

equations. Our predictions are in line with existing measurements in a range of model systems including the squid giant axon and the garfish olfactory nerve. We expect our model to serve as a framework for understanding the physical origins and possible functional roles of these AWs in neurobiology. See arXiv:1407.7600 for details.

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Penetration of Action Potentials during Collision in the Medial Giant Axon of Invertebrates

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It is generally accepted that the collision of two action potentials coming from opposite directions is produced by the mutual annihilation of both signals. The experimental confirmation of this effect was shown by Tasaki in 1949 [1] and experiment is in agreement with the predictions of Hodgkin-Huxley model for action potential propagation [2].

In the current work we performed an analogous experiments to these made by Tasaki but using earthworms *Lumbricus terrestris* and lobsters *Homarus americanus* as an animals models. The collision of two simultaneously generated impulses propagating in orthodromic and antidromic directions were investigated. Also, the collision of compound of action potentials in the medial giant axons of sensory nerve bundle present in walking legs of lobster propagating were observed too. The experiments have been performed in the extracted ventral cords and nerve bundles of walking leg of lobster by using external stimulation and recording. The stimulation voltage was used as a tool to selectively stimulate the small neuronal population of giant axons (5 to 6 neurons).

Surprisingly, the collision of two impulses generated simultaneously, does not result in their annihilation. Instead, they penetrate each other and emerge from the collision without material alterations of their shape or velocity [3]. These results were published in Physical Review Letters X (2014) and are not consistent with expectations from the established HH model. But the findings, could be explained if nerve pulses are "electromechanical" waves - solitons, as suggested by Heimburg and Jackson [4].

1. Tasaki I. *Bioch. Bioph. Acta*, 1949;3:494-497.

2. Hodgkin A.L. and Huxley A.F. *J. Physiol.*, 1952;177:500-544.

3. Gonzalez-Perez A., Budvytyte R., Mosgaard D.L., Nissen S., Heimburg T. *P. Phys. Rev. X.*, 2014;4:031047.

4. Heimburg T. and Jackson A.D. *Proc. Natl. Acad. Sci. USA.*, 2005;102:9790-9795.

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Dynamics of Glycine Receptors and their Interactions with Gephyrin Scaffolds Revealed with High-Density Single Particle Tracking and Bayesian Inference

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The scaffold protein gephyrin plays a critical regulatory role in the transmission of nerve signals in inhibitory synapses. Its interactions with receptors of inhibitory neurotransmitters, such as glycine or GABA, are postulated to be a key molecular mechanism of synaptic formation and plasticity. Previous studies have shown that glycine receptors transiently bind to gephyrin scaffolds inside the synapse, however there is limited knowledge of the strength of this interaction. This is due, in part, to the consensus view that the synapse is a complex and dynamic assembly that is sensitive to a host of stimuli including neuronal activity, hormones, and pathological states. Therefore, a beneficial approach to improve our understanding is to reconstitute gephyrin scaffolds and their interactions with receptors in non-neuronal cells. To this end, in a greatly simplified system we use a transmembrane construction that consists of the large intracellular β -Loop that directly interacts with the gephyrin scaffold, mimicking the actions of the endogenous glycine receptor.

Based on high-density single-particle tracking, we use a robust Bayesian inference approach to spatially map the dynamics inside receptor-scaffold sites in non-neuronal cells. Through adaptive spatial meshing techniques, we are able to conform our maps to highly heterogeneous trajectory and diffusion distributions and, notably, generate whole-cell landscapes of interaction energies. Importantly, we treat the inherently multi-scale nature of receptor motion in a faithful and reproducible manner.

We show that our method allows us to distinguish interactions between different β -Loop mutants, effectively correlating protein-level modifications to quantifiable metrics of receptor dynamics. This is an important advance

that reinforces the treatment of complex biological systems with statistical methods.

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Robust Optical Stimulation of Neuronal Activity using Functionalized Gold Nanoparticles

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It was recently shown that infrared light pulses can directly stimulate neuronal action potentials by quickly heating the cell membrane and inducing capacitive currents (Shapiro, et al., *Nat Commun*, 2012). While useful, this technique works by heating the entire aqueous environment around a cell, rendering it nonspecific and incapable of stimulating specific populations of cells. Furthermore, since water absorbs infrared, the light source must be near the target. Here, we sought to stimulate neurons with visible light, using the ability of 20 nm spherical gold nanoparticles (AuNPs) to absorb green light and convert it into heat. By applying the AuNPs to painted lipid bilayers, we first confirmed that, upon 532 nm pulse stimulation, the membrane capacitance increases following the rate of temperature increase. The resulting currents are well-behaved, being linear with respect to both laser power and membrane potential. We next applied AuNPs to cultured dorsal root ganglion (DRG) neurons and demonstrated action potential initiation with 532 nm pulses of ≤ 1 ms. Finally, we obtained a dramatic improvement of this technique by functionalizing AuNPs with high-avidity ligands for DRG neuron membrane proteins. Non-functionalized AuNPs are cleared away from DRG neurons within seconds of starting a perfusion wash, causing the cells to lose optical excitability. However, AuNPs functionalized with Ts1 neurotoxin, or with anti-TRPV1 or anti-P2X3 antibodies, showed substantial resistance to washout; DRG neurons labeled with these functionalized particles remained light-sensitive after more than 20 minutes of perfusion washing. Furthermore, these particles required a lower concentration and less laser power to confer optical excitability. By appropriate selection of AuNP-conjugated ligand, this technique may enable improved cell-type specificity of neuronal photostimulation, such as ganglion cells in diseased retina where photoreceptors are non-functional. Support: R21-EY023430 and The Beckman Initiative for Macular Research.

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Ligand Fingerprinting in the Membrane Dynamics of Single TrkA and P75NTR Neurotrophin Receptors

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We have sought to investigate the responses of Nerve Growth Factor (NGF) receptors TrkA and P75NTR at the plasma membrane of living neuronal cells by single-molecule imaging and tracking. To this purpose we exploit the acyl carrier peptide and some of its shortened versions (A1 and S6 tags, labeled selectively by two different PPTases) to tag human p75NTR and TrkA. These tags were covalently conjugated to the biotin- or fluorophore-substituted arm of a coenzyme A (CoA) substrate.

This approach allows: (i) a precise control of stoichiometry and site of biotin conjugation; (ii) versatility of the tags used; (iii) studying two interacting molecules with orthogonal fluorolabels, at the single-molecule or single-interaction-complex level. This experimental toolbox is completed by fast microscopy (e.g. TIRF microscopy with a fast EM-CCD), and by a semi-automatic algorithm for the analysis of the trajectories. This novel algorithm separates self-similar from multimodal trajectories, divides the last ones in sub-trajectories, and calculates the combined distributions of parameters measuring the diffusivity, the localization or driftness degree, and/or the number of molecules in tracked spots.

We shall present results on the early response of TrkA upon binding different biologically-relevant ligands (including NGF and proNGF): without ligands, TrkA is present mostly as fast-diffusing monomers; ligand binding results in an increasing number of dimers and oligomers, which are typically slower and/or more confined. Each ligand promotes distinct trajectory patterns at the cell membrane, because of different receptor-binding affinities, intracellular effectors recruited and formation of signalling/recycling endosome precursors. We believe that this imaging toolbox and our results pave the way to the quantitative description of the kinetics, dynamics and stoichiometry of any binary or