Activity-dependent recruitment of AMPA receptors to the postsynaptic compartment by facilitated diffusion in the plasma membrane

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The brain operates by continually transmitting and processing vast amounts of data through complex networks of neurones. This information is transmitted via the release and detection of neurotransmitters at synapses. In the mammalian brain the predominant excitatory neurotransmitter is glutamate that acts at several types of glutamate receptors located at, or near, the postsynaptic density on specialized dendritic protrusions called spines. The ability of neurones to modulate the efficacy of synaptic transmission in response to previous activity at that synapse, termed synaptic plasticity, is fundamental to cognitive processes such as memory and learning. AMPA receptors (AMPARs) are a subclass of glutamate receptor, which mediate nearly all fast excitatory neurotransmission. Synaptic plasticity largely depends on the activity-dependent trafficking of AMPARs into and out of spines. Thus, a major challenge in neuroscience is to better understand how neurotransmitter receptors get to the right synapse at the right time and how their numbers within spines and at the postsynaptic density are maintained and regulated. We recently reported results that offer a mechanistic explanation of how AMPARs can be recruited to spines by synaptic activity. In this addendum we address technical aspects of this work and further discuss the context and implications of our data.

Most excitatory synapses are morphologically characterized by dendritic membrane protrusions named spines that encompass an electron dense postsynaptic density (PSD), a distinct head region and a narrow actin rich neck. The PSD is a microdomain enriched in AMPARs and associated proteins apposed to the glutamate release site. Regulating the number of receptors located at the PSD is a key mechanism that tunes synaptic strength and produces synaptic plasticity that is believed to underlie learning and memory.2,3

Given their importance, synaptic AMPARs are stringently regulated but despite intense investigation the processes by which AMPARs are delivered to and retained at the PSD remain a matter of controversy. For example, it has been proposed from experiments using photoreactive antagonists and electrophysiology that AMPARs are only exocytosed into the plasma membrane at the cell body and undergo lateral diffusion within the membrane the long distances to synapses.4 In stark contrast, real time imaging approaches have suggested that AMPARs undergo intracellular transport and are inserted into the plasma membrane of dendritic shaft close to, but not in, dendritic spines.6 Yet another report suggested that AMPARs are exocytosed directly into the PSD.7

Despite these disparate results for some aspects of AMPAR trafficking, what has been well established is that lateral diffusion in the plasma membrane is a major contributor to the exchange of receptors in and out the PSD.8-10 This gives rise to the interesting question of how membrane topology affects the lateral diffusion of AMPARs and other membrane proteins. In fact, it has been demonstrated that spine geometry is a key determinant of the number of synaptic AMPARs and we have shown previously that membrane protein diffusion in spines is slow compared to lateral

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diffusion on non-spiny membrane.12 It has also been reported that endocytosis at specialised endocytic zones close to the PSD in the dendritic spine and their recycling provides a mechanism to counteract lateral diffusion of AMPARs away from the PSD.13

Taken together these findings suggest that endocytosis and exocytosis, lateral diffusion and membrane topology may all play roles in regulating the mobility of AMPARs and probably other membrane proteins in spines. To test the hypothesis that there is a strong interdependence between these processes we used photobleaching techniques and near real time confocal imaging to visualize super-ecliptic pHluorin tagged AMPAR surface expression and movement.14,15 More specifically, we examined how lateral diffusion is regulated in dendritic spines by blocking dynamin-dependent endocytosis and stimulation of NMDA type receptors (NMDARs). We also performed in silico Monte Carlo simulations of particles undergoing random walks on lattices fitting theoretical spines to test how membrane topology affects protein diffusion. Our data indicated that the membrane topology of spines alone is sufficient to constrain lateral diffusion and that endocytosis within spines can provide a driving force to generate inward membrane flow that can recruit plasma membrane proteins into the spine from the adjacent dendrite.

**Technical Issues, the Third Dimension Problem**

One of our first aims was to learn more on why protein diffusion is affected more in spines compared to non-spiny regions.12 We hypothesized that the shape of the membrane itself is sufficient to produce anomalous diffusion. We therefore constructed a Monte Carlo simulation experiment in which particles randomly explore a given area over time. This provides a useful tool to model elementary diffusion issues16 and our data runs indicated that topological factors produce anomalous diffusion, which should be considered in the interpretation of diffusion experiments. The most accurate technique to record lateral diffusion is single particle tracking (SPT).15 However, this approach, which relies on the tracking of tagged proteins, takes no account of depth since every 3D displacement of the tagged protein is projected on a 2D recording device. Interestingly, according to our modeling, SPT underestimates the topology induced restricted motion and may thus overestimate the number and properties of anchoring events.

Another experimental paradigm widely used to measure diffusion properties is Fluorescence Recovery After Photobleaching (FRAP). This is based on measurement of changes in fluorescence signals from populations, rather than individual, tagged proteins. In a FRAP experiment a given area is photobleached to remove the fluorophore signal and the kinetics of fluorescence recovery in that area reflects the exchange, by mean of diffusion, of non-bleached (fluorescent) proteins with bleached ones.17 The time constant of the recovery is an indicator of the diffusion properties of the tagged protein but, to compare from an experiment to another, and to compute diffusion coefficients, the time constant should be normalized by the area of the bleached zone. In fact, in spine structures it is not obvious that the apparent area (2D projection of the focal plane) faithfully reflects the biological area explored. To test this experimentally we developed a protocol based on calibration curves to measure the membrane area of various recorded spines. This method relies on the assumption that a membrane tagged protein, such as palmitylated GFP (mGFP) that partitions to the inner leaflet of the plasma membrane distributes evenly among a cylindrical surface of non-spiny dendritic shaft region. By sampling this cylinder in a series of cylindrical sections of known length and diameter we were able to correlate the fluorescence signal to an area of membrane of known dimensions. Using this information we can compute the membrane area of complex spiny regions from the fluorescence emitted. This approach allowed us to calculate the precise membrane area in topologically complex regions of dendrites such as spines and thus to accurately assess diffusion constants in FRAP experiments.

** AMPARs in Flow Motion**

A still unresolved question in the field of AMPAR trafficking is the location of their plasma membrane insertion. To address this we used a super ecliptic pHluorin (SEP) tagged fusion of the GluR2 AMPAR subunit to visualize the locations of surface expression. Our results indicate that AMPARs are surface expressed in the plasma membrane of the dendritic shaft (Fig. 1). Our approach does not exclude
the possibility of reinsertion after recycling with the spine head as suggested (Fig. 1). This finding is consistent with other recent data also obtained using the SEP. Interestingly, AMPARs inserted the dendritic membrane appear to undergo relatively rapid diffusion via the spine neck to the PSD. However, in apparent contrast to these observations our modeling data suggests that the topology of the spine prevents a rapid diffusion of receptors into to the spine head (Fig. 1).

To reconcile these results we reasoned that AMPARs may undergo facilitated diffusion to overcome the barrier imposed by spine shape. We hypothesized that endocytosis of spine plasma membrane (and possible fusion with and dispersion in the intracellular endoplasmic reticular spine apparatus) could act as a mechanism to draw plasma membrane for adjacent regions of dendritic shaft and corresponding transmembrane proteins into the spine. To test this we used both chemical and dominant negative strategies to block the activity of the large GTPase dynamin, which plays a critical role in many endocytic pathways. We found that blocking dynamin activity is sufficient to reduce lateral diffusion of both AMPARs and the mGFP into the spine (Fig. 1). Thus, we propose that dynamin activity in the spine head can produce an inward flow of membrane lipids and embedded proteins. In addition, we also found that calcium entry through NMDA receptors facilitates AMPARs diffusion into spines and this effect is occluded by dynamin blockade suggesting a common pathway (Fig. 1).

**The Dead End Theory**

At the EM level the spine neck is enriched with actin filaments but despite investigation by many labs, as yet, only one report has described vesicular trafficking bodies in spine neck. Together with evidence for internalisation of receptors outside spines, there is mounting evidence that dynamin dependant endocytosis and recycling occurs in spine heads themselves. Our results suggest that the disequilibrium between spine endocytosis and reinsertion of material may produce the flow of membrane material. Further, protein degradation mechanisms within the spine head have been shown to be a key mechanism producing plasticity.

AMPARs play critical roles synaptic transmission and the regulation of synaptic activity is of paramount significance for normal brain function. Thus, understanding the mechanisms controlling the synaptic delivery of AMPARs is an important goal. Empirically the concept of dynamin-dependent membrane into spines provides an elegant system that could directly couple synaptic activity to membrane protein recruitment. Of course, much work remains to be done to validate and refine the theories we put forward and many important questions have yet to be resolved.

**References**

1. Harris KM. Structure, development and plasticity of dendritic spines. Curr Opin Neurobiol 1999; 9:343.
2. Song I, Huganir RL. Regulation of AMPA receptors during synaptic plasticity. Trends Neurosci 2002; 25:578.
3. Palmer CL, Cotton L, Henley JM. The molecular pharmacology and cell biology of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. Pharmacol Rev 2005; 57:253.
4. Adesnik H, Nicoll RA, England PM. Photoinactivation of native AMPA receptors reveals their real-time trafficking. Neuron 2005; 48:977.
5. Perensten PV, Henley JM. Characterization of the intracellular transport of GluR1 and GluR2 α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons. J Biol Chem 2003; 278:45325.
6. Yudowski GA, Pathenvedu MA, Lonotudakis D, Panicker S, Thorn KS, Beartie EC, et al. Real-time imaging of discrete excitocytotic events mediating surface delivery of AMPA receptors. J Neurosci 2007; 27:11121-21.
7. Gerges NZ, et al. Dual role of the exocyst in AMPA receptor targeting and insertion into the postsynaptic membrane. EMBO J 2006; 25:1623-34.
8. Ashby MC, De La Rue SA, Ralph GS, Uney J, Collingeidge GL, Henley JM, et al. Removal of AMPA receptors (AMPA) from synapses is preceded by transient endocytosis of extrasynaptic AMPARs. J Neurosci 2004; 24:5172-6.
9. Groc L, Choquet D, AMPA and NMDA glutamate receptor trafficking: multiple routes for reaching and leaving the synapse. Cell Tissue Res 2006; 326:425-39.
10. Tardin C, Cognet L, Bats C, Lounis B, Choquet D. Direct imaging of lateral movements of AMPA receptors inside synapses. EMBO J 2003; 22:4656-65.
11. Matsuzaki M, Ellis-Davies GC, Nemoto T, Miyashita Y, Iino M, Kasa H. Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. Nat Neurosci 2001; 4:1086-92.
12. Ashby MC, Maier SR, Nishimune A, Henley JM. Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology. J Neurosci 2006; 26:7046-55.
13. Lu J, Helton TD, Blanpied TA, Rácz B, Newpher TM, Weinberg RJ, et al. Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer. Neuron 2007; 55:874-89.
14. Ashby MC, Ibaraki K, Henley JM. It’s green outside: tracking cell surface proteins with pH-sensitive GFP. Trends Neurosci 2004; 27:257-61.
15. Jaskolki F, Henley JM. Synaptic receptor trafficking: The lateral point of view. Neuroscience 2009; 158:19-24.
16. Ritchie K, et al. Detection of non-Brownian diffusion in the cell membrane in single molecule tracking. Biophys J 2005; 88:2266-77.
17. Jaskolki F, Mayo-Martin B, Jane D, Henley JM. Dynamin-dependent membrane drift recruits AMPA receptors to dendritic spines. J Biol Chem 2009; 284:12491-503.
18. Park M, Salgado JM, Ostroff L, Helton TD, Robinson CG, Harris KM, et al. Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. Neuron 2006; 52:817-30.
19. Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD. Recycling endosomes supply AMPA receptors for LTP. Science 2004; 305:1972-5.
20. Pak DT, Sheng M. Targeted protein degradation and synapse remodeling by an inducible protein kinase. Science 2003; 302:1368-73.