inhibitors. Future projects are designed to determine whether Myosin-IIA inhibition or Arp2/3 complex activation can ameliorate chronic inflammatory diseases.

Platform: Neuroscience

1354-Plat
Modeling Human Brain Organogenesis using Pluripotent Stem Cells
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Human Induced pluripotent stem cells (hiPSCs) has the potential to generate all cell types of a human body under 2D culture conditions or form organ like structures- organoids, under 3D culture conditions. Brain organoid cultures from human iPSCs have been recently developed to recapitulate the cellular composition and the cytoarchitecture of the developing brain. These hiPSC based model systems offer unique advantages in understanding molecular and cellular mechanisms governing embryonic neural development and in modeling neurodevelopmental disorders, such as brain malformation and neurodevelopmental disorders. I will discuss our recent work using these systems to understand human brain development and neurotropism of SARS-CoV-2.

1355-Plat
Margaric Acid Counteracts Neuronal Mechanical Sensitization
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Sensory neurons innervating the skin rely on mechanosensitive ion channels to transduce mechanical forces into electrical signals. PIEZO2 is the essential transduction channel for touch discrimination, vibration, proprioception and injury-induced tactile pain. Mice and humans lacking Piezo2 experience severe mechanosensory and proprioceptive deficits and fail to develop tactile allodynia (when normal innocuous stimuli induce pain). Bradykinin, a proalgesic agent released during inflammation, potentiates PIEZO2 activity. Hence, molecules that decrease PIEZO2 function could reduce heightened touch responses during inflammation. Previous work in our laboratory demonstrated that enriching the plasma membrane with margaric acid (MA, C17:0) decreases PIEZO1 activation by increasing the rigidity and bending stiffness of the plasma membrane. Here, we show that MA also decreases PIEZO2 function in a dose-dependent manner. PIEZO2 inhibition by MA becomes more apparent when the cytoskeleton is pharmacologically disrupted with latrunculin A. Swapping an intracellular helix between PIEZO1 and PIEZO2 demonstrates that the PIEZO2 beam is a key region tuning MA-mediated channel inhibition. We demonstrate that MA reduces neuronal action potential firing elicited by mechanical stimuli in mice and rat neurons. Moreover, enriching the plasma membrane with MA counteracts PIEZO2 sensitization by bradykinin. We also show that this saturated fatty acid decreases PIEZO2 currents in touch neurons derived from human induced pluripotent stem cells. Our findings report on a natural product that inhibits PIEZO2 function and counteracts neuronal mechanical sensitization and reveal the intracellular beam as a key region for channel inhibition.

1356-Plat
Optogenetic Activation of ERK and AKT Signaling Promotes Axon Regeneration and Functional Recovery in Drosophila
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Neuroregeneration is a dynamic process synergizing the functional outcomes of multiple signaling circuits including the growth factor signaling. Here, we utilized optogenetic systems, optoRaf and optoAKT, to delineate the contribution of the ERK and AKT signaling pathways to neuroregeneration in live *Drosophila* larvae. We showed that optoRaf or optoAKT activation not only enhanced axon regeneration in both regeneration-competent and -incompetent sensory neurons in the peripheral nervous system but also allowed temporal tuning and proper guidance of axon regrowth. Furthermore, optoRaf and optoAKT differ in their signaling kinetics during regeneration, showing a gated versus graded response, respectively. Importantly in the central nervous system, their activation promotes axon regrowth and functional recovery of the thermonociceptive behavior. We conclude that non-neuronal optogenetics target damaged neurons and signaling subcircuits, providing a novel strategy in the intervention of neural damage with improved precision.

1357-Plat
Stochastic Reaction-Diffusion Modeling of Calcium Dynamics in 3D-Dendritic Spines of Purkinje Cells
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Calcium (Ca^{2+}) is a second messenger assumed to control changes in synaptic strength in the form of both long-term depression (LTD) and long-term potentiation (LTP) at Purkinje cell dendritic spine synapses via inositol trisphosphate (IP_3) induced Ca^{2+} release. These Ca^{2+} transients happen in response to stimuli from parallel fibers (PF) from granule cells and climbing fibers (CF) from the inferior olivary nucleus. These events occur at low numbers of free Ca^{2+} requiring stochastic single particle methods when modeling them. We use the stochastic particle simulation program MCell to simulate Ca^{2+} transients within a three-dimensional Purkinje cell dendritic spine. The model spine includes the endoplasmic reticulum (ER), several Ca^{2+} transporters and endogenous as well as exogenous buffer molecules. Our simulations successfully reproduce properties of Ca^{2+} transients in different dynamical situations. We test two different models of the IP_3 receptor (IP_3R). The model with non-linear concentration response of binding of activating Ca^{2+} reproduces experimental results better than the model with linear response due to the filtering of noise. Our results also suggest that Ca^{2+} dependent inhibition of the IP_3R needs to be slow in order to reproduce experimental results. Our approach also allows for studying the influence of Ca^{2+} buffers on IP_3Rs and Ca^{2+} transients and the modeling of pathological conditions like Ataxia, a loss of fine motor control assumed to be the result of malfunctioning information transmission at the granule to Purkinje cell synapse, resulting in a decrease or loss of Ca^{2+} transients. Finally, we propose possible ways of recovering Ca^{2+} transients under Ataxia

1358-Plat
Targeted Sensors for Glutamatergic Neurotransmission
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Optical report of glutamate release is gaining traction as the method of choice for visualizing excitatory synaptic transmission. So far, sensitive genetically-encoded reporters operating with a range of affinities and emission wavelengths were generated, and used to report vesicle fusion. However, without targeting to synapses, the specificity and dynamic range of the fluorescent signal is uncertain compared to sensors directed at vesicles or other synaptic markers. We fused the state-of-the-art reporter iGluSnFR to two glutamate receptor auxiliary proteins in order to target it to postsynaptic sites. The auxiliary proteins retained function and served to localise the reporter to excitatory synapses. Spontaneous miniature currents could be resolved in hippocampal neuronal cultures. In autaptic neurons on micro island cultures, evoked release could be detected quantitatively at tens of synapses in a field of view whilst evoked currents were recorded simultaneously. Analysis of fluorescence responses showed signals were diffraction limited and increased in dynamic range compared to the parent sensor, probably due to the focused localisation reducing activation by background and/or spillover glutamate. These experiments also revealed a specific postsynaptic deficit from Stargazin overexpression, resulting in synapses with normal release but without postsynaptic responses. We could revert this deficit by delaying overexpression. By working at different calcium concentrations, we could resolve release probabilities at individual sites. To do this, we developed an open-source analysis suite for extracting release properties from fluorescent synaptic responses. Taken together, postsynaptic targeting improves several properties of iGluSnFR and should allow quantitative report of neurotransmission from a postsynaptic locus in arbitrary neuronal subtypes.