The 8th Asian Pain Symposium (APS 2019): Summary and Abstracts

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Summary
The 8th Asian Pain Symposium (the 8th APS) was held in Songdo, Incheon of Republic of Korea during December 5 to December 7, 2019. The purpose of this symposium is to promote basic and translational research on pain and related neurological disorders and to advance pain research to a new level in Asian countries. Over 300 pain researchers and physician scientists from Asian and North American countries/regions participated in the 8th APS. There were a total of 91 presentations: 2 plenary lectures, 4 presidential lectures, 44 oral presentations, and 41 poster presentations at the 8th APS. The attendees presented their recent research findings in a wide range of areas including peripheral mechanisms of pain, pain circuit, ion channels and trigeminal mechanisms of pain, role of brain glia in chronic pain and the associated brain dysfunction, pain in the cortex, translational and clinical pain research, neuromodulation for chronic pain, and analgesic targets and drug developments. Many of the studies presented in this symposium have filled important scientific gaps and advanced our knowledge about pain and its effective managements. The 8th APS was organized by Dr. Seog Bae OH, the president of the 8th APS, and by the 8th APS local organizing committee including Dr. Dong Kuk Ahn, Kyungpook National University, Republic of Korea, Dr. Sun Wook Hwang, Korea University, Republic of Korea, Dr. Chul-Kyu Park, Gacheon University, Republic of Korea, and Dr. Yong Ho Kim, Gacheon University Republic of Korea. The 8th APS was kindly sponsored by Incheon Free Economic Zone Organization, Korean Association for the Study of Pain (Korea Chapter, IASP) and Korea Society for Ion Channel Research. During the 8th APS, a council meeting was held and Dr. Xu Zhang (Shanghai Institutes for Biological Sciences, China) and Guang-Yin Xu (Soochow University, China) were elected as the co-president of the 9th Asian Pain Symposium to organize the next symposium in China in 2021. In order to keep a permanent record and to help promote pain research in Asia, we have collected abstracts of oral presentations and published them in Molecular Pain in the order when the presentations were given at the 8th Asian Pain Symposium. Please note that individual authors who are involved in the studies described in each abstract are shown in each abstract, and the publication of the abstracts are agreed by the corresponding authors.

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A subset of dorsal horn inhibitory interneurons has a critical role in mechanical allodynia after nerve injury

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Abstract
Neuropathic pain is caused by peripheral nerve injury (PNI). One hallmark symptom is allodynia (pain caused by normally innocuous stimuli), but its mechanisms are not fully understood. In particular, whether selective stimulation of non-nociceptive primary afferent Aβ fibers indeed evokes neuropathic pain-like sensory and emotional behaviors after PNI is unknown, because of the lack of tools to manipulate Aβ fiber function in awake, freely moving animals. In this study, we used a transgenic rat line that enables stimulation of non-nociceptive Aβ fibers by a light-activated channel (channelrhodopsin-2). We found that illuminating light to the plantar skin of these rats with PNI elicited pain-like withdrawal behaviors that were resistant to morphine. Light illumination to the skin of PNI rats increased the number of spinal dorsal horn lamina I neurons positive to activity markers. Whole-cell recording revealed that optogenetic Aβ fiber stimulation after PNI caused excitation of lamina I neurons, which were normally silent by this stimulation. Moreover, illuminating the hindpaw of PNI rats resulted in the activation of central amygdaloid neurons and produced an aversion to illumination. Thus, these findings provide the evidence that optogenetic activation of primary afferent Aβ fibers in PNI rats produces excitation of lamina I neurons and neuropathic pain-like behaviors. Moreover, we recently identified a new subset of spinal dorsal horn inhibitory interneuron that acts as a critical brake on conversion of Aβ fibers signals into pain. Enhancing activity of these neurons may offer a novel strategy for treating neuropathic allodynia.

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Cytochrome P450c17 modulates the role of progesterone in the development of neuropathic pain

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Abstract
There are numerous studies showing that neurosteroids have profound neuromodulatory activities in the nervous system. The neurosteroid progesterone has neuroprotective properties against a diverse array of nervous system injuries, whereas there is a growing list of negative clinical trials that have failed to show a beneficial effect of progesterone treatment. Here, we investigated whether spinal progesterone has an effect on the development of neuropathic pain in a chronic constriction injury (CCI) model, and whether progesterone-metabolizing enzymes, cytochrome P450c17 and 5-α reductase, are associated with the actions of progesterone. Intrathecal administration of progesterone during the induction phase of neuropathic pain (post-operative days 0–3) facilitated the development of mechanical allodynia and spinal glial fibrillary acidic protein (GFAP) expression on day 1 post-CCI surgery. Phospho-serine levels of P450c17 were increased in the lumbar spinal cord dorsal horn on day 1 post-CCI surgery. Co-administration of the P450c17 inhibitor, ketoconazole with progesterone during the induction phase attenuated the progesterone-induced enhancement of mechanical allodynia and spinal GFAP expression in CCI mice. By contrast, co-administration of the 5-α reductase inhibitor, finasteride with progesterone during the induction phase had no effect on the progesterone-induced enhancement of mechanical allodynia and spinal GFAP expression. Collectively, these results demonstrate that during the induction phase of peripheral neuropathy, progesterone activates spinal astrocytes and enhances the CCI-induced alldynic effect via the activation of spinal P450c17, but not 5-α reductase.

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Chronic hyperglycemia before spinal cord injury increases inflammatory reaction and astrogliosis after injury: Human and rat studies

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Abstract

Traumatic spinal cord injury (SCI) can cause permanent disabilities that seriously reduce quality of life. We evaluated the effects of chronic hyperglycemia before SCI on inflammatory markers and functional recovery after SCI in human patients and a rat model. In the human study, multivariate logistic regression analysis revealed that hemoglobin A1c (HbA1c) values, reflecting average plasma glucose concentration over a three-month period, at admission were a significant risk factor for poor functional recovery. Moreover, patients with chronic hyperglycemia (HbA1c ≥ 6.5%) had high concentrations of inflammatory biomarkers (IL-6 and IL-8) of cerebrospinal fluid after SCI. Consistent with patient findings, chronic hyperglycemia before SCI in rats was associated with increased inflammatory responses and oxygen-free radicals in the spinal cord and blood, thus resulting in poor functional recovery and histologic outcomes. Tight glucose control before SCI decreased the harmful effects of hyperglycemia after SCI in both human and rat studies. Our findings suggest that chronic hyperglycemia before SCI may be a significant prognostic factor with a negative impact on functional and histologic outcomes, highlighting the importance of tight glucose control before SCI.

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Nanocarrier-mediated delivery of CORM-2 enhances functional recovery in a rat model of spinal cord injury

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Abstract
Spinal cord injury (SCI) is a devastating condition of the central nervous system, which may lead to permanent motor and sensory deficits. Carbon monoxide-releasing molecule-2 (CORM-2) has been reported for its anti-inflammatory, anti-apoptotic, and angiogenic properties. However, it has short CO release half-life (~1 min), limits its clinical utility. To overcome this hurdle, we have developed CORM-2 incorporated solid lipid nanoparticle (CORM-2-SLNs) and evaluated its ameliorating effects for preventing blood–spinal cord barrier (BSCB) disruption and endothelial cell death following SCI. After moderate compression injury (compression with 35 g impounder for 5 min) of rat spinal cord, the animals were treated with CORM-2-S and CORM-2-SLNs at an equal dose of 10 mg/kg via intraperitoneal injection for eight consecutive days. Behavior analysis was performed. Animals were sacrificed at different time points and evaluated for whether CORM-2-SLNs prevents BSCB disruption and rescues endothelial cells damage following SCI. CORM-2-SLNs-treated group showed significant reduction of the number of apoptotic cells as well as pro-inflammatory cytokines. Permeability and expression of Evans blue dye extravasation was significantly diminished, and reduced expression of tight junctions proteins following SCI was optimally rescued by CORM-2-SLNs. Likewise, reduced expression of neurotrophic factors after SCI was significantly increased, and the significantly diminished level of endothelial cell markers, rat endothelial cells antigen-1, angiopoietin-1 and platelet endothelial cell adhesion molecules after SCI was optimally stabilized at 21 days. Ultimate effect was significantly improved functional recovery, compared to CORM-2-S. These findings suggest that CORM-2-SLNs could be a potential agent to maintain BSCB integrity following SCI.

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Functional and molecular dissection of nociceptive amygdala

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Abstract
The central nucleus of the amygdala (CeA) is involved in autonomic and emotional responses to aversive stimuli including nociception. In addition, accumulating evidence has revealed that the CeA could change nociception sensitivity. The latero-capsular subdivision of the central amygdala is termed the “nociceptive amygdala” because CeA neurons receive nociceptive inputs from the spinal dorsal horn and trigeminal nucleus via the lateral parabrachial nucleus (LPB), and most of CeA neurons respond to noxious stimuli. It is demonstrated that LPB-CeA synaptic transmission is potentiated in various pain models. We previously reported, using selective optogenetic stimulation, that CeA neurons receive monosynaptic excitatory inputs from the LPB and polysynaptic inhibitory inputs through local CeA GABAergic neurons as well. These results suggest that inputs from the LPB could modulate characteristics of CeA local network and output neurons.¹ We also found that the excitatory synaptic transmission was enhanced in a subset of CeA neurons in inflammatory pain models. Based on this finding, we hypothesized that enhanced nociceptive inputs from the LPB would result in plastic changes in the CeA network and modulation of nociception sensitivity. To test this hypothesis, we used transgenic mice expressing Cre recombinase under calcitonin gene-related peptide (CGRP) promoters and selectively expressed channelrhodopsin2 in CGRP-positive LPB neurons, which are shown to project to the CeA. At four to eight weeks after virus injection, we made acute brain slices containing the CeA from systemic inflammatory model mice, which show hypersensitivity in their hindpaw. Light-evoked responses and membrane properties were recorded from the CeA neurons, and after the recording, slices were immunostained. Combining these results, we characterized the functional and molecular properties of CeA neurons. This attempt could lead to better understanding of how CeA regulates peripheral sensitivity in sustained pain status.

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Declined constitutive activity of mGluR5 in the periaqueductal gray perpetuates abnormal pain

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Abstract
Why and how the pain remains chronically even after recovery from the initial nerve injury is not completely understood. Pain sensation is powerfully modulated by signal processing in the brain, and pain becomes chronic with the dysfunction of the endogenous pain modulatory system. However, the underlying mechanisms are unclear. Here, we unravel the mechanisms underlying prolonged dysfunction of the brain which leads to chronic neuropathic pain. We found that the metabotropic glutamate receptor 5 (mGluR5) in the periaqueductal gray (PAG), the key area of endogenous pain modulation, is constitutively active in a normal condition to maintain an appropriate sensory perception. In the condition of a surge in nerve injury-induced excessive pain signals, the persistent mGluR5 activation was disrupted by Homer1a, an activity-dependently expressed immediate-early gene product. This homeostatic suspension was sufficient to shift the brain circuits from normal to pathological pain state. The decline of mGluR5 activity was associated with a profound reduction of excitability of PAG neurons. Remarkably a single-time blockage of the mGluR5 in the PAG resulted in chronic neuropathic pain-like symptoms even in the absence of the peripheral nerve injury. Studies focus attention on the maladaptive coping of the brain to the peripheral sensation as a basis for understanding and treating chronic pain.

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Brain mechanisms for cognitive evaluation and modulation of pain in humans

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Abstract
Pain is an inherently multi-faceted experience that includes sensory-discriminative, emotional, and motivational components. The perception of pain is variable across individuals, which depends on individuals’ cognitive state when they evaluate afferent nociceptive information, as well as their cognitive modulation of pain processing. By using functional magnetic resonance imaging, we revealed that there is a distinct signature for the encoding of a painful experience in the human brain, and this encoding process involves a strong affective component. Vigilance-related enhancement in the parieto-thalamic attention network allows the prefrontal cortex to estimate the relative intensity differences between noxious stimuli. Moreover, aversive prediction error-related networks interact with pain-processing circuits to underlie stimulus expectancy effects on pain. These cognitive evaluation and modulation mechanisms not only play an adaptive and protective role by providing sensory-discriminative information of painful stimuli for humans to cope with potentially life-threatening situations but also enhance neuroscientific knowledge about top-down cognitive modulation of nociception. They may also serve as the neural basis to decipher the neural mechanisms underpinning the cognitive dysfunctions associated with pain processing in patients with chronic pain in the future.

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Cancer itch: Mouse model establishment and neuronal mechanisms investigation

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Abstract
Chronic itch is a long-lasting and distressing sensation that elicits an intense desire to scratch. Notably, chronic itch can be associated with hematological malignancies such as cutaneous T cell lymphoma (CTCL), and nearly 90% of CTCL patients suffer from severe chronic itch which significantly impairs their quality of life. However, the mechanisms of CTCL-associated itch were few investigated due to the lack of clinically relevant animal models. We developed a chronic itch model of CTCL by intradermal inoculation of CD4+ Myla cells in immune-deficient mice. Our study shows that in this CTCL model, the mice not only show robust tumor growth but also exhibit remarkable ongoing chronic itch for more than two months. Thus, this model provides a powerful tool to investigate the mechanisms of malignancy-associated chronic itch. Our study found that the miR-711 released from malignant T-Cells could itch by directly binding and activating TRPA1, which is also the major component of CTCL-associated itch at the early stage. However, at the late stage of CTCL, itch development is associated with remarkable peripheral neuropathy, blocking miR-711 or downstream TRPA1 cannot attenuate CTCL-associated itch effectively any more. In contrast, blockade of large A-fibers with a combination of flagellin, an agonist of TLR5 with QX-314, or application of gabapentin, a clinical treatment for neuropathic pain, can effectively suppressed chronic itch in the late-phase of CTCL. These findings revealed the different mechanisms of CTCL-associated itch at early stage and later stage, which provide a new clue for drug development targeting CTCL-associated itch.

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Mesocortico-limbic system plays a key role in exercise-induced hypoalgesia: An experimental study

Emiko Senba

Abstract
We have previously shown that voluntary exercise induced exercise-induced hypoalgesia (EIH), which is due in part to the activation of dopamine neurons in the ventral tegmental area (VTA). Activation of nucleus accumbens (NAc) by dopaminergic input may hold the key to EIH. Glutamatergic neurons in the basolateral amygdala (BLA), medial prefrontal cortex (mPFC), and ventral hippocampus (vHipp) also project to the NAc and may contribute to EIH. These brain regions, including VTA, NAc, BLA, mPFC, and vHipp, are called “mesocortico-limbic system” and play a key role in emotional and motivational process in the brain. In this presentation, I will show that this system is deactivated in chronic pain state, while exercise can dramatically activate this system and contribute to EIH. Negative neurons in the BLA were activated by PSL, while positive neurons were activated by exercise and projected to the NAc to promote EIH. PSL activated GABA neurons and deactivated pyramidal neurons in the mPFC, all of which were reversed by exercise. Pyramidal neurons in the mPFC projecting to the NAc and PAG were activated by exercise. Chronic pain patients suffer dysfunction of mesocortico-limbic system because of fear for pain and negative emotion. Exercise and positive feeling can activate the emotional and motivational brain (mesocortico-limbic system) and may reduce chronic pain and suffering.

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Modeling of human peripheral neuropathy by using patient stem cell-derived congruent cell types

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Abstract
Patient-specific human-induced pluripotent stem cells (hiPSCs) hold great promise for disease modeling of genetic disorders; however, alternatives for validation with embryonic stem cells harboring the same disease mutation or utilizing another reprogramming approach from somatic cells of same patients have not yet been employed. Here, we report that a converged disease-relevant phenotype found in Charcot-Marie-Tooth 1A (CMT1A)-hiPSC-derived Schwann cells and two additional congruent CMT1A models using CMT1A-specific human embryonic stem cells (hESCs) and directly converted induced neural crest (iNC). We have devised a defined protocol for the direct derivation and prospective isolation of Schwann cells from hiPSCs, leading us to uncover cell-intrinsic mis-regulated immune signaling in Schwann cells derived from CMT1A-hiPSCs and CMT1A-PGD-hESCs (isolated from embryos with a Preimplantation Genetic Diagnosis of CMT1A), corroborated with Schwann cells of iNC (directly converted from CMT1A fibroblasts). Further confirmatory experiments demonstrated the upregulation of CXCL1 and MCP-1 expression, consistent with data from CMT1A patient nerve biopsies, and could be reversed by gene editing technology for inactivation of PMP22, a disease responsible gene. Our study illustrates the promise of applying hiPSC technology to one of the most common hereditary neuropathies for gaining new insights into human disease pathogenesis and potential treatment, and these results demonstrate the feasibility of verifying disease phenotypes by utilizing the malleability of cellular fates.

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Epigenetic regulations and chronic pain

Guang-Yin Xu

Abstract
To determine how DNA methylation and demethylation homeostasis of p2x7r induced by TET3 in spinal astrocytes contributes to visceral hypersensitivity in a rat model of irritable bowel syndrome, and how the process is reversed by Folic Acid. Visceral hypersensitivity was induced in rats by neonatal colonic inflammation (NCI) and identified by colorectal distention threshold. Methylation-specific polymerase chain reaction and bisulfite sequencing polymerase chain reaction were used to detect the methylation status of p2x7r promoter. The binding of transcription factors to p2x7r promoter was measured by chromatin immunoprecipitation assay and luciferase report gene assay. Patch clamp of spinal slice was used to record spinal synaptic transmission. NCI activates spinal astrocytes. Inhibition of astrocytes by fluorocitrate significantly attenuates visceral pain in adult rats. Ten-eleven translocation 3 (TET3) is upregulated in the spinal astrocytes, and the antagonist dimethylxalylglycine markedly inhibits spinal astrocytes activation and enhances the pain threshold of NCI rats. NCI also significantly upregulates P2X7R by TET3-mediated demethylation of p2x7r CpG island in association with enhanced transcription factor GATA1 binding. P2X7R antagonist A438079 treatment eliminates spinal astrocytes activation and visceral hypersensitivity in NCI rats. Furthermore, the injection of Folic Acid obviously reverses p2x7r promoter demethylation and decreases P2X7R expression. In addition, Folic Acid, A438079, or fluorocitrate treatment also significantly suppresses the frequency of spinal spontaneous excitatory post-synaptic current in NCI rats. These data demonstrate a novel mechanism of DNA methylation/demethylation homeostasis regulated by TET3 and Folic Acid on P2X7R expression in the spinal astrocytes, eventually leading to visceral pain in adult rats with NCI.

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Gut microbia contributes to morphine analgesic tolerance by promoting neuroinflammation in the dorsal root ganglia in mice

Ran Guo¹, Zhi-Hong Wang², Bing Wang², Li-Hua Chen³ and Tong Liu²,⁴

Abstract
Morphine analgesic tolerance remains a significant problem in the management of chronic pain. However, the precise mechanisms underlying morphine analgesic tolerance are still unclear. Recent studies documented that the dysregulation of microbiota–gut–brain axis plays a key role in many kinds of neurological diseases, including neurodegenerative diseases, depression, antinism, and chronic pain. Herein, we proposed a hypothesis that dysregulation of microbiota–gut–brain axis plays a key role in the development of morphine analgesic tolerance in mice. We first demonstrated that 16s rRNA sequencing showed that chronic morphine treatment significantly altered the gut microbial composition and induces preferential expansion of gram-positive communities. Hematoxylin and eosin staining demonstrated mucosal damage with chronic morphine exposure also increased gut permeability and bacterial translocation, which were prevented by propranolol treatment. Macrophage marker F4/80, inflammatory cytokines and chemokines increased in the dorsal root ganglia (DRG) in morphine tolerance mouse model. Gut bacteria depletion prevented chronic morphine treatment-induced upregulation of F4/80, inflammatory cytokines, and chemokine expression in mice. Macrophage depletion in vivo also prevented the development of morphine analgesic tolerance. Chronic morphine induced increase in neuronal hyperexcitability of DRG neurons, which can be partially reduced by macrophage depletion. TLR2 expression in the DRGs was increased in morphine tolerance mouse model. And the development of morphine analgesic tolerance was impaired in TLR2 knockout mice. Intrathecal injection of TLR2 agonist peptidoglycan accelerated the development of morphine analgesic tolerance. Intrathecal injection of TLR2 neutralizing antibody prevented morphine tolerance. Chronic morphine-induced upregulation of F4/80, inflammatory cytokines, and chemokines was abolished in TLR2 knockout mice. Chronic morphine treatment altered gut microbiota community, enhanced intestinal permeability, and promoted macrophage TLR2-mediated neuroinflammation in the DRGs. These mechanisms contributed to the development of morphine analgesic tolerance possible by inducing neuronal hyperexcitability of DRG neurons. Thus, targeting gut microbiota or neuroinflammation in the DRGs may represent a novel strategy for preventing morphine analgesic tolerance.

Keywords
morphine tolerance, gut microbiota, macrophage, TLR2, neuroinflammation

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TREK-1 and TRAAK are principal K⁺ channels at the nodes of Ranvier for rapid action potential conduction on mammalian myelinated afferent nerves

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Abstract
Rapid conduction of nerve impulses are critical in life and rely on action potential (AP) leaps through the nodes of Ranvier (NRs) along myelinated nerves. While NRs are the only sites where APs can be regenerated during nerve conduction on myelinated nerves, ion channel mechanisms underlying the regeneration and conduction of APs at mammalian NRs remain incompletely understood. In the present study, we show that TREK-1 and TRAAK, the thermosensitive and mechanosensitive two-pore domain potassium (K₂P) channels, are clustered at NRs of rat trigeminal Aβ-afferent nerves with density over 3000-fold higher than that on their somas. These K₂P channels, but not voltage-gated K⁺ channels as in other parts of nerves, are required for rapid AP repolarization at the NRs. Furthermore, these channels permit high-speed and high-frequency AP conduction along the myelinated afferent nerves, and loss of function of these channels at NRs retards nerve conduction and impairs sensory behavioral responses in animals.

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Spinal control of parasympathetic neuronal activity by nociceptive afferent fibers

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Abstract
C fibers, small unmyelinated afferent fibers having slower conduction velocity is essential mainly for cutaneous nociceptive responses also innervates within the visceral organs. They terminate in the superficial spinal dorsal horn and send nociceptive information to spinal dorsal horn neurons. Synaptic mechanisms for the spinal nociceptive transmission have been well-studied. However, how nociceptive afferent fibers modulate visceral organ functions is not fully understood. To address this issue, we developed in vivo patch and extracellular recording techniques to detect excitation of the parasympathetic preganglionic neurons in the lumbosacral spinal cord in combination with urinary bladder contraction monitoring and examined synaptic responses in the preganglionic neurons evoked by afferent fibers. Spinal parasympathetic preganglionic neurons in the lumbosacral spinal cord exhibited spontaneous excitatory postsynaptic currents and the synaptic responses were inhibited by CNQX. When a lumbosacral dorsal root of afferent fibers was stimulated, spinal preganglionic neurons evoked excitatory postsynaptic currents which could elicit action potentials, and the afferent fibers were classified into A and C afferent fibers based on their conduction velocities. In vivo extracellular recordings from the lumbosacral parasympathetic nucleus showed that spontaneous firing was detected in vivo with characteristic bursts of firing coinciding with the increases in intravesical pressure during micturition. In vivo whole-cell recordings from spinal parasympathetic preganglionic neurons also showed similar bursts of action potentials associated with the increased intravesical pressure and showed the characteristic morphological features of parasympathetic preganglionic neurons. The in vivo analyses also revealed that the C afferent fibers play an important role on setting the threshold for normal micturition reflex. Furthermore, frequent urination induced by inflammation can be inhibited by a blockade of the afferent conduction through the capsaicin-sensitive fibers. These findings indicate that spinal parasympathetic preganglionic neurons received glutamatergic synaptic inputs from slow-conducting afferent C fibers and spinal parasympathetic outflow from the neurons can be controlled by the feedforward loop mediated through capsaicin sensitive C fibers.

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The amygdala is a hub for pain-induced anxiety, depression, and chronic stress-induced exacerbation of neuropathic pain

Guo-Gang Xing¹, Hong Jiang¹, Lin Chen¹, Ling-Yu Liu¹, Ming-Jia Li¹ and Jie Cai¹

Abstract
The comorbidity between chronic pain and mood disorders, especially anxiety and depression disorders, is prevalent. However, the underlying mechanism is largely unknown. Using a rat model of spinal nerve ligation (SNL)-induced neuropathic pain, we proved that the amygdala plays an important role in neuropathic pain-related anxiety and depression following nerve injury. The results revealed that the loss of GABAergic inhibition is responsible for potentiated plasticity and sensitization of central nucleus of the amygdala (CeA) neurons, which likely underlie the enhanced output of amygdala and neuropathic pain-related anxiety in SNL rats, while long-term depression at the basal lateral amygdala (BLA)-CeA synapse mediated by AMPA receptor internalization underlies the neuropathic pain-related depression. Moreover, the amygdala is also involved in chronic stress-induced depression and stress-induced hyperalgesia (SIH) in rats. Activation of corticotropin-releasing factor (CRF)/CRF receptor type 1 (CRFR1) signaling in the BLA contributes to chronic forced swim stress (CFSS)-induced depressive-like behaviors in rats through potentiating synaptic efficiency at the external capsule (EC)-BLA pathway and sensitizing BLA neurons excitability. Exposure of CFSS to rats resulted in an increased activity of rostral anterior cingulate cortex (rACC) neuronal population, promoted the functional connectivity and the synchronization between rACC and BLA regions, and enhanced the pain-related neural information flow from rACC to BLA, thereby causing the pathogenesis of SIH. In addition, our data demonstrated that CFSS potentiates synaptic efficiency of the BLA-CeA pathway, leading to the activation of GluN2B-containing NMDA receptors and sensitization of CeA neurons, which subsequently facilitate pain-related synaptic plasticity of the parabrachial area-CeA pathway, thereby exacerbating nerve injury-induced neuropathic pain. In summary, our findings suggest that the amygdala is a hub for pain-induced anxiety, depression, and chronic stress-induced exacerbation of neuropathic pain.

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Roles of the insular cortex in the perception and modulation of pain

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Abstract
The insular cortex (IC) forms a distinct, but entirely hidden lobe, situated in the depth of the lateral fissure. Although the IC has been conventionally regarded as a gustatory cortex, it was recently reported that the IC may play a critical role in processing and modulating pain sensation. Numerous researches have suggested that the plasticity of pain-related brain areas contributes to chronic pain states. Recent evidences of clinical and animal studies suggest that the IC is related to the affective-motivational dimension of pain. Because, plastic changes in the IC after nerve injury induced the long-term changes at synaptic level. In order to prove the inhibition of plastic changes in the IC reduces behavioral sensitization caused by nerve injury, synaptogenetic changes were modulated and analyzed in the IC and the molecular mechanisms through glia-orchestrated the formation of synaptic circuits were investigated. The findings of synaptogenetic and morphological evidences suggest potential neurophysiological mechanism of pain modulation synapse in the IC that contributes to the transition from acute to chronic pain in the IC.

Funding
This work was supported by the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT, and Future Planning (NRF-2017R1A2B3005753 and 2019R1I1A1A01059697).

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The asymmetric passive and active roles of the central amygdala in the trigeminal inflammatory pain

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Abstract
Because the face and head is the primary interface to the approaching external world, sensations of these regions, mediated by trigeminal sensory systems, are directly linked with the emotional valence, particularly with aversive negative emotion to detect danger. As such, the novel finding that the trigeminal nociceptive afferents project directly to the lateral parabrachial nucleus (LPB) in the pons are of particular interest because LPB neurons then project directly to the central amygdala (CeA), a site for integration and memory of aversive sensations. Subcutaneous formalin injection results in latent inflammatory pain lasting for more than several days after initial nocifensive behaviors, which was accompanied by widespread sensitization, synaptic potentiation of the LPB-CeC synaptic transmission, and selective activation of the right CeA neurons. Manganese-enhanced magnetic resonance imaging combined with chemogenetic suppression of the right CeA significantly reduced the spontaneous activities in various limbic nuclei. Finally, chemogenetic suppression of the right, but not the left, CeA attenuated the widespread sensitization. Altogether, inflammation triggers asymmetric amygdala plasticity through activation of the LPB, leading to widespread hyperalgesia. These findings, together with recent lines of evidence, suggest that the central amygdala, especially that in the right, is a kernel regulating the intensity of the protective behaviors in response to the long-lasting painful/inflammatory situations.

Funding
Contributions by Mariko Sugimoto, Yuta Miyazawa, Yae K Sugimura, and Yukari Takahashi are acknowledged. Supported by MEXT Japan, Uehara Foundation and Naito Foundation.

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Anoctamins in pain and itch

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Abstract
Anoctamin 1 (ANO1) is a Ca2⁺-activated chloride channel activated by intracellular Ca2⁺ and that mediate numerous physiological functions. ANO1 has 10 isoforms in the family. ANO family has diverse functions. ANO1 and ANO2 is a Ca2⁺-activated chloride channel, whereas ANO6 is a scramblase that disrupts the polarized phospholipids. Surprisingly, ANO9 is a cation channel activated by intracellular cAMP. Recently, molecular structures of ANO1 are well characterized with CryoEM technique. In the present symposium, Anoctamins that are related to pain and itch will be introduced. ANO1 is highly expressed in small sensory neurons, suggesting a possible role in nociception. It colocalized largely with nociceptor markers. Surprisingly, ANO1 is activated by heat over 44°C, a temperature for thermal pain. Specific knock-out of ANO1 from DRG neurons, (Adv/Ano1fl/fl) mice, induces hypoalgesic effects over heat. In addition, ANO1 appears to mediate non-histaminergic itch. The majority of MrgprA3 positive dorsal root ganglion (DRG) neurons co-expressed with ANO1. Ano1-deficient (Adv/Ano1fl/fl) mice showed a significant reduction in scratching behaviors in response to non-histaminergic pruritogens like chloroquine (CQ) or SLIGRL injection, as well as dry-skin condition, but not to histamine injection. These pruritogens activate DRG neurons via ANO1 both in vitro and in vivo. In vivo calcium response of DRG evoked by CQ also disappeared when pruritogens were co-treated with MONNA. These results demonstrate that ANO1 mediates histamine-independent itch signaling in pruriceptors. Recently, we also found that ANO8 is a cation channel that is activated by intracellular Ca2+. Surprisingly, ANO8 is rich in DRG neurons and expressed highly in IB4⁺ neurons. Knock-down of ANO8 in DRG neurons induces hypoalgesic behaviors in mice. Thus, many of ANO channel family genes involved in nociception and itch.

Funding
Supported by the NRF of Korea (2011-0018358).

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Mosquitoes and TRP channels

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Abstract
Temperature and odors profoundly affect the behavior of animals. Transient receptor potential channel, subfamily A, member 1 (TRPA1) functions as a polymodal nociceptor for sensing both vital environmental cues in insects. Mosquitoes are recognized as disease vectors, and many efforts have been devoted to investigations of their host-seeking behaviors and repellents. However, the physiological characteristics of mosquito TRPA1 have not been systematically studied. We identified multiple alternative splice variants of the TrpA1 gene from Anopheles gambiae, Anopheles stephensi, Aedes aegypti, and Culex pipiens pallens mosquitoes. And we performed comparative analyses of the responses of mosquito TRPA1s to heat or chemical stimuli with calcium-imaging and whole-cell patch-clamp methods. Comparison of TRPA1 among four mosquito species from different thermal niches revealed that TRPA1 of Culex pipiens pallens inhabiting the temperate zone had a lower temperature threshold for heat-evoked activation, which was supported by the in vivo heat-avoidance test. Notably, the chemosensitivity of mosquito TRPA1 channels revealed differences not only between variants but also among species. Moreover, we discovered three novel mosquito TRPA1 agonists. Thermal niches selection and evolutionary trajectories significantly affect the functional properties of mosquito TRPA1, which represents a hallmark of the behaviors that may permit the design of improved mosquito control methods. I will also show the molecular mechanisms for the painless pierce by mosquitoes. Mosquitoes pierce using their fascicle, which is a bundle of coherently functioning six stylets. It has been presented that mosquitoes painlessly pierce using a combination of the numbing, the fascicle's serrated design, the vibratory actuation, and the graded and frequency-dependent mechanical properties of the labrum. Here, we discovered that saliva is also involved in the painless pierce and mosquito saliva contains a substance which inhibits TRPV1.

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Abstract

Transient receptor potential vanilloid subtype 1 (TRPV1) is a nonselective cationic channel activated by painful stimuli such as capsaicin and noxious heat, and enriched in sensory neurons of the pain pathway. During inflammation, chemical mediators activate protein kinases (such as PKC) that phosphorylate TRPV1 and thereby enhance its function, with consequent increases in nociceptor sensitization. However, the causal relationships between TRPV1 phosphorylation and pathological pain remain unexplored. To directly investigate the roles of one specific TRPV1 phosphorylation event in vivo, we genetically altered a major PKC phosphorylation site, mouse TRPV1 S801, to alanine. The TRPV1 expression pattern in sensory neurons of S801A knock-in (KI) mice was comparable to that in wild-type (WT) controls. However, sensitization of capsaicin-mediated currents following the activation of PKC was substantially impaired in sensory neurons from KI mice. Thermal hyperalgesia induced by phorbol myristate acetate (PMA) or burn injury in KI was identical to WT. Inflammatory thermal hyperalgesia was only marginally attenuated in KI mice. In contrast, PMA-evoked nocifensive responses and sensitization of capsaicin responses were significantly attenuated in the hindpaws of KI mice. Ongoing pain from inflamed masseter muscle was also reduced in KI mice and was further inhibited by the TRPV1 antagonist AMG9810. These results suggest that PKC-mediated phosphorylation of TRPV1 S801 contributes to inflammation-mediated sensitization of TRPV1 to ligand, but not heat, in vivo. Furthermore, this suggests that interference with TRPV1 S801 phosphorylation might represent one potential way to attenuate inflammatory pain, yet spare basal sensitivity and produce fewer side effects than more general TRPV1 inhibition.
 ASIC1b, an orphan ASIC subtype predominantly expressed in the somatosensory neurons

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Abstract
Acid-sensing ion channels (ASICs) are important acid sensors involved in neural modulation in the central nervous system and pain-associated tissue acidosis in the peripheral nervous system. In mammals, there are at least six ASIC subtypes expressing in the nervous system, including ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4. Among ASIC subtypes, ASIC1b is the most selectively expressed in peripheral sensory neurons. However, the role of ASIC1b is still elusive in terms of its functions and expression profile. Here, we probed the role of ASIC1b in sensory neurons related to acid-induced muscle pain. By generating ASIC1b-knockout mice, we demonstrated an important role for ASIC1b-containing channels in the induction and maintenance of acid-induced chronic mechanical hyperalgesia in a mouse model of fibromyalgia. ASIC1b knockout significantly attenuated the acid-induced hyperalgesia. Likewise, mambalin-1, an inhibitor of ASIC1b-containing channels, dose-dependently inhibited the acid-induced hyperalgesia in wild-type mice. Furthermore, we generated ASIC1b-Cre transgenic mice to examine the acid-induced currents and the co-location of ASIC1b with other ASIC subtypes in ASIC1b-expressing dorsal root ganglion neurons. We concluded that ASIC1b might form a wide variety of heteromeric channels, and the ASIC1b-containing heteromeric channels might be promising targets for the therapeutic treatment of acid-induced chronic muscle pain.

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Synaptic connectivity of substance P-, CGRP-, isolectin B4-immunopositive axon terminals in the rat medullary dorsal horn in the normal condition and following inflammation

Yong Chul Bae

Abstract
Information on the synaptic connectivity of the peptidergic and nonpeptidergic C afferents in the medullary dorsal horn (MDH) may help understand how orofacial nociception conveyed via these C afferents is transmitted to the first relay nucleus of the brain stem. In this study, we examined synaptic connectivity of the substance P (SP)-, calcitonin gene-related peptide (CGRP)-, isolectin B4 (IB4)-immunopositive (+) axon terminals (boutons) in the superficial lamina of the MDH in naïve rat and following CFA application into the rat vibrissa pad. Behavioral assay, serial section electron microscopy, immunocytochemistry, and quantitative analysis were performed for this study. In normal rats, numbers of postsynaptic dendrites were different among SP+, CGRP+, and IB4+ boutons. IB+ boutons frequently, but SP+ and CGRP+ boutons never, received axoaxonic synapse, implying presynaptic inhibition from GABAergic axon terminals. Density of C afferent boutons was significantly higher in CFA-group than control, suggesting the formation of new boutons following inflammation. Fraction of boutons forming synapse with dendritic spine of all SP+, CGRP, and IB4+ boutons was significantly higher in the CFA-group showing thermal hyperalgesia than control. Number of postsynaptic dendritic spine per a SP+, CGRP, and IB4+ boutons was also significantly higher in the CFA-group than control. Bouton volume, mitochondrial volume of SP+, CGRP+ and IB4+ boutons, and head volume and postsynaptic density size of their postsynaptic dendritic spine were significantly higher in the CFA-group than control. These findings suggest that nociceptive information conveyed via SP+, CGRP+, and IB4+ afferents is processed in a distinct manner, respectively, in the MDH and synapses associated with these boutons show structural plasticity under pathologic pain condition.

Keywords
peptidergic, non-peptidergic, axon terminal, synaptic connectivity, structural plasticity, inflammatory pain

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Role of non-neuronal cell activation in orofacial neuropathic pain

Koichi Iwata

Abstract
It is well documented that peripheral nerve injury or tissue inflammation causes a variety of changes in the peripheral (PNS) and central nervous systems (CNS). High-frequency nerve discharges are generated in the PNS and conveyed to the CNS following nerve injury or tissue inflammation, resulting in the various changes of molecular expression in the PNS and CNS. These molecular changes are thought to be involved in the hypersensitivity of the uninjured areas as well as injured or inflamed areas. However, many recent studies have reported that non-neuronal cells, such as glial cells and immune cells also have a role involved in neuropathic and inflamed hypersensitivity. We observed an extensive increase in the satellite glial cell activation in the trigeminal ganglion (TG) after inferior alveolar nerve transection (IANX) rats. Activated satellite glial cells release various molecules such as cytokines or nitric oxide. These molecules are involved in the enhancement of TG neuronal excitability. We also observed accumulation of macrophages in TG following IANX. These macrophages release various cytokines, and the excitability of TG neurons is further accelerated. Trigeminal spinal subnucleus caudalis (Vc) neurons and C1/C2 neurons receiving noxious inputs from the orofacial regions are strongly activated following IANX. Many astroglial cells and microglial cells are activated, and macrophages are accumulated in TG as well as the enhancement of Vc and C1/C2 neuronal activity. These glial cells and macrophages also release various molecules and cause further enhancement of the VC and C1/C2 neuronal activity, resulting in persistent orofacial pain. Finally, I will show you some possible drugs to treat orofacial neuropathic pain patients. In this symposium, I will address some pieces of evidence regarding neuron–non-neuronal cell communication under the pathological condition and discuss the involvement of non-neuronal cells in the orofacial persistent pain mechanism.

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Astrocytic L-lactate signaling facilitates cortex synchrony and decision-making in rats with chronic visceral pain

Ying Li¹,²

Abstract
Human brain imaging studies have revealed the anterior cingulate cortex (ACC) as a key brain region for mediating visceral pain–cognitive interactions. Recently, we characterized impairments of long-term potentiation and spike-field coherence in the basolateral amygdala (BLA)-ACC network in association with a decision-making deficit in rats with visceral hypersensitivity (VH). Now, by combining integrative neurobiological approaches, we show that ACC-reactive astrogliosis and activity-dependent impairment of lactate release occur in VH rats. Exogenous lactate supply rescues chronic-visceral pain-caused impairments of ACC phase locking and decision-making, which can be mimicked by optogenetic activation of ACC astrocytes. Large-scale electrophysiological recordings in free-moving animals during a decision-making task indicate that optogenetic astrocytic activation improves decision-making performance and engages ACC phase locking and BLA-to-ACC information flow. Collectively, these observations support the idea of an “astrocyte-neuron L-lactate shuttle” and suggest that targeting astrocytes may help with cognitive dysfunctions under chronic visceral pain.

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Microglial role in the pathological pain

Hidetoshi Tozaki-Saitoh

Abstract
Microglia, which are pathological effectors and amplifiers in the central nervous system, undergo various forms of activation. There is extensive evidence indicating activation of spinal microglia is a key event for the development of chronic pain after neuronal damage or dysfunction. However, phenotypic and functional change or significance of brain microglia in the chronic pain model was less understood. We have reported several transcription factors which contribute to microglial development have significant role in the activation of spinal microglia. Using CX3CR1-creERT2-mediated microglia selective conditional knockout mice, we investigated whether microglia were involved in the reserpine-induced pain model in which reserpine depletes amines in the nervous system inducing pain hypersensitivity that is used as a model of fibromyalgia. We found that depleting expression of microglial transcription factor, IRF8 and MafB, suppressed the reserpine-induced pain hypersensitivity. In immunohistochemical analysis, any apparent morphological activation was not observed in microglia in the spinal cord and the prefrontal cortex. However, analysis of single-cell transcriptome of prefrontal cortex microglia predicted some phenotypic change in the brain microglia in the reserpine-induced pain model mice. We also found that acute social stress-load induced relapse of pain hyper sensitivity and microglial reactivation. We propose that brain microglia are phenotypically altered in the reserpine-induced pain model and may regulate the development of pain hypersensitivity.

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Astrocyte-mediated synapse remodeling and neuropathic pain

Schuichi Koizumi

Abstract
Glial cells are very sensitive to environmental changes, and then, change their phenotypes into very different ones. As for astrocytes, they become “reactive astrocytes” and contribute to both beneficial and hazardous brain functions. Here, I show that metabotropic glutamate receptor 5 (mGluR5) in the reactive astrocytes in the primary somatosensory cortex is a switch of synapse remodeling and mechanical allodynia. We previously showed that partial sciatic nerve ligation (PSL) activated S1 astrocytes and upregulate mGluR5. mGluR5 is absent in the normal adult astrocytes but is upregulated in the pathological condition. We made astrocyte-specific mGluR5 conditional knockout mice (astro-mGluR5-cKO) and tested whether astrocytic mGluR5 is required for mechanical allodynia. Unlike wild-type control mice, PSL never upregulated mGluR5 in S1 astrocytes. In astro-mGluR5-cKO mice, PSL failed to increase Ca²⁺ signals, production of synaptogenic molecules in S1 astrocytes. PSL also did not cause synapse remodeling in the S1 cortical networks and mechanical allodynia. Thus, we concluded that mGluR5 in S1 astrocytes is a cause of and required for mechanical allodynia. It should be noted that the refractory allodynia could be controlled just by controlling one molecule of astrocytes, mGluR5. I will also talk about a new technique to manipulate S1 astrocytes, which is sufficient to induce synapse remodeling and mechanical allodynia.

Keywords
reactive astrocytes, mGluR5, neuropathic pain

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Potentiation of cortical transmission by calcitonin gene-related peptide in the anterior cingulate cortex

Min Zhuo$^{1,2,3}$

Abstract
The neuropeptide of calcitonin gene-related peptide (CGRP) plays critical roles in chronic pain, especially in migraine. Immunohistochemistry and in situ hybridization studies have shown that CGRP and its receptors are expressed in cortical areas including pain perception-related prefrontal anterior cingulate cortex. However, less information is available for the functional roles of CGRP in cortical regions such as the anterior cingulate cortex (ACC). In this talk, we used 64-electrode array field recording system to investigate the effect of CGRP on excitatory transmission in the ACC. We found that CGRP induced potentiation of synaptic transmission in a dose-dependently manner (1, 10, 50, and 100 nM). CGRP also recruited inactive circuit in the ACC. An application of the calcitonin receptor-like receptor antagonist CGRP8-37 blocked CGRP-induced chemical long-term potentiation and the recruitment of inactive channels. CGRP-induced long-term potentiation was also blocked by N-methyl-D-aspartate (NMDA) receptor antagonist AP-5. Consistently, the application of CGRP increased NMDA receptor-mediated excitatory postsynaptic currents. Finally, we found that CGRP-induced long-term potentiation required the activation of calcium-stimulated adenylyl cyclase subtype 1 (AC1) and protein kinase A. Genetic deletion of AC1 using AC1$^{-/-}$ mice, an AC1 inhibitor NB001 or a protein kinase A inhibitor KT5720, all reduced or blocked CGRP-induced potentiation. Our results provide direct evidence that CGRP may contribute to synaptic potentiation in important physiological and pathological conditions in the ACC, an AC1 inhibitor NB001 may be beneficial for the treatment of chronic headache.

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Reactivation of astrocyte in somatosensory cortex relieves peripheral hypersensitivity

Junichi Nabekura¹, Ikuko Takeda¹ and Kohei Yoshihara²

Abstract
In developmental phase of hypersensitivity after peripheral nerve injury, activation astrocyte rewires the neuronal circuits in the primary somatosensory cortex (S1) of mice and contributes to establishing pathological circuits. In this process, the spines existing before peripheral injury are preferentially replaced with those newly established after the injury. In the maintenance phase, the activity of astrocyte and neuronal plasticity has been reduced, which could be the underlying mechanism in long-term maintenance of pathological circuits.¹ This idea prompts us to challenge astrocyte at the chronic phase to re-introduce an increased plasticity in the S1 circuits to retrieve the pathological circuit. Reactivation of astrocyte in the maintenance phase of chronic pain model mice induced a relief of allostynia in the long-term aspect. This could be the new strategy for the treatment of chronic pain in the clinics.

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Noradrenergic modulation of cerebellar glial activity during nociception

Sang Jeong Kim

Abstract
The cerebellar activation during noxious stimulation and removal of the cerebellum resulting in the somatosensory dysfunction have been revealed in clinical studies. The cerebellum is a part of the pain matrix. However, how the cerebellum takes part in pain processing is still elusive. Here, using two-photon calcium imaging, we showed that Bergmann glia (BG) calcium response in the pain state was mediated by the alpha 1 adrenergic receptor (α1-AR). Interestingly, using the α1-AR genetically suppressed in the BG-specific manner, not only BG calcium response was completely blocked, but somatosensory cortex activity also reduced. Furthermore, capsaicin-induced paw licking behavior was reduced in the BG-specific α1-AR knockdown mice, indicating the BG calcium activity is related to pain behavior. Taken together, we suggest that the pain processing in the cerebellum maybe mediated by BG activity through glial adrenergic signaling and the cerebellum is actively involved in pain processing.

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Decoding of spontaneous pain information from cortical neuronal calcium signals in awake mice with machine learning

Sun Kwang Kim¹

Abstract
Various animal models of pain have been developed for studying its basic mechanisms and evaluating the analgesic efficacies of drugs. Generally, behavioral responses (e.g., licking and withdrawal) have been measured as the quantitative endpoint of pain in those animal models. In such conventional behavioral response paradigms, however, an objective measurement of spontaneous ongoing pain during chronic neuropathic pain, headache, motor impairment, or other CNS diseases is not possible. The primary somatosensory (S1) cortex plays an important role in the perception and discrimination of pain sensation. In the present study, we hypothesized that neuronal activity patterns in the mouse S1 cortex are distinct between pain and non-pain conditions, and that this discrepancy can be used for measuring spontaneous pain and then evaluating the analgesic efficacies of pain killers. To explore this hypothesis, we performed in vivo two-photon calcium imaging in the S1 cortex of awake, head-fixed mice with or without formalin-induced spontaneous pain. We also applied a machine learning to decode spontaneous pain information from the recorded neuronal calcium activity patterns. In this talk, I will explain and discuss in detail what the issues of conventional analyses were and how to achieve the goal of this study.

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The potential role of gene therapy for the treatment of neuropathic pain

Jin Woo Chang

Abstract
Pain is a survival mechanism that serves as a warning sign of ongoing or impending tissue damage. However, neuropathic pain can develop after nerve injury, when deleterious changes occur in injured neurons and along nociceptive and descending modulatory pathways in the central nervous system. Various causes of neuropathic pain have been described, and it is well known that this variability of cause, complexity of symptoms, and pathogenesis make the treatment hard. Current pharmacological, surgical, or interventional modalities available for controlling neuropathic pain showed variable efficacy. In addition, other problems with current therapy include intolerable adverse effects along with the treatment. Recently, emerging modalities, especially gene therapy, have shown potential promise in the management of neuropathic pain. However, there are several aspects that need to be clarified regarding their use including mechanism of action, specific advantages and disadvantages, as well as their practicality for general clinical use. Gene therapy certainly has provided treatment options for diseases that are beyond the reach of traditional approaches. However, still now, only few medical disease, such as spinal muscular atrophy, hemophilia A or B, lipoprotein lipase deficiency, inherited retinal dystrophy, were approved from either U. S. Food and Drug Administration or European Medicines Agency. We underwent multiple studies since 2005, with using recombinant adeno-associated viral vector to deliver GAD 65/67 or GCH1 to relieve pain behavior on the peripheral or central neuropathic pain model. GAD was selected for the strategy of “gain of function” which could release the GABA, and GCH1 was selected for the strategy of “loss of function” to down-regulate GCH1. Also the route for the delivery and direct injection to the peripheral nerve, dorsal root ganglion, or spinal cord were investigated to optimize the benefit. In this session, I will briefly introduce my works of gene therapy for neuropathic pain and also will review the literature regarding current state of gene therapy for the treatment of neuropathic pain.

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The pathomechanism of neuropathic low back pain

Joo Han Kim¹

Abstract
Neuropathic low back pain is the pain arising from nerve roots that innervate the spine and pathological invasive innervation of the degenerative intervertebral discs. Although there are many studies in the literature, it is not well known the successful treatment of this pain due to vague mechanism. Therefore, the author would like to present the pathomechanism of discogenic neuropathic pain according to literature and my own studies. Under normal conditions, the outer layers of the annulus fibrosus (AF) are innervated by sensory nerve endings from the dorsal root ganglion, whereas in patients with discogenic low back pain, sensory nerve endings, and blood vessels are frequently observed in the inner layer of the AF and the nucleus pulposus. Such nerve ingrowth and neovascularization is thought to be one of the major sources of discogenic low back pain and can occur as a consequence of the local production of angiogenic and neurogenic factors in annular injury. Although all of discs are removed by surgical method, the neuropathic low back pain cannot be disappeared by the remodeling of dorsal root ganglion, injury of spinal cord, or somatization. Therefore, I consider that intervertebral disc is involved in the inflammatory reaction and the interactions related to nerve ingrowth and neovascularization, and treatment of nerve transmission is the next step for the control of the neuropathic low back pain.

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Biological approaches to treating chronic low back pain

Inbo Han¹

Abstract
Intervertebral disc (IVD) degeneration is a common, chronic, and complex degeneration process that frequently leads to back pain and disability, resulting in a major public health issue. Since the exact mechanisms underlying IVD degeneration have not yet been fully elucidated and conservative managements appear to be mostly ineffective, current surgical treatment focuses on removal of the pathological disc tissues combined with spinal fusion. The treatment options, however, often produce insufficient efficacy and even serious complications. Therefore, there has been a growing demand and endeavors for developing novel regenerative biology-guided strategies for repairing the IVD via delivery of exogenous growth factors, introduction of therapeutic genes, and transplantation of stem cells or combinations. Mesenchymal stem cells (MSCs) have been considered to hold promise for treating IVD degeneration. However, the different therapeutic efficacy of MSC has been a major problem and so far the derivation of MSCs for use in IVD degeneration has not been optimized. To overcome hurdle of stem cell therapy for IVD degeneration, we have conducted in vitro, pre-clinical, and clinical study using MSCs and describe key strategies for treating IVD degeneration.

Keywords
intervertebral disc degeneration, mesenchymal stem cell

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Current status of spinal cord stimulation

Byung-Chul Son

Abstract
Spinal cord stimulation has been reported to be superior to conservative medical management and reoperation when dealing with pain from failed back surgery syndrome. It has also demonstrated clinical benefit in complex regional pain syndrome, critical limb ischemia, and refractory angina pectoris. Furthermore, several cost analysis studies have demonstrated that spinal cord stimulation is cost effective for these approved conditions. Despite the lack of a comprehensive mechanism, the technology and the complexity in which spinal cord stimulation is being utilized is growing. Newer devices are targeting axial low back pain and foot pain, areas that have been reported to be more difficult to treat with traditional spinal cord stimulation. Percutaneous hybrid paddle leads, peripheral nerve field stimulation, nerve root stimulation, dorsal root ganglion, and high frequency stimulation are actively being refined to address axial low back pain and foot pain. High-frequency stimulation is unique in that it provides paresthesia free analgesia by stimulating beyond the physiologic frequency range. The preliminary results have been mixed and a large randomized control trial is underway to evaluate the future of this technology. Other emerging technologies, including dorsal root ganglion stimulation and hybrid leads, also show some promising preliminary results in non-randomized observational trials. In addition, the role of paddle lead spinal cord stimulation and burst stimulation will be discussed. Spinal cord stimulation has demonstrated clinical efficacy in randomized control trials for the approved indications. In addition, several open label observational studies on peripheral nerve field stimulation, hybrid leads, dorsal root ganglion stimulation, and high-frequency stimulation show some promising results. However, large randomized control trials demonstrating clear clinical benefit are needed to gain evidence-based support for their use.

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Significant therapeutic effects of adult human multipotent neural cells on spinal cord injury

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Abstract
For the successful clinical application of regenerative treatment to spinal cord injury (SCI), safe and ethical stem cells with proved treatment effects and therapeutic mechanisms are required. In this study, we preclinically demonstrated the significant treatment effects and pro-angiogenic paracrine mechanisms of adult human multipotent stem cells (ahMNCs) on SCI. Upon ahMNCs were cultured from voluntarily donated human surgical samples and they did not show any noteworthy preclinical side effects including tumor formation, ahMNCs could be a clinically applicable stem cell therapeutics for SCI patients who have no alternative regenerative treatment modalities.

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Nociplastic pain

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Abstract
“Nociplastic pain” is a new term recently defined by the International Association for the Study of Pain (IASP) as “pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain.”¹ We support the use of this term, “nociplastic pain,” because there are many clinical pain conditions that can be categorized under this term, and the term gives mechanistic clues to many painful conditions that arise from altered nociceptive functions. To study mechanisms underlying nociplastic pain, our lab recently developed an experimental procedure that can transition acute pain into a long-lasting nociplastic pain using a mouse capsaicin model. Specifically, intradermal injection of capsaicin in a mouse causes well-known pain behaviors for a short duration (less than one day). However, when capsaicin injection is followed, 2 h later, by an intermittent brief series of innocuous stimuli (10 min of intermittent vibration or warmth), the pain behaviors last for more than two weeks without clear evidence of tissue damage in the previously capsaicin-injected site. We believe that this is a good model to study the transition of acute pain into persistent or chronic nociplastic pain. In addition, although this nociplastic pain model can be induced in both sexes of mice, there is a clear sexual dimorphism in underlying mechanisms. Specifically, the persistent nociplastic pain in female mice is found to be maintained by continuous peripheral input from the previously capsaicin-injected (original injury) site, whereas the nociplastic pain in male mice is independent of such peripheral input. Experiments to determine the detailed mechanisms of nociplastic pain in both female and male mice are under way in our lab, some of which will be discussed in this presentation.

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The mechanism of spinal cord stimulation in animal model of neuropathic pain

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Abstract
Spinal cord stimulation (SCS) has been shown to be effective in the management of certain neuropathic pain conditions; however, the underlying mechanisms are incompletely understood. Our study showed that the endocannabinoid system, and in particular the CB1 R, plays a pivotal role in the long-lasting and incremental reversal of hyperalgesia induced by repetitive SCS in a peripheral neuropathic pain model. Our another study demonstrated that spinal progenitor cells can be activated by SCS via descending pathways to relieve spinal cord injury-induced neuropathic pain. Further research direction in SCS using neuropathic pain animal model will also be discussed.

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Spinal cord stimulation for managing chronic neuropathic pain

Billy Huh

Abstract
Neuropathic pain is a particular type of pain that is characterized by distinctive symptoms due to damage or dysfunction to the nervous system. Neuropathic pain is challenging to manage with symptoms that persist longer than normal tissue healing of an underlying disease. Spinal cord stimulation (SCS) is used successfully in neuropathic pain. Neuropathic pain affects up to 8% of the population. It is responsible for 30% to 65% of patients seen at pain clinics. The pathophysiology is poorly understood. In severe cases, the quality of life is rated worse than other pain conditions such as heart failure or cancer. The most common indication for SCS in North America is chronic intractable neuropathic pain due to failed back surgery syndrome. Neuropathic back and leg pain can be successfully managed, but the associated back pain may have both neuropathic and nociceptive component, which can be more difficult to treat. Neuropathic pain due to complex regional pain syndrome, which can develop in the distal aspect of a limb, from a minor injury, is another common indication. To a lesser extent, postherpetic neuralgia is also managed with SCS. For cancer survivors, chemotherapy-induced peripheral neuropathy (CIPN) can be debilitating, and SCS has been successfully used for the refractory CIPN. SCS is considered as a pain management therapy only after conventional pain therapies, including pharmacological, nonpharmacological, and surgical treatments, have been tried and have failed. A systematic review of the literature supports the effectiveness of traditional tonic SCS to decrease pain in various neuropathic pain syndromes. However, the quality of the evidence varied from weak to moderate—level 2 evidence for reducing pain, and level 3a evidence for improving functional status and quality of life. Hence, SCS may be considered for patients with chronic, neuropathic pain for whom standard pain treatments have failed, and when there is no indication for surgical intervention to treat the underlying condition. The more recently introduced Burst, dorsal root ganglion (DRG), and high-frequency stimulation has shown promise in clinical practice. Burst stimulation consists of intermittent packets of closely spaced 40-Hz burst stimulation with five spikes at 500 Hz per burst, with a pulse width of 100 msec. In contrast, traditional SCS is delivered in constant or tonic stimulation, at a pulse width of 300 to 500 msec. In a prospective, randomized, double-blind, placebo-controlled study on the effectiveness of burst stimulation in 20 patients with failed back surgery syndrome. For the burst stimulation treatment group, mean numeric rating scale and Short-Form McGill Pain Questionnaire scores were significantly decreased compared with the other treatment groups; these scores did not differ considerably between 500-Hz tonic stimulation and placebo stimulation groups. Hence the advantages of the burst are no paresthesia and possibly superior analgesia. Moreover, the burst stimulation suggests that the quality of the stimulation is improved, a broader area is covered, and the strength of stimulation is not subject to positional variability. The neuronal cells within the DRG are actively involved in signaling in both the central and peripheral nervous systems. Various neuroactive chemicals may be released as a result of nerve injury or inflammation. Studies have shown that the DRG plays an active role in both neuropathic and chronic pain via central sensitization, and electrical stimulation of the DRG may alter its ion channel gene expression. The changes in the DRG caused by nerve injury may be restored to normal via electrical stimulation. The one-year follow-up study on human subjects with back, neck, or foot pain showed that DRG stimulation is comparable to traditional SCS regarding pain relief. The benefits of DRG stimulation include the ability to achieve precise pain–paresthesia

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concordance, including in regions that are typically difficult to target with SCS, and consistently maintain the coverage over time independent of movement or body position. Historically, one of the concerns with SCS is the changes in the stimulation intensity resulting from patient movement. Since the cerebrospinal fluid (CSF) surrounding the spinal cord, any movement by the patient can cause the SCS electrode to alter its proximity to the spinal cord. There is no CSF surrounding the DRG, and stimulation is not subject to the patient's positional changes and can be used to target specific painful dermatomes. These options have never been seen before, and the results were seen in Europe and our own experience with DRG stimulation on a patient with refractory chemotherapy-induced peripheral neuropathy, pelvic, and inguinal pain have been very promising, especially after failing to respond to all pharmacotherapies as well as traditional tonic SCS. In a multicenter, randomized, controlled study comparing high-frequency (HF-10) and traditional low-frequency spinal cord stimulation for the treatment of chronic back and leg pain, more subjects responded to HF10 therapy than traditional SCS (back pain: 76.5% vs. 49.3%; \( P < .001 \); leg pain: 72.9% vs. 49.3% \( P = .003 \)). Also at 24 months, back pain decreased to a greater degree with HF10 therapy (66.9% vs. 31.8%) than traditional SCS (41.1% vs. 36.8%, \( P < .001 \) for non-inferiority and superiority). Leg pain also decreased to a greater degree with HF10 therapy (65.1% vs. 36.0%) than traditional SCS (46.0% vs. 40.4%, \( P < .001 \) for non-inferiority and \( P = .002 \) for superiority). Neuropathic pain is a common health-care problem with some patients who are refractory to standard treatment. Spinal cord stimulation offers a cost-effective treatment with improved long-term outcomes in such patients. Technological advances and increased understanding of the therapy have resulted in better and more reliable SCS therapies.

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Motor cortex stimulation for managing chronic neuropathic pain

Koichi Hosomi¹,² and Youichi Saitoh¹,²

Abstract

Invasive electrical motor cortex stimulation (EMCS) for neuropathic pain was developed by neurosurgeons at Nihon University (Tokyo, Japan) in the early 1990s. Initially, they applied EMCS to post-stroke pain, then patients with other neuropathic pain underwent EMCS. Subdural or epidural electrodes are implanted over the primary motor cortex (M1) via a small craniotomy or burr hole, followed by subcutaneous implantation of a pulse generator, as with the deep brain stimulation. Systematic reviews of EMCS reported that the success rate was approximately 50%. In our case series of 39 patients with EMCS, 15 patients (38%) reported a reduction of at least 30% in pain scores during follow-up, and peripheral neuropathic pain tended to respond better to EMCS than did central neuropathic pain. Recently, few cases, however, undergo EMCS in Japan because patients prefer less- or non-invasive treatments. Repetitive transcranial magnetic stimulation (rTMS) of M1 for treating neuropathic pain emerged from experience with EMCS. We introduced rTMS with a navigation system in 2003 and studied optimal target regions, frequency of stimulation, and duration of effect in patients with neuropathic pain. According to the results from these preliminary studies, we conducted two multicenter blinded randomized controlled trials to assess the safety and efficacy of daily sessions of 5 Hz-rTMS of M1. The first pilot crossover trial succeeded to show that 10 daily 5 Hz-rTMS transiently provided modest pain relief in neuropathic pain patients. However, the second pivotal parallel trial failed to prove that five daily sessions of 5 Hz-rTMS was effective in the primary outcome which was a short-term decrease in a visual analogue scale of pain intensity. The negative results could be caused by the suboptimal stimulus procedure. The dose of stimulation was insufficient, and the duration of treatment period was not enough long. Actually, responders to daily rTMS could benefit from additional long-term weekly sessions in the trial. Based on results from these previous clinical trials, we are investigating the optimal stimulation conditions of rTMS to aim for regulatory approval of the neuropathic pain treatment. rTMS could be established as neuromodulation therapies for clinical practice according to improvement of the treatment protocol.
Intrathecal analgesia for managing chronic pain

Yong-Chul Kim

Abstract
Opioids produce a profound inhibition of the evoked discharge of spinal nociceptive neurons, thereby inhibiting the transmission of pain. Intrathecally administered opioids have no effect on the motor function and autonomic responses. Analgesic effect of opioids is dose dependent and antagonized by naloxone. Technical development in the drug delivery system to the intrathecal area more regularly makes possible intrathecal implantations. Indications of the intrathecal analgesia for managing chronic pain are chronic, intractable pain of malignant and/or benign origin who responsive to opioids: cancer pain, chronic non-malignant pain, opioid responsive during screening trial, no difficulty of catheter advance in the subarachnoid space, inability to control pain with systemic opioids, life expectancy more than three months, opioid responsive during screening trial, more conservative treatments have failed, further surgical intervention is not indicated, no untreated drug addiction, and no compensatory need on psychological evaluations. Recently, baclofen, chemotherapeutic agents, or antibiotics are also used. Contraindication of this treatments are systemic infection, pump cannot be implanted <2.5 cm from the surface of the skin, known allergies to the materials or medications, drug abuse, etc. The procedure of implantation, setting of the pump, clinical efficacy, and complications will be discussed during the lecture.
Drug development strategies of Nav1.7 blocker

Sung-Young Kim

Abstract
Chronic pain represents a critical unmet medical need that afflicts over one hundred million Americans with an extremely high economic cost. But current available therapies lack robust efficacy, carry significant abuse potential, and/or suffer from low tolerability and safety. Nav1.7 controls the passage of sodium ions into sensory neurons, and the target garnered attention after it was found that rare human mutations characterized by a loss of function in Nav1.7 channels resulted in congenital insensitivity to pain. Despite genetic validation, a selective Nav1.7 blocker PF-05089771 failed to demonstrate efficacy in clinical trials. PF-05089771 is a potent and selective Nav1.7 blocker, but it lacks enough target engagement to exhibit efficacy. Looking at current developments, there are various strategies to target Nav1.7 including small molecule, biologics. In this presentation, I will introduce strategies of various Nav1.7 blocker for pain drug development.

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VM202, a DNA-based potential disease-modifying treatment for painful diabetic peripheral neuropathy

Seung Shin Yu

Abstract
A novel concept medicine targeting neuropathy has been developed using gene therapy technologies. The drug, VM202, is a plasmid DNA engineered to express human hepatocyte growth factor when introduced into the body through intramuscular injection. The Phase 3 clinical trial on painful diabetic neuropathy patients has recently been completed in the U.S. This trial consisted of two independent studies, 3–1 with 500 subjects and 3–1B with 101 subjects, with the aim of collecting safety and efficacy data at 9 months and 12 months, respectively. In this trial, VM202 was administered in two rounds of injections at a two-week interval (total 8 mg/round), and this treatment cycle was repeated three months later (i.e., a total of four rounds of injections: on day 0, day 14, day 90, and day 104). VM202 was confirmed to be very safe in both studies. In the 3–1 study results, several anomalies were observed in the pharmacokinetic data, and the cause is currently under investigation. However, the drug's efficacy was statistically meaningful in the Adjusted ITT group which excluded data from patients with obvious errors. In the 3-1B ITT group (N = 101), the pain reduction effect on the VM202 treatment group demonstrated a statistically meaningful difference compared to that on the Placebo group, at 6 months, 9 months, and 12 months. In particular, the effect was higher in the patient group not taking gabapentin or pregabalin, the most commonly used diabetic neuropathy drugs (N = 53). VM202's pain-relieving effect persisted for at least eight months after the drug had completely disappeared in the body. This, along with results from previous preclinical and clinical studies, strongly suggests that VM202 may be a “regenerative medicine” that can regenerate nerves and fundamentally treat painful neuropathy.

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Therapeutic approach for intractable pain: Potential application of transforaminal epidural gene therapy

Daewook Kim¹, Kyung-Ran Kim¹, Yejin Kwon¹, Minjung Kim¹, Min-Ju Kim¹, Yeomoon Sim¹, Hyelin Ji¹, Jang-Joon Park¹, Jong-Ho Cho¹, Heonsik Choi¹ and Sujeong Kim¹

Abstract
Neuropathic pain is a chronic pain state characterized by nerve damage, inflammation, and nociceptive neuron hyperactivity. As the underlying pathophysiology is complex, targeting multiple elements would be a better approach to effective therapy for neuropathic pain. Here, we generated recombinant adeno-associated viruses encoding three therapeutic genes, namely, glutamate decarboxylase, glial cell-derived neurotrophic factor, and interleukin 10, with various combinations. And the efficacy for pain relief was evaluated in a rat spared nerve injury model of neuropathic pain. The maximal analgesic effect was achieved when the adeno-associated virus expressing all three genes was administered into a rat pain model. The combination of two virus constructs expressing the three targets was named KLS-2031 and evaluated as a potential novel therapeutic for neuropathic pain. Single transforaminal epidural injections of KLS-2031 into the intervertebral foramen to target the appropriate dorsal root ganglion produced notable long-term analgesic effects in female and male rats. Furthermore, KLS-2031 mitigated the neuroinflammation, neuronal cell death, and dorsal root ganglion hyperexcitability induced by the spared nerve injury. These results suggest that KLS-2031 represents a promising therapeutic option for refractory neuropathic pain.
Neuron types and their changes during the development of neuropathic pain in primary sensory neurons

Xu Zhang¹,²

Abstract
Peripheral neuropathic pain is a typical phenotype of peripheral nerve injury, which induces noticeable gene regulations in dorsal root ganglion (DRG). The changes of gene profiles in DRG contribute to the development and maintenance of neuropathic pain. Recently, gene profiles of single DRG neurons have been identified by single-cell RNA sequencing (scRNA-seq). To identify the gene expression changes in single DRG neurons induced by peripheral neuropathic pain, we carried out 10X genomics scRNA-seq on dissociated DRG cells of mice with spared nerve injury (SNI) at different time. We revealed three novel neuron clusters induced by SNI, which were characterized by the expression of Atf3, a typical injury marker of DRG neurons. Furthermore, we identified gene expression changes within novel clusters. The upregulated genes were functioned in synapse organization, ion transport and inflammatory response induced by cytokines. These cytokines including Csf1 and Il1b have been reported to play vital roles in neuropathic pain behavior. Finally, we identified the gene regulatory network underlying gene regulations among different DRG neuron clusters. We showed that Atf3 and Crem could be key transcription factors underlying Gal upregulation after SNI while Cpeb1 contributed to Mrgprd downregulation in the neuron-type specific way. In conclusion, our results provided a framework for understanding the mechanisms underlying peripheral neuropathic pain.

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Spontaneous high-current spikes in thalamocingulate pathways signaling normal and pathological pain states

Bai Chuang Shyu

Abstract
Prominent 7–12 Hz oscillations in frontal cortical networks in rats have been reported. However, the mechanism of generation and the physiological function of this brain rhythm have not yet been clarified. Multi-channel extracellular field potentials of the anterior cingulate cortex (ACC) were recorded and analyzed using the current source density method in halothane-anesthetized rats. Spontaneous high-current spikes (HCSs) were localized in the deep part of layer II/III and upper part of layer V of the ACC. The frequency of HCSs in the ACC was 7–12 Hz, with an amplitude of $6.5 \pm 0.76 \text{ mV/mm}^2$ and duration of $55.24 \pm 2.43 \text{ ms}$. The power density significantly decreased ($84.56\% \pm 6.93\%$, $p < 0.05$, t-test) after pinching the hindpaw and significantly increased ($149.28\% \pm 15.96\%$) after treatment with morphine. The suppressive effect of pinching was reversed by naloxone (0.7 mg/kg, i.p.). HCSs coincided with initiation of the depolarization of cingulate neurons and remained in a depolarized upstate. The occurrence of cingulate HCSs was persistently preceded by a hyperpolarization phase and a burst of multiunit spike activity in the medial dorsal thalamic nucleus (MD). Spontaneous field-potential oscillations changed from 10 Hz to a lower band (i.e., $\sim 7.5 \text{ Hz}$) when a central poststroke pain (CPSP) condition was induced. The CPSP group had a higher average coherence coefficient compared with the control group. Our results indicate that spontaneous cingulate cortical HCSs could be initiated by thalamocortical synaptic inputs from the MD and maintained by intracortical neuronal upstate mechanisms in physiological and pathological pain states.

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Electro and other neuro-behavioral assessment of chronic intractable neuropathic pain

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Abstract
Chronic pain develops due to psychosocial factors in addition to physical problems, and is often exacerbated and maintained by the patient’s pain behavior. In a typical example, pain is causing anxiety, scariness, excessive rest, mental depression, and worsening pain, called the Fear Avoidance Model. To escape and recover from this vicious circle of pain requires improvement of anxiety by understanding the pathology, but it is also important that the body has no red flag or critical neurological dysfunction. We evaluated the somatosensory-evoked potential method using Intraepidermal Electrode (hereinafter referred to as IE) for the purpose of excluding significant neurological abnormalities and detecting pain neuronal signaling abnormalities related to maintaining the onset of chronic pain. IE can selectively stimulate each type of nerve by changing various stimulation modes such as Ab fiber stimulation, Ad fiber stimulation, and C fiber stimulation. In this presentation, we show the results of multimode somatosensory-evoked potentials using IE in healthy subjects, and the application and analysis results for cases such as Wallenberg syndrome.
Application of botulinum toxin type A in chronic pain in dentistry

Jo-Young Son¹, Jin-Sook Ju¹ and Dong Kuk Ahn¹

Abstract
Botulinum toxin is a potent neurotoxin produced by the bacterium Clostridium botulinum, which acts by blocking acetylcholine release at the neuromuscular junction. Recent data support the evidences for the use of botulinum toxin type A (BoNT-A) in treatment of several painful states. In the present study, we investigated the anti-nociceptive effects of BoNT-A in a rat model of inflammatory and neuropathic pain in the orofacial area. Experiments were carried out in male Sprague-Dawley rats. Orofacial formalin-induced pain responses and CFA-induced thermal hypersensitivity were observed as an inflammatory pain model. We also examined mechanical allodynia in rat models of trigeminal neuropathic pain and trigeminal neuralgia. Subcutaneous injection of BoNT-A produced significant suppression of formalin-induced nociceptive behavior and CFA-induced thermal hyperalgesia. Intracisternal injection of BoNT-A also produced significant antinociceptive behavior in same animal models. A single injection of 3 U/kg BoNT-A produced prolonged anti-allodynic effects in rats with inferior alveolar nerve injury. Double treatments with low dose of 1 U/kg BoNT-A produced prolonged anti-allodynic effects compared with single treatments. Besides, treatment with BoNT-A on postoperative days 7 and 12, when pain had already been established, also produced prolonged anti-allodynic effects. Although nerve injury increased the levels of Nav1.6, 1.7, 1.8 expression in the trigeminal ganglion on POD 3, subcutaneous injection of BoNT-A down-regulated only Nav1.7 expression. Peripheral administration of BoNT-A produced anti-allodynic effects in a rat model with trigeminal neuralgia by compression of the trigeminal nerve root. This compression of the trigeminal nerve root increased the release of cytokines from the trigeminal ganglion. Peripheral administration of BoNT-A suppressed the cytokine levels in the rats with compression of the trigeminal nerve root. In summary, subcutaneous injection of BoNT-A produced prolonged anti-nociception in orofacial chronic pain. Therefore, BoNT-A is a potential new therapeutic target for chronic pain control in the orofacial area.

Funding
This research was supported by NRF-2017R1A5A2015391 and NRF-2018R1D1A1B07049025. We are grateful to Hugel Inc. (Chuncheon, Republic of Korea) for providing Botulax®.

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