Abstracts of the 7th Asian Pain Symposium

Jianguo G. Gu1, Min Zhuo2, Makoto Tominaga3, Xu Zhang4, Fusao Kato5, Seog Bae OH6 and Bai Chuang Shyu7

Summary

The Asian Pain Symposium (APS) is a main pain research meeting in Asia. Since established in 2000 in Kyoto, five other APSs have been held in different Asian regions including Seoul of Korea in 2004, Fukuoka of Japan in 2008, Shanghai of China in 2011, Okazaki of Japan in 2013, and Suzhou of China in 2015. The 7th Asian Pain Symposium (APS 2017) was held in Taipei of Taiwan during October 26th to October 29th, 2017. The APS 2017 was sponsored by The Ministry of Science and Technology of Taiwan and Institute of Biomedical Science and Neuroscience Program of Academia Sinica and Taiwan Pain Society. The president of the APS 2017 was Dr. Bai Chuang Shyu, Institute of Biomedical Sciences, Academia Sinica, Taiwan. Local organizing committee also include Dr. Jen-Chuen Hsieh, Institute of Brain Science, National Yang-Ming University and Veteran General Hospital, Taiwan, Dr. Wei-Zen Sun, Department of Anesthesiology, National Taiwan University Hospital, Taiwan, and Dr. Chih-Cheng Chen, Institute of Biomedical Sciences, Academia Sinica, Taiwan. Main topics of the APS 2017 included the latest progress of pain research and novel strategies of pain treatments. Symposium attendees presented their interesting and exciting research findings in the areas of 1) basic sensory and nociceptive functions, 2) ion channels and their functions in somatosensory physiology and pain, 3) brain functions and regulations in pain, 4) spinal cord mechanisms of nociception and pain, 5) analgesia and pain regulations, 6) chronic pain mechanisms and treatment, and 7) brain circuits underlying the physiological and pathological pain. There were a total of 29 oral presentations and 23 poster presentations at the 7th APS. A council meeting was held during the 7th APS, and at this council meeting Dr. Seog Bae OH (Seoul National University) was elected as the president of 8th Asian Pain Symposium to organize the next symposium in Seoul, Korea in 2019. In order to keep a permanent record and to help promote pain research in Asia, we have collected abstracts of oral presentations and posted them below in the order when the presentations were given at the 7th Asian Pain Symposium.
Somatosensory neuron types and their functions

Xu Zhang

Abstract

Neuron types are traditionally classified by their morphological, anatomical, and physiological properties. Recently, the single-cell RNA-sequencing has been used to study the neuron types. Using the high-coverage single-cell RNA sequencing and in vivo electrophysiological recording, we analyzed the transcriptome and functions of somatosensory neurons in the dorsal root ganglion (DRG) of mice. Ten types and 14 subtypes of DRG neurons have been identified, including 6 types of mechanoheat nociceptors. We are also analyzing the changes of DRG neuron types and subtypes in the mouse models of chronic pain. Moreover, we investigate the molecular network and mechanism responsible for heat nociception in these mechanoheat nociceptors. Fibroblast growth factor 13 (FGF13), which is a non-secretory protein, was highly expressed in five types of mechnoheat nociceptors. We found that the loss of FGF13 in the mouse DRG neurons selectively abolished the heat nociception. FGF13 interacted with Na\textsubscript{v1.7} and maintained the membrane localization of Na\textsubscript{v1.7} during noxious heat stimulation, enabling the sustained firing of action potentials. The FGF13/Na\textsubscript{v1.7} complex is essential for sustaining the transmission of noxious heat signals. Finally, we suggest that neuron types should be defined based on their transcriptome, morphology, and function. Such a classification of neuron types is important for revealing the pain mechanisms under the physiological and pathological conditions.

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Molecular mechanisms of the sense of touch

Jianguo G Gu

Abstract
The evolution of the sensory systems has let mammals develop complicated tactile end organs to enable sophisticated sensory tasks, including social interaction, environmental exploration, and tactile discrimination. The Merkel disc, a main type of tactile end organs consisting Merkel cells and Aα-afferent endings, is highly abundant in fingertips, touch domes, and whisker hair follicles of mammals. It has high tactile acuity for an object’s physical features such as texture, shape, and edges. Mechanisms underlying the tactile function of Merkel discs are obscured as how Merkel cells transmit tactile signals to Aα-afferent endings leading to tactile sensations. In this talk, I will present our recent study demonstrating that tactile signals are transduced via piezo2 channels and transmitted by serotonin at Merkel discs in whisker hair follicles.

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The TRPM2 ion channel is required for sensitivity to warmth

Chun-Hsiang Tan¹,2 and Peter A McNaughton¹

Abstract
The discovery of the transient receptor potential (TRP) family of ion channels was a significant advancement in our understanding of thermosensation. However, genetic deletion of TRPV1, TRPV2, TRPV3, TRPV4, TRPM8, TRPM3, and TRPA1 has only modest effects on physiological thermal behavior in mice, with the exception of TRPM8, the deletion of which has marked effects on the perception of moderate coolness. In addition, these knockout mice thermoregulate normally. Although TRPV3 and TRPV4 were initially suggested to participate in the detection of non-painful warmth, later findings show that mice deficient in both TRPV3 and TRPV4 show thermal preference behavior similar to wild-type mice on a thermal gradient, and little or no change in acute heat perception indicate that the molecular mechanism responsible for detecting non-painful warmth remains elusive. Recently, we showed that TRPM2 ion channel is required for warmth sensation. We used calcium imaging to identify a population of thermally sensitive somatosensory neurons which do not express any of the known thermally activated TRP channels, including TRPV1, TRPV2, TRPV3, TRPV4, and TRPM3. We then used a combination of calcium imaging, electrophysiology, and RNA sequencing to show that the ion channel generating heat sensitivity in these neurons is TRPM2. Autonomic neurons, usually thought of as exclusively motor, also express TRPM2 and respond directly to heat. Most importantly, mice in which TRPM2 had been genetically deleted showed a striking deficit in their sensation of non-noxious warm temperatures, consistent with the idea that TRPM2 initiates a “warm” signal which drives cool-seeking behavior. These results demonstrate that the molecular mechanism underlying warmth sensation is mediated by TRPM2.¹

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A novel neuro-immune mechanism for the degeneration of sensory afferents following nerve injury

Seog Bae Oh$^{1,2}$

Abstract

Intractable chronic pain such as neuropathic pain frequently manifests features of neuro-inflammatory disease which involve activation of neuroglial cells such as microglia and astrocytes in the central nervous system (CNS) and inflammatory/immune cells in the peripheral nervous system. The cross-talks between neuroglia/immune cells and neuronal cells might play critical roles in the pathophysiology of intractable chronic pain. However, while there have been remarkable advances in our understanding of neuro-glial interaction in the spinal cord (CNS), functional significance of peripheral immune cells following peripheral nerve injury is not fully understood yet. My laboratory is currently studying response and functional role of peripheral immune cells in the context of peripheral nerve injury and neuropathic pain. In this talk, I will discuss how cytotoxic lymphocytes, one of peripheral immune cells, respond to peripheral nerve injury and then interact with sensory neuron processes within peripheral tissue, which may affect sensory dysfunctions frequently produced by peripheral nerve injury.

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Transient receptor potential channels and itch

Makoto Tominaga

Abstract
Itch is an unpleasant cutaneous sensation that can arise following insect bites, exposure to plant ingredients, and some diseases. Interestingly, many mediators of itch involve signaling related to transient receptor potential (TRP) channels. TRP channels, especially thermosensitive TRP channels, are expressed by primary sensory neurons and skin keratinocytes, which receive multimodal stimuli, including those that cause itch sensations. Lysophosphatidic acid (LPA) is an itch mediator found in cholestatic itch patients and it induces acute itch and pain in the experimental rodent models. LPA-induced itch behavior and cellular effects were dependent on TRPA1 and TRPV1, which are important for itch signal transduction. We also found that, among the 6 LPA receptors, the LPA5 receptor had the greatest involvement in itching. Furthermore, we demonstrated that phospholipase D (PLD) plays a critical role downstream of LPA5 and that LPA directly and intracellularly activates TRPA1 and TRPV1. These results suggest a unique mechanism that cytoplasmic LPA produced de novo could activate TRPA1 and TRPV1. Thus, targeting TRPA1, TRPV1, or PLD could be effective for cholestatic itch interventions. Crotamiton (N-ethyl-o-crotonotoluidide) has been used as an anti-itch agent for humans for around 70 years although its mechanism of action remains unknown. We found that crotamiton strongly inhibited TRPV4 channels followed by large currents after crotamiton washout. In mice, crotamiton inhibited itch-related behaviors induced by a TRPV4-selective agonist (GSK1016790A). Comparing single-channel open probabilities and current amplitudes of TRPV4, increases in both parameters were found to contribute to the large washout currents of TRPV4. We examined the possibility of TRPV4 pore dilation with cation replacement experiments and by measuring changes in reversal potentials. Greater cation influxes and changes in reversal potentials upon crotamiton washout were observed, suggesting that the TRPV4 pore dilated in its uninhibited state.

Keywords
TRPA1, TRPV1, TRPV4, itch, lysophosphatidic acid, crotamiton

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Methylglyoxal activation of TRPA1 contributes to diabetic itch in a murine model of type 1 diabetes

Ruo-Xiao Cheng1,2, Yu Feng1,2, Di Liu3, Cun-Jun Su1,2, Teng-Teng Liu2, Yan Zhou2, Bing Wang2, Ya Huang2, Li-Hua Chen3, Ji Hu1, Ru-Rong Ji4,5 and Tong Liu1

Abstract
Itch (pruritus) is a common symptom of skin and many systemic diseases, including diabetes. However, the molecular mechanisms underlying diabetic itch are still largely unknown. Ion channel TRPA1 has been shown to be involved in both acute non-histaminergic and chronic itch. The present study investigated the role of TRPA1 in diabetic itch. Methylglyoxal (MGO), an endogenous carbonyl compound generated as an intermediate during glycolysis, is demonstrated to be elevated in diabetic patients and rodent models. Intradermal injection of MGO was sufficient to evoke scratching behavior in a dose-dependent manner in mice. MGO directly activated TRPA1, but not TRPV1, to induce inward currents and calcium influx in dorsal root ganglia neurons in vitro. Consistently, TRPA1, but not TRPV1, was required for MGO-induced scratching in mice. In streptozotocin (STZ)-induced type 1 diabetic mice, the mechanical itch was found to be significantly increased, but there was no obvious spontaneous scratching behavior. Furthermore, MGO-TRPA1 signaling was also required for this mechanical itch in STZ-induced type 1 diabetic mice. Thus, the results suggested that the activation of TRPA1 by MGO contributes to diabetic itch in a murine model of type 1 diabetes. Targeting this MGO-TRPA1 pathway may be beneficial for chronic itch treatment in diabetes.

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SHANK3 deficiency impairs heat hyperalgesia and TRPV1 signaling in primary sensory neurons

Yong Ho Kim¹,²,³, Qingjian Han³, Yong-Hui Jiang⁴ and Ru-Rong Ji³,⁵

Abstract
Abnormal pain sensitivity is commonly associated with autism spectrum disorders (ASDs) and affects the quality of life of ASD individuals. SHANK3 deficiency was implicated in ASD and pain insensitivity. Here, we report functional expression of SHANK3 in mouse dorsal root ganglion (DRG) sensory neurons and spinal cord presynaptic terminals. Homozygous and heterozygous Shank3 complete knockout (Δe4–22) results in impaired inflammatory and neuropathic pain. Specific loss of SHANK3 in Nav1.8-expressing sensory neurons also impairs heat sensitivity. SHANK3 interacts with transient receptor potential subtype V1 (TRPV1) via proline-rich region and regulates TRPV1 surface expression. Furthermore, TRPV1 signaling, including capsaicin-induced peripheral and central pain, DRG neuronal inward currents, and spinal cord synaptic currents are all substantially reduced in Shank3 haploinsufficiency. Finally, partial knockdown of SHANK3 expression in human DRG neurons also abrogates TRPV1 function. Our findings reveal a peripheral and presynaptic mechanism of SHANK3, which may underlie pain deficits in SHANK3-related ASDs.

Keywords
Autism spectrum disorders (ASD), Dorsal root ganglion (DRG), Heat hyperalgesia, Human sensory neurons, SHANK3, TRPV1
The roles of acid-sensing ion channels in nociception and proprioception

Chih-Cheng Chen

Abstract
Acid-sensing ion channels (ASICs) are a group of proton-gated ion channels belonging to the DED/ENaC family. There are at least six ASIC subtypes that are ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4, all of which are expressed in somatosensory neurons. Among ASIC subtypes, ASIC3 is the most abundant in dorsal root ganglion (DRG) and the most sensitive to extracellular acidification. ASICs have been demonstrated as the major player for acid-induced pain in humans. Accumulating evidence has further shown ASIC3 is the molecular determinants involved in pain-associated tissue acidosis in rodent models. Besides the role of nociception, members of DEG/ENaC family have been demonstrated as essential mechanotransducers in the nematode Caenorhabditis elegans and fly Drosophila melanogaster. ASICs are mammalian homologues of DEG/ENaC and thus may play a role in mechanotransduction. However, the role of ASICs in neurosensory mechanotransduction is disputed. We report here the generation of ASIC3-knockout/eGFPf-knockin mice and subsequent characterization of heterogeneous expression of ASIC3 in DRG neuron subpopulations. Besides the expression in nociceptors, ASIC3 is expressed in parvalbumin-positive proprioceptor axons innervating muscle spindle. We further generated a floxed allele of Asic3 (Asic3f/f) and probe the role of ASIC3 in mechanotransduction in neurite-bearing parvalbumin-positive DRG neurons through localized elastic matrix movements and electrophysiology. Targeted knockout ASIC3 disrupts spindle afferent sensitivity to dynamic stimuli and impairs mechanotransduction in parvalbumin-positive DRG neurons because of substrate deformation-induced neurite stretching, but not to direct neurite indentation. In behavioral tasks, global knockout (Asic3−/−) and parvalbumin-Cre::Asic3f/f mice produce similar deficits in grid and balance beam walking tasks. We conclude that, at least in mouse, ASIC3 is a molecular determinant contributing to dynamic mechanosensitivity in proprioceptors.

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Synaptic long-term potentiation as a cellular model for chronic pain and anxiety

Min Zhuo

Abstract
The anterior cingulate cortex (ACC) and insular cortex (IC) are activated in pain conditions. In this talk, I will discuss increasing evidence from rodent studies that ACC/IC activation contributes to chronic pain states and describe several forms of synaptic plasticity that may underlie this effect. In particular, one form of long-term potentiation (LTP), which is triggered by the activation of N-Methyl-D-aspartic acid receptors and expressed by an increase in \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-receptor function, sustains the affective component of the pain state. Another form of LTP, which is triggered by the activation of kainate receptors and expressed by an increase in glutamate release, may contribute to pain-related anxiety.
Decoding the perception of spontaneous pain from resting-state magnetoencephalography

Po-Chih Kuo¹, Yi-Ti Chen¹, Yong-Sheng Chen¹,², Li-Fen Chen³,⁴ and Jen-Chuen Hsieh³,⁴

Abstract
Decoding the neural representations of physical pain is essential for obtaining an objective assessment as well as understanding of its underlying mechanisms. The complexities involved in the subjective feeling of pain experience make it difficult to obtain a quantitative assessment from the induced spatiotemporal patterns of brain activity with high dimensionality. Most previous studies have investigated the perception of pain by analyzing the amplitude or spatial patterns of brain responses to external stimulation. This study investigated the decoding of endogenous pain perceptions according to resting-state magnetoencephalographic (MEG) recordings. In our experiments, we applied a beamforming method to calculate the brain activity for every brain region and examined temporal and spectral features of brain activity for predicting the intensity of perceived pain in patients with primary dysmenorrhea undergoing menstrual pain. Our results show that the asymmetric index of sample entropy in the precuneus and the sample entropy in the left posterior cingulate gyrus were the most informative characteristics associated with the perception of menstrual pain. The correlation coefficient between the predicted and self-reported pain scores demonstrated the high prediction accuracy. In addition to the estimated brain activity, we were able to predict accurate pain scores directly from MEG channel signals. These findings suggest the possibility of using the proposed model based on resting-state MEG to predict the perceived intensity of spontaneous pain.

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Brain excitability change in migraine: Characterization by magnetoencephalography

Wei-Ta Chen

Abstract

Episodic migraine (EM) may evolve into disabling chronic migraine (CM, monthly migraine days ≥8 and headache days ≥15) with unknown mechanism. Since magnetoencephalography (MEG) is superior to traditional electroencephalography in measuring cortical excitability, we used this neuroimaging tool to explore the role of sensory cortex excitability in migraine chronification. In our visual MEG studies, visual cortical activation to checkerboard stimuli were obtained from patients with EM (with or without aura) and CM. Our findings showed a steady-state ictal-like excitability pattern in CM, in contrast with the dynamic habituation change across the interictal-ictal cycle in EM. Moreover, those CM patients who remitted to EM after treatment showed a corresponding switch of the central excitability pattern with the clinical status of migraine. Recently, we used paired-pulse electrical stimulations to assess the somatosensory cortex excitability in migraine. The findings indicated a baseline hypoexcitability in EM and CM, and an impaired somatosensory gating that showed correlation with migraine frequency. To conclude, sensory cortex excitability is altered in migraine, which may link to the chronification of migraine, and serve as a potential brain signature of this disabling headache disorder.

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Alteration of resting-state networks in patients with fibromyalgia, complex regional pain syndrome, and other functional somatic pain syndrome

Masahiko Shibata¹, Yoshiyuki Watanabe², Hisashi Tanaka² and Shigeyuki Kan¹

Abstract
The aim of this study was to investigate the alterations of spontaneous brain activity in patients with fibromyalgia (FM), complex regional pain syndrome (CRPS), and other functional somatic syndrome (FSS) with pain. A total of 105 subjects participated in this study (FM 16 cases, CRPS 19 cases, FSS 19 cases, and healthy controls 51 cases). They underwent a 5-min resting-state functional magnetic resonance imaging scan. We performed seed-based correlation analysis. Seed regions were the thalamus, hippocampus, amygdala, nucleus accumbens, default mode network (medial prefrontal cortex, posterior cingulate cortex), salience network (anterior cingulate cortex, anterior insula), dorsal attention network (frontal eye fields and intraparietal sulcus), fronto-parietal network (lateral prefrontal cortex [LPFC], posterior parietal cortex [PPC]). We also performed amplitude of low frequency fluctuations (ALFF)/functional ALFF analysis. We will show the differences and similarities of alterations of spontaneous brain activity among these three categories of pain syndrome and discuss about their pathophysiological mechanisms in the brain.

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Imaging signatures of degeneration-induced neuropathic pain in human small fiber neuropathy

Sung-Tsang Hsieh¹,²

Abstract
Degeneration of nociceptive nerves, small fiber neuropathy, is common in general population, such as in peripheral nerve degenerative disease of diabetes mellitus, chemotherapy, and autoimmune diseases, and so forth. Patients always suffer two intuitively paradoxical manifestations: (1) negative (loss-of-function) symptoms of reduced sensitivity to thermal and nociceptive stimuli and (2) positive (gain-of-function) symptoms of neuropathic pain ranging from thermal hyperalgesia to mechanical allodynia. Our group is one of the pioneer laboratories, which developed an innovative approach of skin biopsy and quantitate intraepidermal nerve fiber density (IENF density) as a pathological biomarker of nociceptive nerve degeneration.¹ This skin biopsy-based pathologic examination of nociceptive nerve terminals has become a gold standard for diagnosing small fiber neuropathy, a special type of neuropathy which mainly affects unmyelinated nerve fibers and as the most common form of neuropathy in diabetes. A critical issue is how skin nerve degeneration causes chronic neuropathic pain. In addition to peripheral sensitization due to nerve terminal degeneration, we investigated the contribution of central sensitization underlying chronic neuropathic pain in small fiber neuropathy by performing contact heat-evoked potential (CHEP) and functional magnetic resonance imaging on these patients. The degree of skin innervation, i.e. IENF density, was associated with activations of specific brain areas including the thalamus and the insular cortex.² As a physiology measure of neuropathic pain, CHEP amplitude was associated with IENF density as a biomarker of small fiber neuropathy.³ Integrating skin biopsy as a measure of peripheral nerve degeneration, we showed that patients with heat pain had marked increase in brain activations in the limbic and striatal systems in addition to the activations of somatosensory system.⁴ These observations provide evidence of maladaptive brain plasticity after peripheral nerve injury as new insights on degeneration-induced chronic neuropathic pain.

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Neuronal circuitry for pain processing in the spinal dorsal horn

T Yasaka¹, AJ Todd², BA Graham³ and DI Hughes²

Abstract
Lamina II of the spinal dorsal horn is a major target of nociceptive primary afferents and plays a critical role in both modulating and transmitting incoming sensory information. Despite the importance of this region in processing pain information, our knowledge of the functional organization of lamina II neurons and the circuits they form remains poor. Although these interneurons show morphological, neurochemical, and electrophysiological diversity, it is, nonetheless, of critical importance that we dissect these local circuits in order to understand how this region processes sensory information under both normal conditions and in pathological states. We have recently identified a spinal circuit that might contribute to the development of allodynia (touch-evoked pain) in chronic pain states. We found that vertical cells, which have their cell body in lamina II, dendrites spreading ventrally into laminae II inner (IIi) and III/IV, and axons terminating on lamina I projection neurons, are a principal target of low-threshold mechanoreceptive afferents (LTMRs). Vertical cell dendrites receive significant numbers of inhibitory synaptic inputs in IIi and III/IV, and here we report that many of these inhibitory inputs are derived from cells that express the calcium-binding protein parvalbumin (PV). Since PV cells are also known to be a source of inhibitory presynaptic inputs on to the central terminals of LTMRs (including those which target vertical cell dendrites), we propose that a loss of PV-cell-mediated inhibition allows mechanosensitive afferent input into laminae II-IV to be relayed to pain projecting circuits in lamina I through vertical cells. We conclude that particular types of lamina II neurons have very specific roles in modulating local circuitry under normal conditions, and that disrupting the input–output relation of these spinal circuits has the capacity to result in pathological pain states and altered sensory perceptions.

Keywords
Allodynia, interneurons, local circuits, pain, spinal cord

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Meeting Report

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Modulation of mechanical sensitivity by spinal glial cells

Makoto Tsuda

Abstract
Neuropathic pain is a debilitating chronic pain condition that is caused by a lesion or malfunction of the nervous system. In rodent models of neuropathic pain, peripheral nerve injury (PNI) induces a variety of plastic modifications in synapses, connections, and networks in the spinal dorsal horn (SDH), which contribute to pain hypersensitivity. Mounting evidence indicates that non-neuronal cells in the nervous system are crucial for the PNI-induced pain hypersensitivity. Those include macrophages, T cells, and glial cells. In my talk, I will show our findings focused on the role of astrocytes in the SDH. Results of our laboratory have demonstrated that SDH astrocytes become reactive states after PNI and play a pivotal role in the maintenance of mechanical hypersensitivity. Furthermore, we investigated the effect of SDH astrocyte stimulation on sensory information processing in normal mice by the Designer Receptor Exclusively Activated by Designer Drugs (DREADD) technology. By using a minimally invasive method of the SDH microinjection of an adeno-associated viral vector (AAV), we successfully expressed the excitatory DREADD hM3Dq specifically in SDH astrocytes and confirmed its functional expression. We found that specific stimulation of SDH astrocytes by clozapine N-oxide (CNO) induced transient mechanical hypersensitivity without affecting nociceptive behaviors by other noxious stimuli. Our results suggest a predominant role of SDH astrocytes in modulating mechanosensory information processing.

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Separate spinal substrates transmitting dynamic versus static mechanical pain

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Abstract
Mechanical allodynia is a debilitating symptom associated with millions of chronic pain patients. It exists in two forms, dynamic and static, which are evoked by gentle skin touch and pressure, respectively, but the underlying specific circuits remain unknown. Here, we report that spinal neurons marked by the knock-in Vglut3-Cre are required to transmit dynamic, but not static, allodynia, and these neurons form morphine-resistant polysynaptic pathways to relay inputs from low-threshold Aβ mechanoreceptors to pain output neurons. Static allodynia is mediated separately via a subset of somatostatin lineage neurons that are preserved in VGLUT3 neuron-ablated mice, which form multiple morphine-sensitive and morphine-resistant pathways and relay inputs from both Aβ and C-fibers. Furthermore, acute silencing of VGLUT3 lineage neurons attenuated pre-existing dynamic allodynia. The studies reveal for the first time separate spinal substrates transmitting dynamic versus static allodynia, and identify new cellular targets for treating these forms of mechanical pain.

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DNA demethylation of P2X7 receptors in spinal astrocytes contributes to visceral pain induced by neonatal colonic inflammation in rats

Ping-An Zhang1, Meng Li1, Xue-Lian Liu1, Rui Wu1, Rui-Xia Weng2, Lu Xue1, Chuang-Ying Hu2 and Guang-Yin Xu1

Abstract
Objective: Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders. It is characterized by abdominal pain in conjunction with altered bowel habits. IBS rat model was well established by neonatal colonic inflammation (NCI). However, the pathophysiology of the syndrome remains largely unknown. The aim of the present study is to explore whether and how the epigenetic mechanisms of P2X7 receptors (P2X7R) in the spinal cord contributes to the visceral hypersensitivity induced by NCI.

Methods: Visceral hypersensitivity was identified by colorectal distention (CRD). Methylation-specific PCR and bisulfite sequencing PCR were used to detect the methylation/demethylation status of p2x7r promoter. The binding of transcription factors to p2x7r promoter was measured by chromatin immunoprecipitation assay and Luciferase reporter gene assay in vitro.

Results: (1) Fluorocitrate treatment significantly reduced the CRD threshold in NCI rats. (2) P2X7Rs in the astrocytes were upregulated in spinal dorsal horn of NCI rats, and its antagonist A438079 markedly increased the CRD threshold and inhibited the activation of astrocytes. (3) NCI obviously demethylated p2x7r CpG island. (4) TET2, TET3, and GATA1 were remarkably upregulated, while MBD2 and MBD4 were significantly downregulated in spinal dorsal horn of NCI rats. (5) The binding of GATA1 with p2x7r promoter was dramatically enhanced in NCI rats. However, MBD4 competitively combined to p2x7r promoter with GATA1 in vitro. (6) TRAF6 level in the astrocytes was greatly increased in the spinal dorsal horn of NCI rats. (7) Knockdown of TRAF6 enhanced the CRD threshold and also led to a significant downregulation of TET3, GATA1, and P2X7Rs. (8) Fluorocitrate or A438079 incubation significantly reduced the spontaneous excitatory postsynaptic current (sEPSC) frequency of neurons in the spinal dorsal horn of NCI rats.

Conclusions: Epigenetic regulations by TET3 and MBD4 of P2X7R expression in the spinal dorsal horn were involved in adult visceral hyperalgesia in rats with NCI. Activation of TRAF6 upregulated TET3 and GATA1 expression in the astrocytes of spinal dorsal horn of NCI rats.

Keywords
Irritable bowel syndrome, P2X7Rs, epigenetic regulations, spinal cord, astrocytes, visceral pain

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Neural mechanisms of offset analgesia

Jiro Kurata

Abstract
One feels suffering on increase in pain and relief on decrease in pain. Such nociceptive intensity-dependent dynamics of pain perception are considered physiologically normal responses in terms of strategy to estimate nociceptive stimulus in a timely manner to protect oneself from injury. One feels motivated to escape from nociceptive stimulus on its increase and relieved or even rewarded after successful avoidance or decrease in pain. Being compatible with such basic mechanisms of pain perception, offset analgesia (OA) was first described by Grill and Coghll as a disproportionately large decrease in pain perception following a tiny decrease, usually by 1°C, of thermal nociception on the skin in humans (Figure 1). OA is considered a physiological phenomenon that mediates relief and reward after a decrease in pain, and also contribute to “temporal sharpening” of nociception. Earlier neuroimaging studies revealed possible involvement of periaqueductal gray, a relay center for descending pain modulation, in mediating OA. Patients with neuropathic pain showed absence of OA, implying disturbance of descending pain modulation. In a more general population of patients with chronic pain, we found attenuation of OA in patients depended on the length of sensitizing stimulus and disease duration, and was associated with slower speed of pain perception. We also used simultaneous functional magnetic resonance imaging and OA stimulation, and found that attenuation of OA in patients was associated with decreased activities at multiple areas involved both in descending pain modulatory systems, as well as a decreased connectivity among the default mode network, in patients. Insights from OA studies might thus help to reveal cerebral mechanisms of pain chronification.

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Figure 1 A typical time course of offset analgesia. Adapted from Kobinata H et al.4

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Comprehensive regulation of ion dynamics through transient receptor potential–anoctamin 1 interaction in primary sensory neurons

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Abstract
Transient receptor potential (TRP) channels are calcium-permeable channels except for TRPM4 and TRPM5, and anoctamin 1 (ANO1, also called TMEM16A) is a chloride channel activated by intracellular calcium. Some TRP channels and ANO1 are reported to physically interact on the plasma membrane. Thus, chloride movement is rapidly induced after TRP channel activation within a calcium nano-domain. Several TRP channels expressed in dorsal root ganglion (DRG) neurons are involved in the detections of dangerous environmental stimuli, including noxious heat and natural compounds. Interestingly, TRPV1 was co-expressed with ANO1 in DRG neurons, and capsaicin-induced currents and action potential generation in the isolated small DRG neurons were inhibited by an ANO1-specific inhibitor, T16Ainh-A01. Furthermore, pain-related behaviors in mice subcutaneously injected with capsaicin into their hind paw were also inhibited by T16Ainh-A01. These results suggest that TRPV1–ANO1 interaction enhances acute pain sensation. The exploration of novel inhibitors for ANO1 therefore would be an important strategy to develop analgesic agents. We recently found that menthol strongly inhibited currents of both mouse and human ANO1. Moreover, we identified the core chemical structure for the inhibition. It is isopropylcyclohexane. Although its inhibitory effect was slower than that of menthol, the more hydrophilic compound, 4-isopropylcyclohexanol (4-iPr-CyH-OH), similarly suppressed ANO1 activities. In addition, 4-iPr-CyH-OH inhibited TRPV1-, TRPA1-, TRPM8-, and TRPV4-mediated currents. These results indicate that 4-iPr-CyH-OH has analgesic effects because the four TRP channels are reportedly involved in nociception. Indeed, 4-iPr-CyH-OH reduced capsaicin-induced action potential generation and pain-related behaviors in mice. Thus, 4-iPr-CyH-OH could be an interesting seed chemical for the development of novel analgesics.

Keywords
Transient receptor potential channel, anoctamin, acute pain, 4-isopropylcyclohexanol

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Descending noradrenergic inhibition—An important target of gabapentin action in neuropathic pain

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Abstract
The gate control theory of pain posits a modulatory role of descending systems on spinal cord sensory processing which is important to not only endogenous analgesia but also pharmacological target to treat neuropathic pain. Gabapentinoids are effective in a wide range of animal pain models and in patients with neuropathic pain and have become one of the first choice treatments for neuropathic pain. Because spinal plasticity and sensitization play important roles in neuropathic pain, most laboratory studies have focused on actions of gabapentinoids in the spinal cord, where they reduce primary afferent traffic and excitation of spinal nociceptive neurons, via interaction with α2δ subunits of voltage-gated Ca²⁺ channels. However, a recent clinical study questioned this theory by demonstrating a complete lack of clinical efficacy of intrathecal gabapentin in patients with chronic pain. We and others demonstrated that gabapentin inhibits presynaptic gamma-aminobutyric acid release and induces glutamate release from astrocytes in the locus coeruleus (LC), thereby increasing LC neuron activity and spinal noradrenaline release, and that gabapentin relies on this action in the LC for its analgesia. We also recently discovered that, when neuropathic pain turns into chronic pain, noradrenergic neurons in the LC become less responsive to gabapentin, leading to impaired gabapentin analgesia, and that astrogial glutamate dysregulation is critical to impaired LC response. This presentation will discuss the analgesic mechanisms of gabapentinoids action in the LC, how resistance to gabapentin develops during chronification of neuropathic pain, and translational approach to restore gabapentin analgesia using a clinically available drug valproate.

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Abstract
Using an unbiased LC-MS-MS-based lipomics approach, Dr. Serhan’s group at Harvard Medical School uncovered two families of endogenous lipid mediators, including resolvins (e.g., resolvin E1, resolvin D1, resolvin D2) and protectins (e.g., protectin D1 or neuroprotectin D1) in resolving inflammatory exudates. They are biosynthesized from omega-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid and show remarkable potency in treating inflammation-related diseases in animal models. Recent studies have demonstrated that resolvins and protectin also potently inhibited somatic inflammatory pain in part by modulating TRPV1 and TRPA1 activity in dorsal root ganglion neurons in the somatosensory system. We investigate how lipid mediators, such as resolvins and neuroprotectins, and maresins, derived from omega-3 unsaturated fatty acids control pain by (1) blocking transient receptor potential (TRP) channels, (2) resolving synaptic plasticity, and (3) inhibit inflammation and glial activation. We have shown that resolvins are among the most potent inhibitors for inflammatory pain and TRP channels. We also determine the downstream G-protein-coupled receptors signaling that mediates the potent actions of these lipid mediators.

Keywords
lipid mediators, resolvins, protectins, maresin 1, TRPV1, TRPA1

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The Ying & Yang of opioid tolerance: Translational research from bedside back to bench

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Abstract
Opioid analgesics remain the most effective and widely used analgesics for the management of moderate to severe pain, including cancer pain and chronic non-cancer pain. However, the efficacy of long-term opioid analgesics is progressively attenuated by tolerance, preventing adequate pain relief under stable opioid dosages for chronic pain patients. Classical neuron-centered concepts such as internalization of opioid receptors, upregulation of N-methyl-D-aspartate receptor function, or downregulation of glutamate transporter activity can only partially explain the phenomenon of tolerance. Recent evidence showing glial activation and upregulated inflammatory mediators in the rodent central nervous system has confirmed the pivotal role of neuroinflammation in neuropathic pain or opioid tolerance, or both. However, human evidence is still sparse. Based on our clinical practice, we conducted translational research by investigating the intraspinal cytokine and chemokine profiles of opioid-tolerant patients after research ethic committee approval. Cerebrospinal fluid samples from opioid-tolerant patients and opioid-naive subjects were compared. We found CXCL1, CXCL12, and leukemia inhibitory factor (LIF) were significantly upregulated among the opioid-tolerant patients and positively correlated with the opioid dosage. In laboratory animal experiment, after induction of tolerance by morphine infusion, the spinal cord expression of CXCL1, CXCL12, and LIF was upregulated. Although CXCL1 and CXCL12 infusion alone did not affect baseline tail flick latency, morphine analgesic efficacy dropped significantly after intrathecal infusion of CXCL1 and CXCL12. After establishing tolerance by intrathecal continuous infusion of morphine, tolerance development was accelerated by co-administration of CXCL1 and CXCL12. In parallel, the effect was attenuated by co-administration of CXCL1 or CXCL12-neutralizing antibody or concordant receptor antagonists. On the contrary, although chronic morphine administration still induced LIF upregulation in rat spinal cords, intrathecal injection of LIF potentiated the analgesic action of morphine and delayed the development of morphine tolerance. Upregulation of endogenously released LIF by long-term use of opioids might counterbalance the tolerance induction effects of other proinflammatory cytokines. CXCL1, CXCL12, and LIF are upregulated in both opioid-tolerant patients and rodents. The onset and extent of opioid tolerance was affected by antagonizing intrathecal CXCL1/CXCR2, CXCL12/CXCR4, and LIF signaling and could be novel drug targets for the treatment of opioid tolerance.

Keywords
Chemokine, CXCL1, CXCL12, cytokine, leukemia inhibitory factor, opioid analgesics, intrathecal morphine, tolerance, neuroinflammation, translational research

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Tackling pain associated with rheumatoid arthritis: Proton-sensing receptors

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Abstract
Rheumatoid arthritis (RA), characterized by chronic inflammation of synovial joints, is often associated with ongoing pain and increased pain sensitivity. High hydrogen ion concentration (acidosis) found in synovial fluid in RA patients is associated with disease severity. Acidosis signaling acting on proton-sensing receptors may contribute to inflammation and pain. Previous studies focused on the early phase of arthritis (<5 weeks) and used different arthritis models, so elucidating the roles of different proton-sensing receptors in the chronic phase of arthritis is difficult. We intra-articularly injected complete Freund's adjuvant into mice once a week for four weeks to establish chronic RA pain. Arthritic mice showed long-term joint inflammation and long-lasting bilateral hyperalgesia for at least 12 weeks. Deletion of acid-sensing ion channel 3 (ASIC3) or transient receptor potential/vanilloid receptor subtype 1 (TRPV1) prevented RA disease progression and establishment of hyperalgesic priming, thereby leading to attenuation of the chronic phase of RA pain (>6 weeks or >8 weeks). Mice with T-cell death-associated gene 8 (TDAG8) knockout showed attenuated acute and chronic phases of RA pain. TDAG8 likely participates in the initiation of RA pain and also regulates ASIC3 and TRPV1 to establish hyperalgesic priming.

Keywords
Rheumatoid arthritis, chronic pain, acidosis, TDAG8, ASIC3, TRPV1

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No pain no gain and no protection: Chronic neuropathic pain protects heart from ischemia-reperfusion injury in mice

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Abstract
Myocardial infarction is the leading cause of death worldwide. Restoration of blood flow rescues myocardium but also causes ischemia-reperfusion (IR) injury. Here, we show that in a mouse model of chronic neuropathic pain, IR injury following myocardial infarction is reduced, and this cardioprotection is induced via an anterior nucleus of paraventricular thalamus (PVA)-dependent parasympathetic pathway. Pharmacological inhibition of extracellular signal-regulated kinase activation in the PVA abolishes neuropathic pain-induced cardioprotection, whereas activation of PVA neurons pharmacologically, or optogenetic stimulation, is sufficient to induce cardioprotection. Furthermore, neuropathic injury and optogenetic stimulation of PVA neurons reduce the heart rate. These results suggest that the parasympathetic nerve is responsible for this unexpected cardioprotective effect of chronic neuropathic pain in mice.

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Down-regulation of Protein Arginine Methyltransferases 1 induced hypersensitivity via fragile X mental retardation protein

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Abstract

Objective: PRMT1 is the major isoform of type I protein arginine methyltransferases, which catalyzed the post-translational methylation of arginyl residues. Previous studies found that the PRMTs were involved in the developments of nerve system. However, the function of arginine methyltransferases at the adult stages kept unclear. In the current study, we investigate the role of PRMT1 in the regulation of hypersensitivity induced by peripheral nerve injury.

Methods: The left common peroneal nerve (CPN) of mouse was ligated, and the mechanical allodynia and spontaneous pain were evaluated by Von Frey behavioral assay. The expressions of cingulate PRMT1 were examined after nerve injury, the over-expression or ShRNA targeting to PRMT1 were delivered to the ACC to examine the casualty and necessity for the developments of hypersensitivity.

Results: (1) The expression of PRMT1 in the ACC was downregulated at day 7 after nerve injury or CFA injection. (2) Overexpression of PRMT1 rescued the mechanical allodynia induced by nerve injury, and downregulation of PRMT1 in the ACC decreased PWTs on the normal mice. (3) Downregulation of PRMT1 in the ACC of Fmr1 KO mice failed to decrease PWTs. Mutation the arginine to alanine changed the binding mRNA.

Conclusion: The PRMT1 was involved in the developments of neuropathic pain via fragile X mental retardation protein.

Keywords

ACC, nerve injury, PRMT1, FMRP, hypersensitivity

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Role of calcitonin gene-related peptide in nociception-emotion link and its consequences

Fusao Kato¹, Kei Shinohara¹, Yuya Okutsu¹, Mariko Sugimoto¹, Yae K Sugimura¹ and Yukari Takahashi¹

Abstract
The largest portion of the ascendingly projecting neurons in the superficial layer of the dorsal horn and those in the caudal spinal trigeminal nucleus target the lateral parabrachial nucleus (LPB), from which nociceptive signals course to the central amygdala (CeA). This relay thus serves as the link between the nociception and the emotion. This LPB-CeA projection is principally glutamatergic and shows manifest synaptic potentiation in chronic pain models of various types. The LPB contains calcitonin gene-related peptide (CGRP) and the CeA is rich in its binding sites and receptors, suggesting that CGRP would play a role in modulating the LPB-CeA synaptic transmission. First, we examined whether and how the exogenous CGRP affects the LPB-CeA synaptic transmission in the acute brain slices from mice. While CGRP did not affect the EPSCs mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors evoked by LPB fiber stimulation, it potently increased the amplitude of those mediated by N-methyl-D-aspartate receptors recorded in isolation. Second, we analyzed the synaptic potentiation in the formalin-inflammation model in the mice lacking CGRP (transgenic mice provided through the courtesy of Prof. H. Kurihara). The increase in the amplitude of LPB-CeA EPSCs at 6 h after intraplantar formalin injection in wild-type mice was absent in the mice lacking CGRP, despite only partial deficiency in the acute nocifensive behaviors, suggesting that CGRP is necessary for the full expression of chronic pain-induced synaptic plasticity in the LPB-CeA synapse. Finally, we examined how inactivation of the CGRP receptors in the CeA affects nociceptive behaviors. In the wide-spread sensitization model showing facial inflammation and hind limb tactile allodynia in the bilateral hind limb, micro-injection of the CGRP1 receptor antagonist into the CeA significantly and transiently mitigated the hypersensitization in a similar manner to the pharmacological activation of the inhibitory Designer Receptor Exclusively Activated by Designer Drugs expressed in the CeA GABAergic neurons. Altogether, it is concluded that endogenous CGRP in the amygdala regulates the expression of nociception-associated plasticity and also the chronic pain-related symptoms.

Keywords
Central amygdala, lateral parabrachial nucleus, calcitonin gene-related peptide, Designer Receptor Exclusively Activated by Designer Drugs

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Role of the mesolimbic network in pathological pain and analgesia

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Abstract
Pain is a negative experience, suggesting that pain is negatively correlated with pleasure. On the other hand, the mesolimbic dopaminergic system has been recognized to play a central role in motivated behaviors, including various types of reward and pleasure. However, it is not yet clear whether the mesolimbic dopaminergic system and small molecules released from the nucleus accumbens (N.Acc.) are involved in pain modulation. In this study, we evaluated the pain threshold when we controlled dopaminergic activity using optogenetics and detected small molecules released from the N.Acc induced by pain or the administration of analgesics. We generated transgenic mice expressing channelrhodopsin-2 (ChR2) under the control of the dopamine transporter promoter (DAT-cre/ChR2). Using these mice, we found that the activation of mesolimbic dopaminergic neurons produced analgesia. Next, we generated mice expressing ChR2 in nociceptive neurons via the injection of AAV6-hSyn-ChR2 (ET/TC)-EGFP. Two weeks after AAV injection, we collected the dialysate in the N.Acc after the activation of nociceptive neurons by optical stimulation. We also collected the dialysate in the N.Acc after the systemic administration of morphine. We then performed a comparative metabolome analysis by Fourier transform mass spectrometry. In the present study, some small molecules were dramatically decreased after the activation of nociceptive neurons, whereas they were increased after the systemic administration of morphine in the N.Acc. These findings provide evidence that the mesolimbic dopaminergic system and small molecules released from the N.Acc. could be, at least in part, associated with pain modulation.

Keywords
Dopamine, ventral tegmental area, nucleus accumbens, morphine

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Abstract
Cognitive behavioral therapy, such as environmental enrichment combined with voluntary exercise (EE-VEx), is under active investigation as an adjunct to pharmaceutical treatment for chronic pain. However, the effectiveness and underlying mechanisms of EE-VEx remain unclear. In a mouse model of complete Freund’s adjuvant-induced chronic inflammatory pain, our results revealed that EE-VEx alleviated perceptual, affective, and cognitive dimensions of chronic inflammatory pain. These effects of EE-VEx on chronic pain were contingent on the occurrence of adult neurogenesis in the dentate gyrus in a functionally dissociated manner along the dorsoventral axis: adult neurogenesis in the ventral dentate gyrus participated in alleviating perceptual and affective components of chronic pain by EE-VEx, whereas neurogenesis in the dorsal dentate gyrus was involved in EE-VEx’s cognitive-enhancing effects. Chronic inflammatory pain was accompanied by decreased levels of brain-derived neurotrophic factor (BDNF) in the dentate gyrus, which were reversed by EE-VEx. Over-expression of BDNF in the dentate mimicked the effects of EE-VEx. Our results demonstrate distinct contribution of adult hippocampal neurogenesis along the dorsoventral axis to EE-VEx’s beneficial effects on different dimensions of chronic pain.

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Altered functional connectivity in pain modulatory systems of primary dysmenorrhea: Imaging genetics studies

Shyh-Yuh Wei¹,²

Abstract
Primary dysmenorrhea (PDM), menstrual pain without discernable organic causes, is a prevailing problem in women of reproductive age. As many as 90% of adolescent girls and more than 50% of menstruating women worldwide report suffering from PDM, with 10–20% of them describing their suffering as so severe that it requires absence from school or work. PDM subjects exhibit structural alterations in periaqueductal gray (PAG), which is one of the most important neural substrates of the descending pain modulatory systems (DPMS). As many chronic pain disorders exhibit altered functions in the DPMS, the high comorbidity of PDM with these chronic pain disorders raises the question of whether maladaptive neuroplasticity of the DPMS already occurs in PDM that may predispose the PDM for the development of these functional disorders in later life. Furthermore, brain-derived neurotrophic factor (BDNF) acts as a pain modulator within the PAG, enriched with opioidergic neurons, and the BDNF Val66Met polymorphism contributes towards susceptibility to PDM; nevertheless, few studies have addressed how BDNF Val66Met polymorphism or OPRM1 A118G polymorphism modulates the neural processing of pain. Therefore, our series studies aimed to investigate, firstly, the functional connectivity (FC) of PAG as a window to further elucidate the DPMS of PDM; secondly, the influence of the genotype (the OPRM1 A118G and the BDNF Val66Met polymorphisms) on the DPMS; and thirdly, the association among the genotype, the DPMS (endophenotype), and the severity of PDM (phenotype). In the first study, the PAG of PDM subjects exhibited hyper-FC with the sensorimotor and supplementary motor area during painful menstruation (MENS), whereas it exhibited hypo-FC with the dorsolateral prefrontal cortex and default mode network during MENS or periovulatory phase. We propose that the maladaptive hypo-FC in PDM may underpin the central susceptibility to subsequent development of various functional disorders in later life. In the second study, the AA homozygotes, in comparison to G allele carriers, exhibited hyper-FC between the anterior cingulate cortex (ACC) and PAG that correlated with their spontaneous pain intensity. The AA homozygous PDM demonstrated an active cortical modulation, while the PDM subjects with G allele showed dys-regulated DPMS. This OPRM1 A118G-DPMS interaction is one plausible neurological mechanism underlying the individual differences in pain experience. In the third study, our findings indicate that the BDNF Val66Met polymorphism is associated with the diverse functional expressions of the DPMS in healthy subjects. When confronted with the repeated stress of menstrual pain, the functional dynamics of the DPMS undergo further differential changes that vary with the BDNF Val66Met genotype of the individual. The Val/Val PDM subjects exhibit more adaptive neuroplasticity, while the Met/Met PDM subjects more maladaptive neuroplasticity. In the fourth study, the severe PDM subjects, in comparison to the moderate PDM subjects, exhibited poor physical well-being, higher proportion of Met allele, and a yearly shifting of the PAG-seeded FC from the pain-sensory system to the limbic system. The chronification of pain and the dysregulated function of DPMS suggest maladaptive neuroplasticity in the severe PDM, and may serve as neural markers distinguishing the severe from the moderate type of PDM. There are two pivotal risk factors that may prognosticate the development of chronic pain: genotype (innate mechanisms) and concurrent disease at critical developmental periods (acquired mechanisms). Our data underpin the importance of treating PDM aggressively as early as possible and individualizing analgesic therapy to optimize medical treatment for pain relief.

Keywords
Periaqueductal grey, BDNF Val66Met polymorphism, OPRM1 A118G polymorphism, menstrual pain, functional magnetic resonance imaging

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Reliability and validity of the Athens Insomnia Scale in patients with chronic pain

K Enomoto¹, T Adachi¹, D Inoue¹, T Nishigami¹ and M Shibata¹

Abstract

Objectives: To confirm the psychometric properties of the Athens Insomnia Scale (AIS) among Japanese chronic pain patients.

Methods: A total of 144 outpatients with chronic pain (Mean age (SD) = 53.3 (16.2) years, 60% female) participated. They were asked to complete the questionnaire, including the AIS and pain intensity (Numerical Rating Scale), disability (Pain Disability Assessment Scale), anxiety and depression (Hospital Anxiety and Depression scale), catastrophizing (Pain Catastrophizing Scale), and self-efficacy (Pain Self-Efficacy Questionnaire). To assess participants' sleep disturbance, semi-structured interviews were conducted. If participants have any sleep complaints that included difficulty in initiating sleep, difficulty in maintaining sleep, or early morning awakening, they were considered to have insomnia symptoms.

Results: According to confirmatory factor analyses, two-factor model of the AIS-8 and one-factor model of the AIS-5 showed good-fit to the data (AIS-8: $\chi^2(19) = 36.33$, $p < .05$, standardized root mean square residual (SRMR) = .05, root mean square error of approximation (RMSEA) (90% CI) = .08 (.04–.10), comparative fit index (CFI) = .97, and Akaike’s Information Criterion (AIC) = 2623.30, AIS-5: $\chi^2(4) = 4.01$, $p = .41$, SRMR = .01, RMSEA (90% CI) = .00 (.00–.13), CFI = 1.00, AIC = 1595.53). The AIS had adequate reliability ($\alpha = .66–.89$, intraclass correlation coefficient = .54–.72). Patients with insomnia had higher AIS score than those without insomnia. AIS was positively associated with pain intensity, disability, depression, anxiety and catastrophizing, whereas AIS was negatively associated with pain related self-efficacy. The cut-off value of the AIS-8 was estimated at 8 points (area under the curve (AUC) (95% CI) = .82 (.72–.91), 72% sensitivity and 85% specificity) and the AIS-5 was estimated at 4 points (AUC (95% CI) = .82 (.71–.92), 78% sensitivity, and 70% specificity).

Conclusions: The AIS-8 and AIS-5 had adequate reliability and validity in patients with chronic pain.

Keywords

Insomnia, sleep disturbance, chronic pain, Athens Insomnia Scale

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Meeting Report

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Japanese validation of the Pain Stage of Change Questionnaire

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Abstract

Objectives: The present study sought to evaluate psychometric property of the Japanese version of the Pain Stage of Change Questionnaire (PSOCQ-J) in a sample of adults with chronic pain.

Methods: Two hundred and one adults with chronic pain (mean age (SD) = 61.3 (13.9) years, 66% female) were administered the PSOCQ-J, as well as measures of pain severity and pain interference (Brief Pain Inventory), catastrophizing (Pain Catastrophizing Scale), self-efficacy (Pain Self-Efficacy Questionnaire), and pain coping (Chronic Pain Coping Inventory).

Results: Confirmatory factor analysis revealed a four-factor structure of the PSOCQ-J with items loading on precontemplation, contemplation, action, and maintenance factors showing an acceptable fit ($\chi^2(391) = 812.74, p < .01$, standardized root mean square residual = .08, root mean square error of approximation (90% CI) = .07 (.07-.08), comparative fit index = .78, and Akaike’s Information Criterion = 15438.41). Good internal consistencies ($\alpha = .72–.80$) and moderate to excellent test–retest reliabilities (intraclass correlation coefficient = .57–.69) were observed for the four scales. The Precontemplation Scale had weak to moderate positive associations ($r = .21$ to .49) with negative pain-related outcomes and illness-focused coping. The Action and Maintenance scales had weak to moderate positive associations ($r = .17$ to .44) with self-efficacy and wellness-focused coping. The Contemplation Scale had weak positive associations ($r = .17$ to .31) with pain interference and both illness and wellness-focused copings.

Conclusions: The PSOCQ-J demonstrated sound psychometric property in a sample of Japanese chronic pain patients. The measure can be used to evaluate the role that readiness to self-manage pain may play in adjustment to chronic pain in Japanese chronic pain populations.

Keywords

Readiness to change, self-management, chronic pain

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Gait analysis in mouse models of chronic widespread pain

Lee CH¹, Hsieh SY², Hsieh TH³ and Chen CC⁴

Abstract
Chronic widespread pain is quite unbearable and affecting quality of life in patients but is often difficult to treat. In preclinical studies, many drugs with high efficacy on evoke-pain monitoring platforms (e.g., von Frey and/or hot plate tests) were often failed in clinical trial. Thus, besides evoked pain assessment, methods to evaluate non-evoked pain (e.g., spontaneous pain) responses are urged in preclinical studies. Previous reports have demonstrated gait analysis is a useful method to evaluate spontaneous pain in mouse models of acute pain. However, it is still not known whether gait analysis is good for chronic pain assessment, especially for chronic widespread pain in mouse. Thus, we aimed to identify sensitive parameters of gait analysis for chronic pain assessment. We analyzed mouse gait after treatment of intermittent cold stress (ICS), an animal model of fibromyalgia, which causes mouse wide spread pain. After ICS treatment, mice developed chronic mechanical hypersensitivity at both hind paws and gastrocnemius muscle. We analyzed temporal and special parameters of mouse gait including Stance time (ST), Swing phase time (SWP), Double support time (DS), Walking speed (WS), Step length (StepL), Stride length (StrideL), Base of support (StepW), Print length (PL), Foot angle (FtAng), Intermediary toe spread (ITS), and Toe spread (TS). As compared with naive mice, ICS-treated mice significantly decreased scores in ST, DS, PL, and increased scores in StepW. Interestingly, the StepW parameters of ICS mice could be corrected after intraperitoneal injection of pregabalin, a well-known analgesic drug for fibromyalgia. Taken together, gait analysis might be useful for detecting chronic widespread pain behavior in mice.

Keywords
Chronic widespread pain, spontaneous pain, gait analysis

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Suspension of abnormal pain sensitivity after thalamic hemorrhagic trauma in the P2X7 knockout mice

Shih HC1, Kuan YH1 and Shyu BC1

Abstract
Chronic pain is a serious health challenge in aged society. Patients’ life quality decreased and the cost of medical care increased massively. About 7–15% patients with hemorrhage stroke in lateral thalamus would suffer central post stroke pain (CPSP). But the pathophysiological mechanism of CPSP is not clear. Although a hemorrhage rat model was well established for CPSP research, due to the efficiency to test gene function in transgenic mice, it is valuable for establishing a mouse hemorrhage CPSP model that could help to distinguish more detail mechanisms of CPSP. In this study, we tested the hypothesis that CPSP is caused by P2X7 receptor activation after thalamus hemorrhage damage. Following the same experimental protocol of rat hemorrhage CPSP model, wild-type mice and P2X7 knockout mice were injected with 0.01U/0.2μl type 4 collagenase into ventrobasal complex of the thalamus. After lesion, the mechanical and thermal allodynia appeared in the wild-type mice gradually and kept for 35 days follow lesion. Multiunit activities in medial dorsal (MD) nucleus of thalamus were enhanced and lengthened after noxious electrical stimuli on sciatic nerve in lesion wild-type mice group. In contrast with GABAergic inhibitory effect in sham lesion mice, spontaneous unit activity in MD could be enhanced by muscimol application in wild lesion mice. But in P2X7 knockout mice lesion group, patterns of allodynia in hind limbs enhanced and lengthened noxious activities, and muscimol-enhanced response in MD neuron was not observed. Preliminary results suggest that the activation of P2X7 after thalamus hemorrhage damage is an important factor of the development of CPSP. Our next step is to identify the effects of P2X7 cascades on CPSP.

Keywords
P2X7, central post stroke pain

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Does pre-scratching reduce the itch transmission?

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Abstract

Itch is an unpleasant sensation caused by pruritogen transmitted by sensory neurons to the spinal dorsal horn and then to higher brain center, which eventually induces the desire to scratch. The act of scratching and varying noxious counter stimuli like noxious heat and painful sensations relieve the itch sensation. However, it is not clear whether the noxious stimuli prior to injection of pruritogen could effectively reduce the itch sensation. Here, we observed antipruritic effect produced by brief noxious stimuli applied on mouse skin prior to pruritogen. This effect was accomplished by the application of noxious stimuli (e.g., passive scratching) on the nape skin of mild anaesthetized prior to pruritogen could significantly reduce itch response. We demonstrated that application of noxious stimuli (e.g., passive scratching) on the nape skin of mild anaesthetized prior to pruritogen could significantly reduce itch response. Similar itch reduction was observed in the nape and cheek skin models, when intradermal injection of capsaicin was given prior to pruritogen. The antipruritic effect produced by brief noxious stimuli lasted for more than 20 min. We further demonstrated that passive scratching of mice led to phosphorylation of extracellular signal-regulated kinase in cervical dorsal root ganglion neurons and enhanced c-Fos expression in lamina II of cervical spinal dorsal horn. Taken together, noxious stimuli prior to pruritogen challenges are effective to diminish itch responses by neural modulation at the spinal cord level, although possible involvement of supraspinal control cannot be excluded.

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How distraction modulates pain habituation

Yi-Hsuan Lin and Ming-Tsung Tseng

Abstract
Human pain experiences are shaped by different cognitive modulators. Despite accumulating studies investigating the role of separate cognitive factors, little attention has been paid to their interactions. Among the cognitive factors, habituation and attention are two important modulators that help reduce pain sensation. Aberrance of these processes has been suggested to play important roles in chronic pain. In the present functional magnetic resonance imaging study, we investigated whether and how distraction influenced the process of pain habituation in healthy adults. Participants underwent repetitive painful and non-painful stimuli to the leg skin when their attention toward stimuli was distracted during Stroop tasks. Behavioral results suggested that distraction enhanced the extent of pain habituation. At the neural level, anterior insular cortex participated in the process of pain habituation. Importantly, the periaqueductal gray and the rostral ventral medulla were involved in the modulation of distraction on pain habituation process. In conclusion, these results suggest that distraction recruits the descending pain modulatory system to enhance the pain habituation process, which provides a new insight into the understanding of chronic pain in the future.

Keywords
Pain habituation, distraction, functional magnetic resonance imaging, anterior insular cortex, periaqueductal gray, rostral ventral medulla

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Behavioral interaction between electrically evoked pain and itch in humans

Chun-Yen Chiang¹, Hsin-Yun Tsai¹ and Ming-Tsung Tseng¹

Abstract
Itch and pain are nociceptive sensations, which enable us to avoid potential damage. Both sensations encompassed different cognitive attributes, and the prevailing belief is that pain inhibits itchy sensation. Nevertheless, most researchers used different methods to evoke pain and itch. The interaction between pain and itch, as well as whether their interaction was confounded by different modalities used, remains unclear. In the current study, we used electrical stimulation to elicit painful and itchy sensation and investigated their behavioral interaction. Healthy volunteers received randomized administration of four stimulus types (pain only, itch only, pain and itch, and non-pain, and non-itch) and reported the intensity of the stimulus (how painful and how itchy) and the urge to elicit a motor response (withdrawal for pain and scratch for itch) immediately after the offset of the stimulus. We observed that electrically evoked pain inhibited itch sensation at both the perception and the motor levels, but not vice versa. These preliminary results lead us to conclude that complex interactions in different cognitive dimensions exist between these nociceptive sensations.

Keywords
Pain, itch, nociception, cognition, interaction

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The role of emotion in the effect of expectation on pain

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Abstract
Expectations of pain significantly bias the experience of pain in humans and different emotional states potentially influence this cognitive process. However, how pleasant and unpleasant moods affect pain expectations at the behavioral level remains unknown. Here, we aim to clarify if different emotional states bias effects of expectation on pain at behavioral level. In the current study, we manipulated the expectation of participants toward the upcoming painful stimuli and induced different emotional conditions by using the International Affective Picture System. In preliminary results, we found that both painful sensation and effect of expectation on pain were significantly modulated by picture-evoked emotions. Results obtained from this research will enhance our knowledge on how expectation interacts with emotion to shape human responses to pain.

Keywords
Pain, emotion, expectation, nociceptive stimulation, cognition

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Probing roles of sensory neuron subtypes in nociception via chemo-optogenetics

Jiang BY, Lin SH, Lee CH and Chen CC

Abstract
Clinically, pain can be divided into evoked pain and ongoing pain. Evoked pain is pain triggered by noxious thermal stimuli (thermal nociception), mechanical stimuli (mechanical nociception), or chemical stimuli (chemical nociception) and ongoing pain is the spontaneous pain behaviors without external stimuli such as flinching or guarding behaviors. Accumulated evidence has shown that several nociceptive neuron populations involved in different evoked pain sensation such as thermal nociception, mechanical nociception, and chemical nociception. However, little is known about the nociceptive neuron populations contribute to ongoing pain. In formalin test, we found mechanical hyperalgesia resolved earlier than guarding pain behaviors in mice. Therefore, we hypothesized nociceptive neurons contribute to either evoked or ongoing pain are distinguishable. Here, we have developed a method to activate specific nociceptive neuron subtypes via chemo-optogenetics and probe their roles in evoked pain or ongoing pain. We first generated a Cre-dependent reporter mouse line that carries a luminopsin, a fusion protein of channelorhodosin and luciferase. We then used ctz (coelenterazine), a subtract of LMO3 (luminopsin 3) channel to activate LMO3-positive dorsal root ganglion neurons via peripheral nerve terminals and tested the evoked and ongoing pain behaviors in specific Cre-line::LOM3 mice. Results showed after ctz injection, transient receptor potential cation channel subfamily V member 1 (TRPV1) positive neurons contributed to both evoked and ongoing pain sensation; tyrosine hydroxylase positive neurons involved in evoked pain only. Together, we have established a mouse model that can probe the roles of sensory neuron subtypes in nociception via chemo-optogenetics.

Keywords
Ongoing pain, coelenterazine, luminopsin 3, chemo-optogenetics, TRPV1

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Comparison between platelet-rich plasma and hyaluronic acid treatments for talar osteochondral lesions: A systematic review with a network meta-analysis of randomized controlled trials

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Abstract

\textbf{Background:} Both platelet-rich plasma (PRP) and hyaluronic acid (HA) with or without surgical intervention can enhance healing and improve function in talar OCLs. However, recent studies on OCLs have not thoroughly investigated the effects among PRP, HA, and conventional treatment.

\textbf{Purpose:} To synthesize evidence by comparing the effects (pain score and foot and ankle condition scores) among PRP, HA, and conventional treatment strategies for talar OCLs.

\textbf{Study Design:} Systematic review with network meta-analysis.

\textbf{Methods:} All relevant research articles were included using related terms in the PubMed, EMBASE, Web of Science, ScienceDirect, and Cochrane library databases from their inception to June 2017. The screening criteria for this systematic review were as follows: randomized controlled trials (RCTs) that compared PRP with HA, PRP with control, or HA with control in patients with talar OCLs. The risk of bias in the included studies was assessed using the Cochrane Risk of Bias Tool. Data were extracted and recorded as weighted mean difference and their standard deviations with 95\% confidence intervals (CIs), consistency $H$, and $I^2$ for continuous data in the network meta-analysis.

\textbf{Results:} A total of 1199 references were identified of which 5 RCTs were included in the final synthesis. These studies randomized 197 patients into the PRP, HA, and control groups. PRP caused higher reductions in the visual analog scale score than HA and conventional treatment, and the WMDs were $-1.109$ (95\%CI: $-1.716$, $-0.502$) and $-2.301$ (95\%CI: $-2.825$, $-1.777$). Moreover, PRP improved the American Orthopedic Foot and Ankle Society score more than the other treatment methods, and the WMDs were 12.448 (95\%CI: 7.224, 17.672) and 18.617 (95\%CI: 13.536, 13.698).

\textbf{Conclusion:} PRP reduced pain and improved ankle conditions to a greater extent than HA and conventional treatment. Therefore, PRP might be recommended for the treatment of talar OCLs. Further investigation is required to guarantee the safety and efficacy of different surgical treatments.

\textbf{Keywords}

Osteochondral lesions, talus, platelet-rich plasma, hyaluronic acid

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Central nociceptive transmission modulated by P2X7 in thalamocingulate circuit

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Abstract
Background: The well-known biological energy fuel adenosine triphosphate (ATP) was discovered to act as a neurotransmitter in the early 1950s and first demonstrated involving in stimulation-dependent release from the nervous tissue by Holton and colleagues. Later reports demonstrated that specific ATP-gated ion channels are localized on primary sensory neurons and that activation of these channels mediates ATP-evoked neuronal excitability. Interest in the role of ATP in synaptic transmission started growing since then. Among ATP receptor families, P2X7 receptors differ in many respects from the other subtypes of this family (P2X1–6). The short-time stimulation of P2X7 leads to the expected activation of cationic currents, upon repeated or prolonged ATP application, the opening of a large membrane pore can be detected. In view of the great significance of peripheral P2X7 receptors in pain and inflammation, we set out to investigate the possible role of this receptor in the central modulation of pain signaling in the thalamocingulate pathway.

Materials and methods: Coronal or medial thalamic-anterior cingulate cortex slices of the mouse brain (300–350 μm thick) containing the anterior cingulate cortex (ACC) or both nucleus of medial thalamus (MT) and anterior cingulate cortex were prepared from 21- to 35-day-old BL6/c wild-type mice. Whole-cell patch clamp currents were recorded with an EPC10 amplifier using pClamp (HEKA Elektronik) from a holding potential of −70 mV unless otherwise stated. Extracellular field currents were recorded with 64-channel multichannel electrical array (MEA system) with P2X7 agonist or antagonist perfused into the array chamber at 1 ml/min and 20–25°C. In vivo 16-channel multichannel recordings were done with 8 week old mice anesthetized maintaining at 0.5% halothane inhalation upon high-intensity nociceptive electrical stimulation from the sciatic nerve (SNS Sti) with or without site direct application of P2X7 antagonists into medial-thalamus. The recorded field potentials were calculated with the current source density analysis method, other electrophysiological data were averaged using Signal 4 (Cambridge Electronic), Clampex (Molecular Devices), and Excel (Microsoft) softwares.

Results: 64-channel MEA recordings reveal that the evoked response in ACC upward deflection after 10 ms of MT electrical stimulation, the BzATP treatment group was larger than those evoked in control groups. Whole-cell patch clamp recordings from ACC neurons revealed that extracellular P2X7 agonist BzATP application increased the membrane potential and spike numbers of EPSCs without changing in their amplitudes, whereas the P2X7 antagonist application of A-740003 could reserve/suppress the facilitated neuron activity by BzATP. In vivo medial dorsal site application of selective P2X7 antagonists reveal inhibition of ACC neuron activity in response to nociceptive SNS stimulation and selective P2X7 agonist application facilitate the ACC neuron activity.

Conclusion: In brief, our data obtain valuable information to the controversy regarding the presence of P2X7 in the central nervous system which indicates the clear functional existence of P2X7 alone the thalamocingulate pathway, particularly on the ACC neurons in responses to P2X7 agonist or antagonist applications without or with thalamic inputs and in modulation of the peripheral inputs by SNS stimulation. Modulations of nociceptive transmission in the central thalamocingulate path via P2X7 targeting may contribute a new insight for further anti-nociceptive applications or promote knowledge of the physiological roles of the P2X7 receptors.

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Hypoxia and ATF3 as bio-signatures of persistent pain associated with lumbar radiculopathy

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Abstract
Lumbar radiculopathy due to nerve constriction in proximity to the lumbar part of dorsal root ganglion (DRG) is associated with devastating neuropathic pain. In rat models of lumbar radiculopathy, distal constriction of DRG has shown more severe clinical symptoms and persistent pain than proximal constriction of DRG. However, how the constriction sites cause differential effects of radiculopathy is largely unknown. Here, we used rat models of lumbar radiculopathy to probe how constriction proximal or distal to the DRG differentially affects pain behaviors, the extent of hypoxia, and the damage of DRG neuron subpopulations. As expected, rats with distal spinal nerve injury showed more persistent pain behaviors than those with proximal spinal nerve injury in 50% paw withdraw threshold, incapacitance test, and acetone test. The distal constriction also caused more severe DRG hypoxia than proximal constriction as indicated by optical density of hypoxia-probe 1 staining. In addition, the ratios of ATF3-positive DRG neurons were significantly higher in distal spinal injury group than those in proximal spinal injury group. The ratios of calcitonin gene-related peptide+ or IB4+ or N52+ DRG neuron were not different among three groups. Together, increased hypoxia extent and ATF3 expression in DRGs are potential bio-signature of persistent neuropathic pain in lumbar radiculopathy associated with distal constriction to DRGs.

Keywords
Dorsal root ganglion, spinal nerve injury, constriction, lumbar radiculopathy, pain

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Effects of thalamic hemorrhagic lesions on explicit and implicit learning during the acquisition and retrieval phases in an animal model of central post-stroke pain

Cheng Chung Wang¹, Chung-Lei Huang¹, Hsi-Chien Shih², Bai Chuang Shyu² and Andrew Chih Wei Huang¹

Abstract
Hemorrhagic stroke has many symptoms, including central pain, learning and memory impairments, motor deficits, language problems, emotional disturbances, and social maladjustment. Lesions of the ventral basal complex (VBC) of the thalamus elicit thermal and mechanical hyperalgesia, forming an animal model of central post-stroke pain (CPSP). However, no research has yet examined the involvement of learning and memory in CPSP using an animal model. The present study examined whether VBC lesions affect motor function, conditioned place preference (CPP; implicit memory), and spatial learning (explicit memory) in the acquisition and retrieval phases. The results showed that rats with VBC lesions exhibited thermal hyperalgesia in the acquisition and retrieval phases, indicating that these lesions can induce CPSP. During these phases, the rats with VBC lesions exhibited enhanced (morphine-induced) CPP learning. These lesions did not affect the rats’ total distance travelled, time spent, or velocity in the spatial learning tasks. The lesions also did not affect motor function in the rotarod task. Altogether, VBC lesions resulted in CPSP and facilitated CPP (implicit memory). However, the lesions did not affect spatial learning (explicit memory) or motor function. The relationship between CPSP and learning and memory is important for patients who suffer from such central pain. The implications of the present study may provide insights into helping reduce CPSP and its associated symptoms.

Keywords
Central post-stroke pain, conditioned place preference, spatial learning, Morris water maze, motor function, ventrobasal complex nuclei of the thalamus

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The analgesia efficiency of ultrasmall magnetic iron oxide nanoparticles in mice chronic inflammatory pain model

Yen-Chin Liu¹, Dar-Bin Shieh² and Ping-Ching Wu²,³

Abstract
Seldom research investigated the effects of iron oxide nanoparticles on analgesia. We developed inflammatory pain models via Complete Freund’s adjuvant injection over hind paw in ICR mice. Different doses of magnetite nanoparticles (Fe₃O₄, FeNP) were injected to the paw. Analgesia behavior was checked with von Frey microfilament and thermal irradiation measurement. Paw skin tissues were harvested during the maximal analgesia time point. The presence of activated white cell (CD68, myeloperoxidase) and the free radical (ROS) production were also checked. Western blotting was used to identify the changes of ROS production enzymes. FeNP demonstrated dose-related analgesia effect with significant reduction in inflammatory cells, pro-inflammatory markers, and ROS production in lesion paw. The ROS production enzymes expression were also declined. Our results indicated that local FeNP administration induced significant analgesia via attenuation of inflammatory cell infiltration and pro-inflammatory signaling as well as scavenged microenvironment free radicals in mice inflammatory pain model.

Keywords
Ultra small iron nanoparticles, inflammatory pain, free radical, anti-inflammatory effect

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Calcitonin gene-related peptide as a regulator of the pain-associated synaptic plasticity in the central nucleus of the amygdala

Yukari Takahashi¹, Kei Shinohara¹, Yuya Okutsu¹ and Fusao Kato¹

Abstract
The capsular part of the central amygdala (CeC) is one of the important regions of pain processing because it receives excitatory synaptic inputs from the lateral parabrachial nucleus (LPB), which is the major target of NK1-positive projection neurons in the lamina I of the spinal dorsal horn, and because it projects to various brain regions involved in behavioral, autonomic, and endocrine responses. Lines of evidence indicate that the excitatory synaptic transmission from the LPB neurons to those in the CeC is potentiated in various pain models. In addition to the glutamate, the LPB fiber terminals contain calcitonin gene-related peptide (CGRP) and the CeC is abundant in CGRP-binding sites. In this study, we focused on the role of CGRP in the LPB-CeC synapse for nociception-related excitation of the amygdala neurons. First, we examined the effect of exogenous CGRP to the LPB-CeC synaptic transmission using patch clamp recording in acute brain slices of naïve mice. CGRP significantly increased the amplitude of EPSCs mediated by N-methyl-D-aspartate-Rs in a protein kinase A-dependent manner but not that of EPSCs mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-Rs. The effect is concentration-dependent, antagonist-sensitive, and varied among each CeC neuron. Next, we evaluated the pain behaviors and the LPB-CeC synaptic transmission in CGRP knockout (CGRP-KO) mice after formalin injection to the hind paw. After the formalin injection, the wild-type mice showed, following the acute stereotypic nocifensive behaviors lasting for 1 h, bilateral mechanical allodynia and robust LPB-CeC synaptic potentiation at 6 h after injection. In contrast, despite manifest acute nocifensive behaviors with only a partial and time-limited attenuation, we failed to observe the allodynia and the LPB-CeC synaptic potentiation at 6 h in the CGRP KO mice. From these results, it is suggested that CGRP at the LPB-CeC synapses might play an essential role in determining plastic changes in response to increased nociceptive inputs and also presumably to the inflammatory mediators, thus defining the various altered responses through wide-spread outputs from the amygdala in chronic pain conditions.

Keywords
Central amygdala, lateral parabrachial nucleus, calcitonin gene-related peptide, patch clamp recording

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Do thalamic hemorrhagic lesions affect LiCl-induced conditioned taste aversion, episodic memory, and fear conditioning during the acquisition and retention phases in an animal model of central post-stroke pain?

Alan Bo Han He¹, Ying Hao Yu¹, Hsi-Chien Shih², Bai Chuang Shyu² and Andrew Chih Wei Huang¹

Abstract
Hemorrhagic stroke can induce many behavioral symptoms including central pain and some comorbidity symptoms such as learning and memory dysfunction or motor impairments. The purpose of the present study is to examine whether the ventrobasal complex (VBC) of thalamus lesions affected motor function, implicit memory in LiCl-induced conditioned taste aversion (CTA) and fear conditioning, and explicit memory in episodic memory in the acquisition and retrieval phases. The results appeared that the VBC lesions induced thermal hyperalgesia in the acquisition and retrieval phases to reveal central post stroke pain (CPSP) occurred at the VBC lesions. The VBC lesions did not affect LiCl-induced CTA learning in the acquisition and retrieval phases. Besides, the VBS lesion did not affect that the rats’ total distance traveled to test motor function. The VBC lesions actually disrupt episodic memory regardless of time spent and trial numbers in E-maze task during the acquisition phase. However, the VBS lesion did not affect the spent time, but it decreased trial numbers during the retrial phase in E-maze task. Interestingly, the VBC lesion decreased fear conditioning in the acquisition phase, but it did not affect fear conditioning in the retrieval phase. The present results might be important for CPSP’s major core symptom central pain and its comorbidity symptoms learning memory.

Keywords
Central post-stroke pain, conditioned taste aversion, episodic memory, fear conditioning, motor function, ventrobasal complex nuclei of the thalamus

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Elucidations of thalamic hemorrhagic lesions-induced pain and motor function, anxiety, depression, and conditioned place aversion in an animal model

Alan Bo Han He¹, Kai Chieh Chang¹ and Andrew Chih Wei Huang¹

Abstract
The present study examined whether the ventrobasal complex (VBC) of thalamus lesions changed central pain, anxiety, depression, motor deficit, and pain-induced conditioned place aversion. The results appeared that the VBC lesions could elicit thermal hyperalgesia to reveal the occurrence of central post stroke pain (CPSP). We found that the VBC lesions did not change movement in total distance traveled and max speed. The VBC lesion could increase anxiety behaviors in inside–outside numbers and spent time in inside of the central square in the open field task. Moreover, the VBS lesion decreased struggling time and increased floating time in the forced swimming test. The VBC lesion also induced conditioned place aversion. The present findings indicated that the VBC lesion to intimate the CPSP patients exhibited central pain symptom, anxiety, depression, and conditioned place aversion. The present data should be discussed further. The findings might provide some implications for CPSP in clinic.

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Role of a protein kinase C/extracellular signal-regulated kinase signaling pathway in the transition of acute to chronic pain in acid-induced muscle pain model

Wei-Hsin Chen¹, Sin-Jhong Cheng² and Chien-Chang Chen¹

Abstract

Aim: Chronic pain can be initiated by one or more acute tissue insults to sensitize the neurons into primed state. In the primed state, the basal nociceptive thresholds of the animal are normal, but in response to another hyperalgesic stimuli, the animal develop enhanced and prolonged hyperalgesia. The mechanism of priming leading to the chronic pain is not completely understood.

Methods: We use acid-induced muscle pain model to investigate the mechanism underlying the transition from acute to chronic pain. Two injections of acidic saline (pH 4.0) into gastrocnemius muscle separated by four days produce a persistent bilateral mechanical hyperalgesia lasting up to three weeks.

Results: We demonstrated that phosphorylated extracellular signal-regulated kinase (pERK)-positive neurons in amygdala, spinal cord, and dorsal root ganglion are significantly increased after 1st acid injection. Infusion of U0126, a Mitogen-activated protein kinase kinase (MEK) inhibitor, intrathecally (i.t.) but not intracerebroventricular or intramuscular prevents the development of chronic pain induced by 2nd acid injection. Furthermore, i.t. injection of protein kinase C (PKC) but not protein kinase A blocker also prevents the development of chronic pain and PKC agonist, phorbol 12,13-dibutyrate, is sufficient to induce the prolonged hyperalgesia response after acid injection. We also found that mechanistic target of rapamycin pathway-dependent protein synthesis is required for the priming establishment. Using spinal cord slice preparation, we showed that the synaptic plasticity was enhanced in dorsal horn neurons after 1st acid injection.

Conclusions: These findings reveal that the activation of PKC/extracellular signal-regulated kinase signal pathway and downstream protein synthesis is required for hyperalgesic priming and the consolidation of pain singling.

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Alleviate pain via anterior nucleus of paraventricular thalamus, a locus in the brain

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Abstract
Pain-related diseases are the top leading causes to life disability. Enhanced neuronal reactivity persistently in the central nervous system known as central sensitization is responsible for chronic pain development. Yet, the underlying brain mechanism is not completely understood. Here, we showed that anterior nucleus of paraventricular thalamus (PVA) is important for the development and maintenance of mechanical hyperalgesia in neuropathic and inflammatory pain models in mice. PVA neuronal activity increased after pain induction. An increase of cFos and extracellular signal-regulated kinase (ERK) activity and neuronal excitability of PVA were detected. Activation of PVA neurons by phorbol 12,13-dibutyrate or optogenetic technique is sufficient to enhance mechanical hyperalgesia in naïve mice. In addition, PVA received projection from the central nucleus of amygdala (CeA), a known pain-associated locus. Activation of the right CeA-innervated PVA neurons is enough to induce mechanical hyperalgesia. Furthermore, a time window to block chronic pain maintenance via either an inhibition of ERK activity or an inhibition of neuronal activity with novel Designer Receptor Exclusively Activated by Designer Drugs in PVA was demonstrated in the both pain models. Our data suggest that PVA plays an essential role in the development of chronic mechanical hyperalgesia and inhibition of PVA neuronal activity leads to a long-term alleviation of pain-like behavior after the development of chronic pain.
The roles of TDAG8 involved inflammatory hyperalgesia and experimental rheumatoid arthritis in mice

Shih-Ping Dai¹, Wei-Shan Hsieh¹ and Wei-Hsin Sun¹

Abstract
Chronic pain, resulting from injury, rheumatoid arthritis (RA), and cancer, is often accompanied by inflammation. High concentrations of protons found in inflamed tissues result in tissue acidosis, a major cause of pain and hyperalgesia. The expression of T-cell death-associated gene 8 (TDAG8), a proton-sensing G-protein-coupled receptor, is increased during inflammatory hyperalgesia. Attenuating TDAG8 expression in the spinal cord inhibits bone cancer pain, but whether TDAG8 is involved in inflammatory hyperalgesia or RA pain remains unclear. In this study, we used TDAG8-knockout or -knockdown to explore the role of TDAG8 in pain. Suppressed TDAG8 expression delayed the onset of inflammatory hyperalgesia and shortened hyperalgesic time in mice. In a dual acid-injection model (acid [pH 5.0] injected twice, 5 days apart), shRNA inhibition of TDAG8 shortened the duration of the second hyperalgesia. Similar results were found in TDAG8-deficient mice. The dual administration of TDAG8 agonist also confirmed that TDAG8 is involved in hyperalgia. Mice with TDAG8 knockout showed attenuated acute and chronic phases of RA pain. Accordingly, TDAG8 may participate in inflammatory chronic pain.

Keywords
T-cell death-associated gene 8, rheumatoid arthritis, hyperalgesia

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The cross-talk between peripheral 5-HT3 and acid-sensing ion channel 3 mediates mirror-image pain

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Abstract
Mirror-image pain that occurs in association with a variety of clinic pain syndromes, such as complex regional pain syndrome, rheumatoid arthritis, and chronic migraine, is characterized by increased pain sensitivity of healthy body regions other than the actual injured or inflamed sites. Previous studies proposed that only a high level of peripheral inflammation induces mirror-image pain. 5-Hydroxytryptamine (5-HT), postaglandin E2 (PGE2), and proton are well-known inflammatory mediators resulting in pain and hyperalgesia. Combination of 5-HT with any other mediator produced strong potentiation of pain-related behaviors, while all other combinations only induced additive or sub-additive responses. Administration of 5-HT, PGE2, or acidic buffer (pH5.0, proton) only caused unilateral hyperalgesia, but co-injection of 5-HT with acidic buffer or with PGE2-induced bilateral hyperalgesia (mirror-image pain). Inhibition of 5-HT3 or acid-sensing ion channel 3 (ASIC3) abolished mirror-image pain induced by 5-HT/acid co-injection. Interestingly, administration of 5-HT3 agonist alone induced mirror-image pain. 5-HT3 antagonist, ASIC3 blocker, 5-HT2B agonist, or protein kinase C activator reversed 5-HT3–induced mirror-image pain. The in vitro and in vivo studies suggested that a cross-talk between 5-HT3, and ASIC3-mediated mirror-image pain through activation of satellite glial cells.

Keywords
Serotonin, acid, 5-HT3, mirror image pain, Acid-sensing ion channel 3

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Involvement of advillin in somatosensory neuron subtype-specific axon regeneration and neuropathic pain

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Abstract
Advillin is a sensory neuron-specific actin-binding protein expressed at high levels in all types of somatosensory neurons in early development. However, the precise role of advillin in axon regeneration and neuropathic pain, especially in adulthood, is largely unknown. Here, we revealed advillin expression restricted to isolectin B4-positive (IB4+) neurons in adult dorsal root ganglia (DRG) and located at growth cones and the very tips of filopodia. Advillin knockout (KO) specifically impaired axonal regeneration in adult IB4+ DRG neurons. In the recovery phase of experimental autoimmune encephalomyelitis, a neuropathic pain model, advillin KO disturbed neural plasticity in the spinal cord dorsal horn, especially in IB4+ lamina, and aggravated neuropathic pain, including mechanical hyperalgesia and cold allodynia. Our study highlights a role for advillin in growth cone formation, axon regeneration, and neuropathic pain associated with IB4+ DRG neurons in adulthood.

Keywords
Neuropathic pain, axon regeneration, neural plasticity

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Severe left facial and ear pain possibly originated from left trigeminal nucleus lesion caused by centripetal migration of varicella zoster virus—A case report of MRI image

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Abstract
The varicella zoster virus may reactivate and cause several neurologic diseases, including herpes zoster ("shingles"), post-herpetic neuralgia (PHN), vasculopathy, myelopathy, corneal or retinal lesions, cerebellitis, and zoster sine herpetic. We report a case of an 82 year-old woman who had left facial eruptions and was diagnosed as herpes zoster one month ago. Immediate antiviral agent treatment limited the skin lesion extension. However, the neurological pain at left facial and ear was aggravated severely especially at night. This old lady was referred to Pain clinic and diagnosed as PHN. Magnetic resonance imaging examination revealed a small linear lesion with abnormal SI change in left dorsal cervical cord, medulla, and pons possibly associated with degenerative changes, demyelination, or cell death of left trigeminal nucleus by centripetal migration of varicella zoster virus. This patient is improving after anticonvulsant and antidepressant treatment.

Keywords
Herpes zoster, postherpetic neuralgia, central nervous system involvement of herpes zoster, magnetic resonance imaging examination

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Meeting Report

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