

synaptic transmission is the opening of calcium channels to initiate fusion of a transmitter vesicle. Beside the need to have a fusion competent vesicle at the synaptic membrane, the positioning between voltage gated calcium channels (VGCC) and the vesicle is critical for the success of transmitter release. Here we used sptPALM experiments of mEOS-tagged calcium channels to monitor the organization of $Ca_v2.1$ -channels in the presynaptic membrane. A major determinant of the presynaptic release probability is the stabilisation of VGCC by presynaptic scaffold proteins. Many interactions are described to depend on the interaction between the C-terminal tail of the channel and different domains of scaffold proteins. Alternative splicing of exon 47 result in the expression of either $Ca_v2.1$ -channels that lack a significant proportion (~200 aa) or have the full length of the C-terminus. We used both splice variants to explore their presynaptic dynamics and impact in synaptic transmission. Both channel splice variants are temporal confined in nanodomains, which themselves can travel within the presynaptic membrane. Using optogenetic tools to interfere with the mobility of VGCC in the neuronal membrane enabled us to uncover a contribution of mobile VGCC to synaptic transmission. These findings suggest a flexible organization of VGCC in the presynaptic membrane and offer a very flexible modulation of temporal synaptic transmission. We hypothesize that this molecular flexibility contribute to the temporal coding of information within neuronal networks.

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Novel iGluSnFR Variants Optimised for Rapid Glutamate Imaging

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Intensity-based glutamate-sensing fluorescent reporter iGlu-'sniffer' (iGluSnFR) is a useful tool for neuroscience that has enabled detection of glutamate release from single presynaptic terminals. However the probe's fluorescence rise and decay kinetics appeared too slow to give an accurate readout of glutamate dynamics at the synapse during high frequency bursts. We thus generated novel variants with faster glutamate binding kinetics by mutation of amino acid residues coordinating glutamate at the binding site. Fast variants iGlu_v and iGlu_u have comparable brightness and fluorescence dynamic range to iGluSnFR. The K_d for glutamate measured by equilibrium binding titration at 20 °C is increased from 33 μM (iGluSnFR) to 137 μM and 600 μM (iGlu_v and iGlu_u, respectively). At 34 °C, *in vitro* dissociation rate measured by stopped-flow fluorimetry are increased up to 6-fold from 233 s⁻¹ for iGluSnFR (τ_{off} =4.3 ms) to 1481 s⁻¹ for iGlu_v (τ_{off} =0.7 ms), making iGlu_v the fastest glutamate fluorescent reporter to-date. At single presynaptic terminals stimulated at 100 Hz in hippocampal slice culture, iGlu_u has 5-fold faster "off" rate (τ_{off} =2.6 ms) than iGluSnFR, with the signal returning to baseline between each stimulus, revealing complete clearing of synaptic glutamate between high frequency release events. Glutamate neurotransmission shows pronounced depression during high frequency bursts that can be attributed to a depletion of presynaptic resources or desensitization of postsynaptic receptors. By comparing iGlu_v signals and AMPA receptor currents, we show that synaptic depression during 100 Hz trains is entirely due to reduced glutamate release while the recovery after 500 ms has a postsynaptic component.

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Diffusion-Limited Glutamate Binding to GluBP is Revealed by Novel Fluorescent Probe

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AMPA-s are the fastest responding ligand-gated ion channels with channel opening times in the μs range¹, requiring rapid glutamate binding. Bacterial periplasmic glutamate/aspartate binding protein (GluBP) has structural homology to AMPAR, thus the glutamate binding mechanisms of the two protein families are expected to be similar. However, current indicators of glutamate kinetics record association rate constants in the order of 10⁷ M⁻¹s^{-1(2,3)}, thus may be too slow for the detection of the rapid AMPAR response kinetics.

We have developed a novel glutamate sensor based on GluBP (Fl-GluBP), labelled with an environmentally sensitive fluorophore at a Cys residue near the binding site. The K_d of 600 nM measured for GluBP⁴, is increased to

11 ± 1 μM for Fl-GluBP, at physiological ionic strength, pH 7.5 and 3 °C. Investigating glutamate association kinetics by stopped-flow fluorimetry, biphasic fluorescence increase was detected. 80% of the fluorescence enhancement was too fast to measure. Rates in the range of 200 to 800 s⁻¹, showing saturation, were measured for the second phase, interpreted as an isomerisation. Data for the hyperbolic concentration dependence of isomerisation were best fit to a two-step mechanism in which rapid, diffusion-limited binding is followed by isomerisation: equilibrium constant K_1 of 4.6 × 10⁴ M⁻¹ for step 1, consistent with $k_{+1} \geq 10^9$ M⁻¹s⁻¹ and $k_{-1} \geq 216000$ s⁻¹; k_{+2} of 2220 s⁻¹ and k_{-2} of 229 s⁻¹ for step 2 ($K_{d(overall)}$ 27 μM). We propose that a similar mechanism of diffusion-limited glutamate binding exists for and explains the rapid gating of AMPAR.

¹Li et al., 2003 Biochemistry 42, 12358-12366.

²Miller et al., 1983 J Biol Chem 258, 13665-13672.

³Helassa et al., submitted.

⁴Okumoto et al., 2005 PNAS 102, 8740.

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Single Molecule Translation Imaging of Local Protein Synthesis and RNA Docking Reveals the Regulation of Site Specific Axon Remodeling In Vivo

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Localised protein synthesis (LPS) is known to take place at precise subcellular locations to guide the growth response in neurones, for example in the presence of chemical guidance cues. We show here that LPS is also a major determinant of axon branching in developing neurones. Axon branching is key to maximise the opportunity for post synaptic connections to be made and therefore for the development of neuronal circuitry. We show the use of high resolution live imaging to determine the spatial and temporal dynamics of local protein synthesis in *Xenopus* retinal ganglion cells [1, 2]. We see that LPS and axon branching is tightly regulated by spatial patterns of endogenous RNA granules as they are being trafficked through the neurones. We see that RNA granules dock at sites of branch emergence and that prolonged docking of RNA granules leads to the development of axon branches. Branches can form and retract, depending on granule movement, and branches that remain stable are invaded by RNA granules over prolonged periods. Our results reveal a close relationship between arbor dynamics and RNA trafficking and show that RNA docking predicts sites of branch emergence. Live visualization of β-actin synthesis reveals the rapid accumulation of nascent β-actin in discrete 'hotspots' in branches and at branch points. The demonstration here that axonal arborization is disrupted by a loss of β-actin translation implicates RNA localization and local translation broadly in wiring the nervous system and raises the possibility that axonal, as well as dendritic, arborization defects underlie some neurodevelopmental disorders. [1] Wong HH et al. *Neuron* 95 (4), 852-868 (2017) [2] Strohl, F et al, *Scientific reports* 7, 709 (2017)

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Physicochemical Principles of AMPAR Insertion in Dendritic Spines

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Dendritic spines are small signaling compartments in neurons and house important signaling networks, receptors, and molecules associated with learning and memory. In particular, Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), protein phosphatase 1 (PP1), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) have been identified as molecular markers of memory, suggesting that their spatio-temporal dynamics play an important role in structural plasticity. Furthermore, dendritic spines have characteristic shapes that have been linked to healthy state, disease, aging, and other factors; however, the exact relationship between shape and function remains unknown. To elucidate this relationship, we developed a spatial model of Ca²⁺-influx through N-methyl-D-aspartate receptor (NMDAR), CaMKII/PP1 activation, and AMPAR insertion in a realistic spine geometry. Using this model, we show that i) variables in membrane voltage mediated Ca²⁺-influx, particularly the number of active NMDAR and extracellular Ca²⁺ levels, primarily regulate cytosolic protein dynamics through their impact on Ca²⁺ dynamics. ii) AMPAR dynamics depend on a combination of membrane curvature effects, endoplasmic reticulum (ER) spatial distribution, cytosolic protein concentrations, and stargazin binding. iii) AMPAR levels depend on both exocytosis from cytosolic stores and, more significantly, diffusion of AMPAR from extrasynaptic membrane (ESM) regions on the dendrite.