and BK channels are proximally clustered and functionally coupled in the cultured neurons by using super-resolution imaging and electrophysiological recording techniques. To investigate the Ca_Vs and calcium-activated ion channels' clustering patterns at the super-resolution level, we firstly screened antibodies specific to the different channels. We have examined the expression patterns of the various combinations of the voltage-gated calcium channels ($Ca_V1.3$, $Ca_V2.1$, $Ca_V2.2$, $Ca_V2.3$, $Ca_V3.2$, and $Ca_V3.3$) and calcium-activated ion channels (Bestrophin-1, Ano-1, BK, and SK channels) in the hippocampal neurons. For this screening purpose, expression patterns were examined under the confocal microscope and structured illumination microscopy (SIM) imaging. From the confocal and SIM images, we found that some ion channel combinations can be assigned as "co-localization" and others as "non-colocalization". Further analysis will perform to obtain quantitative results. We are currently planning to visualize ion channels' clustering patterns in the cortical pyramidal neurons by using SIM and their functional consequences by using patch-clamp recordings.

This work was supported by the KBRI Basic Research Program funded by the Ministry of Science and ICT (19-BR-01-02 to H.-H.L.) and by the NRF Brain Research Program funded by the Ministry of Science and ICT (2017M3C7A 1048086 to H.-H.L.).

https://doi.org/10.1016/j.ibror.2019.07.1261

P26.03

Synaptic regulation of intrinsic excitability of hippocampal CA3 pyramidal cells: Ex-vivo study

Kisang Eom, Dong-Gu Lee, Won-Kyung Ho, Sukho Lee*

Seoul National University College of Medicine, Seoul, Republic of Korea

Dynamic regulation of intrinsic excitability has been implicated in memory allocation in the hippocampus. Previously we reported that long-term potentiation of intrinsic excitability (LTP-IE) in hippocampal CA3 pyramidal cells (CA3-PC) is preferentially regulated by mossy fiber (MF) synaptic inputs by Ca2+-dependent activation of protein tyrosine kinase (PTK) and Zn2+-induced inactivation of protein tyrosine phosphatase (PTP). To test if LTP-IE occurs in vivo, we investigate LTP-IE in CA3-PCs expressing c-fos using AAV expressing Robust Activity Marking (RAM) system. One hour after being exposed to novel context, c-fos(+) CA3-PCs exhibited lower input conductance (Gin; c.a. 4 nS) and no further LTP-IE. This high excitability state was restored to the basal level (about 6 nS) over 6 h during which the rats were in the sleep cycle, but not in the awake cycle. Cellular mechanisms that erase the LTP-IE of CA3-PCs (depotentiation) have not been investigated. Consistent with our previous finding that higher cytosolic [Ca2+] is required for activation of PTP than PTK, we found that high frequency somatic firing or strong stimulation of recurrent (A/C) synaptic inputs that evokes excessive Ca2+ transient in CA3-PCs depotentiated the LTP-IE of CA3-PCs evoked by MF synaptic inputs. Our results suggest that learning causes temporal increase in the excitability of CA3-PCs, and that strong activation of A/C fibers that occurs during sleep cycle, probably sharp wave-ripple, may be responsible for the depotentiation of LTP-IE in CA3-PCs.

https://doi.org/10.1016/j.ibror.2019.07.1262

P26.04

Specific motor learning memory traces are affected by SK2 channels-dependent modulation of excitability in cerebellar Purkinje cells

Giorgio Grasselli[1], Henk-Jan Boele[2], Heather K. Titley[1], Nora Bradford[1], Lisa Van Beers[2], Lindsey Jay[1], Chris I. de Zeeuw[2], Martijn Schonewille[2], Christian Hansel[1,*]

[1] *Department of Neurobiology, University of Chicago, Chicago, USA*
[2] *Department of Neuroscience, Erasmus MC, Rotterdam, Netherlands*

Neurons store information content by changing synaptic input weights (i.e., by synaptic plasticity: LTP and LTD). However, accumulating evidence suggests that in addition they use their ability to adjust membrane excitability to reach spike firing ranges that are optimal for specific behavioral learning tasks ('intrinsic plasticity'). This type of adaptation can be observed in cerebellar Purkinje cells that are spontaneously active and enhance their rate of simple spike firing in gain upregulation of the vestibulo-ocular reflex (VOR), but reduce spike firing to enable eyelid closure in delay eyeblink conditioning (EBC). SK2-type, calcium-dependent K^+ conductances are critically involved in the regulation of excitability as they contribute to the afterhyperpolarization (AHP) that is initiated by spike bursts, and are downregulated in activity-dependent plasticity of Purkinje cell excitability ('intrinsic plasticity'). To study the relevance of excitability modulation in cerebellar motor learning, we therefore generated and tested mice with a Purkinje cell-specific SK2 knockout (L7-SK2). Deletion of SK2 channels enhanced Purkinje cell excitability and prevented intrinsic plasticity, while synaptic plasticity remained intact. L7-SK2 mice showed EBC impairment and alterations in locomotion. These findings suggest that cell-autonomous adjustment of membrane excitability to the demands of specific motor behaviors provide a distinct component of cellular learning, in addition to, and separable from, changes in synaptic strength.

https://doi.org/10.1016/j.ibror.2019.07.1263

P26.05

Lactate enhances NMDA receptor responses via two distinct mechanisms

Gabriel Herrera-Lopez[1], Fouad Lemtiri-Chlieh[3], Hanan Mahmood[2], Lorene Mottier[2], Hubert Fiumelli[2], Pierre J. Magistretti[2,*]

[1] *Cinvestav-IPN, Mexico City, Mexico*
[2] *BESE, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*
[3] *Department of Neuroscience, UConn Health, Farmington, USA*

Lactate is known as an energy substrate for neurons. It is also emerging as a trophic signaling molecule regulating higher brain functions such as learning and memory. Lactate was shown to potentiate NMDA receptor signaling and regulate gene expression related to synaptic plasticity.

Using patch-clamp recordings in cultured cortical neurons, we found that lactate enhanced amplitude and inactivation time-constant (tau) of NMDA receptor currents (I_{NMDA}) evoked by brief applications of glutamate and glycine. The effect on the amplitude

was prevented by pre-treating neurons with a MCTs blocker or by including CaMKII inhibitors in the patch pipette, whereas the effect on tau was not.

To characterize the CaMKII dependence, we recorded from two cell lines expressing functional NMDARs: CaMKIIα-expressing HEK293 cells and control HEK293 cells. Lactate's effect on I_{NMDA} amplitude was only seen in CaMKIIα-expressing cells. This enhancement was dependent on MCT transport since its blockade prevented this effect. In contrast, the effect on tau was always seen.

We next interfered with the intracellular conversion of lactate into pyruvate using pharmacological LDH inhibitors and with CaMKIIα binding to NMDA receptors by expressing mutant NMDA receptor subunits. Results showed that blocking LDH or expressing GluN2B mutants prevented the increase in I_{NMDA} amplitude induced by lactate. Loading HEK293 cells with NADH also occluded the effect of lactate on I_{NMDA} amplitude. In contrast, none of these treatments affected the change observed in tau.

Altogether, these results indicate that lactate generates two distinct effects on NMDAR responses. (1) An enhancement of the I_{NMDA} amplitude that requires intracellular uptake of lactate, an increase in the internal NADH/NAD+ ratio through LDH activity, and interaction with CaMKIIα. (2) A change in the inactivation kinetics that is independent of lactate import possibly by directly acting on a yet unidentified extracellular target.

https://doi.org/10.1016/j.ibror.2019.07.1264

P26.06

The relation of functional states of dendritic spines with contents of micro-RNA

Hyun Jin Kim[1], Ik-Bum Park[2], Ki-Bong Sung[1], Ha-Min Lee[1], Jung Hyun Pyo[1], Soyeon Yun[1], Joung-Hun Kim[1,*]

[1] *Department of Life Sciences, POSTECH, Pohang, Republic of Korea*
[2] *Division of Integrative Biosciences and Biotechnology, and Research Institute of Industrial Science and Technology, POSTECH, Pohang, Republic of Korea*

Protein expression is essential for synaptic plasticity including structural changes and these expressions controlled by diverse molecular mechanisms. microRNAs (miRNAs), small non-coding RNA, have emerged as critical regulators for synapse development and plasticity through their control of gene expression. However, we still do not know even the distribution and role of miRNAs at dendritic spines which significant loci of excitatory inputs and synaptic plasticity accompanying structural changes. Brain-specific miR-134s likely regulate the skeletal maturation of dendritic protrusions, but their subcellular distributions and functional impacts have rarely been assessed. Here, we adapted atomic force microscopy (AFM) to visualize in situ miR-134s, which indicated that they are mainly distributed at nearby dendritic shafts and necks of spines. The abundance of miR-134s varied between morphologically and functionally distinct dendritic protrusions, and their amounts were inversely correlated with their postulated maturation stages. In addition, spines exhibited reduced contents of miR-134s when selectively stimulated with beads containing a brain-derived neurotrophic factor (BDNF) or activated loci of protrusions. Altogether, in situ visualizations of miRNAs provided unprecedented insights into the "inverse synaptic-tagging" roles of miR-134s that are selective to inactive/irrelevant synapses and potentially a molecular means for modifying synaptic connectivity via structural alteration.

https://doi.org/10.1016/j.ibror.2019.07.1265

P26.07

The distinct structural and functional heterogeneity of dopamine synapses in the brain

Hyun-Jin Kim, Byungjae Hwang, Byeong Eun Lee, Jieun Lee, Youngeun Lee, Jung-Hoon Park, Jae-Ick Kim*

UNIST, Ulsan, Republic of Korea

Dopamine system is a group of midbrain nuclei and their axonal projections across the entire brain. This system is essential for governing diverse neural functions, including voluntary movement, reward, motivation, mood, attention, learning, and memory. Although the role of neurotransmitter dopamine and dopamine neurons have been intensively studied so far, the precise structure and function of dopaminergic synapses remain unclear in various regions of the brain where dopaminergic axons differentially project. Here, we utilized enhanced confocal imaging and electrophysiology combined with optogenetics to dissect structural and functional heterogeneity of dopamine synapses across the brain. We found that dopaminergic synapses have regional heterogeneity in their distribution and spatial pattern. In addition, these structural features in dopamine synapses were correlated with functional aspects of dopaminergic synaptic transmission in different brain regions. Thus, the differential structure and function of dopamine synapses in distinct brain areas may contribute to the multi-faceted role of dopamine system in a variety of dopamine-related behaviors.

https://doi.org/10.1016/j.ibror.2019.07.1266

P26.08

Functional role of the C-terminal domain of Bestrophin-1, a calcium-activated chloride channel

Dong-Hyun Kim, Junmo Hwang, Hyun-Ho Lim*

Korea Brain Research Institute, Daegu, Republic of Korea

The Bestrophin-1 (BEST1), calcium-activated chloride channels, are widely expressed in a variety of tissues including the brain. Functional calcium-activated chloride channels consist of homo-pentamer of BEST1 protein symmetrically arranged around a central axis, which forms a Cl^--conduction pathway. The N-terminal region (amino acids 1–390) of BEST1 shows highly conserved amino acids among vertebrate orthologues, but the C-terminal region (amino acids 391–585) is rather distantly related. The highly conserved N-terminal region contains membrane spanning transmembrane domains as well as a calcium-binding domain, Ca^{2+}-clasp. Structural studies suggested that direct binding of Ca^{2+} to Ca^{2+}-clasp induce the conformational changes and open the BEST1 channel. However, the structural and functional roles of weakly conserved and structurally disordered C-terminal region of BEST1 channel remain poorly understood. Here, we present a clue for understanding the functional role of C-terminal region with the result of electrophysiological studies on wild-type and mutant BEST1 channels from human and mouse. Interestingly, the results suggest that the C-terminal region could functionally