iGluR 2023

9th Annual Conference | July 8-10, 2023

Northwestern University, Feinberg School of Medicine | Chicago, IL

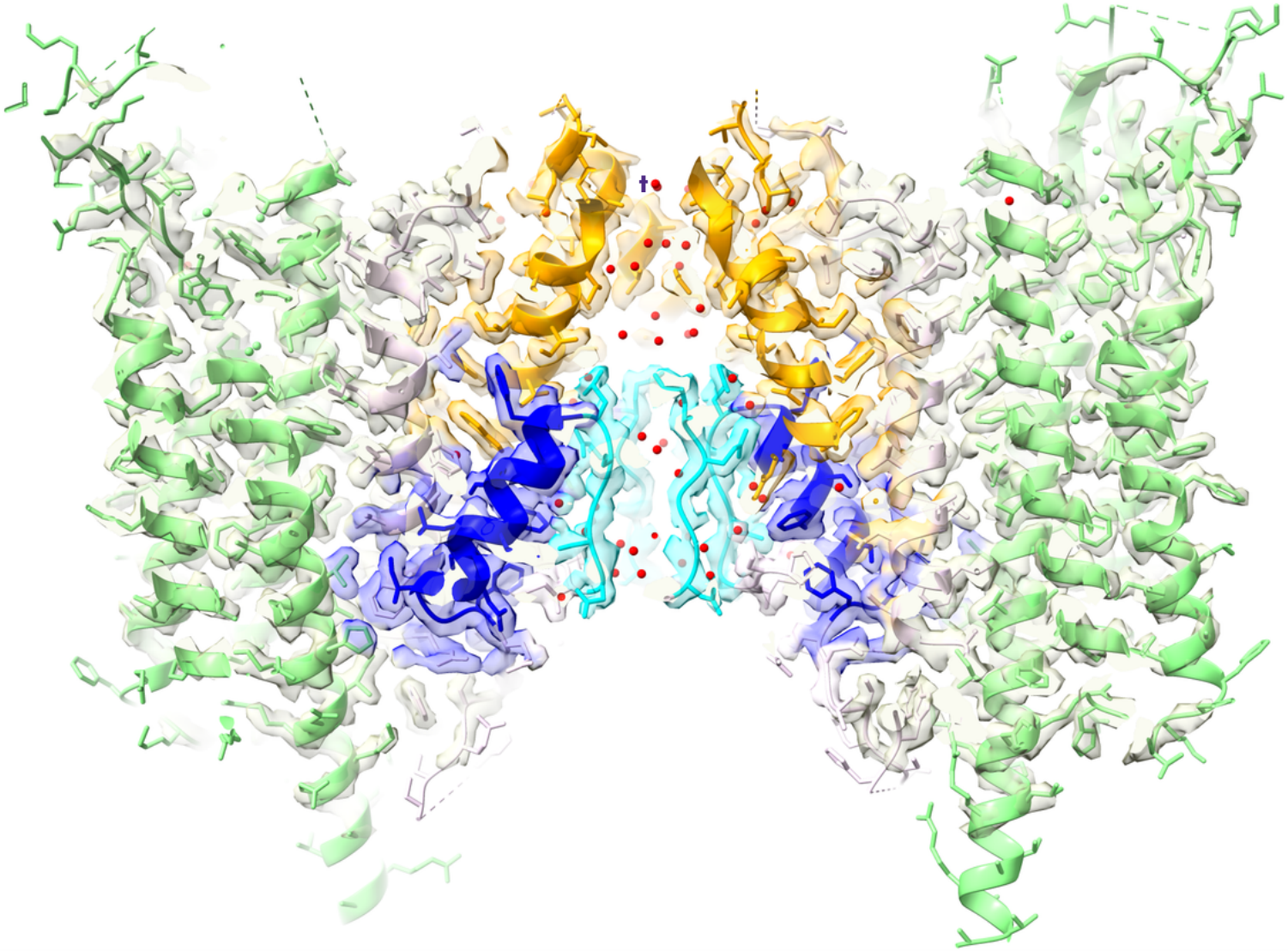

Image by: Dr. Terunaga Nakagawa at Vanderbilt University (2023)

Northwestern Medicine®
Feinberg School of Medicine

SATURDAY - MONDAY, JULY 8-10, 2023
303 E. SUPERIOR ST. CHICAGO, IL 60611

iGluR 2023

DAY 1

Conference Opening | 8:00 AM - 8:15 AM

8:00am - 8:15am — **Welcome remarks from Dr. Geoff Swanson and Dr. Anis Contractor**

Session 1: Philippe Ascher Memorial Session | 8:15 AM - 9:45 AM

8:15am - 8:40am

Linda Nowak, Ph.D.
Cornell University
"Negative Allosteric Modulation of AMPA Receptors"

8:45am - 9:10am

Jon Johnson, Ph.D.
University of Pittsburgh
"Membrane to Channel Inhibition, a Novel NMDA Receptor Channel Blocking Mechanism"

9:15am - 9:40am

Gabriela Popescu, Ph.D.
University at Buffalo
"Allosteric Inhibition of NMDA Receptors by Ketamine"

Session 2: iGluR biogenesis and signaling | 9:50 AM - 11:30 AM
Chair: Ingo Gregor

9:50am - 10:10am

Yael Stern-Bach. Ph.D.
The Hebrew University of Jerusalem
"Characterization of De Novo Mutations in AMPA Receptors Associated with Developmental Cognitive and Motor Dysfunctions"

10:15am - 10:35am

Bernd Fakler, Ph.D.
Albert-Ludwigs-Universität Freiburg
"The Interactome of AMPA-receptors - Protein-coding of Excitatory Neurotransmission and its Plasticity"

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DAY 1

10:40am - 11:00am

Stephanie Gantz, Ph.D.

University of Iowa

"Potentiation of neuronal activity by GluD1 current in brain slices"

11:05am - 11:25am

Villu Maricq, M.D., Ph.D.

University of Utah

"Synaptic Function, Error Correction and the Control of Navigation"

BREAK | 11:30 AM - 11:40 AM

Special Panel: CureGRIN/GRIN2B representatives | 11:40 AM - 12:35 PM

11:40am - 11:50am

Steve Traynelis, Ph.D.

Emory University

11:55am - 12:10pm

Liz Marfia-Ash

President and Founder | GRIN2B Foundation

12:15pm - 12:30pm

Keith McArthur

CEO | CureGRIN Foundation

Group photo, lunch and posters | 12:35 PM - 1:45 PM

12:35pm - 1:45pm

Group Photo | Lunch | Poster Session

Potocsnak Family Atrium

303 E. Superior St.

Chicago, IL 60611

iGluR 2023

DAY 1

Sponsor Presentations | 1:45 PM - 2:10 PM

1:45pm - 2:10pm

RWD Life Science

Scientifica

elements
enabling technologies for Life Science

Session 3: iGluRs and Disease | 2:15 PM - 3:50 PM
Chair: Mike Salter

2:15pm - 2:35pm

Rick Huganir, Ph.D.
Johns Hopkins University
"Regulation of AMPA Receptor Receptors During Synaptic Plasticity in Health and Disease"

2:40pm - 3:00pm

Lonnie Wollmuth, Ph.D.
SUNY Stony Brook
"Zebrafish to study brain development and NMDA receptor disease associated variants"

3:05pm - 3:25pm

Sharon Swanger, Ph.D.
Virginia Tech
"NMDA receptor diversity enables target-specific tuning of corticothalamic excitation"

3:30pm - 3:45pm

Toshihiro Nomura, M.D., Ph.D.
Northwestern University
"Pathogenic mutation in GluK2 kainate receptors elevates neuronal excitability through dendritic mechanisms"

BREAK | 3:50 PM - 4:00 PM

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DAY 1

Session 4: Function of iGluRs | 4:00 PM - 5:35 PM
Chair: Susumu Tomita

4:00pm - 4:20pm

Kasper Hansen, Ph.D.
University of Montana
"Allosteric modulation of GluN3-containing NMDA receptors"

4:25pm - 4:40pm

Kishore Narasimhan, Ph.D.
Creighton University
"Beyond NMDAR Hypofunction: Unveiling GluN2D Subunit's Impact on Schizophrenia-Related Circuitry and Behavior"

4:45pm - 5:05pm

Jesper Sjöström, Ph.D.
McGill University
"Non-Canonical NMDA Receptor Signalling in Neocortical Plasticity"

5:10pm - 5:30pm

Steve DeVries, M.D., Ph.D.
Northwestern University
"Kainate receptor diversity at the mammalian cone photoreceptor synapse"

6:30pm

Dinner | Woodwind
259 E. Erie St., 18th Floor,
Chicago, IL 60611

iGluR 2023

DAY 2

Session 1: Stevens Memorial Session | 8:30 AM - 10:15 AM

8:30am - 8:55am

Lynn Dobrunz, Ph.D.

University of Alabama at Birmingham

"Presynaptic kainate receptors contribute to extremely large short-term plasticity onto somatostatin interneurons in hippocampus"

9:00am - 9:20am

Kim McAllister, Ph.D.

University of California, Davis

"Surprising roles for glutamate signaling, NMDARs, neuroligin/neurexin adhesion, and MHCI molecules in cortical synapse formation "

9:25am - 9:45am

Sam Young, Ph.D.

University of Iowa

"Defining the Molecular Principles of Auditory Information Processing"

9:50am - 10:10am

Yongling Zhu, Ph.D.

Northwestern University

"Intersectional mapping of Glutamate/GABA co-transmission neurons in the brain"

BREAK | 10:15 AM - 10:25 AM

Session 2: Structure of iGluRs | 10:25 AM - 12:00 PM

Chair: Lonnie Wollmuth

10:25am - 10:45am

Vasanthi Jayamaran, Ph.D.

University of Texas Houston

"Partial agonism in iGluRs"

10:50am - 11:05am

Shanti Pal Gangwar, Ph.D.

Columbia University

"Mechanism of kainate receptor regulation by positive and negative allosteric modulators"

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DAY 2

11:10am - 11:30am

Teru Nakagawa, M.D., Ph.D.

Vanderbilt University

"Visualizing putative ions and water molecules in the ion conduction path of the AMPA receptor"

11:35am - 11:55am

Ingo Greger, Ph.D.

MRC Laboratory of Molecular Biology

"Gating modulation in a GluA1 AMPA receptor complex "

Lunch and posters | 12:00 PM - 1:30 PM

12:00pm - 1:30pm

Lunch | Poster Session

Potocsnak Family Atrium
303 E. Superior St.
Chicago, IL 60611

Session 3: Synaptic Structure and function | 1:30 PM - 3:10 PM

Chair: Katherine Roche

1:30pm - 1:50pm

Matt Dalva, Ph.D.

Tulane University

"The nanoscale organization of glutamate receptors at cortical neuron spine synapses"

1:55pm - 2:15pm

Jeff Savas, Ph.D.

Northwestern University

"Notch receptor-ligand binding facilitates extracellular vesicle-mediated trans neuronal communication"

2:20pm - 2:40pm

Susumu Tomita, Ph.D.

Yale School of Medicine

"Synaptic structure and receptor localization"

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DAY 2

2:45pm - 3:05pm

Jacques Wadiche, Ph.D.

University of Alabama at Birmingham

"Diffuse and local actions of glutamate"

BREAK | 3:10 PM - 3:20 PM

Session 4: Synapses and Disease | 3:20 PM - 4:30 PM

Chair: Kim McAllister

3:20pm - 3:35pm

Pushpa Kumari, Ph.D.

Northwestern University

"Fast acting antidepressants: Mechanistic insights and future directions"

3:40pm - 4:00pm

Peter Penzes, Ph.D.

Northwestern University

"A shed neuronal ectodomain, detected in patients' cerebrospinal fluid, modulates glutamate receptors, neuronal network dynamics, and rescues disease-relevant phenotypes"

4:05pm - 4:25pm

David Stellwagen, Ph.D.

McGill University

"TNF-mediated synaptic plasticity in anxiety and disease"

4:30pm

Closing remarks from Dr. Geoff Swanson and Dr. Anis Contractor

ABSTRACTS

Testing AMPA receptor-specific RNA aptamers in conditional ADAR2 knockout ALS model mice

Megumi Akamatsu[1 2], Zhen Huang[1], Shin Kwak[2], Li Niu[1]

1. Department of Chemistry, University at Albany, State University of New York, Albany, NY, USA
2. Department of Neurology, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

Background: The expression level of adenosine deaminases acting on RNA 2 (ADAR2) is reduced in the motor neurons of the majority of sporadic ALS patients. The reduction of ADAR2 triggers the abnormal expression of unedited GluA2 at the Q/R site, which leads to abnormal Ca2+ influx into the motor neurons. An exaggerated increase in Ca2+ results in activation of Ca2+-dependent protease calpain in the cytoplasm, thereby causing TDP-43 pathology in the ADAR2-lacking motor neurons of AR2 mice. Notably, motor neurons devoid of ADAR2 immunoreactivity exhibit TDP-43 pathology (i.e., TDP-43 cytoplasmic mislocalization and aggregation) in AR2 mice, as in sporadic ALS patients. Blocking the exaggerated Ca2+ influx mediated through abnormally expressed Ca2+-permeable AMPA receptors is therefore a potential therapeutic strategy for sporadic ALS. Previous study showed that using perampanel, a non-competitive AMPA receptor antagonist, led to normalization of TDP-43 pathology but with a significant sedative side effect on these mice at a dose that would limit this small molecule compound to be used clinically. RNA aptamers are a new group of potential ALS drug candidates that have better pharmacological properties than small molecule antagonists of AMPA receptors, due to their high potency, selectivity, and water solubility.

Objective: To investigate the efficacy and safety of AMPA receptor-specific RNA aptamers on the ALS phenotype of the AR2 mice.

Methods: ADAR2 cKO mice were used in this study. Two RNA aptamers (FN1040 and FN58) were tested. An aptamer was continuously delivered to the cerebroventricule of the AR2 mice through a cannula connected to an Alzet osmotic pump. Anti-ChAT antibody and anti-TDP-43 antibody were used for immunohistochemistry. Rotarod test was conducted as a measure of motor function.
Results: We first examined the distribution of aptamers using 32P-labeled FN1040 and verified aptamers distributed throughout the brain to the spinal cord. Then we determined the efficacy and the optimum concentration of aptamer in a two-week administration. The efficacy was measured by increase in the number of motor neurons and the normalization of TDP-43 mislocalization in the motor neurons of the AR2 mice. We found both aptamers were efficacious. In a long-term administration of aptamers (three months), both FN1040 and FN58-treated mice showed significant improvement in that the number and the size of motor neurons increased and the motor function improved as compared with vehicle-treated mice. Furthermore, FN1040-treated mice exhibited no detectable side effects even the dose of aptamer used was high.

Conclusion: In this study, we measured the neuroprotective properties of AMPA receptor aptamers in this ALS mouse model. Our results suggest that use of AMPA receptor-specific RNA aptamers is potentially a new therapeutic approach for ALS.

Acknowledgement: This work was previously supported by an R21 and is now supported by an R01 grant from NIH/NINDS.

ABSTRACTS

Cav2.1 current facilitation is critical in regulating synaptic transmission and plasticity at the Calyx of Held/MNTB synapse

Mohammed Al-Yaari[1], Samuel M. Young, Jr.[1 2]

1. Department of Anatomy and Cell Biology, Iowa Neuroscience Institute, University of Iowa, Iowa City, IA 52242, USA
2. Department of Otolaryngology, Iowa Neuroscience Institute, University of Iowa, Iowa City, IA 52242, USA

Sound information encoding within the initial auditory processing stations requires reliable and precise synaptic transmission over a broad dynamic range of sound frequencies. In mammals, the calyx of Held/medial nucleus of the trapezoid body (MNTB) synapse is a critical synapse for encoding sound localization and temporal features of music and verbal communication. The calyx of Held, a glutamatergic presynaptic terminal that arises from the Globular Bushy Cell axon is the sole input driving action potential spiking in the MNTB. Cav2.1 is exclusively the calcium channel subtype in the mature calyx of Held terminal which controls the strength and dynamics of neurotransmission. The alternative splice variant Cav2.1 exon 37a in the calyx undergoes Calcium-dependent facilitation (CDF) while Cav2.1 exon 37b does not. CDF is hypothesized to be important for sustaining synaptic transmission to maintain the input firing rate needed for the encoding of auditory information. Therefore, to test the role of Cav2.1 current modulation in the regulation of precise and reliable synaptic transmission, we expressed either Cav2.1 37a or Cav2.1 37b at the calyx of Cav2.1 knockout mice. we have observed no change in the amplitude of 1st EPSC and onset of EPSCs during high-frequency stimulation (300, 500 Hz), suggesting that neither the calcium current amplitude nor the coupling of Cav2.1 channels has changed when expressing either of the two splice isoforms. Importantly, calyces that express Cav2.1 37a showed facilitation of EPSCs which is comparable to that in wild-type calyces. However, calyces expressing Cav2.1 37b did not show synaptic facilitation, rather they increased the short-term synaptic depression. We here identified distinct roles of calcium current facilitation in the auditory processing neurons, in which the Cav2.1 37a isoform is critical in supporting the natural facilitation of synaptic transmission thus maintaining firing rates over a wide range of sound frequencies. Results of this work and how the lack of CDF impacts the reliability and precision of naturalistic auditory stimulation patterns will be discussed.

Allosteric modulation of GluN1/GluN3 NMDA receptors by orthosteric GluN1 ligands

Carly M. Anderson[1], Nirvan Rouzbeh[1], Andrew R. Rau[1], Avery J. Benton[1 2], Feng Yi[3], Mia R. Johns[1], James S. Lotti[1 2], and Kasper B. Hansen[1]

1. Center for Structural and Functional Neuroscience, Center for Biomolecular Structure and Dynamics, Division of Biological Sciences, University of Montana, Missoula, MT, USA.
2. Department of Biomedical and Pharmaceutical Sciences, Skaggs School of Pharmacy, University of Montana, Missoula, MT, USA.
3. Guangdong-Hong Kong-Macao Greater Bay Area Center for Brain Science and Brain-Inspired Intelligence, Southern Medical University, Guangzhou, China.

Abstract

NMDA-type glutamate receptors are ligand-gated ion channels that are critical to brain function and are implicated in central nervous system disorders. The pharmacological properties of NMDA receptors composed of GluN1 and GluN2 subunits are well-understood compared to those composed of GluN1 and GluN3 subunits. These GluN1/3 receptors display unusual activation properties in which binding of glycine to GluN1 elicits strong desensitization, while glycine binding to GluN3 alone is sufficient for activation. We demonstrate mechanisms by which GluN1-selective competitive antagonists potentiate GluN1/3A and GluN1/3B receptors by preventing glycine binding to GluN1. We show that both CGP-78608 and L-689,560 prevent GluN1/3 desensitization through this same mechanism, but CGP-78608-bound receptors display higher glycine potency and efficacy at GluN3 subunits compared to L-689,560-bound receptors. Furthermore, we suggest that binding of GluN1-selective competitive antagonist or mutations in the GluN1 glycine binding site can promote distinct conformations of the GluN1 agonist binding domain (ABD) that in turn differently influence agonist potency and efficacy at GluN3 subunits. These findings identify the orthosteric binding site in GluN1 as a modulatory site in GluN1/3 receptors and demonstrate intra-subunit allosteric interactions that may be relevant to neuronal signaling in brain function and disease.

ABSTRACTS

Neurogliaform GABAergic Interneurons Show Strong Modulation of GluN2D-containing NMDA Receptors

Chad R. Camp[1], Tue Banke[1], Hao Xing[1], Matt Epplin[2], Russell Fritezmeier[2], Sukhan Kim[1], Jing Zhang[1], Dennis C. Liotta[2], Hongjie Yuan[1], Stephen F. Traynelis[1]

1. Department of Pharmacology and Chemical Biology, Emory University School of Medicine, Atlanta, GA
2. Department of Chemistry, Emory University School of Medicine, Atlanta, GA

GABAergic interneurons make up a small proportion of total cells within neocortical and hippocampal tissue yet exert immense control on circuit excitability and behavior. Hippocampal GABAergic interneuron function can be directly linked to spatial navigation, memory, circuit excitability, and overall network oscillations. Additionally, interneuron dysfunction is a central hypothesis to multiple neuropathological diseases including epilepsy, schizophrenia, and autism. Thus, the therapeutic potential of interneuron modulation is immense. One potential avenue for interneuron-specific control is through activation of GluN2D-containing NMDA receptors. The GluN2D subunit is preferentially expressed in GABAergic interneurons, with little to no expression on glutamatergic principal cells. Recently, we synthesized and characterized a new derivative of the GluN2C/D-selective PAM CIQ, (+)-EU1180-453 (hereafter referred to as EU1180-465) that shows over 15-fold improvement in doubling concentration (i.e., the concentration needed to double a current response) and more than 7-fold improvements in aqueous solubility. Here, we examined the effects of EU1180-465 on circuit excitability and ability to potentiate NMDA receptors on multiple classes of GABAergic interneurons. The GluN2C/D-specific positive allosteric modulator EU1180-453 significantly increased the charge transfer of evoked NMDA receptor-mediated currents onto stratum radiatum interneurons (0.59 pC for baseline vs 0.92 pC for 10 μM EU1180-453; paired t-test, p=0.003; n=13). Interestingly, only 54% (7/13) of these recorded cells showed potentiation above 1.25-fold, while the remaining cells showed no potentiation. Given heterogeneity of interneurons found within stratum radiatum, we hypothesized that the differential effects of EU1180-453 may be driven by lack of GluN2D expression on a particular interneuron subtype. To answer this question, we used genetically driven fluorescent mice to test EU1180-453's efficacy on neuropeptide-Y (NPY) positive neurogliaform cells in stratum radiatum. Neurogliaform cells are unique in that they largely communication via GABAergic volume transmission, with the majority of their presynaptic boutons at considerable distances from their postsynaptic contacts. Moreover, the role of GluN2D-mediated signaling in neurogliaform cells has yet to be examined. We show that synaptic NMDA receptor responses in all NPY-positive neurogliaform cells (n=13/13 cells) showed strong potentiation following EU1180-453 application. Future studies will elucidate the roles of neurogliaform cell potentiation to modulate the tonic GABA current, which has been shown to be critical for determining pyramidal cell excitability. More importantly, these data provide mechanistic detail for novel GluN2D modulators to alter interneuron function and network output.

Disclosures: H.Y. and S.F.T. are co-inventors of Emory-owned intellectual property. S.F.T. is a PI on a research grants from Janssen to Emory, is a member of the SAB for Sage Therapeutics, Eumentis Therapeutics, the GRIN2B Foundation, the CureGRIN Foundation, and CombinedBrain. H.Y. is the PI on a research grant from Sage Therapeutics to Emory. S.F.T. is cofounder of NeurOp, Inc. and Agrithera. D.C.L. is a member of the Board of Directors for NeurOp Inc. D.C.L., and S.F.T. are co-inventors on Emory-owned Intellectual Property that includes positive allosteric modulators of NMDA receptor function.

Funding: This work was supported by the following grants from the National Institutes of Health, National Institute of Neurological Disease and Stroke: NS113530 (C.R.C.), MH127404 (H.Y.), HD082373 (H.Y.), and NS111619 (S.F.T.). The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institute of Health.

ABSTRACTS

Characterization of disease-associated GluN1-M706V mutation effects on NMDA receptor function

Lauren Cornelison[1], Jed T. Syrenne[1], and Kasper B. Hansen[1]

1. Division of Biological Sciences, Center for Structural and Functional Neuroscience, Center for Biomolecular Structure and Dynamics, University of Montana, Missoula, MT

N-methyl-d-aspartic acid receptors (NMDARs) are tetrameric subunit complexes composed of two glycine-binding GluN1 and two glutamate-binding GluN2 subunits (GluN2A-D). Recently, mutations within NMDARs have been shown to significantly contribute to the etiology of encephalopathies, which give rise to sensory, motor, and cognitive impairments. One such mutation in the GluN1 agonist binding domain (ABD), Met706Val, was identified in a patient presenting with a severe neurological phenotype. However, given NMDAR complexity, classification of a mutation as loss or gain of function can be challenging. We sought to elucidate the mechanism by which this mutation impacts receptor function and initially assessed the impact of GluN1-M706V on ligand potency using two-electrode voltage clamp recordings from NMDARs expressed in Xenopus oocytes. We found that the mutation affected the concentration-response relationship of glycine in a GluN2 subunit-dependent manner, and that this phenotype was ameliorated when oocytes were pre-exposed to a saturating concentration of glycine. Fast-application patch-clamp electrophysiology in HEK293 cells was used to evaluate the impact of the mutation on receptor kinetics, as well as the effects of sustained glycine exposure on receptor function. Current amplitudes of mutant receptors in response to glycine application increased with prolonged and repeated exposure to glycine, suggesting that glycine binding may contribute to the recruitment of active receptors in mutant GluN1-M706V/2A, but not wild type GluN1/2A receptors. However, macroscopic response time course was not markedly different between the mutant and wild type receptors. These results suggest that binding of glycine site agonists to GluN1-M706V/2A may improve receptor function through a mechanism akin to pharmacological chaperones. Further characterization of the GluN1-M706V mutation may therefore provide insights into development of therapeutic strategies for patients with similar pathogenic mutations that can be rescued by binding of ligands as pharmacological chaperones.

ABSTRACTS

Characterizing intracellular calcium dependence of triheteromeric NMDA receptors

Quincy Erickson-Oberg

Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA

Aberrant NMDAR Ca2+ flux is linked to myriad nervous system disorders, including Alzheimer's disease (AD). Better understanding of NMDAR function and regulation will yield valuable insights into developing treatments for disorders such as AD. Despite their prevalence and roles in critical brain processes, much remains unknown about NMDARs. Triheteromeric NMDARs (trihets) are of particular interest, as most NMDARs in the brain are thought to be trihets. Formed by two GluN1 subunits and two different GluN2 (commonly GluN2A and GluN2B) or GluN3 subunits, trihets have proved difficult to study. No present method isolates trihet-mediated responses in neurons, and heterologous expression of GluN1/2A/2B trihets requires coexpression of the GluN1, GluN2A, and GluN2B subunits (creating 3 NMDAR subtypes simultaneously). While approaches for isolating trihets have been developed, they require modification of the C-terminal domain, a domain essential for NMDAR regulatory mechanisms including calcium dependent desensitization (CDD). CDD is activated by elevated intracellular Ca2+ and results in decreased NMDAR activity during long exposure to agonists. We have shown that CDD is enhanced by the AD drug memantine (Mem) and proposed that as a result, Mem preferentially inhibits NMDAR populations implicated in disease. We are developing an approach for expressing trihets with unmodified C-terminal domains to electrophysiologically characterize trihet CDD and its role in Mem's mechanism of action. We coexpress GluN1, GluN2B, and mutant GluN2A(T690I) subunits in tsA201 cells. GluN2A(T690I) is used because glutamate potency was reported to be lower for GluN1/2A(T690I) diheteromeric receptors (dihets) than for GluN1/2A(T690I)/2B trihets, allowing minimization of GluN1/2A(T690I) dihet activation. We will use the GluN2B-specific antagonist CP-101,606 to minimize GluN1/2B dihet activation. With this approach we can quantify "contamination" by activation of dihets. GluN1/2B dihet contamination will be measured by applying 10 mM Glu, a saturating concentration for GluN1/2B dihets that does not activate trihets or GluN1/2A(T690I) dihets. Contamination by GluN1/2A(T690I) dihets will be quantified by measuring inhibition by MPX-004, which preferentially inhibits GluN1/2A dihets. Understanding CDD and its role in Mem inhibition of trihets has broad implications for mechanisms of therapeutic drug action.

ABSTRACTS

Quantitative characterization of allosteric NMDA receptor modulation and agonist activity using a two binding site equilibrium model

James S. Lotti[1], Feng Yi[2], Jill C. Farnsworth[1], Kasper B. Hansen[1]

1. Division of Biological Sciences, Center for Structural and Functional Neuroscience, Center for Biomolecular Structure and Dynamics, University of Montana, Missoula, MT;
2. Guangdong-Hong Kong-Macao Greater Bay Area Center for Brain Science and Brain-Inspired Intelligence, Southern Medical University, Guangzhou, Guangdong, China.

N-methyl D-aspartate (NMDA) receptors are a class of ionotropic glutamate receptors critical for healthy central nervous system function. NMDA receptors are composed of two GluN1 subunits which assemble with two GluN2A-D or two GluN3A-B subunits, each with distinct spatial and temporal expression patterns. NMDA receptor dysfunction is implicated in neurodegenerative diseases and psychiatric disorders and many patients with these pathologies may benefit from pharmacological targeting of NMDA receptors. The development of novel NMDA receptor ligands can be facilitated by pharmacological equilibrium models that can quantify allosteric drug action. We developed an equilibrium model to gain insight into the differences in activities of potent GluN2A-selective negative allosteric modulators (NAMs), namely TCN-201, MPX-004, and MPX-007. Using whole-cell patch-clamp of HEK293T cells expressing recombinant GluN1/2A NMDA receptors, we were able to determine the allosteric constant for modulation of agonist binding (α) as well as the allosteric constant for modulation of agonist efficacy (β). Using this model, we determined TCN-201 and MPX-007 share an α of 0.0004, resulting in a stronger allosteric binding modulation than that of MPX-004, with an α of 0.0013. We determined MPX-004 increases the agonist efficacy with a β of 1.22, thus its inhibition is entirely mediated by decreasing the binding affinity of glycine. MPX-007 and TCN-201 behaved as a more typical negative allosteric modulator with β values lower than 1 (0.84 and 0.77, respectively). With α and β determined, we were able to determine the NAM binding affinity using two-electrode voltage-clamp concentration-response experiments on GluN1/2A NMDA receptors expressed in Xenopus oocytes. The binding dissociation constants were determined to be 47 nM for TCN-201, 8 nM for MPX-004 and 2 nM for MPX-007. Thus, the improved GluN1/2A NMDA receptor inhibition of MPX-004 and MPX-007 compared to TCN-201 is due to an increased binding affinity, since TCN-201 had similar (MPX-007) or more inhibitory (MPX-004) allosteric modulation constants (α and β). These findings provide quantitative insights to the activity of these three NAMs and their mechanisms of inhibition, which can improve interpretation of NAM structure-activity relationships and facilitate the development of novel NMDA receptor therapeutics.

ABSTRACTS

Developing GluA3 selective AMPA receptor positive allosteric modulators as a potential novel treatment for schizophrenia

Sean Moran

Broad Institute of MIT and Harvard

Recent exome sequencing efforts directly associated ultra-rare GRIA3 loss-of-function variants, the gene that encodes the GluA3 subunit of the AMPA receptor (AMPARs), with an increased risk of developing schizophrenia. To-date, there is extensive evidence that engaging the AMPAR through positive allosteric modulators (PAMs) can improve cognition in preclinical animal models relevant for the unmet needs of patients with schizophrenia. Unfortunately, current AMPAR PAMs are not subtype selective, demonstrate serious on-target toxicity (seizures) and this AMPAR PAM on-target toxicity may explain the lack of positive results in human clinical trials. Thus, our therapeutic hypothesis is that by selectively targeting GluA3 containing AMPARs, which are the only AMPAR subunits implicated by schizophrenia human genetics, will increase AMPAR PAM efficacy and decrease toxicity. Utilizing homology models, iterative medicinal chemistry efforts based on existing AMPAR PAMs and a high throughput cell-based fluorescence AMPAR assay, we demonstrate that developing a brain penetrant GluA3 selective AMPAR PAMs is feasible and have identified a compound with approximately 10-fold selectivity for GluA3 containing AMPARs over non-GluA3 containing AMPARs. Experiments are ongoing to test our therapeutic hypothesis that a brain penetrant GluA3 specific AMPAR PAMs will demonstrate a larger therapeutic window (more efficacy and/or less toxicity) in rodent preclinical studies compared to non-subunit selective PAMs.

ABSTRACTS

Biophysical and pharmacological characterization of ligand-gated ion channels using automated QPatch technology

David Nagy

Sophion Biosciences Inc., 213 Burlington Road, Suite 105, Bedford, MA

The focus of this set of experiments was to evaluate the performance of multi-hole patch clamp technology in studying fast and slow desensitizing ligand-gated ion channels. We utilized the QPatch system, which allows gigaseal recordings of up to ten cells simultaneously on a single measurement site. Our findings convincingly demonstrate that the multi-hole QPatch experiments exhibit comparable biophysical and pharmacological characteristics compared to the traditional manual patch-clamp and single-hole QPatch experiments.

In this study, we conducted experiments using various ion channels, including the glutamate receptor GluR5, the nicotinic acetylcholine receptor nAChR α1, the acid-sensing ion channel ASIC1a, and the anionic γ-aminobutyric acid receptor A GABA-A α1β2γ2. We investigated parameters such as agonist rise-time, reversal potential, and pharmacological properties on the QPatch HTX in both multi-hole and single-hole modes.

Our results indicate that although the amplitude of the ion channel current is amplified by a factor of 7-10 in the multi-hole configuration, and the success rate in obtaining usable current amplitudes is increased, other essential biophysical properties of these ion channels remain unchanged. This demonstrates the effectiveness of the multi-hole patch clamp technology for studying ion channels while providing valuable insights into their functionality.

ABSTRACTS

Intracellular route of access for membrane to channel inhibition of NMDA receptors by channel blocking drugs

Elizabeth G. Neureiter, Aparna Nigam, and Jon. W. Johnson

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N-methyl-D-aspartate receptors (NMDARs) are highly calcium-permeable tetrameric glutamate receptors that are composed of two GluN1 subunits and two GluN2 (A-D) subunits and/or GluN3 (A-B) subunits. Channel block of NMDARs is a research area with broad implications for treatment of pathological conditions such as Alzheimer's disease and epilepsy. The extensively studied traditional channel block mechanism is exhibited by many compounds such as MK-801 and memantine, and occurs when charged blocker molecules from the extracellular solution enter the open channels of agonist-bound NMDARs, blocking ion flux at the deep site. Our lab recently investigated another channel block mechanism known as membrane to channel inhibition (MCI). We showed that memantine MCI occurs when uncharged memantine molecules in the extracellular solution enter the membrane, and then transit from membrane to open channels of agonist-bound NMDARs through a fenestration, blocking ion flux at the deep site. Here, we investigate properties of MK-801 MCI. We found that the MK-801 MCI IC50s for diheteromeric GluN1/2A (0.85 ± 0.04 μM) and GluN1/2B (0.67± 0.16 μM) receptors are ~60-fold greater than the IC50s for traditional channel block of GluN1/2A (14.4 ± 1.4 nM) and GluN1/2B (13.4 ± 0.4 nM) receptors. In contrast, GluN1/2C and GluN2D receptors show much weaker MCI than GluN1/2A or GluN1/2B receptors: fractional current during MCI by 1 μM MK-801 was 0.97 ± 0.27 for GluN1/2C and 0.95 ± 0.02 for GluN1/2D receptors, suggesting the MK-801 MCI IC50s for GluN1/2C and GluN1/2D receptors are far above 1 μM. We show that the time course of MK-801 MCI onset is significantly different following application of 1 μM MK-801 between GluN1/2A (0.93 ± 0.03 s) and GluN1/2B (1.20 ± 0.05 s) receptors. The time constant of MK-801 exit from the membrane for GluN1/2A (9.27 ± 0.22 s) and GluN1/2B (8.12 ± 0.23 s) are similar. When compared with previous measurements, these data suggest that MK-801 exits the membrane much more slowly than memantine. Previous NMDAR structural modeling identified a putative path (fenestration) that memantine could use to transit from the membrane to the deep site when the channel is open. GluN2A(M630) appeared to be a path-lining residue that forms a constriction in the fenestration, an idea supported by our observation that mutating GluN2A(M630) to a tryptophan residue increased memantine MCI IC50. To test the hypothesis that MK-801 uses the same fenestration as memantine, we compared MK-801 MCI of GluN1/2A and GluN1/2A(M630W) receptors. First, we compared traditional MK-801 IC50s of GluN1/2A (14.4 ± 1.4 nM) and GluN1/2A(M630W) (10.5 ± 1.8 nM) and found no significant difference, indicating that the mutation does not modify MK-801 binding to the deep site. We then compared MK-801 MCI IC50s of GluN1/2A (0.85 ± 0.04 μM) and GluN1/2A(M630W) (0.67 ± 0.002 μM) receptors, and to our surprise, found that the mutation did not increase MK-801 MCI IC50. These results suggest MK-801 and memantine may use different fenestrations to transit from the membrane to the deep site during MCI. Our ongoing characterization of MK-801 MCI will reveal mechanisms that underlie NMDAR MCI.

ABSTRACTS

NMDAR membrane to channel inhibition by extracellular (+)-MK-801

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N-methyl-D-aspartate receptors (NMDARs) are highly calcium-permeable tetrameric glutamate receptors that are composed of two GluN1 subunits and two GluN2 (A-D) subunits and/or GluN3 (A-B) subunits. Channel block of NMDARs is a research area with broad implications for treatment of pathological conditions such as Alzheimer's disease and epilepsy. The extensively studied traditional channel block mechanism is exhibited by many compounds such as MK-801 and memantine, and occurs when charged blocker molecules from the extracellular solution enter the open channels of agonist-bound NMDARs, blocking ion flux at the deep site. Our lab recently investigated another channel block mechanism known as membrane to channel inhibition (MCI). We showed that memantine MCI occurs when uncharged memantine molecules in the extracellular solution enter the membrane, and then transit from membrane to open channels of agonist-bound NMDARs through a fenestration, blocking ion flux at the deep site. Here, we investigate properties of MK-801 MCI. We found that the MK-801 MCI IC50s for diheteromeric GluN1/2A (0.85 ± 0.04 μM) and GluN1/2B (0.67± 0.16 μM) receptors are ~60-fold greater than the IC50s for traditional channel block of GluN1/2A (14.4 ± 1.4 nM) and GluN1/2B (13.4 ± 0.4 nM) receptors. In contrast, GluN1/2C and GluN2D receptors show much weaker MCI than GluN1/2A or GluN1/2B receptors: fractional current during MCI by 1 μM MK-801 was 0.97 ± 0.27 for GluN1/2C and 0.95 ± 0.02 for GluN1/2D receptors, suggesting the MK-801 MCI IC50s for GluN1/2C and GluN1/2D receptors are far above 1 μM. We show that the time course of MK-801 MCI onset is significantly different following application of 1 μM MK-801 between GluN1/2A (0.93 ± 0.03 s) and GluN1/2B (1.20 ± 0.05 s) receptors. The time constant of MK-801 exit from the membrane for GluN1/2A (9.27 ± 0.22 s) and GluN1/2B (8.12 ± 0.23 s) are similar. When compared with previous measurements, these data suggest that MK-801 exits the membrane much more slowly than memantine. Previous NMDAR structural modeling identified a putative path (fenestration) that memantine could use to transit from the membrane to the deep site when the channel is open. GluN2A(M630) appeared to be a path-lining residue that forms a constriction in the fenestration, an idea supported by our observation that mutating GluN2A(M630) to a tryptophan residue increased memantine MCI IC50. To test the hypothesis that MK-801 uses the same fenestration as memantine, we compared MK-801 MCI of GluN1/2A and GluN1/2A(M630W) receptors. First, we compared traditional MK-801 IC50s of GluN1/2A (14.4 ± 1.4 nM) and GluN1/2A(M630W) (10.5 ± 1.8 nM) and found no significant difference, indicating that the mutation does not modify MK-801 binding to the deep site. We then compared MK-801 MCI IC50s of GluN1/2A (0.85 ± 0.04 μM) and GluN1/2A(M630W) (0.67 ± 0.002 μM) receptors, and to our surprise, found that the mutation did not increase MK-801 MCI IC50. These results suggest MK-801 and memantine may use different fenestrations to transit from the membrane to the deep site during MCI. Our ongoing characterization of MK-801 MCI will reveal mechanisms that underlie NMDAR MCI.

ABSTRACTS

Allosteric Modulation of NMDA Receptors, Probing the Link Between Potency And Efficacy Modulation.

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Classical kinetic models of NMDA receptors (NMDARs) separate the binding of agonist and channel gating into two distinct steps and predicts that the enhancement of open probability will shift the equilibrium to activated states and therefore enhance agonist EC50. There is a growing number of NMDAR PAMs now in the literature with a complex array of activities, some potentiate maximal activation, some enhance agonist potency, and some that have both effects. For example, the tetrahydroisoquinoline CIQ is a positive allosteric modulator (PAM) of GluN2C- and GluN2D-containing NMDARs, increasing the current response to maximal effective concentrations of agonist by 3-fold, however surprisingly, CIQ did not detectably alter glutamate potency at GluN2D. Whereas a CIQ analogue, (S)-EU1180-55, which also potentiates GluN2B-containing NMDARs has a perplexing array of modulation activity. (S)-EU1180-55 enhances the response of GluN2D-containing NMDARs by 3-fold but again has no detectable effect on glutamate EC50 for GluN2D-containing NMDARs. In contrast, (S)-EU1180-55 increased the response of GluN2B-containing NMDARs to maximally concentrations of agonist by 2-fold and increased glutamate potency by 1.6-fold. To explore why this PAM, and the many other NMDAR PAM that have been published over the past 10 years, showed variable actions on agonist potency in the various NMDARs, we modelled the efficacy (potentiation) and the ability to allosteric agonist coupling (enhancement of agonist potency) using modified de Castillo and Katz models of channel function that separated binding and gating steps with a variable number of steps. In very simplistic models, evaluation of the shift in EC50 as a function of open probability revealed that the PAM-induced changes in agonist potency following an increase in efficacy are only detectable when the open probability is greater than 0.1 (for modest increases in maximal potentiation 2-4 fold). For exceptionally low open probability receptors such as GluN2D, doubling open probability from 0.01 to 0.02 does not detectably alter the EC50. However, the relationship between open probability and allosteric agonist coupling becomes more complex with more accurate models of NMDAR function. Thus, understanding how PAM modulation behaves (i.e. agonist potency effects primarily or by potentiating the maximal response with minimal effects on agonist potency) is critical for modulator development and for assessing clinical utility.

ABSTRACTS

Neuronal glutamate transporters control reciprocal inhibition and gain modulation in D1 medium spiny neurons

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Understanding the function of glutamate transporters has broad implications for explaining how neurons integrate information and relay it through complex neuronal circuits. Most of what is currently known about glutamate transporters, specifically their ability to maintain glutamate homeostasis and limit glutamate diffusion away from the synaptic cleft, is based on studies of glial glutamate transporters. By contrast, little is known about the functional implications of neuronalglutamate transporters. The neuronal glutamate transporter EAAC1 is widely expressed throughout the brain, particularly in the striatum, the primary input nucleus of the basal ganglia, a region implicated with movement execution and reward. Here, we show that EAAC1 limits synaptic excitation onto a population of striatal medium spiny neurons identified for their expression of D1 dopamine receptors (D1-MSNs). In these cells, EAAC1 also contributes to strengthen lateral inhibition from other D1-MSNs. Together, these effects contribute to reduce the gain of the input-output relationship and increase the offset at increasing levels of synaptic inhibition in D1-MSNs. By reducing the sensitivity and dynamic range of action potential firing in D1-MSNs, EAAC1 limits the propensity of mice to rapidly switch between behaviors associated with different reward probabilities. Together, these findings shed light on some important molecular and cellular mechanisms implicated with behavior flexibility in mice.

ABSTRACTS

Regulation by intracellular calcium of native NMDA receptor inhibition by memantine

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Memantine is a drug currently approved in the US for treatment of Alzheimer's disease and vascular dementia and in clinical trials for several other neurological and psychiatric conditions. Its major mechanism of action is based on channel block of NMDA receptors (NMDARs). Previously we showed that memantine exhibits an intriguing mechanism of action: it enhances desensitization of NMDARs by stabilizing a Ca2+-dependent desensitized receptor state. Thus, memantine inhibits NMDARs more effectively under conditions that permit an increased influx and intracellular buildup of calcium, an effect shown in both expression systems and native receptors (Glasgow et al., 2017).

In the present study, we further investigated the impact of intracellular calcium concentration ([Ca2+]i) on the inhibitory effects of memantine on NMDARs. Utilizing specially designed Ca2+-buffered pipette solutions, we demonstrated that the memantine IC50 decreases as [Ca2+]i increases, a phenomenon not reproduced by Mg2+ or ketamine-mediated NMDAR inhibition. In addition, of the four diheteromeric NMDARs, only GluN1/2A receptors displayed Ca2+-dependent block by memantine. Next, we investigated [Ca2+]i-dependent memantine block in native neocortical NMDARs. Effects of memantine on NMDAR excitatory postsynaptic currents (EPSCs) were assessed in pyramidal neurons from mouse prefrontal cortical slices. NMDAR EPSCs were evoked by extracellular stimulation at the border of layer VI and white matter. NMDAR block by memantine (10 μM) was investigated under two conditions: (a) low [Ca2+]i, with 10 mM BAPTA in the intracellular solution and (b) high [Ca2+]i, with 50 mM free Ca2+ in the intracellular solution. Mg2+ (0.5 mM) and glycine (10 μM) were included in the extracellular solution. NMDAR EPSCs were isolated by addition of GABAA, AMPA and kainate receptor inhibitors. Remarkably, whereas under low [Ca2+]i conditions 10 μM memantine decreased the amplitude of the response by only 19 ± 9%, under high [Ca2+]i conditions the decrease was 42 ± 17% ($p = 0.017$). This effect was reproduced using repetitive extracellular stimulation.

While overactivation of NMDARs is pathological, basal NMDARs signaling is critical for neuronal health. Thus, as a next step, we assessed how memantine affects basal synaptic NMDAR signaling compared to ketamine at their corresponding "neuroprotective" concentrations. Cell death induced by transient exposure to NMDA was quantified by lactate dehydrogenase colorimetric assays in rat neuronal/astrocyte mixed cortical cultures. Both memantine and ketamine had a neuroprotective effect by reducing cell death, with neuroprotective EC50 of 2.98 and 1.75 mM, respectively. The effects of memantine and ketamine at their respective neuroprotective EC50 on synaptic NMDAR function then was assessed by measuring inhibition of NMDAR mini amplitude in cortical cultures. Ketamine had a stronger effect on NMDAR minis amplitude (reduction by 36 ± 8%) than memantine (reduction by 12 ± 3%) ($p < 0.001$). Relative sparing of synaptic NMDAR signaling by memantine may add to its clinical utility.

In conclusion, our study demonstrates that memantine blocks native as well as recombinant NMDARs more effectively as intracellular Ca2+ concentration increases. Our results support exploration of NMDAR Ca2+-dependent block as an important target for drug development.

ABSTRACTS

Postsynaptic nanoblocks in excitatory synapses for transcellular alignment

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The nanoscale organization of proteins within synapses is critical for maintaining and regulating synaptic transmission and plasticity. Here, we use cryogenic electron tomography to directly visualize the in situ three-dimensional architecture and supramolecular organization of transsynaptic alignment of pre-cleft-postsynaptic components in their native cellular context in both synaptosomes and synapses from rat primary cultured neurons. High-resolution electron microscopy and quantitative analyses reveal that release sites align with adhesion molecules, receptor clusters, and postsynaptic density (PSD) nanoblocks as a physical, transsynaptic, nano-alignment. Additionally, it has been determined that the PSDs contain different-sized, membrane-associated, subsynaptic protein nanoblocks. Furthermore, large nanoblocks are formed by small nanoblocks positioned close enough together. Lastly, the tomograms of synaptosomes allow us to detect the glutamate receptor-like particles by subtomogram averaging at resolutions of 24 Å and 26 Å. The results of this study provide a more comprehensive understanding of synaptic ultrastructure and demonstrate that PSD is organized into subsynaptic nanoblocks to form transcellular alignment.

ABSTRACTS

Ykt6 modulation is necessary for hippocampal plasticity

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Synaptic plasticity is one of the foundations of the current understanding of learning memory. One of the mechanisms that allow synaptic plasticity is the glutamate receptor trafficking that allows appropriate insertion and/or removal of the receptors as needed. While these receptors rely on exocytosis/endocytosis for their rapid insertion at postsynaptic terminals, the contribution of distal secretory outposts in the dendrites that has remarkably different local protein synthesis and trafficking distinct from the conventional anterograde somatic transport is still being explored. In this regard, we recently learned that a crucial cytosolic SNARE protein, Ykt6, may play a pivotal role in trafficking the glutamate receptors to facilitate the proper insertion of the receptors so that the synaptic transmission can occur. With Ykt6 knocked-down hippocampal neurons, we observed that GLuA1 receptor at the surface level was diminished, suggesting that the GluA1 surface expression is in part controlled by Ykt6. Furthermore, we have observed alterations in dendritic arborization and glutamatergic transmission with Ykt6 modification. Taken together, these results suggest that proper Ykt6 modulation is necessary for the correct phenotype of hippocampal synaptic plasticity to occur.

ABSTRACTS

Functional characterization of patient variants in a kainate receptor subunit gene (GRIK5) that associated with autism-spectrum disorder

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Kainate receptors (KARs), a family of ionotropic glutamate receptors, have diverse roles in the central nervous system where KAR subunits (GluK1-5) assemble to form cation-permeable channels. Mutations in the GRIK2 gene, which encode the GluK2 KAR subunit, is known to underlie profound neurodevelopmental disorders. The GRIK5 gene encodes the GluK5 protein, which acts as a component subunit within heteromeric KARs that modulate synaptic activity and play roles in various physiological processes in the brain. We recently identified 10 individuals diagnosed with autism spectrum disorder (ASD) harboring potentially pathogenic missense GRIK5 variants. Here we characterized the whole-cell electrophysiological properties of recombinant GluK5-containing KARs mutated to contain patient variants.. Two mutants (N638T and T636S) domain demonstrated gain of function. These residues occur in a highly conserved region (SYTANLAAF) of the M3 pore-forming domain that is shared among iGluRs. We did not detect functional differences in other GluK5 variants, suggesting that disruption of biogenesis or protein-protein interactions might contribute to aberrant function in native neurons. Our findings reveal that human pathogenic GRIK5 variants can modify channel function and may be causative for ASDs. These findings provide insights into the important role of KARs in the early nervous system development and highlight the need for further exploration in native neurons.

ABSTRACTS

Notch receptor-ligand binding facilitates extracellular vesicle-mediated trans neuronal communication.

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Neuron-to-neuron communication has historically been thought to be mediated by neurotransmitters and electrical signals. However, extracellular vesicles (EVs) are emerging as facilitators of intercellular communication in the central nervous system. Despite this, some skepticism regarding the importance of neuron derived EVs has persisted since many aspects of their biology remain elusive. The goal of this research is to investigate how synaptic activity modulates the release of EVs from neurons, to characterize their protein cargo, to determine their receptors, and to study their function.

We examined N-methyl-d-aspartate (NMDA) receptor mediated synaptic strengthening using a glycine-based chemical stimulation paradigm and electrophysiology to confirm enhanced synaptic transmission. Cultured rodent neurons and knock out mice were used as model systems. EVs were isolated using size exclusion chromatography and analyzed with negative staining electron microscopy, antibodies, and mass spectrometry. Individual EV particles were characterized using antibody chips with interferometric and fluorescent microscopy.

We found that activation of synaptic NMDA receptors led to phosphorylation of the ESCRT and membrane scission factor, Alix, at Serine 717 (S717) by protein kinase A, which facilitated neuronal EV release from dendritic spines. Unexpectedly, biochemical analysis revealed high levels of Notch1 and Notch2 proteins in activity-induced EVs. Deletion of the Pdcd6ip gene, which codes for the Alix protein, abolished synaptic activity-induced EV release. Overexpression of wild type Alix or Alix S717D but not Alix S717A restored synaptic activity-induced EV release in Pdcd6ip-/- neurons. Furthermore, we found that synaptic activity-induced EVs were selectively bound and internalized by neurons through surfaced-expressed Notch ligands such as Jag1. In the recipient neurons, Notch became activated and translocated into the nucleus. Finally, Conditional Alix knock out mice have significantly reduced Notch signaling activation in adult hippocampal CA1 and CA3 regions and reduced excitatory synaptic protein expression. Our results indicate that Notch ligand-receptor binding specifies cell specific targeting of EVs in the CNS.

ABSTRACTS

Neuron-specific protein expression at striatal excitatory synapses

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Combinatorial expression of postsynaptic proteins underlies synapse diversity within and between neuron types. Thus, characterization of neuron-type-specific postsynaptic proteomes is key to obtaining a deeper understanding of discrete synaptic properties and how selective dysfunction manifests in synaptopathies. To overcome the limitations associated with bulk measures of synaptic protein abundance, we developed a biotin proximity protein tagging probe to characterize neuron-type-specific postsynaptic proteomes in vivo. We found Shank3 protein isoforms are differentially expressed by direct and indirect pathway spiny projection neurons (dSPNs and iSPNs). Studies in mice lacking Shank3 gene exons 13-16 (Shank3B-/-) revealed a robust postsynaptic proteome alteration and dendritic spine impairment in iSPNs. Dissimilar Shank3 proteoforms were expressed in Shank3B-/- iSPNs and dSPNs. Overexpression of Shank3E in Shank3B-/- iSPNs significantly increased dendritic spine density and size. We report unexpected cell-type specific synaptic protein isoform expression which could play a key causal role in specifying synapse diversity and selective synapse dysfunction in synaptopathies.

ABSTRACTS

MOTOR PHENOTYPE OF A MOUSE MODEL OF A HUMAN KAINATE RECEPTOR GENETIC DISORDER

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Deleterious mutations in ionotropic glutamate receptor genes are causative for numerous non-syndromic neurodevelopmental disorders (NDDs). Individuals with NDDs caused by a point mutation in the glutamate receptor ionotropic, kainate subunit 2 (GRIK2 c.1969 G>A, GluK2 p.A657T) exhibit imbalance and uncoordinated/ataxic gait. Kainate receptors are expressed throughout the mammalian CNS and play distinctive roles in mediating cellular excitability, synaptic transmission, and synaptic development. We are working to identify the mechanistic basis for this impaired motor control using a mouse model – GluK2(A657T) – with an analogous G>A mutation in Grik2. We first tested the hypothesis that GluK2(A657T) animals display motor deficits analogous to those observed in the human disorder.

We probed GluK2(A657T) heterozygous mice and their wild type (WT) littermates of both sexes for ataxia and motor deficits using the Guyenet ataxia phenotyping system along with open field, balance beam, and rotarod testing. Guyenet tests for ledge balance, kyphosis (spine curvature), and hindlimb clasping were scored on a scale of 0-3 of increasing severity to obtain a composite ataxia score. The composite score on the three tests was significantly higher in the GluK2(A657T) (n=11) compared to WTs (n=15) (Glu2(A657T): 1.7 ± 0.5; WT: 0.4 ± 0.4, unpaired two-tailed t-test, $p<0.0001$).

In 10-minute open field tests, GluK2(A657T) mice traveled less (849 ± 314cm, n=12) compared to WT (1122 ± 293 cm, n=16) (unpaired two-tailed t-test, p= 0.026). GluK2(A657T) mice (n=12, WT, n=17) did not demonstrate deficits in balance beam performance (paw slips: Glu2(A657T): 0.41 ± 0.47; WT: 0.94 ± 1.10, Mann-Whitney test, p=0.1999, latency to cross: Glu2(A657T): 4.8 ± 0.89s; WT: 4.2 ± 0.67s, unpaired two-tailed t-test, p=0.0789), but did display an increase kyphosis scored on a binary of 0: absent, 1: present and tail posture scored from 0-2 increasing with rigidity (kyphosis: Glu2(A657T): 0.75 ± 0.399; WT: 0.08 ± 0.26, Mann-Whitney test, $p<0.0001$, tail posture: Glu2(A657T): 1.9 ± 0.22; WT: 0.71 ± 0.61, unpaired two-tailed t-test, $p<0.0001$). There were no significant differences found in rotarod performance. We plan to supplement these data with more rigorous characterization of gait and balance beam traversal differences using a machine learning tracking analysis.

Our results confirm that heterozygous GluK2(A657T) mice display postural alterations and an ataxic phenotype, making them suitable models for the analogous human disorder motor deficits. We anticipate this model will yield insight into the role of kainate receptors in genetic NDDs and guide the development of therapeutics.

This study is funded by NINDS (R01NS105502) and GMCMD-T32 (5T32NS041234-20).

ABSTRACTS

Enhancement of hippocampal interneuron excitability by a novel NMDA receptor positive allosteric modulator

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The N-methyl-D-aspartate (NMDA) receptors are best known for their role mediating a slow component of excitatory synaptic transmission, and have been shown to play important roles in multiple brain functions, as well as in various neurological conditions. Here we describe the actions of a novel positive allosteric modulator (PAM), EU1622-A, that has multiple effects on NMDARs, including enhancement of the maximal response, prolongation of response time course, enhancement of agonist potency, and reduction single channel conductance. EU1622-A is a pan- PAM that potentiates NMDAR function with sub-micromolar potency, with strongest effects on GluN2C- and GluN2D-containing NMDARs. We evaluated effects of this PAM on both CA1 pyramidal cells and CA1 interneurons in whole-cell current clamp and voltage clamp recordings in acutely prepared P17-P22 hippocampal slices. Although this PAM potentiates evoked-NMDAR EPSCs on both CA1 pyramidal cell and interneurons, we show that the PAM enhances interneuron excitability to a greater extent than for principal cells. This appears to result from depolarization of interneurons, increased spike firing, and enhanced NMDAR-mediated current charge transfer in interneurons. By contrast, 1622-A did not detectably depolarize CA1 pyramidal cells, but did have modest effects when bicuculline was used to block GABA receptor signaling that might arise from enhanced interneuron activity within the slice. This data supports the idea that the NMDAR PAM 1622-A can enhance interneuron function with modest effects on the CA1 pyramidal cells.

Key words: GluN2C, GluN2D, NMDA receptor, CA1, pyramidal cell, interneuron.

ABSTRACTS

Modulation of GluA2 AMPA-subtype iGluRs by auxiliary subunits TARP gamma5 and cornichon-2

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Fast excitatory neurotransmission in the mammalian central nervous system is mediated in large part by AMPA-subtype ionotropic glutamate receptors (iGluRs) that are activated by agonist glutamate. AMPA receptor (AMPAR) function is regulated and finely tuned by auxiliary subunits, membrane proteins that associate with the transmembrane domain region of the receptor. Here we present cryo-EM structures of GluA2 AMPAR complexes with inhibitory g5 and potentiating cornichon-2 (CNIH2) auxiliary subunits. CNIH2 appears to destabilize the desensitized state of the complex by bringing the upper lobes in ligand-binding domain dimers closer to one another. At the same time, CNIH2 stabilizes the binding of polyamine spermidine to the selectivity filter of the closed ion channel. Nevertheless, CNIH2 and, to a lesser extent, g5 reduce the polyamine block of the open channel and reduce the potency of the antiepileptic drug perampanel that inhibits the synaptic complex allosterically by binding to sites in the ion channel extracellular collar. These findings illustrate the fine-tuning of AMPAR synaptic complexes, which represent neuromodulatory and therapeutic targets, in an auxiliary subunit-dependent manner.

ABSTRACTS

GluN2-specific NMDA receptor modulation in different neuronal populations using GluN1 glycine site agonists

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N-methyl-D-aspartate (NMDA)-type receptors are ligand-gated ion channels that mediate excitatory synaptic transmission throughout the central nervous system (CNS). Due to their role in the pathophysiology of numerous CNS disorders, pharmacological modulation of NMDA receptor function is being actively pursued for therapeutical intervention in several diseases. Most native NMDA receptors are composed of two glycine/D-serine binding GluN1 and two glutamate binding GluN2 subunits and require agonist binding to all four subunits for activation. GluN1 is encoded from a single gene, whereas four different genes produce the GluN2 subunits (GluN2A-D) that endow NMDA receptor subtypes with distinct functional properties and variation in expression profile among neuronal cell types. Modulation of NMDA receptors subtypes with different GluN2 subunit composition through glycine site agonists has not been fully explored, presumably because the glycine binding GluN1 subunit is conserved among the receptor subtypes. Here, we characterize novel glycine site agonists, AICP, UA3_10, and UA3_2_3 at recombinant diheteromeric (GluN1/2A-D) and triheteromeric (GluN1/2A/2B, GluN1/2B/2D and GluN1/2A/2C) NMDA receptors and demonstrate that these glycine site agonists display variation in activity with unique GluN2 subunit-dependent differences in agonist efficacy. Furthermore, we demonstrate that the agonists can modulate responses from native NMDA receptors by replacing glycine/D-serine binding to GluN1 and thereby change agonist efficacy in a GluN2-specific manner. Evaluation of the agonists in different subtypes of neurons suggest that NMDA receptor modulation can be predicted from the expression of GluN2 subunits in these neurons and the observed agonist efficacy at the respective recombinant triheteromeric NMDA receptor subtypes. Taken together, our evaluation of newly identified compounds suggests that the GluN1 glycine binding site can be targeted for modulation of specific NMDA receptor subtypes in distinct brain regions and neuronal circuits. By leveraging these differential effects, it may be possible to develop novel pharmacological agents for therapeutic intervention of NMDA receptor-related disorders.

ABSTRACTS

Early developmental deletion of forebrain Ank2 causes seizure-related phenotypes by reshaping the synaptic proteome

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Rare genetic variants in ANK2, which encodes ankyrin-B, are associated with neurodevelopmental disorders (NDDs), however, their pathogenesis is poorly understood. We find that mice with prenatal deletion in cortical excitatory neurons and oligodendrocytes (Ank2-/-:Emx1-Cre), but not with adolescent deletion in forebrain excitatory neurons (Ank2-/-:CaMKIIα-Cre), display severe spontaneous seizures, increased mortality, hyperactivity, and social deficits. Calcium imaging of cortical slices from Ank2-/-:Emx1-Cre mice shows increased neuronal calcium event amplitude and frequency, along with network hyperexcitability and hypersynchrony. Quantitative proteomic analysis of cortical synaptic membranes reveals upregulation of dendritic spine plasticity-regulatory proteins and downregulation of intermediate filaments. Characterization of the ankyrin-B interactome identifies interactors associated with autism and epilepsy risk factors and synaptic proteins. The AMPA receptor antagonist, perampanel, restores cortical neuronal activity and partially rescues survival in Ank2-/-:Emx1-Cre mice. Our findings suggest that synaptic proteome alterations resulting from Ank2 deletion impair neuronal activity and synchrony, leading to NDDs-related behavioral impairments.

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