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REVIEW

SYNAPTIC RECEPTOR TRAFFICKING: THE LATERAL POINT OF VIEW

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Abstract—Activity dependent modification of receptors in the post-synaptic density is a key determinant in regulating the strength of synaptic transmission during development and plasticity. A major mechanism for this recruitment and removal of postsynaptic proteins is the lateral diffusion in the plane of the plasma membrane. Therefore, the processes that regulate this lateral mobility are of fundamental importance. In recent years significant progress has been achieved using optical approaches such as single particle tracking (SPT) and fluorescence recovery after photobleach (FRAP). Here, we provide an overview of the principles and methodology of these techniques and highlight the contributions they have made to current understanding of protein mobility in the plasma membrane.

Key words: synapse, receptor, diffusion, FRAP, single particle tracking.

Contents

In 1827, the botanist Robert Brown first described that pollen particles moved haphazardly in water under the microscope (Brown, 1828). This phenomenon, later termed brownian motion (for review see (Nelson, 2001)), is the erratic displacement of a particle due to random impacts with smaller particles (i.e. atoms and small molecules). More than a century later, the first description of a membrane protein (rhodopsin) undergoing lateral mobility was published (Poo and Cone, 1974). This work demonstrated that in the fluid matrix of the lipid bilayer proteins are constantly moving. More recently, using modern techniques in biophysics, it has been shown that both recombinant glycine receptors and metabotropic glutamate receptors diffuse in the plane of neuronal plasma membrane by oscillating between confined and free brownian motion (Meier et al., 2001, Serge et al., 2002). Subsequently, the first evidence of native neurotransmitter receptors (glycine receptors and AMPA receptors) laterally diffusing in and out of synapses was provided in 2003 (Dahan et al., 2003, Tardin et al., 2003).

Protein trafficking to post-synaptic sites is a key mechanism regulating synaptic efficiency and many studies have focused on the mechanisms governing protein trafficking to and from the plasma membrane via exocytosis and endocytosis (for examples see (Perestenko and Henley, 2003, Shi et al., 1999)). This can be viewed as a 'vertical' process since it involves the movement of proteins from inside the cell to the membrane and vice versa. Exocytosis and endocytosis have been used extensively to explain many aspects of protein recruitment and removal underlying synaptic plasticity (Sheng and Lee, 2001). However, there is still only rather limited evidence to support the hypothesis that exocytosis and/or endocytosis occurs within the spine (Blanpied et al., 2002, Lu et al., 2007, for comment see Jaskolski et al., 2007) or more specifically within the post-synaptic density (PSD) (Gerges et al., 2006). An attractive complementary hypothesis for the delivery of at least some, and most likely the majority, of membrane proteins to the post-synaptic site is the 'horizontal' process of lateral diffusion within the plane of the membrane (Triller and Choquet, 2005). Thus, although there is still no widely accepted consensus as to the details and precise locations of membrane insertion, this process most likely involves the exocytosis of membrane proteins destined for the postsynaptic compartment into the dendritic membrane in the vicinity of synapses, followed by their subsequent lateral diffusion to the PSD (Yudowski et al., 2007).

The emergence of lateral mobility as a key mechanism regulating synaptic protein targeting originates mainly from the application of recently developed imaging techniques. There is now a substantial body of evidence to support lateral diffusion and here we review the techniques that have been used to study the contribution of this phenomenon to neurotransmitter receptor trafficking.

SINGLE PARTICLE TRACKING (SPT)

SPT involves real time imaging of a small probe linked to a protein of interest. The development of high sensitivity detection devices that enable the detection of single par-

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Abbreviations: ANQX, 6-azido-7-nitro-1,4-dihydroquinoxaline-2,3-dione; FCS, fluorescence correlation spectroscopy; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein; mGFP, membrane-anchored version of green fluorescent protein; mGluR5, metabotropic glutamate receptor; MSD, mean square displacement; PSD, postsynaptic density; QD, quantum dot; SPT, single particle tracking.

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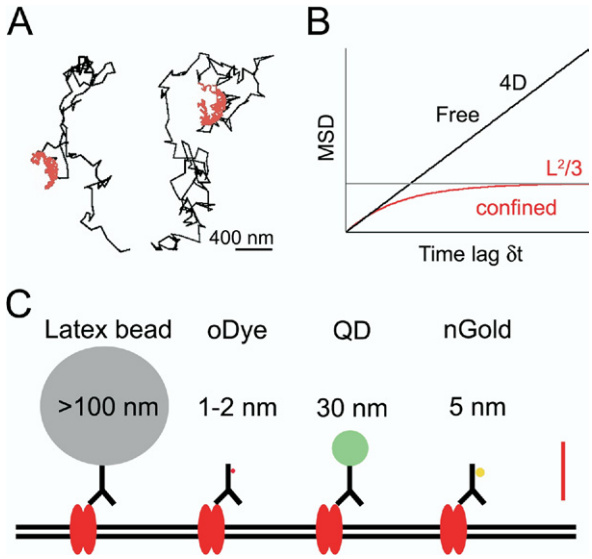

Fig. 1. SPT. (A) Sample traces of particles undergoing brownian motion in 2D oscillating between free motion (black, diffusion coefficient, D=0.5 $\mu m^2/s$) and confined motion in a circular sub-domain (red, D=0.02 $\mu m^2/s$). Generated by computer based Monte Carlo simulations of particles erratically exploring one of four possible positions at each time step (termed random walks). (B) Typical curves for MSD (area per time unit vs. time lag). For free motion the MSD grow linearly with time intervals, the slope is 4D where D is the microscopic diffusion coefficient. When a particle is confined, the MSD grow asymptotically to reach $L^2/3$, where L^2 is the area of the sub-domain. (C) Scale pictogram of various probes used in SPT, the latex bead diameter is 100 nm, 30 nm for the QD, 5 nm for the nano particle of gold (nGold) and 1–2 nm for the organic dye (oDye), the red scale bar=50 nm (average wide of the synaptic cleft).

ticles was a significant advance that allowed detailed analysis of membrane lateral diffusion. For example, SPT has revealed the remarkable erratic displacement of individual receptors in the neuronal plasma membrane (Meier et al., 2001, Serge et al., 2002).

As the movement of a protein in the plane of the plasma membrane is in the range of μm per second, SPT requires exquisitely accurate instrumentation. High-speed cameras are now readily available commercially but optical imaging still has intrinsic limitations in terms of spatial resolution at the μm level. Therefore, data acquired for motion capture in SPT are often subjected to a sub-pixel position calculation technique (Tardin et al., 2003). This post acquisition analysis of raw video frames allows the trajectories of individual particles to be tracked at very high resolution over time (Fig. 1A).

To extract diffusion parameters from the trajectories of proteins of interest it is useful to compute the mean square displacement (MSD, for detailed formulas and fitting see (Kusumi et al., 1993, Bannai et al., 2006)). Briefly, the MSD can be described as the mean area covered by the particle during a given time of observation. For a particle freely moving in the plane of the membrane, the MSD increases in a linear manner with time and its slope is 4D, where D is the microscopic diffusion coefficient (Fig. 1B, area per time unit time). If the particle is confined to a sub-domain of the plasma membrane then the MSD grows asymptotically to reach a limit defined by the mean area of sub-domains (Fig. 1A/B, red trajectories) (Kusumi et al., 1993). In the case of confinement, the initial linear part of the curve can be used to calculate the microscopic diffusion coefficient as for free diffusion. For example, SPT experiments have been used to demonstrate that the metabotropic glutamate receptor (mGluR5) and the inhibitory glycine ligand-gated ion channel receptor are anchored by Homer and Gephyrin proteins respectively (Meier et al., 2001, Serge et al., 2002). These studies showed for the first time that neuronal membrane proteins can be confined in distinct subdomains.

When the computed MSD grows faster than 4D, the observed particle is undergoing facilitated rather than free diffusion. As yet, the only receptors which were shown to undergo driven motion is the mGluR5 and GABA-A receptors (Serge et al., 2003, Bouzigues et al., 2007) where microtubule polymerization has been proposed to generate the driving force. It has also been reported that AMPA receptor activation facilitates the diffusion of the membrane-anchored version of green fluorescent protein (mGFP) in dendritic spines (Richards et al., 2004) although the mechanisms underlying this facilitation remain unclear. In addition to demonstrating that neurotransmitter receptors move in the plane of the plasma membrane, SPT has also demonstrated that a significant proportion (30–50% for AMPA and NMDA receptors) of surface expressed receptors are immobile (Tardin et al., 2003, Groc et al., 2004, 2006). The transition between mobility and anchorage may be a key parameter regulating receptor stabilization in front of synaptic release sites. For example, it has been reported that calcium influx immobilizes AMPA receptors and may act to constrain receptors at the PSD during periods of synaptic activity (Borgdorff and Choquet, 2002).

Perhaps the most exciting potential application for SPT is the direct visualization of receptor entry into and exit from specialized plasma membrane domains such as the post synaptic density. To realize this potential, however, it is necessary to effectively and reliably stain synaptic contacts. This has been accomplished to some extent but notwithstanding the availability of a wide variety of both presynaptic and postsynaptic markers, no absolutely selective probes that do not stain other neuronal domains have been identified thus far. For example, mitochondria are highly enriched in presynaptic compartments and the scaffolding protein Homer 1C is a good marker for the postsynaptic density (Tardin et al., 2003, Bats et al., 2007) but labeling for both of these markers is present elsewhere in the neuron.

A common feature of all current SPT probes is their requirement to be coupled to an antibody, which either recognizes an epitope-tagged recombinant receptor or is directed against the native receptor of interest. Thus a significant limitation to SPT is the range of antibodies available to specifically bind the extracellular domain of a protein. Another critical and potentially limiting consideration for the interpre-