.015[a] 0.029[b]	0.18	>99 (D > 0.0001 μm/s)	Hippocampal neurons culture (14–20 DIV)	Renner et al. (2009a)

[a]Median of diff.-coeff in inhibitory neurons
[b]Median of diff.-coeff in excitatory neurons; in some cases, data were only given as graphs indicated by (~)

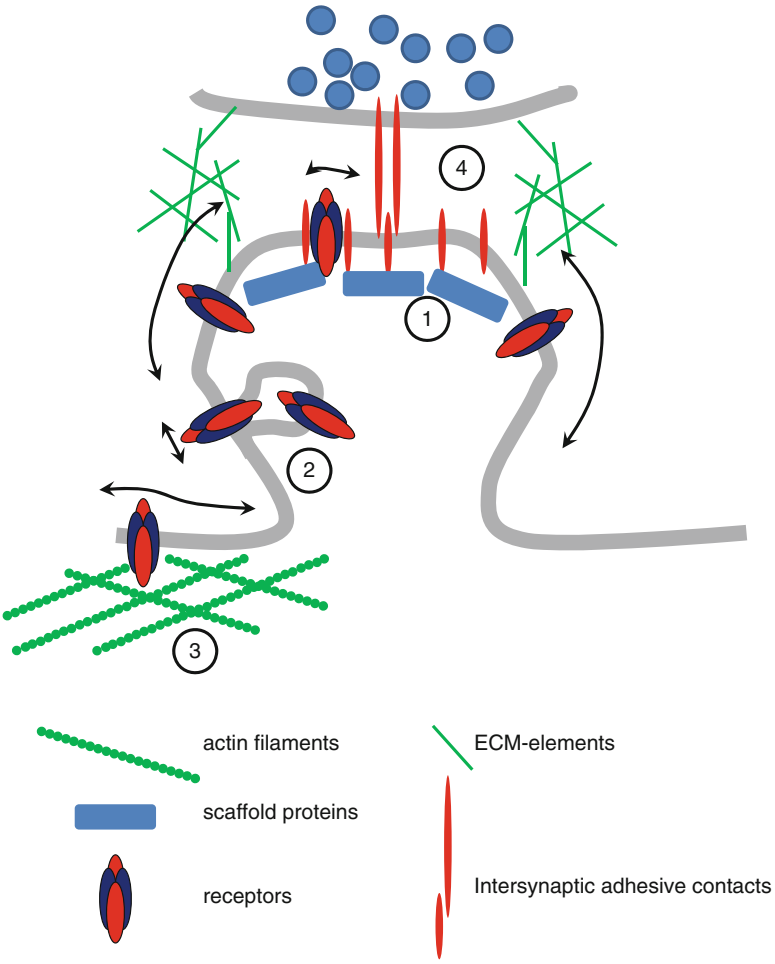

Fig. 9.2 Sketch of the dynamic organization of a spiny postsynaptic density: (*1*) intracellular scaffold proteins can interact with receptors and postsynaptic elements of adhesive contacts, (*2*) specialized zones like endocytotic pits do have a stabilization effect on diffusive receptors and will hinder them to escape from the synapse, (*3*) intracellular cytoskeleton is an important structure to regulate the surface traffic outside the postsynaptic density, and (*4*) intercellular structures like the extracellular matrix of adhesive contacts will have a passive, and, perhaps, also an active, impact on the mobility of receptors in and out of the PSD

In general, the macroscopic mobility of synaptic transmembrane molecules fulfills the characteristics of free and anomalous Brownian diffusion as evidenced by the linear or sublinear relationship of the mean square displacement (MSD) plots over time interval, respectively. For ionotropic receptors of inhibitory and excitatory synapses, it has been demonstrated by SPT (see Table 9.1) as well as electrophysiological measurements (Adesnik et al. 2005; Heine et al. 2008a; Thomas et al. 2005; Tovar and Westbrook 2002; Young and Poo 1983) that they

are in a continuous exchange between the synaptic and extrasynaptic membrane. Therefore, the number and density of those crucial postsynaptic molecules depend on the regulation of diffusion properties and the capacity of a synapse to trap these receptors inside the postsynaptic density (PSD, Fig. 9.2). Molecular interactions between receptors, scaffold proteins below the inner surface of the membrane, and elements of the cytoskeleton are mechanisms to enrich molecules in the PSD. In order to clarify the contribution of a particular interaction to the stabilization of the molecule outside and inside the synapse, the surface mobility is a sensitive readout. The cytoskeleton has been identified to control the diffusion of proteins as probed by the disruption of microtubules and actin filaments (Charrier et al. 2010; Gu et al. 2010; Lee et al. 2009; Renner et al. 2009a; Rust et al. 2010). Inside synapses, major scaffold proteins are identified for AMPA-, NMDA-, GABA-, and Gly-receptors, which contribute to the local organization of these receptors in the synaptic membrane (Choquet 2010; Newpher and Ehlers 2008; Triller and Choquet 2008). As an example, interacting partners for AMPAR in the glutamatergic synapse and their surface dynamic as a functional variable of synaptic transmission are discussed below (see also Choquet 2010; Gerrow and Triller 2010).

AMPARs are composed of four subunits (GluA1–4) and expressed as heteromeric tetramers in the neuronal membrane (Collingridge et al. 2004; Hollmann and Heinemann 1994). The different length of the intracellular C-terminus, the identification of PDZ-binding domains at the end of those termini, and the different types of PDZ-binding domains, type 1 GluA1 and type 2 for GluA2 (Shi et al. 2001), suggested a direct link between AMPARs and intracellular scaffold proteins with multiple PDZ domains like PSD95, GRIP, and PICK (Feng and Zhang 2009; Kessels and Malinow 2009). The different splice variants and subunits of AMPAR, in particular, GluA1 and GluA2, are often assembled as heterodimers and crucial for plastic changes as studied extensively in hippocampal synapses by the use of plasticity protocols to induce long-term potentiation or depression (LTP, LTD) (Kessels and Malinow 2009). Supported by a number of experimental findings, this has led to the hypothesis that the capacity of the postsynaptic side is defined by the number of PDZ domains and the composition and phosphorylation state of the AMPARs that predicts their affinity to the intracellular scaffold (Malenka 2003). Knockout of the GluA1-subunit impairs the induction of long-term potentiation (LTP) in classical high-frequency stimulation paradigm (Zamanillo et al. 1999). GluA1-subunit containing AMPARs are preferentially in cooperated into the synapse during LTP induction as compared to GluA2/GluA3 heterodimers with a shorter C-terminus and a different PDZ-binding domain (Shi et al. 2001). In line, a point mutation within the PDZ-binding domain of GluA1 to disrupt the affinity to the PDZ domain impairs LTP (Hayashi et al. 2000). After LTP induction, synapses are reconsolidated by the gradual loss of GluA1/GluA2-containing receptors which are replaced by GluA2/GluA3 receptors. The disruption of the endo-exocytosis of AMPA-receptors leads to a reduction of postsynaptic receptor population and an impairment of LTP (Ehlers 2000; Park et al. 2004). In parallel to these findings, a transient increase of calcium or induction of chemical LTP was demonstrated to immobilize receptors in the PSD (Borgdorff and Choquet 2002; Tardin et al. 2003).

Contradictory to these experiments, studies in knockout mice lacking GluA2- and GluA3-subunits show no impairment in LTP or LTD (Meng et al. 2003). Furthermore, the specific deletion of the complete PDZ-binding domain of the GluA1- or GluA2-subunit did not disturb basal transmission or LTP induction (Kim et al. 2005; Panicker et al. 2008). Both findings speak against a model that subunit-specific PDZ-binding domains of AMPARs alone are critical for the traffic of the receptor to the synapse and plastic changes of their strength.

The discovery of additional subunits of AMPAR, named transmembrane AMPA receptor regulatory proteins (TARPs, Chen et al. 2000) helped to resolve this discrepant results. Initial investigations of the stargazer mutant mouse lead to the discovery of stargazin, which was believed to be a subunit of calcium channels but has much more dominant function in the traffic of AMPAR in the cerebellum (Chen et al. 2000). TARPs enhance the surface expression of AMPAR (Priel et al. 2005; Tomita et al. 2005), bind directly to PSD95 (Schnell et al. 2002), change pharmacological properties of AMPAR (Milstein and Nicoll 2008), alter kinetic properties of AMPAR (Kato et al. 2010; Milstein et al. 2007; Morimoto-Tomita et al. 2009; Priel et al. 2005; Tomita et al. 2005), and modulate the diffusion properties in the synaptic membrane (Bats et al. 2007; Opazo et al. 2010). The knockout of the dominant TARP isoform γ8 in the hippocampus results in impaired LTP similar to those seen for the GluA1 knockout (Rouach et al. 2005). Following the surface mobility of TARPs together with AMPARs by SPT revealed that AMPARs without this additional subunit stay much shorter inside the PSD and spontaneous postsynaptic currents almost disappear. In addition, the interruption of the PDZ-binding domain of TARP (γ2, stargazin) but not of the GluA2-subunit disrupts the confinement of receptors in the synapse (Bats et al. 2007). By tagging stargazin, the diffusion coefficients were not different in comparison to AMPAR, which implicates that a large population of AMPAR is associated with stargazin (Bats et al. 2007). In order to prove the idea that TARPs stabilize AMPAR in the PSD, Sainlos et al. (2011) developed cell-permeable biomimetic divalent ligands to disrupt AMPAR stabilization in the PSD. Those ligands do specifically bind to PSD95 type 1 PDZ domains and similar PDZ domains of PSD95 like MAGUKS (SAP102, SAP97). Those peptides do indeed disrupt the stabilization of an AMPAR subpopulation but only transient. Application of the biomimetic ligands has a time-dependent effect on AMPAR diffusion but reduces EPSC amplitude to about 40% of the control. Monomeric ligands were not effective (Sainlos et al. 2011). The temporal effect on diffusion can be seen as a loss of stabilization sides in the synapse and a readjustment of the tightly controlled equilibrium between surface and intracellular pool of receptors controlled by endocytotic zones close to the PSD (Blanpied et al. 2002; Petrini et al. 2009; Racz et al. 2004). Endocytic zones have been identified to be a stabilization side for mobile receptors in the periphery of the PSD controlling the local surface population of AMPAR (Petrini et al. 2009). The milder effects of these biomimetic tools point to the multivalent interactions within the PSD to control receptor mobility. The transient change of receptor diffusion therefore supports the trap diffusion model and indicates the dynamic organization of AMPAR within the PSD.

To be plastic, such local trapping of receptors inside the synapse must be modular. Global manipulation of synaptic activity has profound effects on the receptor mobility in the neuronal membrane (Groc et al. 2004; Tardin et al. 2003). A disruption of presynaptic transmitter release revealed that receptor stabilization inside the PSD is activity dependent (Ehlers et al. 2007). Artificial modulation of the mobile population of synaptic AMPAR in parallel to electrophysiological measurements of synaptic responses demonstrated a significant contribution of mobile receptors to basal synaptic transmission (Choquet 2010; Heine et al. 2008a). In case of fast repetitive activation (>10 Hz) of the postsynaptic receptors, the responsive population declines faster if receptors are immobilized in the postsynaptic membrane. Liberating receptors by digestion of the extracellular matrix has opposite effects (Frischknecht et al. 2009; see also Frischknecht and Gundelfinger within this book). Recordings of synaptic responses confirmed that receptor mobility is a variable of short-term plasticity (Choquet 2010; Heine et al. 2008a). The kinetic properties of AMPAR, low affinity to glutamate (Featherstone and Shippy 2008), fast desensitization, and slow recovery from desensitization (Jonas et al. 1993), do support the finding that a fast exchange of glutamate-bound and glutamate-unbound receptors will influence the postsynaptic responsiveness to high-frequency transmitter release. In addition, it has been shown that the synaptic population of AMPARs is not saturated by a single transmitter vesicle (Liu et al. 1999; McAllister and Stevens 2000), supporting the possibility of a fast (within ms) exchange of glutamate-bound receptors by free receptors within the synapse.

Another kinetic property of AMPAR is the steady state desensitization in the presence of micromolar concentrations of glutamate (Featherstone and Shippy 2008; Raman and Trussell 1995). Investigating the interaction between AMPAR and stargazin, Morimoto-Tomita et al. (2009) propose that the association of GluA1 and stargazin is critical for a steady state current evoked by micromolar ambient glutamate concentrations. Furthermore, the dissociation of the receptor-TARP complex occurs at glutamate concentrations above 100 μM and leads to a faster and more complete desensitization as well as slower recovery from this conformational state. Hence, the described dissociation of the complex within a few ms proposes a more complex picture as seen by only looking at AMPAR diffusion. Liberated AMPAR may diffuse away from the scaffold-bound stargazin and stick to their next neighbor TARPs outside the focal plane of glutamate release. With the invention of new imaging techniques, we will be enabled to gain dynamic information for a large population of receptors simultaneously (Giannone et al. 2010; Manley et al. 2008). One might postulate that different activation states of AMPAR will show different diffusion kinetics and will allow further insights in dynamic association and dissociation of receptor complexes.

Focusing on a key molecule for plasticity in glutamatergic synapses, the calcium/calmodulin-dependent protein kinase II (CaMKII; Lisman et al. 2002), Opazo et al. (2010) have demonstrated that CaMKII induces a phosphorylation-dependent stabilization of stargazin that dominates the stability of AMPAR in the PSD and that has similar physiological consequences like artificial cross-linking of AMPAR in the postsynaptic membrane (Heine et al. 2008a). Taken together, this

underscores the importance of local molecular dynamics in the plasma membrane for synaptic transmission and plasticity.

The discovery of other subunits associated with AMPAR in different brain regions, as cornichons (Schwenk et al. 2009), CKAMP44 (von Engelhardt et al. 2010), and SynDIG1 (Kalashnikova et al. 2010), will probably further extend the dynamic view of the AMPAR as an association point for many interacting molecules that might tune the surface mobility and kinetic properties of AMPAR.

Despite the strong interactions of surface molecules with the PSD as discussed above for AMPARs and seen for GABAA-, Glycin-, and NMDA-receptors as well (Bannai et al. 2009; Bard et al. 2010; Charrier et al. 2010; Dumoulin et al. 2009; Jacob et al. 2005; Muir et al. 2010; Tretter et al. 2008; Tretter and Moss 2008) other factors inside and outside the membrane contribute to a dynamic surface organization of signaling molecules (Fig. 9.1). The transient stabilization of receptors in the synapse leads to the idea that other mechanisms in addition to the intracellular anchors will contribute to maintain the concentration of signaling molecules within the synapse. Such mechanisms could include a different lipid composition (Allen et al. 2007), extracellular structures, like components of the extracellular matrix (Dityatev et al. 2010; Gundelfinger et al. 2010, Frischknecht and Gundelfinger in this book), repulsive or attractive intermolecular forces induced by charge differences (electrodiffusion (Savtchenko et al. 2000) or stable transmembrane molecules acting as diffusion obstacles (Kusumi et al. 2005)).

9.3 Molecular Crowding Confines Molecules Inside the Synapse

The existence of cholesterol/sphingolipid microdomains (lipid rafts) within the dendritic membrane was proposed to influence the membrane stabilization of AMPAR at synapses (Hering et al. 2003). Characterization of the diffusion properties of two lipid raft markers, glycophosphatidylinositol-anchored green fluorescent protein (GFP-GPI) and cholera toxin (beta-subunit binds to G_{M1}) in the postsynaptic membrane revealed confined diffusion inside the synapse (Renner et al. 2009a, b). Both raft markers are not enriched in the postsynaptic membrane of excitatory or inhibitory synapses, despite the confined diffusion within the synapse. Interestingly, lipid diffusion is twofold slower in inhibitory than in excitatory synapses, indicating a stiffer organization of inhibitory postsynaptic membranes (Renner et al. 2009a). Manipulation of the cytoskeletal integrity by depolymerization of F-actin leads to an even higher diffusion of fast-diffusing lipids in the postsynaptic membrane. The acceleration of lipid diffusion in the outer leaflet of the membrane can be explained as a decrease in the apparent viscosity of the membrane resulting from a weaker stabilization of transmembrane proteins in the absence of F-actin filaments. The molecular manipulation of actin polymerization by the actin depolymerization factor (ADF) n-cofilin had no effect on cholera toxin

diffusion, confirming the rather indirect effect of the actin skeleton within the PSD (Rust et al. 2010).

A partial extraction of cholesterol from the membrane does not dramatically change receptor or lipid diffusion inside synapses. The confined diffusion without enrichment of lipids inside synapses speaks in favor for a situation where the density of obstacles is the main source of confinement for small molecules like lipids as seen in the axonal initial segment and synapse (Nakada et al. 2003; Renner et al. 2009a, b), rather than a specific population of saturated lipids like those described in lipid rafts. The noncovalent interactions of synaptic receptors with the PSD, as described above, have a significant contribution to the crowded environment keeping a homeostatic concentration of receptors (Santamaria et al. 2010; Shouval 2005). Other forces beside lateral diffusion and intracellular binding affinities manipulating local molecular density are electrostatical interactions between molecules and transient electric fields generated by the opening of receptors after neurotransmitter binding (Fromherz 1988; Poo et al. 1979). For small cortical synapses, the contribution of electric forces has only been investigated theoretically, suggesting that AMPAR will be clustered during trains of high-frequency stimulation ($>$20 Hz) in opposite to the presynaptic release side (Savtchenko et al. 2000). In order to test this idea experimentally, SPT with high spatial and temporal resolution could give an answer (Kusumi et al. 2005). However, the mainly used video-rate acquisition (30 Hz) is not sufficient to follow fast motions. Increasing the temporal resolution is often paid by the loss of spatial resolution and needs further methodological development.

9.4 Membrane Structures Contribute to Local Confinement

Apart from the scaffold protein interaction and packed organization within the postsynaptic membrane, the question occurs how the receptor concentration is maintained within the synapse if each dissociation from an interaction partner results in increased mobility. It appears therefore reasonable to assume that specific structures around the synapse will prevent the escape of proteins over time. Models of the synaptic membrane organization have suggested barriers in the periphery of the PSD (Holcman and Triller 2006; Schuss et al. 2007) with restricted numbers of open gates that allow both the flux of molecules and the retaining of a critical local population inside the synapse. Investigations of the endo-exocytotic cycle and diffusional membrane organization made clear that first the source and sink of receptors are located outside the PSD (Blanpied et al. 2002; Jacob et al. 2009; Jaskolski et al. 2009; Kennedy et al. 2010; Lu et al. 2007; Yudowski et al. 2006), the distance between the PSD and clathrin-coated endocytotic pits does regulate the number of available receptors (Lu et al. 2007), exocytosis occurs within a specific membrane compartment close to the PSD (Kennedy et al. 2010) or within the dendritic shaft and soma (Adesnik et al. 2005; Jaskolski et al. 2009; Makino and Malinow 2009; Yudowski et al. 2006), and transient receptor stabilization in

clathrin-coated endocytotic pits modifies the synaptic responsiveness to activity changes (Jacob et al. 2009; Petrini et al. 2009).

The microscale organization of the perisynaptic membrane has profound consequences for long-term synaptic plasticity. Dendritic exocytosis of AMPAR has been identified as essential for LTP maintenance (Makino and Malinow 2009). The molecular players were recently identified and will help to further explore the molecular network underlying changes in synaptic plasticity. Receptor exocytosis occurs within local clusters of the t-SNARE protein syntaxin4 (Kennedy et al. 2010). Syntaxin4-mediated exocytosis is regulated by the interaction with F-actin filaments (Band et al. 2002), which do control the association with the vesicular SNARE protein synaptobrevin2 (VAMP2) (Jewell et al. 2008). The mobile organization of syntaxin4 aggregates shortly before exocytosis, and the interaction with the vesicular SNARE protein is controlled by the local depolimerization of F-actin (Gu et al. 2010; Kennedy et al. 2010). As mentioned above, the actin depolymerization factor n-cofilin has little effect for the synaptic membrane organization but influences spine morphology, exocytosis, and extrasynaptic surface mobility of AMPAR which seems also to be regulated by the phosphorylation status of n-cofilin (Gu et al. 2010; Rust et al. 2010). Similar mechanisms were reported for acetylcholine receptors in the *Xenopus* neuromuscular junction (Lee et al. 2009). Once exocytosed, the majority of receptors are confined close to the synaptic density (Kennedy et al. 2010). Membrane curvature and the intracellular condensation state of the actin filaments are proposed to hinder diffusion escape from the PSD (Holcman and Triller 2006) and hence allow receptors to become incorporated in the PSD. ADF/cofilins are responsible for the dynamic surface organization of syntaxin4-containing exocytotic zones around the synapse. The structures that cause such restricted mobility still need to be identified. Septins are very likely candidates as demonstrated for the developmental switch from microdomains to nanodomains in the presynaptic terminal of the calyx of Held (Yang et al. 2010).

Knockout mice for n-cofilin show no difference in synaptic transmission and short-term plasticity but have impaired late LTP and LTD as well as deficits in associative learning (Rust et al. 2010). The concept of the membrane microdomain organization around the synapse is interconnected by lateral surface diffusion of proteins between these domains that regulate synaptic plasticity and contributes to the spatial isolation of individual synapses.

At the presynaptic side, the very efficient and fast endo-exocytotic coupling is discussed to be a directed surface diffusion/flow of vesicular proteins (Haucke et al. 2011) within the presynaptic membrane. Its role would be to bridge the space between vesicle fusion and vesicle retrieval in the periphery of the active zone and to prevent an enlargement of the presynaptic terminal. At the postsynaptic side, volume changes in dendritic spines are reported to be associated with the insertion of new AMPAR and the induction of LTP (Kopec et al. 2007; Makino and Malinow 2009), suggesting a less tight coupling between membrane insertion and membrane retrieval. Such structural plasticity may interfere with the adhesion between pre- and postsynaptic membranes. Adhesion molecule surface organization may provide an insight in such dynamic processes.

9.5 Surface Dynamic of Adhesion Molecules as Modulator of Synaptic Molecular Organization

As shown in other cellular systems, the formation and maintenance of focal intercellular contacts, like synaptic junctions, depend on the density and mobility of adhesive partners on both membranes (Chan et al. 1991). It is reasonable to assume that primarily the density of cell adhesive molecules (CAM) will determine the strength of the formed contact. However, the mobility of CAMs in both membranes strongly accelerate the formation of the contact formation (Chan et al. 1991).

Many synaptic CAMs have been found, and the list of their intra- and extracellular binding partners is still growing (for further review, see Dalva et al. 2007; Tallafuss et al. 2010). Beside their known function to tether pre- and postsynaptic membranes, the capacity of transsynaptic signaling of CAMs is an important variable of synaptic plasticity (Futai et al. 2007; Stan et al. 2010). During synaptogenesis, the lateral recruitment of adhesion molecules to focal intercellular contacts is well accepted and used in many approaches to demonstrate the adhesive function of the molecule by the capacity to corecruit other synaptic molecules. For example, a prominent pair of synaptic adhesion molecules, neuroligins (postsynaptic) and neurexins (presynaptic), has been studied by the use HEK cells expressing either neuroligin (Scheiffele et al. 2000) or neurexin (Dean et al. 2003; Graf et al. 2004) cocultured with neurons, demonstrating the binding ability and recruitment of pre- and postsynaptic elements like transmitter vesicles (Scheiffele et al. 2000) or postsynaptic scaffold proteins (PSD95; Irie et al. 1997), Gephrin; (Poulopoulos et al. 2009), NMDA- and AMPA-receptors, respectively. Another assay was to use neurexin-coated beads to recruit neuroligin (Heine et al. 2008b; Nam and Chen 2005) or the simple application of Fc-tagged β-neurexins and subsequential crosslinking by Fc-specific antibodies (Barrow et al. 2009). It can be assumed that at least during synaptogenesis, adhesive molecules are expressed in the outer membrane and able to form preliminary intercellular contacts that will be consolidated following their establishment. However, the direct investigation of the surface distribution and dynamic of adhesive molecules as well as their intracellular trafficking is little understood, due to the methodological problem of interference with the intercellular binding partners by fluorescent labeling without interference with the function of these proteins.

Using transsynaptic enzymatic biotinylation to ensure functional surface labeling of heterophilic contacts between β-neurexin and neuroligin 1, Thyagarajan and Ting (2010) proposed a very local synapse-specific recycling of both molecules, which is modulated by neuronal activity. A similar local trafficking has been proposed for N-cadherin endocytosis (Tai et al. 2007).

Recent work by Fu and Huang (2010) directly addressed the question of subcellular distribution and surface accumulation of neurexins in the axon of parvalbumin-positive interneurons in cultured organotypic brain slices. Using pHluorin (pH-sensitive variant of GFP, Miesenbock et al. 1998) tagged α- and β-neurexins within organotypic slices, they conducted FRAP experiments to probe the dynamic of neurexins in the axonal membrane. β-Neurexins have been seen mostly clustered

within the axonal terminal, whereas α-neurexins are nearly equally distributed in the synaptic and axonal membrane and exchange between neighboring synapses. Despite the differences in surface distribution, both probes recover to 100% after local photobleaching within several minutes. Modulating presynaptic activity by the blocking of sodium channels with TTX or disrupting transmitter release with tetanus toxin did decrease the mobility of β-neurexins but did not influence the mobility of α-neurexins (Fu and Huang 2010). The described functions of these two forms of Neurexins are different and seem to be reflected in the surface dynamic. β-Neurexins possess a short extrasynaptic domain and bind to postsynaptic neuroligin 1 and 2 involved in the establishment and maintenance of specific synaptic contacts depending on the splice isoforms (Dalva et al. 2007; Tallafuss et al. 2010). Whereas, α-neurexins are recognized to organize the number or localization of presynaptic calcium channels (Missler et al. 2003; Zhang et al. 2005) and have only moderate binding affinities to neuroligin 2 in GABAergic synapses. The side of interaction between α-neurexin and N- and P/Q-type channels is still not known. It is conceivable that the differences in surface mobility could be due to a difference in function of neurexin isoforms and splice variants in synapse maturation and plasticity. Another explanation comes from the discovery of new postsynaptic binding partners of β-neurexins, suggesting a stronger anchoring of β-neurexins due to the multivalent interactions with other adhesion molecules (de Wit et al. 2009; Ko et al. 2009) or subunits (α1) of GABAA-receptors (Zhang et al. 2010). The application of SPT to investigate the mobility of adhesion molecules in the synapse will contribute to the validation of biochemically identified binding partners and might help to measure association and dissociation constants in a more physiological cellular environment (Saint-Michel et al. 2009).

9.6 Conclusions

The molecular noise within biological membranes has been documented as an important variable for immunological synapses, which have a lifetime of a few hours at most. The function of neuronal synapses as structural elements for learning and memory suggests a much more stable organization. However, molecular lifetime, nonequal distribution of binding partners, activity-driven changes of binding affinities, and concentrations argue against a rigid structural organization. The advent of imaging techniques that allow resolving single-molecule level shed new light in the nm-scale organization of synapses. The combination of such imaging techniques with physiological assays will clarify the functional impact of molecular motion in processes like learning and memory. As reviewed here for AMPAR synaptic transmission and short-term plasticity, initial changes of synaptic integration in the millisecond time window depend on the mobility of signaling molecules. Therefore, molecular flexibility, which is partially reflected in the differential diffusion of transmitter receptors, has a fundamental impact on neuronal network function.

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