www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

UNDERSTANDING THE BRAIN
&
SMART THERAPEUTIC ADVANCES

The 9th UK-KOREA Neuroscience Symposium

Johnson Auditorium,
Korea Institute of Science and Technology,
Seoul,
Korea

1-2 September 2016

UKorea
www.ukorea.ac.uk

BIS Department for Business Innovation & Skills
MRC Medical Research Council
wellcome trust
Ministry of Health and Welfare
KHIDI Korea Health Industry Development Institute
ibs 기초과학연구원 Institute for Basic Science

UKorea
www.ukorea.ac.uk

The 9th UK-KOREA Neuroscience Symposium

2016 Programme Committee

John O'Keefe
(Chair of Committee)
UCL

Kei Cho
Univ. of Bristol

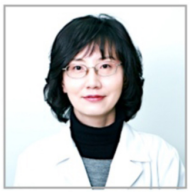
Morgan Sheng
Genentech, USA

Eunjoon Kim
IBS-KAIST

John Isaac
Wellcome Trust

Kyungjin Kim
KBRI, Korea

Seong-Gi Kim
IBS



Inhee Mook-Jung
Seoul Nat. Univ.

Hee-Sup Shin
IBS-KIST

Plenary Speaker: Professor Morgan Sheng (FRS)
Vice-President, Neuroscience, Genentech, USA

I am so glad and honoured to give a plenary talk at the 9th UK-Korea Neuroscience Symposium in Seoul.

Neuroscience truly is an important and interdisciplinary science that requires collaborations from basic research through to clinical studies. I strongly believe that the 'UKorea' Symposium facilitates the development of a unique multidisciplinary collaboration and lays the foundations for a complementary and stable neuroscience research consortium for Korea and the UK.

Through my plenary talk, I will discuss ***"Genes, Microglia and Alzheimer's Disease"***

BIS Department for Business Innovation & Skills
MRC Medical Research Council
wellcome trust
Ministry of Health and Welfare
KHIDI Korea Health Industry Development Institute
ibs 기초과학연구원 Institute for Basic Science

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

A distance-dependent gradient in presynaptic release probability along tapering dendrites

Fredrico Grillo, Guilherme Neves, Gema Vizcay, Roland Fleck and Juan Burrone

Centre for Developmental Neurobiology, King's College London

Correspondence and requests for collaboration should be addressed to ***juan.burrone@kcl.ac.uk***

Neurons receive a large number of heterogeneous synaptic inputs all along their vast dendritic trees. Although recent advances have shed light on the biased distribution of postsynaptic compartments and their role on neuronal integration, very little is known about the distribution of presynaptic boutons along dendrites. Here, we show that the structure and function of presynaptic boutons along the thin tapering basal dendrites of CA1 pyramidal neurons decreases with distance from the soma. Recordings of EPSCs measured electrophysiologically in response to trains of stimuli delivered at varying locations in the stratum oriens of acute slices showed that distal inputs facilitate more than proximal inputs, suggesting a distance-dependent change in release probability. In agreement with our functional data, we find that the size of the active zone (as well as the volume) of presynaptic terminals also decreased with distance to the soma, as measured by 3D reconstructions of dendrites obtained from serial block-face scanning electron microscopy (3 view). In parallel, we find that postsynaptic spines and their postsynaptic densities closely followed the distribution of presynaptic boutons, decreasing in size with both distance and dendrite diameter. We propose that the changes in synaptic transmission dynamics (short-term plasticity) linked to the graded distribution of release probability fine-tunes different sections of dendrite to respond to specific input frequencies. Our findings describe a novel form of distance-dependent distribution of synaptic properties that allow neurons to efficiently sample across large frequency domains.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

The N-end rule pathway in proteasomal and lysosome degradation

Yong Tae Kwon

Seoul National University

Correspondence and requests for collaboration should be addressed to ***yok5@snu.ac.uk***

The N-end rule pathway is a proteolytic system in which single N-terminal amino acids of short-lived proteins function as a class of degradation signals (degrons), called N-degrons. One of N-terminal degrons (N-degrons), arginine (Arg), can be generated by ATE1 R-transferase-mediated arginylation of N-terminal aspartate (Asp) and glutamate (Glu). The resulting Arg is recognized and directly bound by the UBR box of a family of N-recognins (UBR1, UBR2, UBR4, and UBR5) which promote ubiquitination and proteasomal degradation of N-degron-bearing substrates. Since its inception in 1986, the N-end rule pathway has been characterized as a subset of the ubiquitin-proteasome system (UPS), which mediates selective proteolysis of regulatory cytosolic and nuclear proteins. In this lecture, I introduce known functions and mechanisms of the N-end rule pathway. In addition, I show our recent discoveries that the pathway regulates the metabolic stability of endoplasmic reticulum (ER)-residing proteins through stress-induced N-terminal arginylation, that the N-terminally arginylated ER protein BiP/GRP78 migrates to the cytosol and induces an allosteric conformational activation of the autophagic adaptor p62/SQTSM through its N-end rule binding to p62, and that the ligand-bound p62, in turn, activates autophagy. Finally, I show recent data indicating that p62 acts as an autophagic N-recognin for a large number of cytosolic proteins' C-terminal proteolytic fragments bearing N-terminal Arg. Our results suggest that the N-terminal Arg residue acts as a ligand that links various stresses, such as proteasomal inhibition, to autophagic induction through its binding to p62 and possibly other N-recognins.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

From experience to plasticity via MSK1

Bruno G. Frenguelli

School of Life Sciences,
University of Warwick, Coventry,
UK

Correspondence and requests for collaboration should be addressed to ***b.g.frenguelli@warwick.ac.uk***

In the 1940s Donald Hebb showed that rats kept in his home as pets performed better on cognitive tests than rats raised in standard laboratory cages. The beneficial effects of such experimental environmental enrichment have since been shown to extend to neuronal structure, synaptic function, plasticity, and indeed to impact upon a number of cognitive domains. However, the molecular mechanisms underpinning these effects have remained elusive, but seem to involve, at least in part, BDNF. We have previously shown that mitogen- and stress-activated kinase 1 (MSK1), an enzyme downstream of BDNF TrkB receptors, is necessary to translate enrichment into enduring changes in spine density and synaptic transmission in hippocampal area CA1. More recently, we have studied the influence of enrichment on synaptic plasticity and cognition and their dependence on MSK1. We found that whilst both wild-type mice and MSK1 kinase-dead (KD) mutant mice responded to enrichment by performing better on tests of spatial working and reference memory compared to standard-housed mice, cognitive flexibility and performance in a water maze probe trial were impaired in both the enriched and standard-housed mutants. In addition, LTP in area CA1 was selectively enhanced by enrichment in wild-type mice but not MSK1 KD mice. These data suggest that MSK1 is necessary for the full expression of the benefits of environmental enrichment. Strategies targeting MSK1 may thus be of value in the many congenital, acquired and age-related neurological conditions improved by exposure to an enriched environment.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Synaptic function of Intellectual disability related factor, CRBN

Se-Young Choi

Seoul National University

Correspondence and requests for collaboration should be addressed to ***sychoi@snu.ac.uk***

Intellectual disability is one of the most common cognitive disorders; however, its molecular mechanisms remain poorly understood. Mutations in the cereblon (CRBN) gene cause intellectual disability in humans. We studied the role of CRBN in synaptic structure and function. Crbn knockout animals showed an obvious deficit in spatial memory. However, the brain and dendrite structures and synaptic plasticity were fairly normal in Crbn knockout animals. Presynaptic excitatory, but not inhibitory, neurotransmitter release was impaired in Crbn knockout animals, mostly due to altered activity of the BK channel, a large-conductance Ca2+-activated K+ channel. Treatment with thalidomide, a teratogenic drug that inhibits CRBN, mimicked the synaptic and cognitive phenotypes of Crbn knockout animals, which were rescued by a BK channel inhibitor. Our results provide a potential explanation for the possible involvement of BK channel abnormality in intellectual disability, as well as in the presynaptic and cognitive impairments caused by CRBN mutations.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

In vivo imaging of mitochondrial transport in the rTg4510 mouse model of tauopathy

*J. D. Johnson[1], R. Lees[1], D. Ursu[2], J. S. Jackson[2], M. J. O'Neil[2], M. C. Ashby[1]

[1] School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK
[2]Eli Lilly and Co., Windlesham, UK

Correspondence and requests for collaboration should be addressed to ***jj13331@bristol.ac.uk***

The pathological accumulation of the microtubule stabilizing protein tau is associated with a number of diseases, including Alzheimer's Disease (AD). Tau is hyperphosphorylated, becoming aggregative, and spreads throughout the brain forming intracellular neurofibrillary tangles. Uncovering the leading functional elements that underpin the gross synapse loss and cell death observed in AD is crucial in slowing down or reversing this disease. Mitochondria are critical for neuronal well-being and maintenance of synaptic function. Mitochondrial dysfunction can lead to decreases in ATP production, increases in damaging Reactive Oxygen Species, disruption in calcium buffering and apoptosis control. The exact changes and the time course of mitochondrial dysfunction and its relationship to synapse loss in AD patients and animal models, is unknown. Here, the rTg4510 mouse, which expresses a repressible form of human tau containing the P301L mutation linked to human frontotemporal dementia with parkinsonism-17 (FTDP-17), is used to assess mitochondrial changes associated with onset of neurodegenerative pathology. Longitudinal in vivo two-photon microscopy is used to investigate changes in mitochondrial dynamics along the time course of pathological progression. rTg4510 mice and control littermates were transduced with an AAV driving expression of mitochondrial-targeted fluorescent protein in a subset of excitatory cortical neurons. The distribution and motility of axonal mitochondria was imaged in head-fixed, anaesthetized subjects. Mitochondrial location, morphology and speed of movement were measured is the same axons repeatedly over disease progression. These insights will define when and how the axonal mitochondria, which are crucial for presynaptic function, are affected by pathological malfunction of tau.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

A spontaneous mutation of *Nrxn3* in 129S1/SvImJ enhances empathic fear behavior

Arie Kim

University of Science and Technology

Empathy is an important emotional process that involves the ability to recognize and share emotions with others. In observational fear learning (OFL) task, a mouse is conditioned for fear to the context where it observes a conspecific demonstrator receiving aversive stimuli. We have recently reported that empathic fear response is highly variable among 11 inbred mouse strains, and innate differences in conditioned fear, anxiety, locomotor activity, sociability and preference for social novelty are not significantly correlated with OFL among those strains. However, the genetic factors underlying variability in empathic fear remain to be determined. Intriguingly, we have found that mice of the 129S1/SvImJ (129S1) strain, exhibit a marked increase in OFL, as compared to another 129S substrain, 129S4/SvJaeJ (129S4). Through genetic and molecular analyses, a nonsynonymous mutation of arginine to tryptophan (R498W) in Neurexin 3 (Nrxn3) was identified as a causative variant. This mutation occurs at a residue that is well conserved among mammalian species and is predicted to be deleterious to the protein by in silico databases. We have further confirmed that knock-in mice with the R498W mutation by the CRISPR/Cas9 system show increased OFL. Taken together, we propose that Nrxn3 is an important regulator in neural circuits of OFL. These works also demonstrate the validity of the approach to utilize substrains to identify genes and alleles regulating social behaviors.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Genetically encoded voltage indicator imaging of GABAergic cell classes in the mouse brain

Song, C. [1], Power, E. [2], Potapov, K. [2], Piscopo, D. M. [3], Niell, C. [3], Empson, R. M. [2] and Knopfel, T. [1]

[1]Division of Brain Sciences, Department of Medicine, Imperial College London, UK
[2]Department of Physiology, University of Otago, New Zealand
[3]Department of Biology, University of Oregon, United States

Correspondence and requests for collaboration should be addressed to ***chenchen.song@imperial.ac.uk***

GABAergic neurons contribute to diverse neurophysiological and behavioural functions despite constituting only approximately 20-30% of the neuronal populations in the brain. The functional roles of GABAergic neurons in shaping cerebral and cerebellar circuit dynamics had been technically difficult to investigate using traditional imaging approaches. The fast spiking properties and subthreshold activity of cortical GABAergic neuron populations and constant, rhythmic firing of cerebellar Purkinje neurons means that satisfactorily detecting their behaviour using the popular calcium imaging approach is challenging. Therefore, directly monitoring the voltage activity of these cells is necessary. Furthermore, in the cortex and cerebellum, classical voltage-sensitive dye imaging is dominated by activity from the glutamatergic neuronal population. Genetically encoded voltage indicators (GEVIs) targeted to GABAergic cell population offer a solution to this problem.

We expressed two GEVIs (VSFP Butterfly 1.2 and chimeric VSFP Butterfly) in mice to successfully use optical imaging to monitor GABAergic interneuron circuit dynamics in the brain. We first establish transgenic approaches to achieve controlled targeted indicator expression in defined GABAergic neuronal populations. Two strategies created specific expression of VSFP Butterfly 1.2 in cerebellar Purkinje cells (VGat-Cre; Pcp2-tTA; Ai78), and of chimeric VSFP Butterfly in GABAergic neurons across cortical layers (VGat-Cre; ztTA; chiVSFP).

We performed *ex vivo* and *in vivo* imaging respectively from these mice. *Ex vivo* imaging from sagittal cerebellar slices demonstrated robust parallel fibre and climbing fibre synaptic responses specifically from cerebellar Purkinje neuron dendrites. *In vivo* voltage imaging of cortical GABAergic neuronal population demonstrated distinct circuit dynamics during pentobarbiturate-induced slow wave sleep.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Differential patterns of CS-evoked neural activation dependent on training amount: Implication for a shift in nature of associatively-activated event representation over the course of conditioning

Hea-jin Kim[1,2], Hae-Young Koh[1,2] *

[1] Center for Neuroscience, Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul
[2] Department of Neuroscience, Korea University of Science and Technology (UST), Daejeon

In associative learning, a conditioned stimulus (CS) drives to activate an internal representation of the unconditioned stimulus (US) with which it is paired. This CS-evoked representation of a US can substitute for the actual US itself in the acquisition of an aversion to that US, which is called representation-mediated taste aversion (RMTA). In our previous study, we have shown that in mice, RMTA occurs only transiently in the early stage of the initial CS (odor) → US (sugar) conditioning, and CS → nausea pairing can no longer establish an aversion to the US later with extended initial conditioning. It is suggested that the nature of CS-evoked US representation changes over the course of training, in that CS-evoked US representation contains the sensory component available for RMTA learning with a minimal initial training, but no longer does with an extended initial training. In this study, we report an evidence for this change of nature of CS-evoked US representation by showing differential patterns of CS-evoked neural activation in brain regions (e.g. insular cortex, IC; nucleus accumbens, NAcc, etc.) with expression level of c-Fos protein. In the minimal training condition, rewarded odor (+Odor) induced significantly higher level of c-Fos expression than unrewarded odor (–Odor) in IC, which includes gustatory cortex, and also in NAcc, which is involved in motivation processing. In the extended training condition, +Odor induced significantly higher level of c-Fos expression than –Odor in NAcc, but not in IC. These results suggest that CS-evoked US representation contains both the perceptual and motivational components with a minimal training, and becomes less perceptual with an extended training. This study would contribute greatly to better understanding of associatively-activated event representation in the field of learning theory.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Mechanisms and consequences of neuronal protein SUMOylation in health and disease

Jeremy Henley

School of Biochemistry, Centre of Synaptic Plasticity, University of Bristol, UK

Correspondence and requests for collaboration should be addressed to ***j.m.henley@bristol.ac.uk***

Post-translational protein modifications are integral components of signalling cascades that enable cells to efficiently, rapidly and reversibly respond to extracellular stimuli. These modifications play crucial roles in the CNS where the communication between neurons is particularly complex. Protein SUMOylation is a critically important post-translational protein modification that participates in the regulation of nearly all aspects of cellular physiology. SUMO modification is a highly dynamic and transient process that, depending on the target protein, either enhances or hinders protein-protein interactions to alter substrate localisation, function and/or stability. Hundreds of different proteins are SUMO substrates and the mechanisms and protein targets of SUMOylation are activity-dependently controlled and highly sensitive to cell stress. In neurons, SUMOylation is involved in processes ranging from neuronal differentiation and synapse formation to regulation of synaptic transmission and mitochondrial function. We are particularly interested in how SUMOylation of proteins outside the nucleus impacts on:

- Synaptic function and plasticity
- Responses to cell stress and neuroprotective mechanisms

More specifically, we study how SUMOylation of:

- presynaptic proteins controls neurotransmitter release
- postsynaptic proteins alters receptor trafficking and synaptic responsiveness
- mitochondrial proteins regulates mitochondrial morphology, function and apoptotic signalling

Unsurprisingly given the core pathways that are regulated, dysfunction of protein SUMOylation is implicated in a many different diseases including epilepsy, autism and Alzheimer's disease. I will outline the SUMO system and discuss recent discoveries that illustrate some of the roles of SUMOylation in healthy and diseased neurons.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Determination of synapse formation sites through guidance cues during neuronal wiring

Won-Jong Oh

KBRI

Correspondence and requests for collaboration should be addressed to ***ohwj@kbri.re.kr***

Precise synaptic connections are essential for the proper formation of functional circuits and the generation of complex behavior and cognitive function. During development, axons first navigate long distances to reach their target regions and then select an appropriate synaptic partner as well as a specific location on this postsynaptic cell to establish a synapse. However, very little is currently known about the precise mechanisms underlying synaptogenesis in complicated neuronal circuit of the mammalian brain. We discovered that in vivo interaction between traditional axon repulsive cue, Semaphorin 3E (Sema3E), and its receptor, Plexin-D1, determines synaptic specificity in thalamo-striatal circuits of basal ganglia system. Sema3E is secreted by presynaptic thalamo-striatal projection axons while Plexin-D1 is selectively expressed by only one subtype of postsynaptic neurons, the direct pathway medium spiny neurons (MSNs) in striatum. Using a combination of mouse genetics, electrophysiology, imaging and behavioral approaches, we uncovered that Sema3E-Plexin-D1 signaling normally restricts the number of thalamo-striatal synapses formed onto direct pathway MSNs. Moreover, using in vitro co-culture system, we found that distinct subcellular localization of Plexin-D1 in dendrites determines the sites of thalamo-striatal synapse formation. Together, this pre- and post-synaptic coding by Sema3E and Plexin-D1 serves as molecular recognition system to control specific synaptic connections within the complex circuitry of the brain.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Diversity of homeostatic and Hebbian plasticity properties in cortical neurones

Kevin Fox[1], Stanislaw Glazewski[2], Stuart Greenhill[3]

[1]School of Biosciences, Cardiff University, Cardiff UK
[2]School of Life Sciences, Keele University, Keele UK
[3]School of Health Sciences, Aston University, Birmingham, UK

Correspondence and requests for collaboration should be addressed to ***foxkd@cardiff.ac.uk***

Not all neurones are created equal when it comes to plasticity. Either by virtue of their different afferent pathways or their different synaptic receptors, neurones in different layers of the cerebral cortex show different degrees and types of synaptic plasticity. For principal neurons in the adult somatosensory cortex, layer 4 is relatively aplastic while layer 2/3 and layer 5 are highly plastic. Recent studies show that the diversity does not end there. The two major subdivisions of layer 5 pyramidal cells, regular spiking (RS) and intrinsic bursting (IB) cells also show different types of plasticity and rely on Hebbian and homeostatic mechanisms to different degrees. RS neurones show experience-dependent depression following whisker trimming, which slowly recovers homeostatically back to baseline despite the maintained deprivation. The homeostatic rebound is TNFα dependent. Potentiation of spared whisker responses is absent in these cells. In contrast, IB cells do show potentiation of spared whisker responses comprising both TNF α and α-CaMKII-autophosphorylation dependent components (1). As LTP is absent in α−CaMKII-autophosphorylation mutants (2) and synaptic upscaling is absent in TNF α knockouts (3), these findings suggest the two synaptic mechanisms are distributed differently between the two cell types. In the light of these results, we looked again at plasticity in layer 2/3. Previous studies had shown that LTP and experience-dependent potentiation are absent in the barrel cortex of α−CaMKII-autophosphorylation mutants(2). Our present studies show that in addition, following depression of deprived whisker responses, layer 2/3 cells show a homeostatic rebound that is prevented by a soluble TNF α scavenger. In summary, all three cortical cell types show varying degrees of TNF α dependent homeostatic plasticity, but, while layer 2/3 cells show both Hebbian depression and potentiation, in layer 5 Hebbian depression and potentiation are segregated between RS and IB cells respectively.

References

1. Greenhill, S. D. et al. Neuron 88, 539-552, (2015).
2. Hardingham, N. et al. J Neurosci 23, 4428-4436, (2003).
3. Stellwagen, D. & Malenka, R. C. Nature 440, 1054-1059, (2006).

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Dynamic Axonal Translation in Developing and Mature Visual Circuits

Hosung Jung

Yonsei University

Correspondence and requests for collaboration should be addressed to ***hjung@yuhs.ac***

Local mRNA translation mediates the adaptive responses of axons to extrinsic signals, but direct evidence that it occurs in mammalian CNS axons in vivo is scant. We developed an axon-TRAP-RiboTag approach in mouse that allows deep-sequencing analysis of ribosome-bound mRNAs in the retinal ganglion cell axons of the developing and adult retinotectal projection in vivo. The embryonic-to-postnatal axonal translatome comprises an evolving subset of enriched genes with axon-specific roles, suggesting distinct steps in axon wiring, such as elongation, pruning, and synaptogenesis. Adult axons, remarkably, have a complex translatome with strong links to axon survival, neurotransmission, and neurodegenerative disease. Translationally co-regulated mRNA subsets share common upstream regulators, and sequence elements generated by alternative splicing promote axonal mRNA translation. Our results indicate that intricate regulation of compartment-specific mRNA translation in mammalian CNS axons supports the formation and maintenance of neural circuits in vivo.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Genes, Microglia and Alzheimer's Disease

Morgan Sheng

Genentech, USA

Correspondence and requests for collaboration should be addressed to ***sheng.morgan@gene.com***

Alzheimer's disease (AD) afflicts five million people in the US, and millions more globally, but its pathogenesis remains poorly understood at the molecular and cellular level. Our approach is to start from human genetics. Increasingly, human genetics point to microglia cells as playing a central role in risk of AD.

Genetic variants in TREM2 (Triggering Receptor Expressed on Myeloid cells 2) greatly increase the risk of Alzheimer's disease (AD)—~3-fold increase in risk, which is on par with the largest genetic risk factor for AD, apolipoprotein E4 (APOE4). TREM2 is specifically expressed in myeloid cells and only by microglia in the brain--implicating innate immunity mechanisms in the pathogenesis of AD. I will present evidence that links TREM2—biochemically and functionally--with two other major AD risk genes, APOE and APOJ, which encode apolipoproteins. By revealing the cell biological function of TREM2 in microglia, our findings can explain why mutations in TREM2 increase beta-amyloid accumulation and elevate the risk of AD.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Genetic studies on empathic fear behaviour in mice

Sehoon Keum

IBS

Correspondence and requests for collaboration should be addressed to ***sdukeum@ibs.re.kr***

Empathy is an important emotional process that involves the ability to recognize and share emotions with others. Deficits in empathy manifest in a variety of disorders such as autism, schizophrenia, alexithymia, as well as psychopathy. We have developed an observational fear learning (OFL) behavioral assay to measure empathic fear in mice. In the OFL task, a mouse is conditioned for fear to the context where it observes a conspecific demonstrator receiving aversive stimuli. We have further demonstrated that the affective pain circuits including the anterior cingulate cortex, the midline and intralaminar thalamus and the amygdala play a crucial role in OFL. However, despite recent association studies showing that genetic factors account for both change and continuity in empathy, identification of genes controlling empathy has been largely limited in humans. Though the use of forward and reverse genetic approaches, we have identified that phospholipase C beta4 and neurexin-III are involved in modulation of OFL. I will present how we have identified these genes and underlying neural mechanisms for empathic fear behaviors.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Temporal dynamics of neuronal activity engaged in the expression and persistence of hippocampal representation of space

David Dupret

MRC Brain Network Dynamics Unit, University of Oxford

Correspondence and requests for collaboration should be addressed to ***david.dupret@pharm.ox.ac.uk***

How does the brain support the emergence of internal representations of the external world and what are the mechanisms underlying the persistence of a limited subset of these representations? In this talk I will present a series of experiments that address these central questions of neuroscience by monitoring and manipulating neuronal representation of space in the mouse hippocampus. First, I will present recently published work that establishes how a spatial representation can be artificially modified to influence the behaviour associated with a drug-place memory. I will then describe ongoing experiments that demonstrate a central role of reverberating activity during sleep/rest behaviour in the consolidation of newly-acquired place representations. Finally, I will present preliminary data revealing a possible network mechanism underlying the translation of the neural representation of space in the hippocampal-accumbens axis into behavioural performance. Our findings highlight how short-timescale neuronal dynamics support the expression of internal representation of space and their translation into actions.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Whole-cell recordings in freely moving rodents

Doyun Lee

IBS

Correspondence and requests for collaboration should be addressed to ***leedoyun@ibs.re.kr***

The brain controls all of our body's functions including mental processes such as learning and memory. Information processing within the brain is carried out by an enormous number of neurons that are wired together and communicate with each other by sending and receiving electrical signals. Within such a network, each neuron receives thousands of synaptic inputs from other neurons and processes these inputs through various computational mechanisms within the neuron to determine whether it generates outputs or not. Therefore, understanding information processing inside neurons in an awake, working brain is a fundamental step for linking cellular level computations to higher level brain functions. In this talk I will present recent advances in whole-cell intracellular recording techniques that can be applied to animals engaged in free behavior, thus allowing precise measurement and manipulation of electrical signals within a single neuron in the fully functioning brain. I will also present how these techniques have been applied to study the cellular mechanisms of the spatially tuned spiking activity of CA1 neurons ("place cells") in the hippocampus, a critical brain structure for spatial information processing and memory.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Reactivation of developmental synaptic plasticity following peripheral nerve injury

John Isaac

Wellcome Trust

Correspondence and requests for collaboration should be addressed to ***j.isaac@wellcome.ac.uk***

Recent studies have shown that thalamocortical (TC) inputs remain plastic in adulthood after the developmental critical period has closed. However, the mechanism of such post-critical period plasticity remains unknown. Here we show that silent synapses and long-term potentiation (LTP) are transiently restored at spared TC inputs in rodent primary somatosensory (S1) barrel cortex following unilateral infra-orbital nerve lesion in 4-6 week-old rats. The reappearance of LTP and silent synapses leads to a persistent strengthening of the spared TC input and is preceded by a transient re-expression of synaptic GluN2B-containing NMDA receptors. Furthermore, GluN2B-containing NMDA receptor activity *in vivo* is required for the re-emergence of TC plasticity. The plasticity mechanisms activated by infra-orbital nerve lesion are indistinguishable from those during the critical period, suggesting that a reactivation of a developmental synaptic plasticity program occurs following sensory denervation.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Externally and internally driven transformations of the hippocampal cognitive map

Julia Krupic

University College London

Correspondence and requests for collaboration should be addressed to ***j.krupic@ucl.ac.uk***

The hippocampus is a key brain region for representing space. Grid cells and place cells are one of the main functional cell types in the hippocampal formation. It has been suggested that grid cells represent the internal metric for space while place cells are coding the animal's location. In my talk, I will show that an external stimulus, namely the geometry of the enclosure, has a profound effect on grid cell symmetry making the grid less regular and non-homogeneous. This suggests that the internal metric can be distorted in polarized enclosures. I will present some preliminary data showing that an animal's ability to estimate distances can be compromised by the geometry of the enclosure. Importantly none of the existing theoretical frameworks based on path integration hypothesis can explain the effect of the geometry of the enclosure on grid cells. I will propose an alternative theoretical framework where grid cell firing patterns can result from competition between place cells and boundary cells. This model captures many of our experimental findings. Finally, I will also present how the information about an animal's self-motion cues and visual cues are combined in place cells and how putting these two types of information in conflict affects the animal's perception of self-location.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Reconstructing neural representations of dynamic visual objects

Won Mok Shim

IBS / Sungkyunkwan University

Correspondence and requests for collaboration should be addressed to ***won.mok.shim@dartmouth.edu***

Contrary to our seamless percept of visual objects in motion, raw sensory data are often only partial and impoverished. To interact with moving objects in our environment, our visual system rapidly fills in extensive details, creating an enriched representation of dynamic objects. How does the visual system reconstruct these representations of visual objects that are not present in the retinal input but are interpolated during kinetic transformations? Using fMRI techniques with a forward encoding model and functional connectivity, we investigated whether and how interpolated visual features of an object in transition are reconstructed in the visual cortex. We showed that “intermediate” visual features of an object engaged in motion are represented in population-level feature-selective tuning responses in early visual cortex and that motion and shape information in high-level visual areas interact with these feature responses in early cortical processing. Our findings provide insights into how early visual cortex functions as a buffer where bottom-up and top-down information converge, forming the percept of a coherent dynamic visual object.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Representations of retrieved face information in visual cortex

Sue-Hyun Lee

KAIST

Correspondence and requests for collaboration should be addressed to ***suelee@kaist.ac.uk***

Despite the high similarity of human faces, we can easily recognize and discriminate dozens of faces based on our memories and can retrieve how people look. Here, we asked how retrieved face information is represented in cortex? To address this question, we performed an event-related functional magnetic resonance imaging (fMRI) experiment, comprising separate perception, learning and retrieval sessions. Using multivoxel pattern analyses, we found 1) that face-selective and object-selective areas showed category (faces or shoes) specific patterns during both retrieval and perception, 2) that neither face nor object-selective areas showed patterns specific to individual faces or shoes during perception, but 3) that face-selective areas showed specific patterns of response to individual faces during retrieval. Taken together, these results suggest that retrieval of face information generates more discriminative neural responses for individual faces than that evoked by perception of the very same faces.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Combined Use of Functional Neuroimaging and Non-invasive Brain Stimulation in Stroke Neurorehabilitation

Yun-Hee Kim

Samsung medical Center / Sungkyunkwan University

Correspondence and requests for collaboration should be addressed to ***yunkim@skku.edu***

Modulation of neuroplastic activities of perilesional cortex or interconnected network regions may enhance the rehabilitative outcome and functional restoration after stroke, therefore, the methods of modulating neuroplasticity are crucial topics in neurorehabilitation research. Noninvasive brain stimulation (NBS) is one of recently developed techniques to modulate neural plasticity in a noninvasive manner and consequently to enhance neural recovery. The most popular noninvasive methods of neuromodulation include transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS). By TMS, rapidly changing magnetic field induces electric current on the cortical surface that activates neuronal element of the cortex and repetitive TMS (rTMS) modulates the excitability of cortical neuron as frequency dependent manner. In the other hand, tDCS induces excitability changes of human motor cortex by weak DC stimulation through glutamatergic and membrane mechanisms. Diverse factors such as individual skull and cortical morphology, lesion location and severity, genetic polymorphism, etc. are considered as the intrinsic factors affecting individual response variability to NBS. The modulating effect of NBS can expand to the interconnected subcortical network areas beyond the site of cortical stimulation. Multimodal functional neuroimaging methods such as functional MRI, PET, and functional near infrared spectroscopy can help to demonstrate the network spreading effect of NBS. Neural plasticity after stroke can be seen from microscopic to macroscopic levels. Combined use of functional neuroimaging modalities with neuromodulatory interventions such as NBS and various rehabilitation treatments may unveil characteristic reorganization of large-scale neural network after neurorehabilitation of stroke patients.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Towards a molecular understanding of amyloid propagation using super resolution microscopy and multiparametric imaging

Claire H. Michel, Dorothea Pinotsi, Na Yu, Laurie Young, Clemens F. Kaminski, Gabriele S. Kaminski Schierle

Department of Chemical Engineering and Biotechnology, University of Cambridge, UK

Correspondence and requests for collaboration should be addressed to ***gsk20@cam.ac.uk***

Understanding the formation and propagation of aggregates of the Alzheimer disease-associated Tau and Amyloid β protein *in vivo* is vital for the development of therapeutics for this devastating disorder. Using two colour super-resolution imaging we show that there is heterogeneity in the growth rates of individual amyloid fibrils which can be attributed to a structural polymorphism. We have developed this technique further for its application in samples of CSF and interstitial fluid from patients. Furthermore, using our live-cell aggregation sensor in neuron-like cells, we demonstrate that we can follow amyloid formation and propagation in live neuronal cells and we have developed a method that permits the study of exogenous amyloid protein seeding endogenous protein present in normal cells. Our data thus indicate a greater pathological risk than hitherto suspected for extracellular soluble amyloid protein. Moreover, our techniques offer new capabilities in the study of amyloid growth dynamics at the molecular level which will be important for the development of novel therapeutic strategies.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Somatic Mutations Disrupting Brain Connectivity

Jeong-Ho Lee

KAIST

Correspondence and requests for collaboration should be addressed to ***jhlee4246@kaist.ac.kr***

The goal of the human neurogenetics field is to generate new questions and provide unexpected insight into neurobiology by discovering disease-causing mutations in genes that link mechanism of brain development to pathogenesis. With genetic revolution like the release of human genome sequence and the development of next-generation sequencing (NGS) technology over the last decade, we sought to translate this revolution into mono- or polygenic and complex neurodevelopmental disorders to provide better understanding of the developing human brain (Lee et al., Science 2012; Martinez et al., J Med Genet 2013; Valente et al., Nat Genet 2010). Especially, de novo somatic mutations in focal areas are well documented in diseases such as neoplasia but are rarely reported in neurodevelopmental disorders. Compared to other organs in human body, brain has a unique feature of complex networks to form enormous connections between different areas and neurons, thereby allowing higher cognitive functions of human brain. Therefore, if there is any abnormal change of neural activity in a focal area of brain possibly due to somatic mutations, it could make a harmful affect on the neuronal functions in connected brain regions and then disrupt the normal function of the entire brain. As the proof of this concept, we and other group recently identified brain somatic mutations in patients associated with hemimegalencephaly (an enlargement of one side of brain hemisphere) (Lee et al., Nat Genet 2012; Poduri et al., Neuron 2012). In this symposium, I would like to present our recent study about ‘brain somatic mutations’ found in patients with focal cortical dysplasia type, which is the most important cause of focal intractable epilepsy in children.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Problematic proteostasis in fragile X syndrome

Emily Osterweil

Centre of Integrative Physiology, University of Edinburgh

Correspondence and requests for collaboration should be addressed to ***emily.osterweil@ed.ac.uk***

Excessive cerebral protein synthesis is believed to be a core pathophysiology in fragile X syndrome (FXS), a neurodevelopmental disorder with a high incidence of autism and intellectual disability (ASD/ID). Although there is a growing consensus that regulation of protein synthesis is important for treating FXS, the mRNAs that are differentially translated have yet to be identified, and the way this contributes to alterations in neural function is as yet unknown.

Efforts to identify proteins that are excessively translated in FXS have been limited by several factors, including insufficient sensitivity to identify low-expression targets, and an inability to probe for new translation in a cell-type specific manner. To resolve these issues, we are using Translating Ribosome Affinity Purification (TRAP) and RNA sequencing to isolate mRNAs that are selectively associated with translating ribosomes in CA1 pyramidal neurons in the FXS mouse model (*Fmr1*$^{-/y}$). This approach has lead to the identification of a specific population of mRNAs that are differentially translating in *Fmr1*$^{-/y}$ versus wild type CA1 neurons. Surprisingly, a significant overexpression of mRNAs encoding subtypes of the muscarinic acetylcholine receptor (mAChR) is seen in the ribosomes of *Fmr1*$^{-/y}$ CA1 neurons. Subsequent experiments revealed a significant overexpression of the m1 and m4 mAChR subtypes, which may contribute to alterations in hippocampal function in the *Fmr1*$^{-/y}$ mouse. These results show that a cell-type specific translation profiling approach can reveal novel information about the pathophysiology of FXS.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Glucocorticoids activate a synapse weakening pathway culminating in tau phosphorylation in the hippocampus

Jee Hyun Yi[1,5], Christopher Brown[1,2,5], Garry Whitehead[1], Seongmin Choi[1,2], Thomas Piers[1,2,3], Young Seok Lee[1,4], Celia Martinez Perez[1], Philip Regan[1,3], Daniel J. Whitcomb[1,3] and Kwangwook Cho[1,3]

[1]Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, School of Clinical Sciences, Faculty of Health Sciences, University of Bristol, UK
[2]Chonnam-Bristol Frontier Laboratory, Biomedical Research Institute, Chonnam National University Hospital, South Korea
[3]Centre for Synaptic Plasticity, University of Bristol, UK
[4]Department of Life Sciences, Imperial College, UK
[5]These authors contributed equally to the work

Correspondence and requests for collaboration should be addressed to ***JeeHyun.Yi@bristol.ac.uk***

A wealth of evidence suggests that the stress hormones glucocorticoids (GCs) modulate neuronal function and can induce neurodegeneration and cognitive deficits. GCs have been shown to facilitate long-term depression (LTD) and inhibit long-term potentiation (LTP), suggestive of aberrant synapse weakening. However, the molecular mechanisms behind these effects are not fully understood. We hypothesised that glycogen synthase kinase-3 beta (GSK-3β), known to be important in synapse weakening, would be sensitive to modulation by GCs, and this would be central to GC-induced synaptic impairment. Western blot and electrophysiological analysis of acute rat hippocampal slices treated with corticosterone suggests that GCs activate GSK-3β by means of caspase-3-mediated cleavage of Akt1, which ultimately causes aberrant tau phosphorylation. By expressing phospho-null human tau mutants in cultured hippocampal slices, we found that phosphorylation of tau at serine 396 specifically is critical for synapse weakening and the impairment of LTP that is caused by GCs. Together these findings unveil a molecular crossover between GCs and synapse weakening signals, and indicate the potential for stress-induced priming of neurodegeneration.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Synthetic glucocorticoid treatment induces prolonged activation of glucocorticoid receptors in the hippocampus, dysregulates clock gene expression and impairs memory and learning processes in the rat

Matthew Birnie[1], Rebecca Demski-Allen[2], Ben Flynn[1], Yvonne Kershaw[1], Gareth Barker[2], E. Clea Warburton[2], Stafford L. Lightman[1]& Becky L. Conway-Campbell[1]

[1]School of Clinical Sciences, University of Bristol
[2]Department of Physiology and Pharmacology, University of Bristol

Correspondence and requests for collaboration should be addressed to ***matthew.birnie@bristol.ac.uk***

Chronic treatment with the synthetic glucocorticoid (GC) prednisolone has been reported in association with many detrimental health effects. In addition to well-documented adverse metabolic effects, there is evidence for memory impairments in these patients. Cell experiments have shown that synthetic GCs such as methyl-prednisolone (MPL) cause an alteration in timing of glucocorticoid receptor (GR) activation and in contrast to the rapid pulsatile GR activation associated with natural GC hormones corticosterone and cortisol, synthetic GCs including MPL induce a prolonged GR activation profile. We report that with three-day treatment in adrenal-intact rats, 1mg/ml MPL in drinking water supressed endogenous corticosterone secretion but induced significant GR activation during the circadian peak as well as the nadir, consistent with prolonged MPL induced GR activation. We show that locomotor activity and core body temperature, as well as 24h mRNA expression of Period1 and Bmal1 clock genes display a significant dysregulation of circadian rhythm in the MPL treated rats. Interestingly, MPL treatment also resulted in impaired hippocampal-dependent memory when rats were tested in an object location task. MPL treated rats were able to discriminate between novel and familiar in the object location test following a 1h delay. However at a 6h delay, MPL treated rats were not able to discriminate. Therefore, we conclude that chronic MPL treatment impairs hippocampal-dependent spatial memory following a 6h delay, but not a 1h delay. Therefore, our experimental model may provide an understanding for determining the underlying mechanisms of memory deficits in patients treated with prednisolone and other synthetic GCs.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

C9FTD/ALS (GA) RAN translated protein is in the core of seeding process for DPR aggregation and toxicity

Youn-Bok Lee[1], Pranetha Baskaran[1], George Gomez[1], Claire Troakes[1], Agnes Nishimura[1], Boris Rogelj[2], Leonard Petrucelli[3], Sarah Guthire[1], Christopher E Shaw[1]

[1]Department of Clinical Neuroscience, Centre for Neurodegeneration Research, Institute of Psychiatry, King's College London
[2]Department of Biotechnology, Jožef Stefan Institute, Slovenia
[3]Department of Neuroscience, Mayo Clinic Florida, USA

Correspondence and requests for collaboration should be addressed to ***younbok.lee@kcl.ac.uk***

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are devastating neurodegenerative disease, sharing neuropathological phenotypes. Hexanucleotide (GGGGCC; G4C2) repeat expansion in intron 1 of C9ORF72 is the major genetic cause of FTD/ALS. Repeat associated non ATG (RAN) translation is found in hexanucleotide repeat expansion in C9ORF72 gene (C9RAN). C9RAN includes poly (GA, GP, GR, PA, and PR) which form neuronal inclusions though out the central nervous system however the contribution to disease pathogenesis is unknown. We show that among the C9RAN proteins, the expression of poly (GA) is the one activated cell death in dose dependent manner, which accompanied with TDP-43 cleavage. Dual expression of poly (GA) against poly (GP, GR, PA, PR) showed that GP and PA are sequestered by poly (GA) however GR and PR were rare in cell culture model. Similarly, the poly (GP) and poly (PA) sequestrations by poly (GA) are detected on human postmortem brain section. Interestingly, dual expression with poly (GA) and poly (PA) ameliorated the toxicity through competing the poly (GA) aggregation formation. These results confirmed by in_vivo chick embryo by electroporation study that poly (GA) was the most toxic protein among the C9RANs.

Taken together our data provide evidence that poly (GA) cross talk with other type of C9RAN proteins may be a modifier of pathogenic status of C9FTD/ALS.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

CYFIP2: A potential link between Aβ and tau pathologies in Alzheimer's disease

Anshua Ghosh[1], Sachin Tiwari[1], Keiko Mizuno[1], Claire Troakes[1], Tibor Hortobagyi[1,2], Karl Peter Giese[1]

[1]Department of Basic and Clinical Neuroscience, King's College London UK
[2]Departmentof Neuropathology, Institute of Pathology, Univeristy of Debrecen, Hungary

Correspondence and requests for collaboration should be addressed to ***anshua.ghosh@kcl.ac.uk***

Alzheimer's disease (AD) is histopathologically characterised by Aβ plaques and tangles comprising hyperphosphorylated tau; however it is in fact synaptic degeneration that best correlates with the cognitive impairment, making its understanding critical in the development of clinical treatment. Early changes in the AD brain involve alterations in protein synthesis at synaptic sites that may be dependent on RNA-binding proteins such as FMRP and its interactors. Previous work from our group has found that the *C*ytoplasmic *F*MRP-*I*nteracting *P*rotein 2 (CYFIP2) is reduced by about 50% in the AD post mortem hippocampus when normalised for synaptic loss. CYFIP2 is a highly conserved protein that is abundant in synapses and developmentally expressed. While not much is known about its precise physiological role in the brain, it has been proposed to have functions in regulating local protein synthesis and modulating cytoskeletal dynamics. Using CYFIP2 heterozygous knockout mice to model the condition, we find that reducing CYFIP2 increases post- transcriptionally the expression of FMRP-regulated proteins such as Amyloid Precursor Protein (APP) and αCaMKII in hippocampal synapses. CYFIP2$^{+/-}$ mice also have increased BACE1 protein in hippocampal synapses, and elevated $A\beta_{1\text{-}42}$ in total hippocampi. Additionally there is evidence for increased tau phosphorylation in hippocampal synapses of these mice. Taken together, reducing CYFIP2 in the mouse brain is sufficient to increase amyloid production and tau phosphorylation, recapitulating two key aspects of the disease. Therefore reduced CYFIP2 expression may be a key mediator of early changes in the AD brain and a potential link between Aβ and tau pathologies.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

New insights into the pathophysiology of Alzheimer's disease: Phosphorylation of PKCζ and p47phox promotes synapse weakening

Dong Hyun Kim[1,3], *Thomas M. Piers[1,3,4], Jee Hyun Yi[1], Philip Regan[1], Daniel J. Whitcomb[1,2] and Kwangwook Cho[1,2] *

[1]School of Clinical Sciences, Faculty of Medicine and Dentistry, University of Bristol, UK
[2]Centre for Synaptic Plasticity, University of Bristol, UK
[3]These authors contributed equally to this work
[4]Current address: Therapeutic Innovation Group, UCL Institute of Neurology, London, UK

Correspondence and requests for collaboration should be addressed to ***thomas.piers@ucl.ac.uk***

Emerging studies suggest that apoptotic signalling cascades, GSK-3β and reactive oxygen species (ROS) are involved in synapse weakening pathways (SWP). However, it remains to be shown how the SWP is first initiated. We found aberrant phosphorylation of PKCζ (pPKCζ) in Alzheimer's disease (AD) temporal cortex, a kinase linked with activation of the NADPH oxidase complex that induces ROS production. This finding was associated with a reduction in total GluA2 protein expression in the membrane compartment of AD temporal cortex tissue, consistent with previously reported AD pathophysiology data. To explore a specific molecular mechanism of pPKCζ in synapse weakening, we utilised an amyloid-beta (Aβ)-induced synapse-weakening model in rat hippocampal neurons. We found that PKCζ-activated NADPH oxidase 2 (NOX2) is critical for the activation of SWP molecules, and the Aβ-induced synapse weakening in CA1 hippocampal neurons. Furthermore, PKCζ-dependent phosphorylation of p47phox, a NOX2 regulatory subunit, is required for long-term depression (LTD), a physiological mechanism of synapse weakening that is aberrantly hijacked by Aβ and AD pathogens. Our data provides new insights into pPKCζ-dependence, and PKCζ-regulated p47phox phosphorylation, as key upstream determinants of aberrant synapse weakening, and may shed new light on mechanisms of AD pathophysiology.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

The novel role of postsynaptic density protein, DLG2, in neurogenesis

Eunju Jenny Shin, Meng Li, Adrian Harwood

Neuroscience and Mental Health Research Institute, Cardiff University, UK.

Correspondence and requests for collaboration should be addressed to ***shine@cardiff.ac.uk***

Postsynaptic density (PSD) proteins play a critical role in brain function and a number of PSD genes have been found to be associated with increased risk of neuropsychiatric disorders. So far, roles of PSD proteins during neural development have received little attention, probably due to their stereotyped roles in mature synapses. The current study hypothesised that PSD proteins will contribute to early neural development and neurogenesis. We particularly focussed on proteins in DLG (disk large) family of membrane associated guanylate kinases since *de novo* deletions of *DLG2* has been found in psychiatric patients and drosophila DLG is expressed in neural precursor cells and involved in asymmetric cell division to give rise to neurons. To this end, $DLG2^{-/-}$ hESCs were derived using the new genome editing technology called CRISPR/Cas9 system. $DLG2^{-/-}$ hESCs were differentiated into $nestin^{+}$ neural precursors and gave rise to cortical projection neurons. However, specific cortical populations were affected by DLG2 deficiency. The proportion of $TBR1^{+}$ layer 6 cortical neurons was less in $DLG2^{-/-}$ cultures and the percentage of $CTIP2^{+}$ layer 5 cortical neurons was higher compared to the wildtype counterpart. These results show, for the first time, a role of the DLG group of proteins in neurogenesis. The underlying mechanism of DLG2's involvement in cortigoneurogenesis is under investigation and will be presented in the meeting. Unveiling the role of DLG2 during neurogenesis will let us understand more about neural development itself and give us a clue to uncover underlying mechanisms of neurodevelopmental diseases.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Homeostatic scaling of Kainate Receptors

Sonam Gurung, Ash Evans, Kevin Wilkinson and Jeremy Henley

School of Biochemistry, University of Bristol

Correspondence and requests for collaboration should be addressed to ***sg9823@bristol.ac.uk***

Kainate receptors (KARs) are excitatory amino acid receptors that play key roles in regulating neurotransmitter release, neuronal excitability and neuronal network activity. Their functions are required for neuronal development and differentiation, and their dysfunction is strongly implicated in epilepsy, autism and neurodegenerative diseases(1).

We have shown previously that surface expression of GluK2 subunit-containing KARs is dynamically and activity-dependently regulated. Sustained agonist activation of KARs causes their down regulation via endocytosis and lysosomal degradation, which likely represents a neuroprotective mechanism in conditions of excitotoxicity. Relatively brief agonist activation of KARs, however, causes an initial decrease followed by a sustained increase in surface expression, which may act to increase neuronal excitability and potentiate network activity(2).

To further explore the plasticity of KARs we are now investigating how they are involved in homeostatic scaling, the mechanism by which neurons alter their synaptic transmission to compensate for changes in network activity. The role of AMPA receptors in homeostatic scaling has been extensively studied(3) but there are, as yet, no reports on KARs.

Using dispersed cultured rat hippocampal neurons our data show that, similar to AMPA receptors, GluK5 and GluK2-containing KARs also undergo robust homeostatic scaling. In response to decreased synaptic activity following TTX treatment there is a significant increase in the surface expression of KARs. We are currently investigating the localisation of the inserted receptors, and defining the signalling pathways, trafficking mechanisms and possible cross-talk between KARs and AMPARs involved in these processes.

References:

1. Lerma J & Marques JM. Neuron 2013; 80:292-311
2. Martin S et al. J Biol Chem 2008; 283:36435-36440
3. Turrigiano GG. Cell 2008; 135:422-435

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Cell type specific analysis on mRNA translation in fragile X syndrome

Seo, S. S.*, Thomson, S. R.*, Dando, O., Barnes, S. A., Kind, P. C., Bear, M. F., Osterweil, E. K.

Centre of Integrative Physiology, University of Edinburgh, UK

Correspondence and requests for collaboration should be addressed to ***sseo@exseed.ed.ac.uk***

Fragile X Syndrome (FXS) is a neurodevelopmental disorder with a high incidence of autism, epilepsy, and intellectual disability (ID). Many of the neurological symptoms associated with FXS are thought to be the results of excessive neuronal protein synthesis [1]. This includes the exaggeration of long-term synaptic depression (LTD) downstream of metabotropic glutamate receptor 5 (mGluR5) in the CA1 region of the hippocampus. Previous work has shown that the exaggeration of LTD, and many other phenotypes, can be rescued by reducing the rate of protein synthesis [2-4]. However, the mRNAs that are aberrantly translated are yet to be identified. Identification of aberrantly translating mRNAs could give insights into the pathophysiology of FXS and reveal new therapeutic targets.

Previous attempts to identify newly made proteins in FXS have been limited by both insufficient sensitivity to low yield proteins and a lack of cell type specificity. To overcome these issues, we have used Translating Ribosome Affinity Purification (TRAP) to isolate translating mRNAs from hippocampal CA1 pyramidal neurones in the FXS mouse model (*Fmr1*$^{-/y}$). We have combined TRAP with RNA sequencing (RNAseq) to produce a list of differetially translating mRNAs in *Fmr1*$^{-/y}$ CA1 pyramidal neurones.

Our analysis revealed an overexpression of mRNAs encoding specific subtypes of muscarinic acetylcholine receptor (mAChR) in *Fmr1*$^{-/y}$ CA1 pyramidal neurons. Further qPCR and immunofluorescence analyses of isolated CA1 neurons confirm these findings at the translating mRNA and protein level. Ongoing investigations in the hippocampus of the *Fmr1*$^{-/y}$ mouse have revealed perturbed cholinergic function, which is consistent with the changes observed in the RNAseq. Together, these results show that cell type specific analysis of mRNA translation reveals novel information about the identity of mistranslating proteins in the *Fmr1*$^{-/y}$ mouse model.

1. Bear, M.F., K.M. Huber, and S.T. Warren. Trends Neurosci, 2004. 27(7): p. 370-7.
2. Dolen, G., et al. Neuron, 2007. 56(6): p. 955-62.
3. Osterweil, E.K., et al. Neuron, 2013. 77(2): p. 243-50.
4. Osterweil, E.K., et al. J Neurosci, 2010. 30(46): p. 15616-27.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Astrocytic μ-opioid receptor in CA1 hippocampus drives opioid seeking behavior through glutamatergic system

Jaekwang Lee[1]*, Kyung-Seok Han[1,2]*, Jin Young Bae[4], Min-Ho Nam[1,3], Dong Ho Woo[1], Jayoung Kim[1], Junsung Woo[1], Elliot Kwong[1], Heeyoung An[1,5], Sea Hyung Park[1], Tae-Yong Choi[6], Eunju Kim[1], Eun Mi Hwang[1], Se-Young Choi[6], Yong Chul Bae[4]**, and C. Justin Lee[1,2,5]**

[1]Center for Neural Science and Center for Functional Connectomics, Korea Institute of Science and Technology, Seoul
[2]Neuroscience Program, University of Science and Technology (UST), Daejeon
[3]Department of Pathology, College of Korean medicine, Kyung Hee University, Seoul
[4]Department of Anatomy and Neurobiology, School of Dentistry, Kyungpook National University, Daegu
[5]KU-KIST Graduate School of Converging Science and Technology, Seoul
[6]Department of Physiology and Dental Research Institute, Seoul National University School of Dentistry, Seoul

Exogenous opioids such as morphine are the most effective pain medication but chronic use and misuse result in opioid addiction. The mechanism of development of opioid seeking behavior is thought to involve enhanced neuronal network activity of dopaminergic neurons in ventral tegmental area (VTA) and nucleus accumbens (NAc) through GABAergic disinhibition. Although hippocampus has been implicated in drug addiction, its role in development of opioid seeking behavior, especially the role of glia in this region, has been largely unexplored. Here, we report that CA1 hippocampal astrocytes highly express functional μ-opioid receptor (MOR), and activation of MOR by an agonist causes a glutamate release through TREK-1 channels from these cells. The released astrocytic glutamate increases synaptic transmission at the Schaffer-collateral-to-CA1-pyramidal-neuron synapses via activation of presynaptic metabotropic glutamate receptor 1 (mGluR1), and enhances the threshold of synaptic plasticity. In a conventional test of context-dependent drug seeking behavior, astrocyte-specific gene-silencing of MOR in CA1 hippocampus impairs agonist-induced conditioned place preference (CPP), whereas astrocyte-specific over-expression of MOR in CA1 hippocampus of MOR knockout mice fully rescues the impaired CPP. Our study proposes MOR and associated glutamatergic system in astrocytes of CA1 hippocampus as a potential therapeutic target for preventing development of opioid seeking behavior without compromising the analgesic effect.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Cell type-specific proteome labeling by genetic code expansion

Eunjin Kim

Department of Medical Science
The Graduate School, Yonsei University

Analyzing gene expression of specific cells in a living organism is crucial in understanding complex biological systems. It has not been possible, however, to isolate the proteome of specific cells in vertebrates. Genetic code expansion is a rising technique to label newly synthesized proteins. We expanded the genetic code of mammalian cells by introducing the pyrrolysyl-tRNA/tRNA synthetase system of *Methanosarcina mazei*. An alkyne-modified pyrrolysine analog specifically incorporated into the de novo proteome of these cells. Using the alkyne-azide cycloaddition, we could visualize these proteome in situ and after biochemical purification. These results provide a promising direction for a new technique to isolate the proteome of a specific cell type from a living animal.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

BDNF-TrkB-PLCγ1 signaling in CA2 hippocampus is essential for face-associated episodic memory

Jeongyeon Kim[1], Yong Ryoul Yang[2], Jaekwang Lee[1], Jae-Young Jung[1], Pann-Ghill Suh[2], C. Justin Lee[1]*

[1]Center for Neuroscience and Center for Functional Connectomics, Korea Institute of Science and Technology (KIST), Seoul

[2]School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, Republic of Korea

Recently, CA2 of hippocampus has been implicated as a critical brain region for social memory. However, it still remains unclear how CA2 stores social memory. Here, we show that CA2 plays a critical role in associating an experience with individual face, which is the most essential social information. Using transgenic mouse (PLCγ1$^{f/f}$) with floxed phospholipase Cγ-1, the downstream signaling molecule of the BDNF-TrkB pathway, we disrupted BDNF signaling in CA2 specifically with adeno-associated virus expressing Cre under Amigo2, a CA2 specific promoter (AAV-Amigo2::Cre). Theta-burst stimulus induced long-term potentiation at EC layer II input to the CA2 was impaired in AAV-Amigo2::Cre injected mice. To examine the face-associated episodic memory, we developed a new behavioral model of face-associated fear conditioning test using face as a conditioned stimulus. Face-associated avoidance behavior was impaired in AAV-Amigo2::Cre injected mice, whereas novel object recognition was not impaired. Our study suggests that hippocampus is involved in face recognition and may provide new insights on how to tackle face-related disorders such as autism, prosopagnosia, and dementia.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Ultrasonogenetic neuromodulation via TRPA1 channel in astrocyte

Soo-Jin Oh, Hyun-Bum Kim, Kyungmin Ko, Jungmoo Lee, Sungmin Han, Sungon Lee, Inchan Youn, Il-Joo Cho, Ji-Ho Park, Yong Chul Bae, and C. Justin Lee

Korea Institute of Science and Technology (KIST), Seoul

Brain stimulation strategies have been shown to be remarkably effective for treating a number of neurological and psychiatric conditions including Parkinson's disease, dystonia, essential tremor, epilepsy, chronic pain, and major depression as well as a number of other conditions. Considering the limitations in currently implemented approaches to stimulate brain, ultrasound is the improved, focused and noninvasive stimulation method for intact brain. Although the use of ultrasound for functional neuromodulation has been demonstrated by several studies, the underlying mechanism remains unclear. Based on the previous reports observing that ultrasound increased Ca2+ of neurons and astrocytes and ultrasound induced delayed neuronal responses, we hypothesized that ultrasound-induced activation of mechano-sensitive Ca2+ channel in astrocytes indirectly elicit neuronal activity by Best1 mediated gliotransmitter release. Firstly we tested whether several mechano-sensitive Ca2+ channels expressed in astrocytes respond to ultrasonic stimulation by Ca2+ imaging experiment. Ultrasound-induced Ca2+ increase was observed only in TRPA1 channel expressing HEK293T cells. Ca2+ responses induced by ultrasonic stimulation were dramatically decreased by the treatment of selective blocker for TRPA1 channel or gene silencing with TRPA1-shRNA in cultured astrocytes. We measured ultrasound induced neural activity change in rat organotypic hippocampal slice culture using a multi-electrode array (MEA). Ultrasound was capable of stimulating robust neuronal firing which was abolished by astrocyte-specific expression of TRPA1-shRNA, and treatment of TRPA1 blocker. Ultrasound-induced neuronal firing was interrupted by the treatment of Best1 blocker or NMDAR blocker as well. Finally, we observed decreased tail movement responses of TRPA1 KO or Best1 KO mice evoked by transcranial ultrasonic stimulation. Taken together, ultrasonic stimulation increases intracellular Ca2+ mediated by mechano-sensitive TRPA1 channel in astrocytes and elevated Ca2+ induces the Best1-mediated release of glutamate and D-serine that target NMDAR.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

DGKζ mediated regulation of PKCα is required for cerebellar long-term synaptic depression

Dongwon Lee[1], Yukio Yamamoto[2], Eunjoon Kim[1] and Keiko Tanaka-Yamamoto[2]

[1] Center for Synaptic Brain Dysfunctions, Institute for Basic Science, Daejeon
[2] Center for Functional Connectomics, Korea Institute of Science and Technology, Seoul

Long-term synaptic plasticity is controlled by balanced actions of positive and negative regulators at synapses. Protein kinase Cα (PKCα) is a critical mediator that induces long-term depression (LTD) at cerebellar parallel fiber-Purkinje cell synapses. However, the precise regulation of PKCα for LTD is not well understood. Here, I investigated the role of diacylglycerol kinase ζ (DGKζ) – a kinase that physically interacts with PKCα as well as postsynaptic density protein 95 (PSD-95) family proteins and functionally inhibits PKCα by metabolizing diacylglycerol (DAG) – in the regulation of cerebellar LTD. In Purkinje cells of DGKζ-deficient mice, LTD was impaired and PKCα was less localized in dendrites and synapses. This impaired LTD was rescued by virus-mediated expression of wild-type DGKζ, but not by a kinase-dead mutant DGKζ or a mutant lacking the ability to localize at synapses, indicating that both the kinase activity and synaptic anchoring functions of DGKζ are required for LTD. In addition, experiments using another DGKζ mutant as well as immunoprecipitation analysis revealed an inverse regulatory mechanism, in which PKCα phosphorylates, inactivates, and then is dissociated from DGKζ, is required for LTD. These results indicate that DGKζ is targeted to synapses, through its interaction with PSD-95 family proteins, to facilitate synaptic localization of PKCα, but maintains PKCα in a minimally activated state by reducing local DAG until its activation and release from DGKζ during LTD. Such local and reciprocal regulation of positive and negative regulators may contribute to the fine tuning of synaptic signaling.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Synaptic adhesion molecule IgSF11 interacts with PSD-95 and regulates AMPA receptor-mediated synaptic transmission and plasticity

Seil Jang

Institute for Basic Science, Daejeon

Synaptic adhesion molecules regulate synapse development and plasticity through mechanisms including trans-synaptic adhesion and recruitment of diverse synaptic proteins. We report here that the immunoglobulin superfamily member 11 (IgSF11), a homophilic adhesion molecule preferentially expressed in the brain, is a novel and dual-binding partner of the postsynaptic scaffolding protein PSD-95 and AMPAR glutamate receptors (AMPARs). IgSF11 requires PSD-95 binding for its excitatory postsynaptic localization. In addition, IgSF11 stabilizes synaptic AMPARs, as shown by IgSF11 knockdown-induced suppression of AMPAR-mediated synaptic transmission and increased surface mobility of AMPARs, measured by high-throughput, single-molecule tracking. IgSF11 deletion in mice leads to suppression of AMPAR-mediated synaptic transmission in the dentate gyrus and long-term potentiation in the CA1 region of the hippocampus. IgSF11 does not regulate the functional characteristics of AMPARs, including desensitization, deactivation, or recovery. These results suggest that IgSF11 regulates excitatory synaptic transmission and plasticity through its tripartite interactions with PSD-95 and AMPARs at postsynaptic sites.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Cell type-specific translatome profiling of cortical neural progenitors in the developing brain

Jane Jung[1] and Hosung Jung[1,2]

[1]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul
[2]Department of Anatomy and Brain Research Institute, Yonsei University College of Medicine, Seoul

Neural progenitors in the developing brain migrate over long distances to their final destination and make synapses with their appropriate targets. During this process, neural progenitors undergo dramatic morphological and functional changes that require coordinate gene expression. Because different classes of neurons and glia develop asynchronously, it had not been possible to analyze gene expression profiles of a specific class of cells while they develop *in vivo*. Using the RiboTag mouse which has an hemagglutinin (HA) epitope taggable rpL22 allele and the DCX-CreER^{T2} mouse in which activatable Cre recombinase is expressed in neural progenitor, we will label ribosomes in new born neural progenitors at different developmental stages and profile their gene expression by TRAP (Translating Ribosome Affinity Purification). Analysis of ribosome-bound mRNAs (thus translating mRNAs) from a specific subset of neural progenitors developing in an intact mouse brain will provide the basis to understand what genes are coordinately expressed at key steps during cortical development. Furthermore, comparing translatomes of normal and diseased brains will provide novel insights into the pathogenesis of neurodevelopmental disorders.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Non-cell-autonomous mechanism of Parkinsonism through glial GABA

Min-Ho Nam[1,4†], Jun Young Heo[1-3†], Hyung Ho Yoon[5†], Jeongyeon Kim[1†], Yu Jin Hwang[6], Dong Ho Woo[1], Ji Ae Lee[7], Hyun-Jung Park[4], Eunmi Hwang[1], Seonmi Jo[1,8], Sue H. Huh[9], Jae Y. Jeong[9], Kyung I. Kim[9], Neil W. Kowall[10-12], Junghee Lee[10-12], Hyunjoo J. Lee[13], Il-Joo Cho[13], Young Soo Kim[6], Sun Ha Paek[14], Byung K. Jin[9], Gi Ryang Kweon[2,3], Insop Shim[4], Onyou Hwang[7], Hoon Ryu[6,10-12], Sang Ryong Jeon[5*], C. Justin Lee[1,15*]

[1]Center for Neuroscience and Center for Functional Connectomics, KIST
[2]Department of Biochemistry, College of Medicine, Chungnam National University,
[3]Infection Signaling Network Research Center, Chungnam National University School of Medicine
[4]Department of Science in Korean Medicine, Graduate School, College of Korean Medicine, Kyung Hee University,
[5]Department of Neurological Surgery, Asan Medical Center, University of Ulsan College of Medicine
[6]Center for Neuro-Medicine, Brain Science Institute, KIST
[7]Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine
[8]Department of Biological Sciences, KAIST
[9]Department of Biochemistry & Molecular Biology, Department of Neuroscience, Neurodegeneration Control Research Center, School of Medicine, Kyung Hee University
[10]Boston University Alzheimer's Disease Center, Boston University School of Medicine
[11]Department of Neurology, Boston University School of Medicine
[12]VA Boston Healthcare System
[13]Center for BioMicrosystems, Brain Science Institute, KIST
[14]Department of Neurosurgery, Seoul National University Hospital
[15]KU-KIST Graduate School of Converging Science of Technology, Korea University

Parkinsonism is a clinical syndrome of movement abnormalities seen in Parkinson's disease (PD), which has been attributed to a cell-autonomous mechanism of dopaminergic neuronal death in the substantia nigra pars compacta (SNpc). Although reactive gliosis is considered to be a prominent feature of PD, its role in pathogenesis has remained elusive. Here we show that aberrantly synthesized GABA from reactive astrocytes tonically inhibits neighboring dopaminergic neuronal firing in SNpc, resulting in reduction of dopamine production and release, leading to Parkinsonism. Pharmacological and genetic inhibition of monoamine oxidase B and putrescine acetyltransferase, the key enzymes of astrocytic GABA synthesis, restored the pathological changes. These findings are mimicked by optogenetic modulation of dopaminergic neuronal firing. Additionally, brain samples of patients with PD showed numerous GABA-positive reactive astrocytes which are inversely correlated with the number of remaining dopaminergic neurons in SNpc. Our study proposes that glial GABA is inextricably linked to Parkinsonism, which can arise even before substantial dopaminergic neuronal death.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Bongwoori 2, a genetically-encoded voltage indicator, gives large signal-to-noise fluorescence changes for action potentials

Sungmoo Lee[1,2], Arong Jung[2], Tristan Geilleur[2], Bok Eum Kang[2], Yoon-Kyu Song[1,3] and Bradley J. Baker[2]

[1] Department of Transdisciplinary Studies, Graduate School of Convergence Science and Technology, Seoul National University
[2] Center for Functional Connectomics, Korea Institute of Science and Technology
[3] Advanced Institute of Convergence Technology, Suwon

Bongwoori is a genetically-encoded voltage indicator (GEVI) capable of optically resolving evoked action potentials with 60Hz firing frequency. Previously, we reported that changing the linker length and modifying the amino acid composition affected the voltage-sensing properties of voltage indicators. In this work, eight constructs with varied linker compositions were generated. A construct with positively charged amino acids showed increased fractional fluorescence value of ≥ 50% upon a stepped voltage pulse. For optimization of responsive voltage range and kinetics, three mutations were introduced to the voltage-sensing domain resulting in a better probe, Bongwoori-Pos6. To further improve the optical signal size, we conducted an arginine scanning experiment putting a positive charge at every amino acid position between the voltage-sensing domain and the FP. One of these constructs, Bongwoori-2, showed a larger signal size than Bongwoori at all tested voltage ranges. In whole cell current clamp recording on cultured primary neurons, both Bongwoori-Pos6 and Bongwoori-2 resolved evoked action potentials successfully. However, due to its responsive voltage range centered at near -30 mV, Bongwoori-Pos6 was not optimal for action potential recording. The larger signal size and tuned voltage range for action potential imaging for Bongwoori-2 enabled a single pixel recording to exhibit a 20% change in fluorescence during an action potential. These probes will facilitate the optical mapping of brain activity.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

The Endoplasmic Reticulum: a cytoplasmic nervous system – Imaging intercellular membrane potentials

Masoud Sepehri Rad, Lwarence B. Cohen, Bradley J. Baker

Center for Functional Connectomics, Korea Institute of Science and Technology

In eukaryotic cells, the endoplasmic reticulum (ER) is the largest continuous membrane-enclosed network which surrounds a single lumen. Using newly designed genetically encoded voltage indicators (GEVIs), we were able to show that there is a direct electrical interaction between plasma membrane (PM) and ER. The optical signal of the GEVI in the PM is very consistent from trial to trial. However, the ER often exhibits a dynamic signal that can vary both spatially and in signal size. This dynamic behavior of the internal signal suggests that voltage may also stress the ER causing it to remodel and change its resistance. While the physiological consequences of the ER sensing voltage are unclear, the power of optical imaging stands out since it would be impossible to reliably apply an electrode inside the ER. Our findings further suggest that the ER may be capable of transferring an electric signal from the plasma membrane to nuclear envelope.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

Functional circuitry mapping from different neuronal populations in the hippocampus with a fluorescent protein

Ryuichi Nakajima and Bradley J. Baker

Center for Functional Connectomics, Korea Institute of Science and Technology

To map out the circuitry in the brain, it is essential to clarify the functional connections among many different types of cells. For this purpose, optical imaging offers a powerful approach. For mapping brain activity, genetically-encoded calcium sensors such as GCaMP have been used quite often. However, these calcium sensors have critical problems in response to speed and lack of the ability for detecting inhibitory synaptic inputs. As the signal size and speed of genetically encoded voltage indicators (GEVIs) improve, the age of GEVIs has matured to the point that changes in membrane potential can now be observed optically in the brain. ArcLight, a GEVI that gives one of the largest optical signals, was expressed in mice hippocampus using human synapsin promoter or by a floxed version to limit expression in either CaMK2-positive excitatory cells or parvalbumin-positive inhibitory cells via viral injections. Excitatory and inhibitory fluorescent signals were observed in response to electrical stimulation of the Schaffer collateral axons in CA1. Different strata of the hippocampus exhibited different neuronal responses to field stimulus. Pharmacological studies also revealed that the ArcLight signal was able to resolve pre/postsynaptic activities with a single trial. ArcLight and similar probes are becoming a powerful paradigm for functional mapping of brain circuitry.

www.ukorea.ac.uk/pnf

PROCEEDINGS OF

THE UK-KOREA NEUROSCIENCE FORUM

The mechanism of voltage-induced fluorescence change inspires ratiometric genetically encoded voltage indicators.

Bok Eum Kang, Leticia Leong, Yoon Kyung Kim, Bradley J. Baker

Center for Functional Connectomics, Korea Institute of Science and Technology

The introduction of a negatively charged amino acid residing on the outside of the β-barrel structure of a pH-sensitive fluorescent protein (FP) improved the voltage-dependent optical signal of a genetically-encoded voltage indicator (GEVI). We have recently reported that the dimerization of this FP is important for optically detecting changes in the membrane potential leading to the hypothesis that the movement of the voltage-sensing domain drags the negative charge along the β-barrel causing a change in fluorescence. To test this hypothesis, an aspartic acid scan was performed on the exterior residues along the 11th β-strand of the FP. As the negative charge was moved along the β-strand the polarity of the optical signal switches from decreases in fluorescence upon membrane depolarization to increases in fluorescence upon membrane depolarization. To test the possibility that the protonation state of the chromophore was affected by the movement of the negative charge, voltage-clamp fluorometry was performed with excitation at 390 nm followed by excitation at 470 nm resulting in the development of several ratiometric GEVIs that may be able to optically report absolute changes in membrane potential.