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Review

Dynamics of surface neurotransmitter receptors and transporters in glial cells: Single molecule insights

Silvia Ciappelloni[a,b,c,1], Ciaran Murphy-Royal[a,b,c,1,2], Julien P. Dupuis[b,c], Stéphane H.R. Oliet[b,c], Laurent Groc[a,b,*]

[a] Interdisciplinary Institute for Neuroscience, CNRS UMR, 5297, Bordeaux, France
[b] University of Bordeaux, Bordeaux, France
[c] Neurocentre Magendie, Inserm U1215, Bordeaux, France

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ABSTRACT

The surface dynamics of neurotransmitter receptors and transporters, as well as ion channels, has been well-documented in neurons, revealing complex molecular behaviour and key physiological functions. However, our understanding of the membrane trafficking and dynamics of the signalling molecules located at the plasma membrane of glial cells is still in its infancy. Yet, recent breakthroughs in the field of glial cells have been obtained using combination of superresolution microscopy, single molecule imaging, and electrophysiological recordings. Here, we review our current knowledge on the surface dynamics of neurotransmitter receptors, transporters and ion channels, in glial cells. It has emerged that the brain cell network activity, synaptic activity, and calcium signalling, regulate the surface distribution and dynamics of these molecules. Remarkably, the dynamics of a given neurotransmitter receptor/transporter at the plasma membrane of a glial cell or neuron is unique, revealing the existence of cell-type specific regulatory pathways. Thus, investigating the dynamics of signalling proteins at the surface of glial cells will likely shed new light on our understanding of glial cell physiology and pathology.

1. Introduction

The lateral diffusion of proteins at the cell surface has been well-described and established over several decades. This process has now been demonstrated to occur in a wide variety of cells, including cells from the central nervous system such as neurons [1–6], astrocytes [7–10] and microglia [11]. Surface diffusion is a thermodynamic process by which proteins, inserted into the membrane by exocytosis, move in a Brownian manner and potentially interact with a vast number of other proteins and lipid rafts which may impede their diffusion in the membrane [12]. This impedance, through protein–protein interactions has been suggested to be the main mechanism by which neurotransmitter receptors are retained in the post-synaptic density (PSD) of synapses. Quite surprisingly, the turnover of surface receptors in stable structures, such as the PSD, appears to be higher than previously thought, with receptor dwell times in the order of minutes rather than the hours, days, and years believed necessary for memory retention [1]. This process of receptor lateral diffusion plays a pivotal role in basal synaptic transmission as well as forms of synaptic plasticity [13,14]. Furthermore, the surface diffusion of specific glutamate receptors is directly modulated by physiological challenges such as those associated with stress [15–17], suggesting that adaptations of the brain cell network under these conditions involve a fast redistribution of membrane receptors. Thus, there is a consensus that the surface diffusion of neurotransmitter receptors, together with the exo/endocytosis cycling, plays a key role in synaptic and network plasticity in the healthy brain. Despite our wide ranging knowledge of the conditions and constraints of surface diffusion of many different proteins in neurons, where fast

Abbreviations: P2 × 7, purinergic receptor P2 × 7; mGluR5, metabotropic glutamate receptor 5; DAT, dopamine transporter; AQP4, aquaporin-4; DOPE, 1,2-dioleoyl-sn-*glycero*-3-phosphoethanolamine; GLT-1, glutamate transporter-1; $Ca_v1.2$, L-type voltage-gated calcium channels; KCC2, potassium/chloride cotransporter 2; NMDAR, *N*-methyl-D-aspartate receptor; αNa,K-ATPase, sodium-potassium adenosine triphosphatase; D1R, dopamine receptor 1; $GABA_AR$, γ-aminobutyric acid receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; A7-AchR, α7 nicotinic acetylcholine receptors; GlyR, glycine receptor; Bkca, calcium-activated potassium channel; Eag1, ether-à-go-go 1 potassium channel; CB1R, cannabinoid receptor 1

* Corresponding author at: Interdisciplinary Institute for Neuroscience, CNRS UMR, 5297, Bordeaux, France.
E-mail address: laurent.groc@u-bordeaux.fr (L. Groc).

[1] These authors contributed equally to this work.

[2] Current address: Hotchkiss Brain Institute, University of Calgary,3330 Hospital Drive NW, Calgary, AB, Canada.

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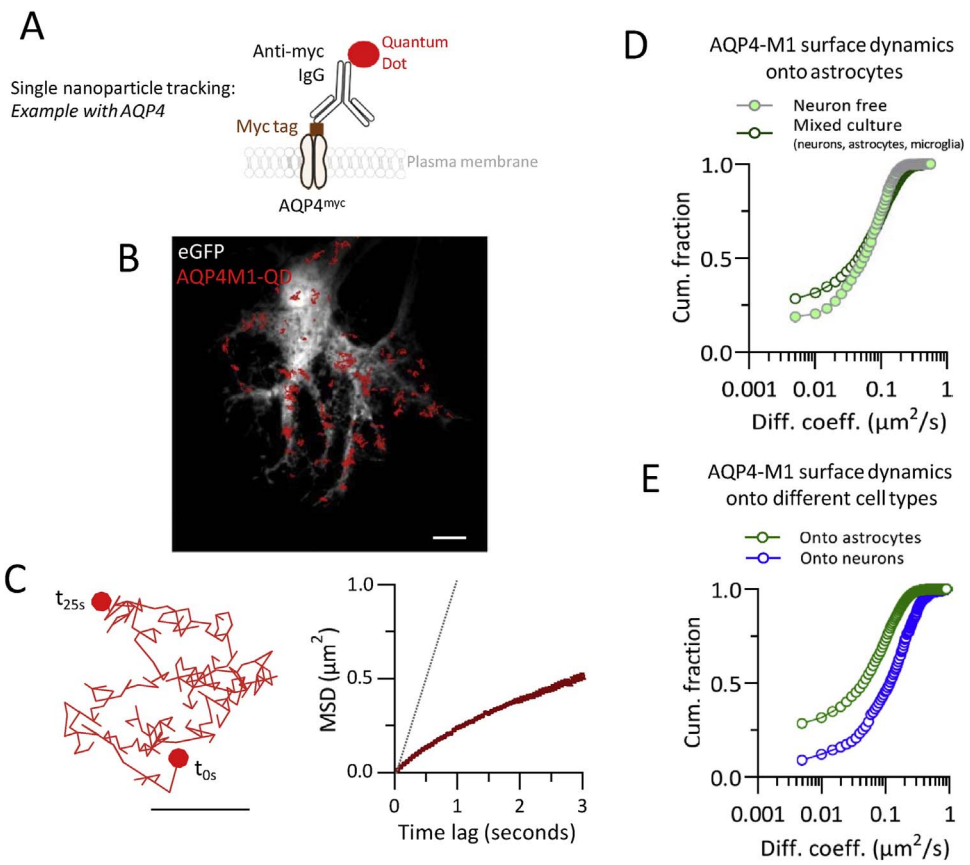

Fig. 1. Single-particle tracking Quantum Dot of surface over-expressed AQP4M1myc on astrocytes in mixed hippocampal culture cells. (A) Schematic description of the experimental procedure to track surface AQP4myc by spt-QD. (B) eGFP- expressing astrocyte with AQP4-QD trajectories (red). Scale bar 5 µm. (C) Left: representative of a 25-s-long single AQP4 trajectory surface diffusion. Scale bar, 500 nm. Right: mean square displacement of AQP4 in mixed hippocampal culture, shows a negative curve characteristic of confined movement. (D) Cumulative distribution of AQP4 diffusion coefficient in neuron-free or mixed culture. (E) Cumulative distribution of AQP4 diffusion coefficient on astrocytes or neurons in mixed culture, showing that AQP4 "artificially" expressed on neurons has a higher numbers of mobile proteins compared to overexpressed AQP4 on astrocytes. Even thought AQP4 is not naturally expressed on neurons, the presence of water channel could have induced shape modifications (swelling and shrinking). However no visible changes were found. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adaptations are key to normal function, our understanding of the surface diffusion occurring at the surface of glial cells is still in its infancy.

Astrocytes are highly ramified cells with extensive branching of fine processes throughout the neuropil. Each astrocyte process contains several microdomains, classically defined by highly localised calcium activity, which can function independently to the rest of the cell and influence nearby synapses and the vasculature. These microdomains on astrocyte processes are highly specialised in terms of structure and function. For instance, perivascular endfeet formed by astrocytic process are enriched with dystrophin associated protein complex (DAPC) and α-syntrophin that are now considered key factors for the molecular assembly stabilization, and concentration of receptors at the end-foot [18]. Another interesting feature of the astrocyte is its branching through the numerous fine-processes that surround synapses, it has been demonstrated that these processes can be tens of nanometers in size, well below the diffraction limit of light [19]. It has been suggested that these astrocytic process regulate several pre- and postsynaptic functions through, the release of gliotransmitters triggered by the local activation of astrocytic neurotransmitter receptors [20,21]. Although the mechanism underlying the anchoring of these receptors and transporters on astrocyte processes near synapses remains largely unknown, specific scaffold proteins as well as the shape of individual processes have been proposed to contribute to the stabilization of receptors/transporters [22–24]. As in neurons, transmembrane proteins located in the plasma membrane of astrocytes diffuse laterally and are dynamically regulated in different cell compartments [7,8,25]. Indeed, their diffusion has been demonstrated to be directly regulated by cell activity (neuronal as well as glial) in both physiological and pathological conditions [7,8,25,26]. Here, we review the literature describing the surface dynamics of neurotransmitter receptors and transporters located on glial cells, emphasizing the unanswered questions, technical advances, and challenges facing the field.

2. How to track membrane transporters?

Several approaches have been used to monitor the lateral trafficking of proteins on neuronal membranes each with distinct advantages and limits. These methods were then applied to investigate the diffusion of receptors, transporters, and channels in astrocytic plasma membranes. There are two main procedures used to track diffusion of a protein: ensemble measurements and single particle/molecule tracking (SPT). Regarding the first method, a well-described approach is the fluorescent recovery after photobleaching (FRAP) [27]. This approach was used to determine the lateral mobility of numerous neurotransmitter receptors [28–37] and neuronal transporters such as GABA transporter GAT-1 [38]. This technique takes advantage of the fact that most fluorophores are irreversibly bleached by incident light of very high intensity. A defined region of the sample is photobleached with high intensity light and these molecules are subsequently replaced by non-bleached molecules over time. The proportions of fluorescent molecules that can participate in this exchange indicate the mobile fraction. The fraction of molecules that cannot exchange between bleached and non-bleached regions is called the immobile fraction. Fluorescence microscopy is often a question of compromise: FRAP technique allows the collection of extensive information regarding the dynamic processes occurring in our sample due to the fast acquisition of images, but at the same time delivering limited spatial information, due to the signal being obtained from a single layer of plasma membrane (~200 nm). This is a limitation because cells are not flat and have various shapes and contours, a limitation that will currently apply to all 2D-imaging approaches. Furthermore it only gives an estimation of the mobility of a group of proteins. Nevertheless, FRAP experiments are commonly performed on laser scanning microscopes, making it an accessible method for determining the diffusion of a population of surface proteins. Another way to measure the average mobility of a protein population is the fluorescence correlation spectroscopy (FCS), which is based on the analysis