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Poster #1

Dysfunctional somatodendritic AMPA receptor signaling in cerebellar interneurons in Fragile X syndrome

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Fragile X syndrome (FXS) is the most common single gene cause of inherited intellectual disability and autism. Affected individuals and preclinical mouse models such as Fmr1-/- knockout (Fmr1-/-) mice show hyperexcitable brain networks. Despite the severity of hyperexcitability, the mechanism underlying this phenotype is poorly explored. In FXS, excitatory-inhibitory (E/I) balance is disrupted and considered one of the major causes of hyperexcitability in FXS neurons. To understand this mechanism in the Cerebellum, we first explored the excitatory and inhibitory synapses at the stellate cells (SC), a major interneuron type in the molecular layer of the Cerebellum. Briefly, Granule cells (GC) send excitatory afferents, otherwise known as parallel fibers (PF), to the cerebellar molecular layer and form excitatory synapses with SC. These PF-SC synapses are essential for properly regulating Purkinje cells (PC), the only group of neurons connecting the Cerebellum to other brain regions. Furthermore, SC forms inhibitory synapses with adjacent SCs, making them a strong candidate for studying the E/I system in the brain. To understand the glutamatergic transmission, we stimulated the parallel fiber with a range of current pulses (2 V - 24 V) using a stimulation electrode placed at the molecular layer and recorded the excitatory postsynaptic potentials (EPSP) at the SC soma with a recording electrode.

Here we demonstrate that the loss of FMRP alters dendritic signaling and excitability in cerebellar molecular layer interneurons (stellate cells). We observed a larger but briefer excitatory postsynaptic potential (EPSP) in SC in Fmr1-/- mice following excitatory parallel fiber (PF) stimulation. We demonstrated that the change in EPSP halfwidth is primarily caused by an increased GABA transmission. While probing the underpinning mechanism behind the enhanced peak amplitude, we found that the levels of A-type and TEA-sensitive delayed rectifier potassium channel (Kdr) currents are diminished or absent in Fmr1-/- mice. Interestingly, we identified a delay in activation of Kdr currents in Fmr1-/- SC. Further blocking Kdr currents using TEA reproduced the Fmr1-/- like EPSP in WT SCs, and the SC is hyperexcitable in Fmr1-/- mice. Re-introduction of an N-terminal fragment of FMRP (FMRP1-297) rectified the elevated EPSP amplitudes and associated hyperexcitability in SC of Fmr1-/- mice while having no effect in WT mice, confirming that this mechanism is regulated by the FMRP. This provides insight into the function of the FMRP in regulating this mechanism in a translation-independent manner.

Poster #2

Distinct disease-associated variants in TRIO drive distinct anatomical, physiological, and behavioral phenotypes

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De novo mutations and ultra-rare damaging variants in TRIO are associated with a range of the neurodevelopmental disorders, including autism (ASD) and schizophrenia (SCZ). In neurons, TRIO regulates Rac1, RhoG, and RhoA small-GTPases to control neuronal migration, morphogenesis, synapse development and function. However, it remains unclear whether and how discrete TRIO variants differentially impact these neurodevelopmental events. We previously demonstrated that disease-associated TRIO variants have discrete impacts on TRIO biochemical functions. Here, we investigated the impact of disease-associated TRIO variants associated with ASD, SCZ and one de novo mutation in an individual with bipolar disorder: TRIO+/K1431M, TRIO+/K1918X, TRIO+/M2145T, respectively. We find synaptic area is reduced in layer-5 cortical synapses of TRIO+/K1431M mice and dendritic arbors are smaller in layer-5 pyramidal neurons (L5-PNs) of TRIO+/K1918T mice. Our single-cell recordings indicate that all TRIO heterozygous mutants have decreased basal-excitatory signaling in L5-PNs. Additionally, we find different changes of the presynaptic release in TRIO+/K1431M and TRIO+/M2145T L5-PNs. TRIO+/K1431M and TRIO+/K1918T mice exhibit significant deficits in rotarod performance and in nestlet shredding. Novel object recognition reduced in TRIO+/K1918X and TRIO+/M2145X mice, while TRIO+/K1431M mice show social preference impairment. Together, our data show that heterozygosity for distinct TRIO variants causes distinct anatomical, physiological, and behavioral phenotypes.

Poster #3

Structural insights into binding of therapeutic channel blockers in NMDARs

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N-methyl-D-aspartate receptors (NMDARs) have long been considered drug targets for treating neurodegenerative diseases and neuropsychiatric disorders due to their critical roles in brain development and functions. Among the different drug candidates, NMDAR channel blockers have drawn many clinical interests in their potential to treat depression, Alzheimer's disease, and epilepsy. However, the capability of inducing schizophrenia-like psychosis hinders the clinical applications of these NMDAR channel blockers. Despite the extensive efforts on understanding the binding mechanism of the channel blockers, it remains unclear due to the lack of high-resolution structural insights into the blocker binding site in the transmembrane domain. Here, we conduct a structure-based comparative analysis of the binding of three clinically essential channel blockers, phencyclidine, S-(+)-ketamine, and memantine. We obtain cryoEM structures of GluN1a-2B NMDAR in the absence and presence of the blockers at 2.5-3.5 Å around the transmembrane blocker binding site and analyze the blocker binding modes through MD simulation. Our results reveal that the binding of channel blockers is governed by a series of hydrophobic interactions with the pore-lining residues along with the cluster of threonine residues at the channel gate.

Poster #4

Ion permeation path and activation of AMPA receptor in complex with TARPg-2 resolved at sub-2.5Å resolution

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The mechanism for ion permeation in the AMPA-type ionotropic glutamate receptors (AMPAR) remains elusive. Here, the open pore architectures of the calcium permeable AMPAR in complex with a gain-of-function TARP□-2 are investigated under different ionic conditions at sub-2.5Å resolution by cryo-EM, revealing ion-specific permeation paths and water binding pockets. We propose the open gate is a high affinity binding site for divalent cations, which may influence divalent cation permeability. Putative densities of ions and water accumulate in the upper selectivity filter, where putative ion ions may simultaneously coordinate to the pore-lining carbonyls and water. The conformational variation of the selectivity filter provides insights into the mechanism for regulation of conductance.

Poster #5

Proteostasis of N-methyl-D-aspartate Receptors containing disease-associated variants within the GluN2B subunit

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N-methyl-D-aspartate receptors (NMDARs) are gated by the primary excitatory neurotransmitter, glutamate. They play essential roles in neuronal formation, maturation, as well as central nervous system function. As such, the GRIN genes that encode the GluN subunits of NMDARs are highly intolerant to genetic variation and are thereby more likely to result in disease states. In this study, 25 mutations within the GluN2B subunit classified as pathogenic or likely pathogenic in the literature were generated for screening to determine their impacts on receptor homeostasis. In order for these receptors to gain their physiological function, they must first be properly folded and assembled within the endoplasmic reticulum and trafficked to the plasma membrane. Here, we expressed NMDARs containing select variants within the GluN2B subunits in HEK293T cells. Cells were harvested, lysed, and subjected to SDS-PAGE and Western blotting to determine the impact of variants on the expression and aggregation propensity of GluN2B subunits. After initial screening, variants that demonstrated decreased expression were transfected into HEK293T cells which were then treated with novel proteostasis regulators to determine if they could influence the expression of NMDARs. It was found that variants within the GluN2B subunit differentially impact the expression of the receptors and select variants are more prone to forming aggregates within the cell. Further, treatment with proteostasis regulators showed significant increases in receptor expression containing loss-of-function variants.

Together, these results indicate that disease-associated variants within the GluN2B subunit impair the overall expression and stability of these receptors. Additionally, the effects on receptor expression observed after treatment with the proteostasis regulators provide a potential targeted pharmacological therapeutic approach to restoring the expression and signaling of NMDARs containing GluN2B subunit harboring disease-associated variants.

Poster #6

Pannexin: a modulator of P2X7 receptor-mediated calcium influx

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Pannexins are the major ATP release channel in many cell types, including astrocytes. Extracellular ATP acts as a ligand for purinergic P2X and P2Y receptors. The opening of ATP-gated P2X7 receptor (P2X7R) causes an influx of Ca2+, which in turn activates Pannexin-1 (Panx1). The concerted activities and functional coupling of P2X7R and Panx1 require their spatial proximity. Earlier reports have suggested physical interaction between Panx1 and P2X7R. Based on their interaction, we hypothesized that Panx1 might influence the function of P2X7R. The effect of Panx1 on P2X7R-mediated intracellular Ca2+ rise was studied. Panx1 was found to attenuate P2X7R-mediated intracellular Ca2+ rise in HEK-293 cells. The inhibition was also observed in rat cortical astrocytes. Panx1-knocked down astrocytes exhibited a significantly higher rise of P2X7R-mediated intracellular Ca2+. To identify the regions of Panx1 associated with P2X7R-inhibition, different deletion mutants were generated by removing amino acids sequentially from the C-terminus of Panx1. The ability of different truncated Panx1 to inhibit P2X7R-mediated Ca2+ influx was tested. The region from Leu350 to Cys426 was found to be crucial for inhibiting P2X7R. Like full-length Panx1, the C-terminus alone could also attenuate the Ca2+ influx through P2X7R. When expressed in astrocytes and HEK, the Panx1-CT played an anti-apoptotic role in P2X7R-mediated cell death. The interplay and transition between the pro-cell death role of WT Panx1 and the anti-cell death function of its C-terminus are determined. One of the characteristic features of P2X7R is the gradual increase of current with the increased frequency of agonist application. We found that co-expression of Panx1-CT with P2X7R significantly reduced the current generated at every consecutive stimulation. However, co-expression of full-length Panx1 reduced the currents at initial stimulations, followed by gradual augmentation. This could indicate the association of Panx1 with P2X7R in the formation of high conductance pore. So, Panx1 initially inhibits P2X7R current through its C-terminus, but in the continuous presence of an activator, it turns into an enhancer. WT Panx1 and Panx1-CT have opposite effects on P2X7R-mediated cell death, wherein it was significantly reduced by Panx1-CT and enhanced by WT Panx1. Panx1-CT significantly decreased mitochondrial depolarization, mitochondrial and cytosolic ROS generation and Caspase 3 activation, whereas WT Panx1 increased them. This inhibitory modulation of P2X7R possibly plays an essential role in physiology and pathophysiology.

Poster #7

Distinctive modulatory effects of human Neto2 splice variants on kainate receptor function

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Pore-forming, ligand-gated ionotropic glutamate receptors (iGluRs) display channel kinetics in heterologous expression systems that contrast with native neuronal iGluRs. The discovery of iGluR auxiliary subunits resolved these disparities. To date, several iGluR auxiliary proteins have been identified and their modulation effects are well reported. However, the impact of auxiliary subunits splice variants on the biophysical properties of iGluRs remains elusive.

Two auxiliary subunits have been identified for the iGluR subfamily of kainate receptors (KARs) - neuropilin and tolloid-like proteins 1 and 2 (Neto1 and Neto2). Neto2 has two splice variants, and of which, variant 1 has an extra seven amino acids stretch between the CUB1 and CUB2 interface. Interestingly, the open access database (GTEx) suggests variant 2 is predominantly expressed within the central nervous system. KARs modulation by Neto2 variant 1 is well studied, however, much work remains to be done regarding Neto2 variant 2.

In the present study, we identify the distinctive modulatory effects of Neto2 splice variants on the functional properties of KARs. In addition, our initial AlphaFold structure predictions suggest that variant 1 has a more compact CUB1/CUB2 structure than variant 2 despite possessing a longer linker. Understanding these diverse modulatory effects on KAR function could aid the development of KAR-specific modulators.

Poster #8

The Impact of Ionotropic Glutamate Receptors on Trans-Synaptic Nanostructure

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Information processing in the brain relies on the dynamic and precise organization of molecular ensembles at the excitatory synapse. Proteins subserving the mechanism of synaptic transmission are heterogeneously organized on the nanometer scale. Presynaptic proteins such as Munc13 are found in ~80 nm diameter subsynaptic regions of high local density. These presynaptic nanoclusters (NCs) are aligned across the synapse with NCs of proteins such as PSD-95 and ionotropic glutamate receptors (iGluRs), forming a trans-synaptic nanocolumn. Our lab established that this nanocolumn is the preferential site of action potential evoked release and that dispersal of AMPARs from the nanocolumn to the remainder of the synapse specifically reduces the strength of the response to evoked release. This highlights how the nanocolumn is critical for synaptic transmission and underscores the importance of knowing what generates and regulates this alignment. We reasoned that a mechanism underlying trans-synaptic alignment would involve a protein that could act as either a structural or functional marker of successful transmission. One underappreciated possibility that embodies both aspects are the iGluRs themselves. First, either AMPARs or NMDARs could trigger downstream mechanisms that sample receptor activation and direct receptor positioning. Second, most intriguingly, both AMPARs and NMDARs have an extended extracellular structure with distal N-terminal domains that could nucleate direct or indirect interaction with presynaptic proteins. iGluRs could also organize scaffold NCs through multivalent interactions, either through NMDAR c-tail or AMPAR TARP binding. Previous studies show that receptor structure, particularly that of the AMPAR subunit GluA2 and its N-terminal domain, influences synaptogenesis and presynaptic maturation. Thus, we hypothesized that the iGluRs align the trans-synaptic nanocolumn. To test this, we generated both GluA2 and GluN1 CRISPR knockouts in dissociated rat primary hippocampal neuronal culture. Here we report preliminary observations that loss of iGluRs seem to impact trans-synaptic nanostructure of scaffold proteins within the synapse. On-going experiments seek to expand upon this observation and elucidate the structural or functional influence of iGluRs. The organization of iGluRs at the synapse is typically thought to be downstream of pre-existing scaffold structure. This study instead tests an unorthodox view of AMPA and NMDA receptors: that they actively shape the structure of synapses and thus may dictate their own activation.

Poster #9

Auxiliary subunit regulation of AMPA receptor assembly

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AMPARs meditate the majority of fast excitatory synaptic transmission. Regulation of the number and composition of AMPARs at the postsynaptic membrane are responsible for the process of learning and memory, and receptor dysregulation is implicated in various brain disorders. AMPARs associate with auxiliary subunits that can affect their functional activity, yet how the availability of receptors is regulated by auxiliary subunits is unclear. Here, we addressed the role of auxiliary subunits in the assembly of AMPARs using a combination of blue native gel-electrophoresis, immunocytochemistry, and electrophysiology. We find that co-expression of CNIH-2 and CNIH-3 with AMPAR subunits enhances the tetramerization process, whereas co-expression with other auxiliary subunits including TARP γ-2, TARP γ-8, and GSG1-L have little or no effect. To study the basis for this, we mutated key residues in the AMPAR transmembrane domain which attenuates tetramerization and co-expressed these AMPARs with auxiliary subunits. Destabilized tetramerization leads to increased receptor dimers and significantly reduced tetramer formation. We found that when AMPAR mutants are co-expressed with CNIHs, the tetramer fraction was efficiently rescued compared to co-expression with other auxiliary subunits. Our results suggest that auxiliary subunits influence receptor tetramerization through the transmembrane domain with CNIHs capable of stabilizing the transmembrane region and rescuing disrupted receptor assembly. In contrast, other auxiliary subunits demonstrate a much weaker influence on tetramerization compared to CNIHs. We conclude that CNIHs differ from other auxiliary subunits in their ability to influence early stages of AMPAR biogenesis.

Poster #10

Architecture and structural dynamics of the heteromeric GluK2/K5 kainate receptor

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Kainate receptors (KARs) are members of the ionotropic glutamate receptor (iGluRs) family and bind the neurotransmitter L-Glutamate to mediate synaptic transmission and plasticity in the brain. KARs are implicated in major neurological diseases, including epilepsy, stroke, mood disorder, autism, schizophrenia, and migraine pain. Native KARs primarily exist as heteromers in the brain. Due to the lack of structures of any heteromeric KARs in physiologically relevant functional states, long-standing questions remain about their architecture and mechanism of ion channel gating in response to L-Glutamate at the synapse. We optimized the biochemistry of GluK2/K5, proposed to be the dominant KAR in the brain, and solved the structures in apo, antagonist-bound and agonist-bound states using cryo-EM single-particle analysis. Our extensive image classifications show that the receptor has 2:2 stoichiometry with GluK2 subunits at the center of the amino-terminal domain and GluK5 subunits at the periphery. The ligand-binding domain is arranged as two heterodimers with GluK5 subunits proximal to the ion channel. The major structural rearrangements of the GluK2 subunits in the agonist-bound state likely corresponds to desensitization. The linkers connecting the transmembrane and ligand-binding domains allow the receptor to accommodate the dramatic conformational changes exhibited during channel gating. Current resolution of the ligand-binding domain limits our insights of ion-independent gating of GluK2/K5 heteromer. Future studies taking advantage of the recent advances of atomic resolution cryo-EM will require understanding the ion regulatory mechanism. Our work provides the first heteromeric KAR structure in the brain to accompany the structures of the dominant forms of other families of iGluRs, AMPARs and NMDARs, and proposes a gating mechanism that is likely conserved across KARs and unique among the iGluRs. Our study enables more comprehensive investigation across the iGluR family and provides a structural framework for precision targeting of drugs to specific receptor subtypes.

Poster #11

Direct Visualization of Triheteromeric NMDA Receptors

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Glutamatergic signaling via NMDA receptors (NMDARs) is critical in synaptic plasticity, brain development, excitotoxicity, and numerous degenerative and cognitive disorders. Each NMDAR comprises four subunits: two obligatory GluN1 subunits and two GluN2A-D or GluN3 subunits. Importantly, NMDARs with different GluN2 compositions display strikingly different biophysical characteristics, and unique protein interactions and signaling. Thus, identifying how neurons control abundance of specific NMDAR subtypes has been a longstanding goal in neuroscience. Unfortunately, our understanding of these mechanisms has been crucially restricted by inability to visualize one of the key classes of NMDARs in neurons, triheteromeric receptors that contain GluN1 and two different GluN2 subunits. Indeed, in many parts of the brain including the hippocampus, one of the most common subtypes of NMDARs is triheteromeric containing both GluN2A and GluN2B. However, the subcellular distribution of triheteromeric NMDARs remains mysterious and the mechanisms controlling their trafficking to and from synapses remain almost totally unknown. Here, we introduce a new method for direct visualization of triheteromeric NMDARs in neurons using bimolecular complementation. We tagged the N-termini of GluN2A and GluN2B subunits with two components of split fluorescent proteins or of a split HaloTag enzyme that is catalytically active only when the two parts are brought within close proximity. Thus, after transfection of these tagged subunits, standard fluorescent imaging can be used to detect specifically NMDARs containing both GluN2A and GluN2B subunits in a single receptor complex. Whole-cell recordings demonstrate that activation of split-tagged NMDARs by glutamate, as well as inhibition by ifenprodil are unaltered by the presence of the tags. Further, the split-tagged subunits do not undergo detectable interreceptor complementation, and neurons expressing the complementing receptor subunits displayed no morphological abnormalities. Using this tool, we find that triheteromeric receptors traffic to synapses and display unique subcellular trafficking characteristics. Moreover, we can utilize the unique permeability properties of HaloTag ligands to directly test surface trafficking and compartment-specific organization of triheteromeric NMDARs. These probes will fill longstanding gaps in our knowledge of NMDARs and lay necessary groundwork for investigation of other aspects of triheteromeric NMDAR trafficking in healthy neurons and disease models.

Poster #12

A subunit-specific regulation of cluster gating in NMDA receptors

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NMDA receptors (NMDAR) are ionotropic glutamate receptors that mediate fast synaptic transmission and synaptic plasticity. Dysfunction of NMDARs has been found in numerous neurological diseases including Alzheimer's disease, Intellectual disability, epilepsy, autism, and schizophrenia. A key function of NMDARs is to convert agonist binding into channel opening, hereby referred to as gating. The NMDARs broad role in neuronal function is reflected in the complexity of its gating function, with several open and closed states, and gating modes. More fundamentally, gating in NMDARs can be broadly divided into periods of high opening activity, or clusters, interspersed by periods of no activity, or inter clusters. NMDARs are obligate hetero-tetramers, formed by two glycine-binding GluN1 subunits and typically two glutamate-binding GluN2 (A-D) subunits. The ion channel pore consists of two transmembrane segments (M1 and M3), a reentrant pore loop (M2) and a peripheral M4. Here, we show that a conserved glycine within the M4 transmembrane segment distinctly disrupt the intracluster and cluster activities in a subunit dependent fashion. Disruption of the GluN1 M4 conserved glycine, profoundly reduce activity within a cluster. Conversely, disruption of the GluN2 M4 hinge has comparatively little effect on cluster activity, instead largely preventing the channel from entering into clusters. MD simulations further reveal that disruption of the GluN1 and GluN2 conserved glycine's collapse distinct portions of the channel pore, with GluN1 mutants collapsing the M2 loop at the bottom of the pore, and GluN2 mutants collapsing the M3 helix bundle at top. We hypothesize that the distinct NMDAR kinetics are mediated by two gates: an external gate at the M3 bundle crossing regulated by the GluN2 M4 as well as pre-M1, and an inner gate at the M2 pore loop regulated by the GluN1 M4. The findings that control of cluster gating can be regulated from the peripheral M4 segment via action at distinct channel pore sites, revealing a promising drug target.

Poster #13

Regulation of GluN2B-NMDARs by the Actin Cytoskeleton

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N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels composed of transmembrane GluN1, GluN2 (A-D), and GluN3 (A-B) subunits that mediate Ca2+ influx into the dendritic spine. Importantly, the unique intracellular tail of the GluN2B subunit is essential for learning and memory. Furthermore, autism spectrum disorder (ASD) and schizophrenia (SCZ) patients display rare genetic mutations within the GluN2B intracellular tail. My overall goal is to elucidate a novel mechanism by which GluN2B tails contribute to the function of GluN2B-containing NMDARs (GluN2B-NMDARs). Previous experiments showed that actin-targeting drugs impact NMDAR activity, but the underlying mechanisms are unknown. My preliminary data strongly support the primary hypothesis of this project: a direct interaction between actin filaments and the GluN2B intracellular tail regulates NMDAR activity. The objective of my project is to elucidate the biochemical basis for an interaction between the GluN2B tail and actin filaments and determining how this interaction regulates NMDAR function.

Poster #14

Structural insights into binding of therapeutic channel blockers in NMDA receptors

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The N-methyl-D-aspartate receptor (NMDAR) is a crucial component underlying the mechanistic basis of synaptic plasticity. As such, NMDARs are a key pharmacological target for the treatment of Alzheimer's disease, mood disorders and pain. Despite this, a lack of high-resolution NMDAR structures has precluded assessment of the precise binding modes of therapeutically beneficial uncompetitive antagonists such as ketamine or memantine. Using single-particle cryo-electron microscopy, we determined the Glutamate and Glycine bound GluN1-2B NMDARs bound to ketamine, phencyclidine and memantine with local resolutions of 2.5-3.5Å at the channel blocker site. Additionally, by performing electrophysiology and MD simulations in the presence of an applied electric field, we examined the precise configurations and components that differentiate therapeutic (ketamine/memantine) and non-therapeutic (PCP) NMDAR channel blockers. Crucially, we observe that interactions of channel blocking drugs are facilitated by two motifs, namely the hydrophobic and threonine 'rings' of the NMDAR transmembrane domain, where binding of channel blockers enables the formation of a hydrophobic dome at the blocker binding site. Additionally, we show that agitation of the threonine ring is associated with changes in Koff for all the ligands, a critical mechanistic determinant of therapeutic benefit.

Poster #15

Structure of the Arabidopsis thaliana glutamate receptor-like channel GLR3.4

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Glutamate receptor-like channels (GLRs) play vital roles in various physiological processes in plants, such as wound response, stomatal aperture control, seed germination, root development, innate immune response, pollen tube growth, and morphogenesis. Despite the importance of GLRs, knowledge about their molecular organization is limited. Here we use X-ray crystallography and single-particle cryo-EM to solve structures of the Arabidopsis thaliana GLR3.4. Our structures reveal the tetrameric assembly of GLR3.4 subunits into a three-layer domain architecture, reminiscent of animal ionotropic glutamate receptors (iGluRs). However, the non-swapped arrangement between layers of GLR3.4 domains, binding of glutathione through S-glutathionylation of cysteine C205 inside the amino-terminal domain clamshell, unique symmetry, inter-domain interfaces, and ligand specificity distinguish GLR3.4 from representatives of the iGluR family and suggest distinct features of the GLR gating mechanism. Our work elaborates on the principles of GLR architecture and symmetry and provides a molecular template for deciphering GLR-dependent signaling mechanisms in plants.

Poster #16

Cryo-EM reveals variable mechanisms of desensitization of AMPA receptor complexes with type II TARP- ɣ5 and GSG1L

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AMPA receptors (AMPARs) mediate the majority of excitatory neurotransmission. Their surface expression, trafficking, gating, and pharmacology are regulated by auxiliary subunits (figure below). Of the two types of TARP auxiliary subunits, type II TARPs serve suppressive functions. We present cryo-EM structures of GluA2 AMPAR in complex with type II TARP ɣ5, which reduces steady-state currents, increases single-channel conductance, and slows recovery from desensitization. GluA2-ɣ5 complex shows maximum stoichiometry of two TARPs per AMPAR tetramer, being different from type I TARPs but reminiscent of the auxiliary subunit GSG1L. Desensitization of both GluA2-GSG1L and GluA2-ɣ5 complexes is accompanied by rupture of LBD dimer interface, while GluA2-ɣ5 but not GluA2-GSG1L LBD dimers remain two-fold symmetric. Different structural architectures and desensitization mechanisms of complexes with auxiliary subunits endow AMPARs with broad functional capabilities.

Poster #17

Disruption of grin2B, an ASD-associated gene, produces social deficits in zebrafish.

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Disease-associated missense mutations in NMDAR subunits have been found for a variety of neurodevelopmental disorders, including Autism Spectrum Disorders (ASD), epilepsy and schizophrenia. These missense mutations are present in all subunits and vary broadly in functional changes, and structural location. How such wide-ranging alterations in NMDAR function impact neurodevelopment and contribute to neurodevelopmental disorders is poorly understood, in part due to the myriad developmental roles of NMDARs. Here, we establish the relevancy of the zebrafish model to study developmental roles of NMDAR. We demonstrate that zebrafish NMDARs display similar structural and functional properties to human NMDARs. To further study subunit-specific developmental roles, we generated fish lacking each of the most widely expressed NMDAR subunits (GluN1 and GluN2A-D). Notably, zebrafish lacking all NMDARs (*grin1*$^{-/-}$) survive until 10 days post fertilization (dpf), and zebrafish lacking all functional GluN2B (*grin2B*$^{-/-}$) survive into adulthood. These viabilities exceed that of murine models and afford the unique opportunity to interrogate developmental roles of NMDARs and GluN2B-containing receptors. Here, we specifically focus on GluN2B, encoded by the high-confidence ASD-associated gene *GRIN2B*. Zebrafish are highly social creatures with many robust behaviors. This permits the study of social preference, deficits of which are highly prevalent in ASD. Unlike wild-type fish, which develop a strong social preference by 3 weeks post fertilization (wpf), *grin2B*$^{-/-}$ fish at this age exhibit significantly reduced social preference. This phenotype is specific for GluN2B, as frameshift mutations in obligatory GluN1 subunit paralogues *grin1a* or *grin1b* or removal of GluN2A, does not generate replicate this deficit. Notably, the lack of GluN2B does not result in a broad disruption of neurodevelopment, as *grin2B*$^{-/-}$ larvae have wild type spontaneous locomotion and gross anatomical brain structure, and exhibit learning capabilities. Whole-brain imaging of *grin2B*$^{-/-}$ larvae revealed a reduction in inhibitory neurons in the subpallium and associated areas. The zebrafish subpallium contains putative homologues to the amygdala, septum, and striatum – regions whose dysfunction is associated with ASD in humans. Together, these findings highlight the unique opportunity to study, in zebrafish, the roles of GluN2B in development and disease etiology and afford a system for future examination of the role of GluN2B in the circuits that generate social preference. Recent advances in genome editing technology can also facilitate the study of developmental effects of individual missense mutations *in vivo* in the zebrafish model.

Poster #18

Structure and function of CALHMs

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Biological membranes of many tissues contain channels with large pores that permeate a wide variety of ions and metabolites. Examples of these so called large pore channels include the connexin, innexin, pannexin and the calcium homeostasis modulator protein (CALHM) families. To maintain cell viability, it is critical for the gating and permeability of these big pores to be tightly regulated. Initially thought to resemble NMDARs, CALHMs are the most recently discovered member of large pore channels. Here we explore the structures and functions of large pore channel members through cryo-EM, electrophysiology and molecular dynamics simulations.

Poster #19

Continuous Protein Dynamics and Heterogeneity from New Single Particle CryoEM Analyses using Computational Biophysics and Zernike Polynomials

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CryoEM provides many 2D projection images of molecular machines rapidly frozen in all possible conformational states, potentially allowing us to reconstruct their dynamics and conformational landscapes. Several methods are under development and show some success, but there is still room for improvement.

The Scipion workflow engine allows the use of multiple methods to better understand biological systems and improve methods. We illustrate this for two such continuous heterogeneity methods that we are involved in developing.

One is our new method that uses 3D Zernike polynomials to calculate possible motions from CryoEM maps and optimises their coefficients to describe transitions to other maps, images, and atomic structures. The other uses normal modes to solve a similar problem and is called hybrid electron microscopy normal mode analysis (HEMNMA).

We present new developments in this area including a Scipion plugin for the ProDy Python package for Protein Dynamics.

Poster #20

Alternative splicing of GluN1 gates glycine site–dependent nonionotropic signaling by NMDAR receptors

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(Abstract not provided – head to the poster in-person to check it out!)

Poster #21

Functional analysis of pathological variants on the GluA2 AMPA receptor subunit

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Glutamate-gated ion channels (iGluRs) mediate the vast majority of fast excitatory transmission at chemical synapses of the central nervous system. AMPA-type iGluRs (AMPARs) are key players in this neuron-to-neuron signaling and are fundamental to brain physiology. AMPARs form as homo- and heterotetramers from any 4 different subunits named GluA1-A4. Due to RNA editing, the GluA2 subunit confers unique characteristics on AMPARs since it possesses a positively-charged Arg residue at the apex of the pore region, the so called Q/R site. Other AMPAR subunits, GluA1, A3 and A4, whose RNA is not subject to editing possess a neutral Gln at this position. GluA2-lacking AMPARs exhibit large single channel conductances, appreciable divalent permeability and high affinity block by cytoplasmic polyamines whereas GluA2-containing AMPARs have low conductance channels, lack divalent permeability and polyamine block. The most abundant AMPARs in the mammalian brain form as heteromers of GluA1/A2 or GluA2/A3 revealing that the GluA2 subunit fulfills a privileged role. A recent study (Salpietro V et al. 2019) has identified several GluA2 mutations in patients affected by neurodevelopmental disorders. The mutations are proposed to have a great impact on the patient's cognitive abilities due to an abnormal gating function of the GluA2 subunit, however, whether these point mutations also affect ion permeation and channel block has not been investigated. In our present study, we performed electrophysiological recordings using the patch-clamp technique on outside-out membrane patches to examine the permeation properties of these GluA2 pathological variants. We transiently transfected HEK293T cells with heteromeric AMPAR formed by GluA1 and GluA2 pathological forms and focused on GluA2 variants localized close to or in the Q/R position. We analyzed the effect of R607G, R607E and D611N variants in receptor kinetics as well as the consequences of these mutations in polyamine sensitivity and ion permeation. The present research adds new and valuable information to our growing understanding of AMPAR physiology and pathology as well as revealing the mechanistic effects of GluA2 pathological variants on the pore region and ion permeation.

Poster #22

Engineering Adhesion Proteins for Probing Synapse Organization

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In the central nervous system (CNS), cell adhesion molecules localize to synapses to control synapse formation and shape synaptic properties. These synaptic adhesion molecules (SAMs) come from diverse gene families that share little homology at the primary sequence level. Despite a lack of homology, many SAMs drive the formation of synaptic contacts when overexpressed in cultured neurons. How do diverse SAMs share a functionally similar relationship to synapse formation, despite lacking common functional domains? We hypothesized that high-affinity adhesion might be a common property of SAMs that facilitated their effects on synapse formation. We tested this hypothesis by generating a novel synaptic adhesion pair with no vertebrate homologues, dubbed 'Barnoligin' and 'Starexin.' Barnoligin and Starexin form a specific adhesion complex with one another, but not with other synaptic proteins. The reconstitution of the Barnoligin-Starexin adhesion complex in cultured neurons drives the formation of synapses, and the effect on synapse formation is directional, with Barnoligin driving only postsynaptic assembly and Starexin driving only presynaptic assembly. A GPI-anchored version of Starexin forms a functional adhesion complex but does not drive synapse assembly, demonstrating that in addition to adhesion, intracellular signaling is also required for SAM-driven synapse formation. We present Barnoligin and Starexin as tools for probing adhesion complexes broadly and for specifically perturbing patterns of synaptic connectivity.

Poster #23

Alternative splicing shape the functional properties of AMPA receptor signaling complexes

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BACKGROUND AND AIM: AMPA-type ionotropic glutamate receptors (AMPARs) are fundamental for fast excitatory neurotransmission across all brain regions. These cation-permeable channels consist of four pore-forming subunits, each of which can be alternatively spliced. Alternative splicing occurs in the ligand-binding domain (LBD) at a region called the 'flip/flop cassette', generating two splice variants: flip and flop. The expression pattern of flip and flop in the CNS is highly controlled, both spatially and temporally. Furthermore, flip and flop isoforms exhibit distinct pharmacological and kinetic properties and also differ in their responsiveness to allosteric modulators. Recent work from our lab has shown that alternative splicing of AMPARs unexpectedly dictates the intrinsic nanoscale mobility of the apo state. In the absence of agonist, flop receptors are inherently more mobile than flip receptors, which underlies differences in the time course of channel activation and sensitivity to allosteric regulation. In the brain, AMPARs do not act alone; they form signaling complexes with auxiliary proteins which modify their functional behaviour. Given this, we wondered whether AMPAR alternative splicing can also impact modulation by auxiliary subunits.

RESULTS: We find that one of the most prominent auxiliary subunits, transmembrane AMPAR regulatory protein gamma-2 (TARP g2) is unable to regulate the time course of desensitization of flop receptors. In contrast, another important auxiliary subunit, Cornichon-3 (CNIH-3) is completely unaffected by alternative splicing. Our data suggest that this distinction can be explained by both structural and kinetic mechanisms. TARPs and CNIHs target different AMPAR gating modes, where TARPs act to slow desensitization via LBD interactions, while CNIHs primarily affect deactivation via the transmembrane region. Since alternative splicing also targets the AMPAR LBD to regulate desensitization, the flip/flop cassette dominates and acts as a master switch to selectively override TARP function. In addition, we show that other auxiliary protein families, namely GSG1L and CKAMP44, are unaffected by alternative splicing. Importantly, we extend our findings to the native system and demonstrate that the flip/flop cassette regulates fully- and partially-TARPed AMPARs in the cerebellum.

CONCLUSIONS: Altogether, this work establishes that AMPA receptor alternative splicing is a complex regulatory mechanism that, in coordination with auxiliary subunit association, fine-tunes and diversifies synaptic transmission.

Poster #24

NMDA receptor mutation dysregulates neuroblast proliferation generating supernumerary neurons in the forebrain of zebrafish larvae

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Normal brain development depends upon precise spatiotemporal regulation of neural stem and progenitor cells (NSPC). N-Methyl-D-Aspartate receptors (NMDAR) are glutamate-gated cation channels known to play essential roles in dynamic neurodevelopmental events yet their role in the regulation of neurogenesis is not fully understood. Some studies indicate suppression of NMDAR-mediated signaling promotes neurogenesis, while other studies support the opposite view.

To probe the role of NMDARs in neurogenesis we developed a mutant zebrafish line that lacks all NMDAR-mediated signaling (*grin1* double mutants). These fish survive far beyond the comparable age of rodent knockout models, thereby providing a singular opportunity to examine the role of NMDARs in all stages of neurodevelopment.

We performed detailed quantification of 12 different forebrain cell populations in 3 days post fertilization (dpf) zebrafish. We found that relative to wild type fish, *grin1* double mutants show significantly increased cell densities in the anterior regions of the forebrain. At 5dpf, increased cell densities were observed in all forebrain cell populations. We performed Immunohistochemistry (IHC) against GFAP and PSA-NCAM to ensure that these increased cells were neurons and not glial cells. Morphometric analysis demonstrated that increased neuronal density occurs without any gross anatomical changes to the brain. Activated caspase-3 IHC indicated that the supernumerary neurons did not result from diminished programmed cell death. We then assayed the NSPCs using IHC to determine the origin of dysregulation. We find that *grin1* double mutants have a higher percentage of mitotic neuroblasts, as indicated by PCNA expression, which inappropriately amplifies the neuronal population.

These data suggest that NMDAR signaling is required for suppression of neurogenesis in the neuroblast transit amplifying cell populations, without which proliferation progressively increases unchecked. Furthermore, as NMDAR mutations are correlated with neurodevelopmental diseases (NDD), as are supernumerary neurons, this work also suggests an unexplored mechanism of NMDAR-mediated NDD etiology.

Poster #25

A novel role for NMDA receptors in neural crest development

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The neural crest (NC) is a migratory, pluripotent cell type of neuroepithelial origin that gives rise to a host of neural and non-neural tissues including the autonomic, enteric, and peripheral nervous systems, as well as cartilage, cardiac and pigment cells. Given the ubiquity of NC-derived cells, and their implication in multiple disease states, a more thorough understanding of their regulation is essential.

N-Methyl-D-Aspartate receptors (NMDAR) are glutamate-gated cation channels that play a key role in excitatory calcium transmission. Numerous mutations in multiple NMDAR subunits are associated with neurodevelopmental diseases (NDD) though disease etiology is not fully understood. Furthermore, their part in NC development and the role that subunit mutations may play in NC atypia has been virtually unexplored. Surprisingly, we found that zebrafish mutants that lack the obligatory NMDAR subunit (*grin1* double mutants) show defects in multiple NC-derived tissues.

We assayed craniofacial cartilage and pigmentation, both NC-derived populations, in *grin1* double mutants and wild type controls, at 5 days post fertilization (dpf). Craniofacial cartilage was examined using two methods. First, zebrafish larvae were embedded in paraffin and serially sectioned in 5µm coronal sections; sections were Nissl stained for visualization and assessed for the presence of ectopic cartilage growth. Additionally, whole-mount embryos were stained with Alcian Blue dye for histological analysis of craniofacial cartilage morphology. We found that although craniofacial abnormalities are a rare occurrence in wild type fish – appearing in less than 15% of fish assayed and only in very mild forms – 100% of *grin1* double mutants assayed displayed severe craniofacial abnormalities. Live imaging of 6dpf zebrafish larvae revealed that *grin1* double mutants display hyperpigmentation over the entire anterior to posterior body axis.

Our work is the first to demonstrate a role for NMDAR-mediated signaling in the regulation of NC-derived cell types. Furthermore, though NDDs are considered disorders of the brain, the atypical development associated with these diseases is not restricted to the CNS. NDDs are often associated with craniofacial abnormalities; autonomic, enteric, and peripheral nervous system dysfunction' and sensory system abnormalities, which implies participation of neural NC abnormalities in disease etiology as these systems all derive from the NC. Thus, our work also suggests that NMDAR dysfunction may lie at the root of multiple NDD phenotypes deriving from non-CNS tissues.

Poster #26

The N-Terminal Domain of the NMDA Receptor Modulates Dynamic Ca^{2+} Permeability

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NMDA receptors (NRs) are glutamate-gated ion channels distinguished by their ability to produce slow, Ca2+ rich excitatory currents. The resulting Ca2+ flux is essential for fundamental aspects of synaptic development and plasticity, and when in excess, drives excitotoxicity. In physiological conditions, it is believed that the relative Ca2+ permeability of the channel is stable and therefore, the amount of fluxed Ca2+ is proportional to the measured ionic current. Here, we show that endogenous receptor modulators, such as extracellular pH and Zn2+, whose concentrations vary with developmental stage, brain region, and activity level, control the channel relative Ca2+ permeability. Similarly, synthetic ligands that act allosterically through the N-terminal domain, such as ifenprodil, modulate the NR relative Ca2+ permeability. These results suggest that the depolarization function of NRs, which is largely mediated by Na+ currents, can be separated from their plastic function, which is largely mediated by Ca2+. Further understanding this phenomenon may aid future NR drug-design, providing novel pharmacological targets that function to alter neuronal Ca2+ flux without disrupting the vital excitatory function of these receptors.