Oral Neuroscience 2017

Saturday, August 26th, 2017

Program & Abstract

Venue

Osaka University

Graduate School of Dentistry

Osaka, Japan

大阪大学
OSAKA UNIVERSITY

Remarks

All attendance including oral or poster presenters, chairmans and staffs, please attend with wearing casual (informal) clothes (e.g., no jacket, no tie).

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Acknowledgments

This symposium is partly supported by “Grant for inter-University Symposia FY2017” from Osaka University.

Organizing Committee

Atsushi Yoshida*, Dept. of Oral Anatomy and Neurobiology, Osaka Univ.
Mikihiho Kogo, First Dept. of Oral and Maxillofacial Surgery, Osaka Univ.
Satoshi Wakisaka, Dept. of Oral Anatomy and Developmental Biology, Osaka Univ.
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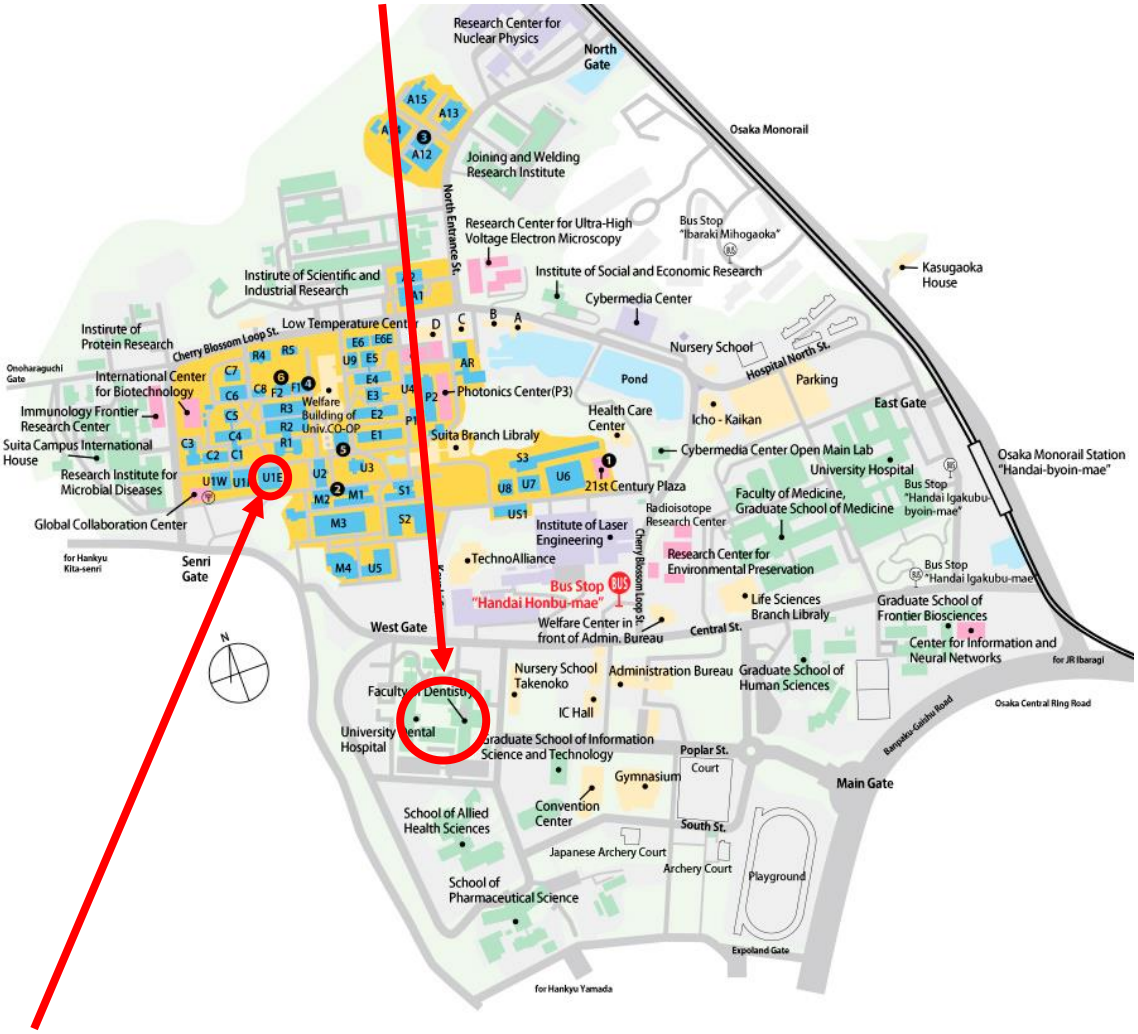
Access

Oral Session & Poster Session: Yumikura memorial hall (Building F, 5F)

Osaka University Graduate School of Dentistry

1-8 Yamadaoka, Suita, Osaka, Japan 565-0871



Information exchange meetings (18:00-)
La Scena, Faculty of Engineering, GSE Common East 15F
(about five minutes' walk from Dental faculty building)

Oral presentation information

As you prepare for your oral presentation at Oral Neuroscience 2017, please find important information concerning your oral presentation.

Presentation time

- ✓ The time allowed for the slide presentation of Lectures is 25 minutes including 7 minutes for discussion.
- ✓ The time allowed for the slide presentation of Special Lectures and Plenary Lecture are 45 minutes including 7 minutes for discussion.

Please bring your presentation on your PC and/or USB flash Drive.

- ✓ Please check a monitor output terminal of your PC. If you are using Sony VAIO, Macintosh, or other types of PC with a special format monitor output terminal, please bring a D-sub 15 pin conversion adaptor with you.
- ✓ Be sure to bring an AC adaptor.
- ✓ Please adjust your computer settings so it does not revert to screensaver or energy-saving mode during your presentation.
- ✓ If your presentation data includes links to movies, graphs, or similar data, please be sure to save these files and check their operation in advance.
- ✓ Audio cannot be used during presentation.
- ✓ After your presentation, please receive your computer from the PC staff.

If you do not use your own PC, please make your presentation using Power Point.

- ✓ Power point 2013 (Windows) or Power Point 2011 (Mac OSX) is available on your presentation.
- ✓ You use standard fonts (e.g. Times Roman, Arial) in your presentation.
- ✓ Include your ppt (pptx) file and any linked files (e.g. movie file) in the same folder.
- ✓ Test your presentation on a separate PC.

Please bring your PC to the PC staff at break times or below times and check your presentation before your presentation.

Morning session: 9:15-9:45 Afternoon session: 12:50-13:10

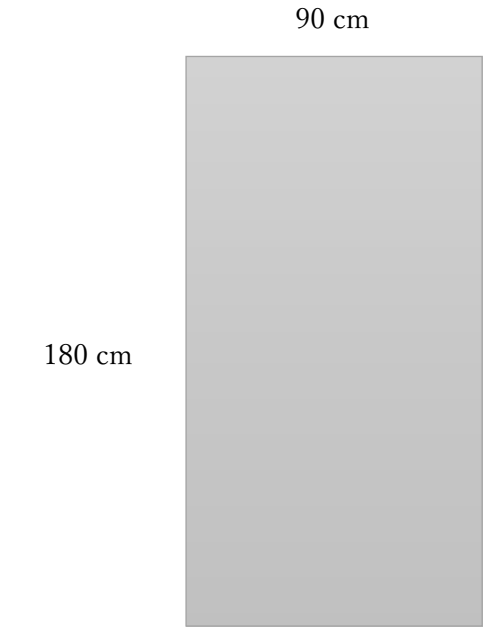
- ✓ We appreciate if you bring your data (USB drive) before the onset of morning session.

Poster presentation information

As you prepare for your poster presentation at Oral Neuroscience 2017, please find important information concerning your poster presentation.

Presentation time: 12:10-13:30 and 14:45-15:15

- ✓ Area of poster presentation: Height 120 cm x Width 90 cm
- ✓ Thumbtacks will be supplied on site.
- ✓ Please mount your poster on the board of your poster number by noon.
- ✓ Posters must remain on display until the end of your poster session.
- ✓ Posters must be removed by 17:50 following your poster presentation.
- ✓ No photography is permitted in the poster sessions.



Oral Neuroscience 2017

Yumikura memorial hall (Dental faculty building F, 5F)

09:55- Opening Remarks Atsushi Yoshida

Session 1	Chair	Takafumi Kato Chiho Kudo

10:00- [Lecture-1] Response characteristics of identified primary afferents in the rat vibrissal system

Takahiro Furuta (Graduate School of Medicine, Kyoto University)

10:25- [Lecture-2] Gustatory and somatosensory characteristics of receptive fields of rat chorda tympani geniculate ganglion neuron

Yusuke Yokota (Graduate School of Dentistry, Osaka University)

10:50- [Lecture-3] Role of neuron-glia interaction in tongue neuropathic pain

Ayano Katagiri (Nihon University School of Dentistry)

Session 2	Chair	Tomio Inoue Yuji Masuda

11:20- [Lecture-4] Involvement of TRPM8 in mediating the superior laryngeal nerve activity and facilitating the triggering of swallowing reflex

Mohammad Zakir Hossain (Matsumoto Dental University)

11:45- [Lecture-5] Central modulation of swallowing and breathing via serotonin and substance P

Tadashi Yamanishi (Graduate School of Dentistry, Osaka University)

Lunch and Poster Session (12:10-13:30)

Session 3	Chair	Kazuhiro Takuma Masayuki Kobayashi

13:30- [Lecture-6] Coordinated movement of the tongue in oral motor functions
Kiyomi Nakayama (Showa University School of Dentistry)

13:55- [Lecture-7] Subthalamo-pallidal interactions in goal-directed actions
Yoshihisa Tachibana (Kobe University Graduate School of Medicine)

14:20- [Lecture-8] Development of high-speed and scalable whole-brain imaging system at subcellular resolution
Kaoru Seiriki (Graduate School of Pharmaceutical Sciences, Osaka University)

Coffee Break and Poster Session (14:45-15:15)

Special Lecture	Chair	Satoshi Wakisaka Mikihiko Kogo

15:15- Neural changes in brain reward and feeding-inhibitory systems associated with binge-like sucrose overconsumption
Yasunobu Yasoshima (Graduate School of Human Sciences, Osaka University)

16:00- A role of glial cells in the development of trigeminal neuropathic pain
Dong-Kuk Ahn (School of Dentistry, Kyungpook National University)

Plenary Lecture	Chair	Atsushi Yoshida

16:50- Ultrastructural basis for processing of craniofacial sensory information in the brain stem
Yong Chul Bae (School of Dentistry, Kyungpook National University)

Closing Remarks Mikihiko Kogo

18:00- Information exchange meeting at the La Scena, GSE Common East 15F

Poster presentations

[Poster-1]

Acute footshock stress can modulate the vigilance states and jaw muscle activities in guinea pigs

Hiroyuki Yano[1,2,3], Hiroshi Yano[1,2,3], Yoshio Ueno[1,2], Makoto Higashiyama[3], Narikazu Uzawa[1], Atsushi Yoshida[2], Takafumi Kato[3]

[1]Department of Oral and Maxillofacial Surgery II, [2]Department of Oral Anatomy and Neurobiology, [3]Department of Oral Physiology, Osaka University Graduate School of Dentistry

[Poster-2]

Slow inward rectifying currents participate in control of bursting behavior in mesencephalic trigeminal neurons

Susumu Tanaka[1], Saori Yamada[1], Akifumi Enomoto[2], Soju Seki[1], Tadataka Tsuji[1], and Mikihiko Kogo[1]

[1]1st Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry, Osaka University

[2]Department of Oral and Maxillofacial Surgery, Kinki University School of Medicine

[Poster-3]

Development of resurgent and persistent sodium currents in mesencephalic trigeminal neurons

Akifumi Enomoto [1,2], Soju Seki [2], Susumu Tanaka[2], Kohji Ishihama[2], Tadashi Yamanishi[2], Mikihiko Kogo[2], Suguru Hamada[1]

[1]Department of Oral and Maxillofacial Surgery, Kindai University Faculty of Medicine

[2]1st Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry, Osaka University

[Poster-4]

Expression of transient receptor potential ankyrin 1 and vesicular glutamate transporters in the rat trigeminal ganglion

Yun Sook Kim, Sung Kook Kim, Yong Chul Bae

Department of Anatomy and Neurobiology, School of Dentistry, Kyungpook National University, South Korea

[Poster-5]

Expression of vesicular glutamate transporter 1 (VGLUT1) and VGLUT2 in the sensory root and soma of the rat trigeminal ganglion in the normal condition and following inflammation

Yi Sul Cho, Jin Young Bae, Hye Min Han, Youn Gyung Kim, Yong Chul Bae

Dept. Anatomy and Neurobiol, School of Dentistry, Kyungpook National University, Korea

[Poster-6]

$P2Y_2$ receptor in goblet cells is involved in dry-eye pain in rats

Shiori Sugawara[1,2], Lou Mikuzyki[1,2], Hiroto Saito[2], Ayano Katagiri[2], Koichi Iwata[2]

[1] Tokyo Medical and Dental University Department of Psychosomatic Dentistry Graduate School,

[2] Department of Physiology, Nihon University School of Dentistry

[Poster-7]

Gingerol and shogaol, the herbal contents, relief oral ulcerative mucositis-induced pain through sodium channel blockage

Suzuro Hitomi[1], Kentaro Ono[1], Izumi Ujihara[1], Kiyoshi Terawaki[2], Chinami Matsumoto[2], Yuji Omiya[2], Kiyotoshi Inenaga[1]

[1] Division of Physiology, Kyushu Dental University, Fukuoka, Japan

[2] Tsumura Research Laboratories, Kampo Scientific Strategies Division, Tsumura & Co., Ibaraki, Japan

[Poster-8]

Blockade of glial EphA4 attenuates mechanical allodynia in rats with trigeminal neuropathic pain

Jo-Young Son[1], Jin-Sook Ju[1], Song-Hee Kang[1], Min-Kyoung Park[2], Min-Kyung Lee[3], Dong-Kuk Ahn[1*]

[1] Department of Oral Physiology, School of Dentistry, Kyungpook National University, Daegu, Korea, [2] Department of Dental Hygiene, Kyung-Woon University, Gumi, Korea, [3] Department of Dental Hygiene, Dong-Eui University, Busan, Korea

[Poster-9]

Distribution differences of thalamic and parabrachial projection neurons receiving C-fiber afferents in the trigeminal spinal subnucleus caudalis and upper cervical spinal cord

Hiroto Saito[1], Ayano Katagiri[2] and Koichi Iwata[2]

[1] Department of Prosthodontics, Nihon University School of Dentistry, [2] Department of Physiology, Nihon University School of Dentistry

[Poster-10]

GABAergic neuronal membrane potential responses to dental pulp stimulation in the rat insular cortex: An *in vivo* targeted whole-cell patch-clamp recording study

Shota Murayama[1,2], Bunnai Ogiso[2], Masayuki Kobayashi[1]

[1]Department of Pharmacology and [2] Department of Endodontics, Nihon University School of Dentistry, Tokyo, Japan

[Poster-11]

Nicotine suppresses synaptic potentiation in layer V pyramidal neurons of the insular cortex

Hiroki Toyoda, Hajime Sato, Tsutomu Kawano, Dong Xu Yin and Takafumi Kato

Department of Oral Physiology, Osaka University Graduate School of Dentistry, Suita, Japan

[Poster-12]

Differential gene expression patterns in iPSC-derived neurons from monozygotic twin cases with treatment-resistant schizophrenia and discordant for clozapine responses

Takanobu Nakazawa and Kazuhiro Takuma

Department of Pharmacology, Graduate School of Dentistry, Osaka University

Abstract
Oral Session

Response characteristics of identified primary afferents in the rat vibrissal system

Takahiro Furuta
Department of Morphological Brain Science, Graduate School of Medicine, Kyoto University

When compared with the visual or auditory systems, the peripheral transduction and coding mechanisms that underlie tactile sensation are as yet poorly understood. Here we exploited the unique morphology of the rat vibrissal (whisker) array to investigate coding and transduction properties of primary tactile afferents. Specifically, we performed in vivo intra-axonal recording and labeling experiments to quantify the responses of four types of identified mechanoreceptors in the vibrissal follicle. After accounting for three-dimensional vibrissal geometry, a simple, three-parameter mechanical model explained many features of the neural responses. Results also revealed a distinct anatomical basis for the difference between the responses of RS-Merkel and lanceolate endings. The present study systematically bridges between the architecture of the tactile sensing apparatus, the response properties of identified primary afferents, and the mechanics that describe touch. Aspects of the model could be applied to the study of more complex mechanical input.

Key Words: Mechanoreceptor, Peripheral nervous system, System neuroanatomy

Lecter-2

Gustatory and somatosensory characteristics of receptive fields of rat chorda tympani geniculate ganglion neuron

Yusuke Yokota[1], Archana Kumari[2], Charlotte M. Mistretta[2], Mikihiko Kogo[1] and Robert M. Bradley[2]

[1]The First Department of Oral and Maxillofacial Surgery, Osaka University Graduate School of Dentistry, Suita, Osaka, Japan, [2]Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI, United States

Primary afferent neurons of the chorda tympani (CT) convey information to the central nervous system about sensory properties of food. Previous investigations of the CT have emphasized responses to stimulation of the anterior tongue with chemicals. We studied the receptive field properties of chorda tympani geniculate ganglion (GG/CT) neurons using extracellular recordings and stimulation of the tongue with chemicals (Yokota and Bradley 2016). However the CT also responds to thermal and tactile stimulation of the tongue and we have investigated the cold and tactile responses of GG/CT neurons. Eleven GG/CT neurons have been isolated that responded only to cold stimulation. Receptive field size of these units was 2.1 ± 0.3 (mean, S.E.) fungiform papillae, significantly smaller than the average field size of chemical-responding units (5.3 ± 0.5 papillae; n=8). The cold-responding papillae were located at the tongue tip. We have also isolated GG/CT neurons that innervate fungiform papillae responding exclusively to tactile stimulation (n=12). These units responded to stroking the tongue dorsum and also have small receptive fields (2.3 ± 0.2 papillae). Receptive fields of these units were evenly distributed over the entire anterior tongue; however they tended to be localized more along the midline region than at tongue margins. Detailed analysis of the receptive field of GG/CT neurons that responded to both taste stimuli and cold water revealed that only a subset of the fungiform papillae making up the receptive field respond to the cold stimuli, whereas the other papillae were unresponsive to cold (n=9). With chemical, thermal and mechanical stimuli demonstrated unique multisensory characteristics of GG/CT neurons. Our finding that fungiform papilla receptive fields have a multisensory function suggests a unique role in sensory input to the central nervous system.

Key Words: geniculate ganglion neurons, multisensory, receptive field

Role of neuron-glia interaction in tongue neuropathic pain

Ayano Katagiri and Koichi Iwata
Nihon University School of Dentistry Department of Physiology

Iatrogenic trigeminal nerve injuries remain a common and complex clinical problem. Especially lingual nerve injury is known to cause severe persistent pain in the tongue, and the patients complain of various functional deficits in the mouth such as speech, mastication and many other daily activities due to tongue pain. Satellite glial cell (SGC) activation, associated phosphorylation of extracellular signal-regulated kinase (ERK) and neuropeptide expression in the trigeminal ganglion (TG) are known to be involved in trigeminal neuropathic pain related to trigeminal nerve injury. However, the involvement of these molecules in orofacial neuropathic pain mechanisms is still unknown. Phosphorylation of ERK1/2 in lingual nerve crush (LNC) rats was observed in SGCs. To evaluate the role of neuron-SGC interactions in tongue neuropathic pain, calcitonin gene-related peptide (CGRP)-immunoreactive (IR), phosphorylated ERK1/2-IR and glial fibrillary acidic protein (GFAP)-IR cells in the TG were studied in LNC rats.

In LNC rats, mechanical and heat hypersensitivity of the tongue and SGC activation was observed. The number of CGRP-IR neurons and neurons encircled with pERK1/2-IR SGCs was significantly larger in LNC rats compared with sham rats. The percentage of large-sized CGRP-IR neurons was significantly higher in LNC rats. The number of CGRP-IR neurons, neurons encircled with pERK1/2-IR SGCs and neurons encircled with GFAP-IR SGCs was decreased following CGRP receptor blocker CGRP8-37 or mitogen-activated protein kinase/ERK kinase 1 inhibitor PD98059 administration into the TG after LNC. Reduced thresholds to mechanical and heat stimulation to the tongue in LNC rats were also significantly recovered following CGRP8-37 or PD98059 administration.

The present findings suggest that CGRP released from TG neurons activates SGCs through ERK1/2 phosphorylation and TG neuronal activity is enhanced, resulting in the tongue hypersensitivity associated with lingual nerve injury. The phenotypic switching of large myelinated TG neurons expressing CGRP may account for the pathogenesis of tongue neuropathic pain.

Key Words: Lingual neuropathic pain, satellite glial cells, CGRP

Involvement of TRPM8 in mediating the superior laryngeal nerve activity and facilitating the triggering of swallowing reflex.

Mohammad Zakir Hossain[1], Shumpei Unno[1], Hiroshi Ando[2], Yuji Masuda[3] and Junichi Kitagawa[1]
[1]Department of Oral Physiology, [2]Department of Biology, [3]Institute for Oral Science, Matsumoto Dental University, 1780 Gobara, Hirooka, Shiojiri, Nagano 399-0781, Japan.

The larynx and associated laryngopharyngeal regions are important areas for swallowing, respiration, and phonation and are sources of vital reflexes like swallowing reflex. Superior laryngeal nerve (SLN) that supply these regions plays an important role in triggering swallowing reflex. SLN has unique responses to chemical stimuli, the underlying mechanism of which has not yet been fully elucidated. Transient receptor potential (TRP) channels may play a role in mediating the SLN response to the chemical stimuli. Besides, delayed triggering of swallowing reflex is a common disorder in aged population and following cerebral vascular accidents that increases the incidence of pulmonary aspirations. Our objectives were to understand whether transient receptor potential melastatin-8 (TRPM8) channels are active in larynx and associated laryngopharyngeal regions and whether they are involved in mediating SLN activity and triggering swallowing reflex. TRPM8 expressions observed in the nodose-petrosal ganglionic complex where cell bodies of the afferents of SLN are located. Most of the TRPM8 were expressed on non-myelinated neurons. Topical application of a TRPM8 agonist, menthol (12.5mM, 25mM and 50mM) in the SLN innervated regions, dose dependently increased the whole nerve activity of the SLN, while, at a high dose (100mM), there was an initial increase followed by a decrease of the SLN activity. Highest nerve response to menthol was observed at the dose of 50mM and the response was reduced by the prior application of a TRPM8 blocker. In addition, topical menthol application facilitated the triggering of swallowing reflex that was significantly attenuated by prior application of the TRPM8 blocker. Furthermore, transection of SLN, completely abolished the menthol evoked swallowing reflexes. These findings suggest that TRPM8 channels are involved in mediating SLN activity and facilitating the triggering of swallowing reflex. TRPM8 channel agonists can be tested for management of dysphagia and reduction of incidence of pulmonary aspiration.

Key Words: TRPM8 channels; Superior laryngeal nerve; Swallowing reflex

Central modulation of swallowing and breathing via serotonin and substance P

Tadashi Yamanishi, Tetsuya Seikai, Hironobu Kobashi, Takeshi Togawa, Takahide Kondo, Hidehiko Koizumi, Mikihiko Kogo

Department of Oral and Maxillofacial Surgery, Osaka Women's and Children's Hospital, Osaka, Japan, First Department of Oral and Maxillofacial Surgery, Osaka University Graduate School of Dentistry, Osaka, Japan

Brainstem serotonin (5-HT) and substance P (SP) modulate activity of many neural circuits in the mammalian brain, but in many cases precise mechanisms have not been resolved. We analyzed actions of raphé neurons on respiratory network activity in neonatal rat medullary slices in vitro, and found that at basal levels of activity, excitation of the respiratory network via simultaneous release of 5-HT and SP was required to maintain inspiratory motor output in both the neonatal and juvenile systems. The midline raphé obscurus contained spontaneously active 5-HT neurons, some of which projected to the pre-Bötzinger complex (pBC) of the kernel circuit for respiratory rhythm generation, and colocalized 5-HT and SP. Experimentally augmenting raphé obscurus activity increased motor output by simultaneously exciting pre-BötC and motor neurons. We also found that 5-HT, but not SP, can transform the electrophysiological phenotype of some pre-BötC neurons to intrinsic bursters, providing 5-HT with a role in promoting rhythm generation. We conclude that both 5-HT and SP modulate in an excitatory way the kernel circuit required for generation of respiratory motor output through spontaneously active raphe neurons. On the other hand, our study on the brainstem network for generation of swallowing activity revealed that 5-HT and SP also work together to modulate swallowing activity but in a different way. We analyzed swallowing activity experimentally induced in working heart brainstem-spinal cord preparations (WHBP) of juvenile rats, and found that exogenous application of 5-HT into the nucleus tractus solitaries (NTS), the central pattern generator for swallowing activity, inhibited generation of swallowing activity whereas SP enhanced induction of swallowing activity. These results suggest a reciprocal role of 5-HT and SP on initiation of swallowing activity.

Key Words:

Coordinated movement of the tongue in oral motor functions

Kiyomi Nakayama[1], Satoshi Tachikawa[2], Yoshiaki Ihara[3], Shiro Nakamura[1], Ayako Mochizuki[1], Takehiko Iijima[2], Koji Takahashi[3], Tomio Inoue[1]

[1]Department of Oral Physiology, [2]Department of Perioperative Medicine Division of Anesthesiology, [3]Department of Special Needs Dentistry Division of Oral Rehabilitation, Showa University School of Dentistry, Tokyo, Japan

The tongue can move freely and plays important roles in oral motor functions such as suckling, chewing, swallowing, respiration, and speech. Since the tongue is attached to the hyoid, mandible, and pharyngeal wall, the tongue should move in concert with the movements of those structures. We have investigated coordination between tongue and these structures during suckling-like activity and respiration. To examine the neural mechanisms underlying coordination between the activity of motor nerves innervating the tongue and jaw muscles during suckling-like activity, rhythmic activity in the hypoglossal nerve that is coincident with rhythmic activity in the ipsilateral trigeminal motor nerve was induced by application of N-methyl-D-aspartate to the neonatal mice brainstem-spinal cord preparations. Complete left/right separation of the preparation or partial midline transection of the preparation from the anterior inferior cerebellar artery to the junction of the vertebral artery only abolished activity in the trigeminal motor nerve. These results suggest that the neuronal network contributing to coordinated activity of the jaw/tongue muscles is located on both sides of the preparation and sends motor commands to contralateral trigeminal motoneurons across the midline, whereas inputs are sent to the ipsilateral hypoglossal motoneurons. Arterially perfused decerebrate rat preparations were used to investigate coordinated movement of the upper airway muscles including the tongue muscles, infrahyoid muscles, cricothyroid and inferior pharyngeal constrictor muscles, and laryngeal abductors and laryngeal adductors during respiration. The preparations exhibited stable inspiratory activity in the phrenic nerve, with efferent nerves innervating the upper airway muscles (the hypoglossal nerve, a branch of the cervical spinal nerve, external branch of the superior laryngeal nerve, and the recurrent laryngeal nerve) under normocapnic conditions. During hypercapnia, pre-inspiratory discharges appeared in all nerves innervating upper airway muscles. Such coordinated activity in the pre-inspiratory phase during hypercapnia contributes to dilation of the upper airway and may facilitate ventilation.

Key Words: Hypoglossal nerve, Trigeminal nerve, Upper airway

Subthalamo-pallidal interactions in goal-directed actions

Yoshihisa Tachibana
Division of System Neuroscience, Kobe University Graduate School of Medicine, Kobe, Japan

To achieve a goal-directed action, we choose one of candidate actions by suppressing the other. The basal ganglia (BG), which consist of the striatum, globus pallidus, subthalamic nucleus, and substantia nigra, are thought to regulate the goal-directed action. First, I will talk about the neuronal processing in BG circuitry, which can facilitate and suppress motor actions. Then, I will focus on abnormal subthalamo-pallidal interactions in primate models of BG disorders such as Parkinson's disease (hypokinetic disorder) and hemiballism (hyperkinetic disorder). In addition to the motor function, I have also been studying cognitive/motivational functions of BG. In the latter part of my talk, I will present neuronal data recorded from the subthalamic nucleus and the ventral pallidum in the primates while performing behavioral tasks related to reward-directed actions.

Key Words: basal ganglia, motor actions, reward

Development of high-speed and scalable whole-brain imaging system at subcellular resolution

Kaoru Seiriki[1,2], Atsushi Kasai[1], Hitoshi Hashimoto[1,3,4]

[1]Laboratory of Molecular Neuropharmacology, Graduate School of Pharmaceutical Sciences, Osaka University,[2]Interdisciplinary Program for Biomedical Sciences, Institute for Academic Initiatives, Osaka University, [3] Molecular Research Center for Children's Mental Development, United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University and University of Fukui, Suita, [4]Division of Bioscience, Institute for Datability Science, Osaka University.

Whole-brain imaging and systems analyses of entire brains at subcellular resolution are prerequisites for understanding the mechanisms underlying brain function and dysfunction. Recent advances in whole-brain imaging techniques, such as light-sheet microscopy with tissue-clearing techniques and serial sectioning and imaging methods, have successfully visualized various brain structures and activation patterns in rodents; however, it is still challenging to detect single cells and long-range axonal projections throughout the whole brain due to imaging speed at high resolution. Here, we developed a high-speed whole-brain imaging system at subcellular resolution, named FAST (block-face serial microscopy tomography). This system achieved to acquire a whole mouse brain image at subcellular resolution in 2.4 hours, which can facilitate multiple brain imaging and subsequent statistical comparison. We performed a multivariate analysis of whole-brain activation patterns at single-cell level using Arc-dVenus reporter mice in which destabilized fluorescent protein dVenus is driven by activation-dependent gene Arc promoter. In the activation mapping study, we focused on disease-related activation patterns induced by pharmacological blockade of *N*-methyl-D-aspartate receptor (NMDAR), which causes psychotomimetic effects in both human and rodents. Although the neurobiological mechanisms have been investigated using rodents as a model of schizophrenia, brain regions associated with behavioral deficits are not well identified due to the ubiquitous expression of NMDAR. Our analysis successfully identified NMDAR antagonist-induced activation in some brain regions including the anterior olfactory area and orbitofrontal cortex. We further applied FAST system to non-human primate brains, and imaged the entire cells in a marmoset brain hemisphere and neuronal projections in a whole marmoset brain. These results indicate that FAST system could identify anatomical and functional features in the whole brain from rodents to non-human primates, and will contribute to understanding anatomical and functional brain networks at systems level.

Key Words: Whole-brain imaging, subcellular resolution, non-human primate

Special Lecture

Neural changes in brain reward and feeding-inhibitory systems associated with binge-like sucrose overconsumption

Yasunobu Yasoshima
Division of Behavioral Physiology, Graduate School of Human Sciences,
Osaka University, Suita, Japan

Binge overconsumption is one of eating disorders in human. It has features such as excessive intake of palatable foods for a short period of time and excessive overconsumption in the absence of hunger. To investigate neural mechanisms underlying the eating disorder, we produced a mouse model of overconsumption of a sucrose solution. Sucrose overconsumption is suggested to be associated with elevation of extracellular dopamine (DA) level in the brain reward system. However, it remains unsolved how DA is elevated in the binge-like behavior. We first focused on ghrelin-related signals in the ventral tegmental area (VTA). To examine ghrelin involvement in binge-like sugar overconsumption, we assessed the effects of systemic and central blockade of ghrelin signaling. C57BL/6J male mice received limited access to a 0.5 M sucrose solution and standard chow for 10 days. After the regimen, intraperitoneal injections of [D-Lys3]-GHRP-6 (DG-6), an antagonist of ghrelin receptors (growth hormone secretagogue receptors), significantly suppressed the binge-like behavior. Intra-VTA infusions of DG-6 also suppressed the expression of the binge-like behavior. Next, we examined alteration in the feeding-inhibitory mechanisms in the mice showing the binge-like behavior. We compared visceral responses in the brainstem nuclei such as the nucleus tractus solitarius (NTS) and parabrachial nucleus (PBN) to intra-gastric infusions of sucrose or intraperitoneal injection of peptide YY between the mice with and without the binge-like behavior. c-Fos-like immunereactivity was used as neuronal activation marker. Our data showed that Fos-like immunoreactive cells in the NTS and PBN after the visceral stimulation were significantly less in mice with sugar bingeing than mice without binge. Taken together, the binge-like sucrose overconsumption in mice may be due both to enhanced activity of the brain reward system via ghrelin signals and blunting of central visceral processing to produce satiety/satiation-related signals.

Key Words: Binge-like sucrose overconsumption, brain reward system, visceral processing

Special Lecture

A role of glial cells in the development of trigeminal neuropathic pain

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Generally, pain is classified into two categories: acute and chronic pain. owever, the underlying mechanisms of acute and chronic pain are totally different. Chronic pain is a kind of pain disorder. In clinic, it is hard to cure chronic pain by conventional analgesics after pain has been established. Because there are several underlying mechanisms participated in the development of chronic persistent pain; peripheral mechanisms, central mechanisms, peripheral or central sensitization, pain modulation system. Recently, glial cells also participate in the development of chronic pain. Especially, microglia and astrocyte play a critical role in the transmission or modulation of pain. My first two topics show evidences for participation of glial cells in the development of trigeminal neuropathic pain. The next two topics handle with underlying cellular mechanisms of the development of trigeminal neuropathic pain. First, we demonstrated that a role of EphA4 in the development of trigeminal neuropathic pain. Our current findings show that a nerve injury induced by mal-positioned dental implants produces significant mechanical allodynia as well as up-regulation of astrocytic EphA4 expression in the medullary dorsal horn (MDH). An early treatment protocol with EphA4-Fc, an EphA4 inhibitor, significantly attenuated mechanical allodynia and reduced the up-regulated EphA4 expression. Moreover, intracisternal administration of EphA4 siRNA produced anti-allodynic effects and reduced the upregulated EphA4 expression. These results suggest that early blockade of EphA4 signaling in the astrocytes inhibited development of trigeminal mechanical allodynia after nerve injury. In the second experiment, we also demonstrated a role of VEGF in in the development of trigeminal neuropathic pain. Intracisternal infusion of VEGF antibody significantly attenuated mechanical allodynia produced by mal-positioned dental implants. Intracisternal injection of a VEGF R1 inhibitor or a VEGF R2 inhibitor produced inhibition of mechanical allodynia. Western blotting analysis reveals that nerve injury produced up-regulation of VEGF expression in the MDH. Double staining data showed that VEGF R2 co-localized with astrocyte cells but VEGF R1 found in vessels stained positive with BBB in the MDH. In addition, inferior alveolar nerve injury increased extravasated Evans's blue and NaF level which was blocked by intracisternal infusion of VEGF antibody. Moreover, intracisternal administration of VEGF siRNA produced anti-allodynic effects and reduced the upregulated VEGF expression. These results suggest that central VEGF pathway play a critical role in the development of trigeminal neuropathic pain. In summary, our results suggest that modulation of glial cells is a new potential therapeutic target for neuropathic pain control including the orofacial area.This research was supported by the National Research Foundation of Korea (NRF) and funded by the Ministry of Science, ICT and Future Planning. (2012M3A9B6055414).

Key Words: glial cell, trigeminal neuropathic pain

Plenary Lecture

Ultrastructural basis for processing of craniofacial sensory information in the brain stem

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Neural informations are processed and transmitted to the postsynaptic neurons at the synapse. If we can label specific neurons and axons of known function and analyze their fiber type and synaptic organization at the synapse, we can understand how the specific neural information conveyed via the specific neuron is processed and transmitted to the postsynaptic neurons. In this symposium I would like to present our recent findings on the central and peripheral processing of the craniofacial sensory information. TRPM8-mediated craniofacial cold information and taste sensation via chorda tympani afferent is transmitted via distinct type of fiber and processed in a distinct manner at the 1st relay nucleus in the brainstem. Trigeminal primary somatosensory neurons receive glycine-mediated presynaptic modulation via extrasynaptic homomeric glycine receptor whereas soma-dendrites of the brainstem neurons receive postsynaptic glycinergic inhibition via heteromeric, subsynaptic glycine receptor. We also demonstrated for the first time preferential localization of $P2X_3$ to the fine astrocytic processes in the trigeminal caudal nucleus, provided evidence that $P2X_3$ in the astrocytic fine process plays a role in the enhanced pain response in a rat model of peripheral nerve injury, and that the expression of astrocytic $P2X_3$ may be regulated by glutamate released from terminals of primary afferent neurons via astrocytic mGluR5.

Key Words: Trigeminal, ultrastructure, sensory processing

Abstract
Poster Session

Poster-1

Acute footshock stress can modulate the vigilance states and jaw muscle activities in guinea pigs

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Experimental stress is known to modulate vigilance states in the laboratory animals. The aim of this study was to investigate the effects of acute footshock stress (FS) on non-functional jaw muscle activities in association with the changes in vigilance states in the freely-moving animals. Methods: Guinea pigs were surgically prepared for chronic recordings of electroencephalogram, electro-occulogram, and electromyograms of neck and masseter muscles in free-moving conditions. Recordings were made from 10:30 to 12:30 on the two days. During recording, animals were cable-connected and housed in a footshock chamber in the sound-attenuated box. On the first day, animals did not receive FS. On the second day, FS was given to the animals for 30 minutes from 10 o'clock. The following variables were analyzed for a two-hour period after FS on the second day and during the same time period on the first day. Vigilance states were defined as wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. Rhythmic masticatory muscle activity during NREM sleep (RMMA) and non-functional repetitive masseter activity during wakefulness (NFRMA) were scored. These variables were compared between the first (non-FS condition) and the second (FS condition) days.Results: Compared to non-FS condition, the percentage of wakefulness was significantly higher ($p < 0.05$) while that of NREM sleep was significantly lower ($p < 0.05$) in the FS condition. No significant change was found for the percentage of REM sleep. RMMA significantly increased from non-FS to FS condition ($p < 0.05$) while NFRMA significantly decreased from non-FS to FS condition ($p < 0.05$).Conclusion: These results suggest that two types of non-functional jaw muscle activities, one occurring during NREM sleep and another during wakefulness, can represent distinct manifestations of stress-related jaw motor reactions under the altered vigilance states after acute FS.

Key Words: sleep, footshock stress, rhythmic masticatory muscle activity

Poster-2

Slow inward rectifying currents participate in control of bursting behavior in mesencephalic trigeminal neurons

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Mesencephalic trigeminal neurons (MTNs) exhibits bursting activities as intrinsic membrane property and previous studies have revealed that critical involvement of 4-AP sensitive non-inactivating K^+ current and persistent sodium current (I_{NaP}) for those activities. Inward rectification manifesting as a depolarizing sag is also common membrane property of many neurons in CNS and is prominent in MTNs. Past study revealed h-current (I_h) underlying this membrane property contributes to control of resting membrane potential and the subthreshold and firing behavior such as low frequency resonance or repetitive firing activities by mediating intra-spike frequency. Given the critical role of I_h in maintain spike train discharge characteristics as shown in I_{NaP}, I_h could participate in control of intrinsic bursting activities by influencing spike frequency control. Therefore, in the present study, we examined the precise role of I_h on the properties of bursting activities in detail using specific I_h blocker.
Sprague-Dawley rats (P10-12) were employed to make coronal brainstem slices containing the mesencephalic trigeminal nucleus and whole-cell patch-clamp recordings were performed to examine a possible role of inward rectifying currents on the intrinsic bursting behavior. External $BaCl_2$ for the reduction of fast inward rectifying current component, I_{Kir}, showed amplification of bursting, while application of ZD 7288, selective blocker of slow inward rectifying current component, I_h, prominently suppressed bursting activities by decrease of burst duration and prolonged cycle duration. Voltage sag elicited by hyperpolarizing current pulses and subsequent post-inhibitory rebound (PIR) were completely abolished after ZD application. Further investigation revealed that I_h could participate in the regulation of bursting behavior by hasten the repolarizing phase of late AHP following each action potential during bursting.

Key Words: slow inward rectifying currents, intrinsic bursting, mesencephalic trigeminal neurons

Poster-3

Development of resurgent and persistent sodium currents in mesencephalic trigeminal neurons

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Sodium channels play multiple roles in the formation of neural membrane properties in mesencephalic trigeminal (Mes V) neurons and in other neural systems. Mes V neurons exhibit conditional robust high-frequency spike discharges. As previously reported, resurgent and persistent sodium currents (I_{NaR} and I_{NaP}, respectively) may carry small currents at subthreshold voltages that contribute to generation of spike firing. These currents play an important role in maintaining and allowing high-frequency spike discharge during a burst. In the present study, we investigated the developmental changes in tetrodotoxin-sensitive I_{NaR} and I_{NaP} underlying high-frequency spike discharges in Mes V neurons. Whole-cell patch-clamp recordings showed that both current densities increased one and a half times from postnatal day (P) 0-6 neurons to P7-14 neurons. Although these neurons do not exhibit subthreshold oscillations or burst discharges with high-frequency firing, I_{NaR} and I_{NaP} do exist in Mes V neurons at P0-6. When the spike frequency at rheobase was examined in firing Mes V neurons, the developmental change in firing frequency among P7-14 neurons was significant. I_{NaR} and I_{NaP} density at -40 mV also increased significantly among P7-14 neurons. The change to an increase in excitability in the P7-14 group could result from this quantitative change in I_{NaP}. In neurons older than P7 that exhibit repetitive firing, quantitative increases in I_{NaR} and I_{NaP} density may be major factors that facilitate and promote high-frequency firing as a function of age in Mes V neurons.

Key Words: Oral-motor activity, TTX-sensitive sodium current, mesencephalic trigeminal neurons

Poster-4

Expression of transient receptor potential ankyrin 1 and vesicular glutamate transporters in the rat trigeminal ganglion

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Transient receptor potential ankyrin 1 (TRPA1) is activated by noxious cold (<17 ℃) and diverse peripheral stimuli. TRPA1 plays an important role in nociceptive hypersensitivity following inflammation. In previous electrophysiological studies, presynaptic activation of TRPA1 localized at the central terminals of primary afferent fiber facilitates glutamate release onto superficial dorsal horn neurons, suggesting that glutamatergic transmission plays a key role in the TRPA1-mediated nociception. However, it is not known whether TRPA1-mediated nociceptive input depends on different types of vesicular glutamate transporters (VGLUTs) mediating glutamatergic signaling. To investigate these questions, we examined the coexpression of TRPA1 and VGLUT1 or VGLUT2 in the rat trigeminal ganglion (TG) after complete Freund's adjuvant (CFA) application to rat vibrissa pad using microscopic fluorescent immunohistochemistry. Many TRPA1+ neurons in the TG expressed VGLUT2, while few expressed VGLUT1. In addition, TRPA1+ neurons expressing VGLUT2 (VGLUT2+/TRPA1+) neurons were mostly small- and few medium-sized. Following trigeminal inflammation, the proportion of TRPA1+ and VGLUT2+ neurons of all TG neurons increased significantly as well as some VGLUT2+/TRPA1+ neurons, whereas that of VGLUT1+/TRPA1+ neurons was unchanged.

Our findings suggest that an increase in the number of TRPA1+ neurons expressing VGLUT2, but not only TRPA1 or VGLUT2, may play a role in mediating hypersensitivity after trigeminal inflammation, while TRPA1+ neurons expressing VGLUT1+ may contribute to acute pain and acute mechanosensation; it means that TRPA1-mediate distinct nociceptive mechanism may be involved in the specific type of VGLUTs-mediated glutamatergic signals.

Key Words : TRPA1, VGLUT1, VGLUT2

Poster-5

Expression of vesicular glutamate transporter 1 (VGLUT1) and VGLUT2 in the sensory root and soma of the rat trigeminal ganglion in the normal condition and following inflammation

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Detailed informations on the expression of vesicular glutamate transporter 1 (VGLUT1) and VGLUT2 in the various types of axons and somata of the primary sensory neurons may help understand role of the VGLUTs in various types of the somatosensation. In the present study, to understand involvement of VGLUT1 and VGLUT2 in acute and inflammatory pain, we investigated expression of VGLUT1 and VGLUT2 in the soma and peripheral sensory root of the trigeminal ganglion in the normal condition and following CFA-induced inflammation by light and electron microscopic immunohistochemistry and quantitative analysis.

Most of the VGLUT1-immunopositive (+) axons and VGLUT2+ axons were unmyelinated and small myelinated Aδ nociceptive fibers (71.8% and 97.3% in the VGLUT1+ and VGLUT2+ axons, respectively). Remaining about 28% of VGLUT1+ and 2.7% of VGLUT2+ axons were large myelinated Aβ fibers. Fractions of VGLUT1+ and VGLUT2+ small-sized soma of all soma (<400 um2 in cross-sectional area) were significantly increased in the CFA group than control ($P<0.05$). Fractions of VGLUT1+ and VGLUT2+ unmyelinated axon of all axon were significantly increased, whereas fractions of VGLUT1+ and VGLUT2+ small and large myelinated axon were not changed in the CFA group than control. $P<0.05$).

Our findings suggest that VGLUT1 as well as VGLUT2 is involved in the acute pain, and that VGLUT1 and VGLUT2 expressed in the unmyelinated axons may contribute increase in glutamate signaling following inflammation.

Key Words : VGLUT, nociceptive fiber, Inflammation

$P2Y_2$ receptor in goblet cells is involved in dry-eye pain in rats

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The main symptom of dry syndrome including dry eye (DE) is eye discomfort and ophthalmalgia due to a significant reduction of tear volume. The cornea is innervated by the first branch of the trigeminal nerve, and the density of C-fibers innervating cornea is the highest in human. Clinically P2Y2 receptor agonist, 3% diquafosal sodium (Diquas ®) is administrated to the eye for the acceleration of tear secretion. A pharmacological action of diquafosal sodium has been reported to accelerate lacrimation by binding to a P2Y2 receptor in goblet cells. We hypothesized that administration of diquafosal sodium to the eye caused the enhancement of lacrimation, resulting in the attenuation of the central sensitization of trigeminal nociceptive neurons associated with ophthalmalgia.We administrated diquafosal sodium daily to the eye for 4 weeks in dry eye model rats receiving exorbital gland resection as the following schedule: 1) Diquafosal sodium administration was started just after exorbital gland resection, and 2) the other group was that diquafosal sodium was applied on day 14 after exorbital gland resection. The tear volume was significantly recovered, and the number of eye blinks against the hypertonic saline application to the eye was decreased by diquafosal sodium administration compared with vehicle-applied DE rats in both these two groups. The number of phosphorylated extracellular signal-regulated kinase-immunoreactive cells and cFos-immunoreactive cells in trigeminal subnucleus caudalis (Vc) was also significantly decreased in the late diquafosal-treated DE rats.
These findings suggest that the enhancement of lacrimation by application of P2Y2 receptor agonist, diquafosol sodium to the eye may attenuate the sensitization of Vc nociceptive neurons in DE rats in the late administration group.
Acknowledgments: Keio Univ. Sch. Med Prof. Kazuo Tsubota.
Conflict of interest: This study is funded by Santen Pharmaceutical Co.

Key Words: diquafosol sodium (Diquas ®), ophthalmalgia, central sensitization

Poster-7

Gingerol and shogaol, the herbal contents, relief oral ulcerative mucositis-induced pain through sodium channel blockage

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It is well known that oral ulcerative mucositis (OUM)-induced pain is persistent and intractable in cancer patients treated with chemo-radiotherapy, resulting under-nutrition and low quality of life. Recently, hangeshashinto (HST), a traditional Japanese medicine, has been reported to be effective on the OUM-induced pain. However, the ingredient-based mechanism that underlies its pain-relieving activity remains unknown. In the present study, to clarify the analgesic mechanism of HST on OUM-induced pain, we investigated putative HST ingredients showing antagonistic effects on Na^+ channels in vitro and in vivo. A screen of 21 major ingredients using automated patch-clamp recordings in channel-expressing cells showed that [6]-gingerol and [6]-shogaol, two components of a Processed Ginger extract, considerably inhibited voltage-activated Na^+ currents. These two ingredients inhibited the stimulant-induced release of substance P in cultured rat sensory neurons. A submucosal injection of a mixture of [6]-gingerol and [6]-shogaol increased the mechanical withdrawal threshold in healthy rats. A swab application of a mixture of [6]-gingerol and [6]-shogaol in the OUM region induced sufficient analgesia of OUM-induced mechanical or spontaneous pain when co-applied with a Ginseng extract containing abundant saponin. The Ginseng extract demonstrated an acceleration of substance permeability into the oral ulcer tissue without an analgesic effect. These results suggest that Na^+ channel blockage by gingerol/shogaol plays an essential role in HST-associated analgesia of OUM-induced pain. This pharmacological mechanism provides scientific evidence for the usage of this herbal medicine in a clinical setting to treat cancer patients suffering OUM-induced pain.

Key Words: Oral ulcerative mucositis; Traditional Japanese Medicine; Sodium Channel

Poster-8

Blockade of glial EphA4 attenuates mechanical allodynia in rats with trigeminal neuropathic pain

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Previous studies reported that ephrin A receptor 4 (EphA4), a member of the Eph family of receptor tyrosine kinases, is an important role in axonal reorganization and synaptic plasticity on various neuronal damages or nerve injury. However, there has been no evidence of participation of EphA4 in the development of neuropathic pain in the orofacial area. Thus, the present study investigated a role of EphA4 in the nociceptive processing in rats with inferior alveolar nerve injury.

Sprague-Dawley male rats were anesthetized with ketamine (40 mg/kg) and xylazine (4 mg/kg). Under anesthesia, the left lower second molar was extracted, followed by the placement of a mini-dental implant to intentionally injure the inferior alveolar nerve. Mechanical allodynia was monitored after mal-positioned dental implant surgery.

Our current findings show that a nerve injury induced by mal-positioned dental implants produces significant mechanical allodynia as well as up-regulation of astrocytic EphA4 expression in the medullary dorsal horn. Although daily treatment with EphA4-Fc, an EphA4 inhibitor, did not produce prolonged anti-allodynic effects when pain is already established, an early treatment protocol with EphA4-Fc significantly attenuated mechanical allodynia and reduced the up-regulated EphA4 expression in the medullary dorsal horn. Inferior alveolar nerve injury increased D-serine-positive signals in astrocytes and blockade of EphA4 reduced the upregulated D-serine-positive signal. Degradation of D-serine by D-amino acid oxidase produced significant anti-allodynic effects in rats with trigeminal neuropathic pain. Moreover, intracisternal administration of EphA4 siRNA produced anti-allodynic effects and reduced the upregulated EphA4 expression.

These results suggest that early blockade of EphA4 signaling, which is mediated by the release of D-serine in the astrocytes of the medullary dorsal horn, inhibited development of trigeminal mechanical allodynia after nerve injury.

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Key Words : trigeminal neuropathic pain, Ephrin A receptor 4 (EphA4), mechanical allodynia

Distribution differences of thalamic and parabrachial projection neurons receiving C-fiber afferents in the trigeminal spinal subnucleus caudalis and upper cervical spinal cord

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Second-order neurons in trigeminal subnucleus caudalis (Vc) and upper cervical spinal cord (C1) receiving C-fiber afferents ascend their axons to the ventral posteromedial thalamic nucleus (VPM), medial thalamic nuclei (MTN) and parabrachial nuclei (PBN) and are critical for craniofacial pain processing. The contribution of each region to trigeminal nociception was assessed by the expression of phosphorylated extracellular signal-regulated kinase-immunoreactive (pERK-IR) and neurokinin 1 receptor (NK1)-, which is the receptor for substance P, IR neurons expressed with fluorogold (FG)-labeled projection neurons in Vc-C1.
FG (3%) was injected into the right VPM, MTN or PBN in male rats. On day 7 after FG injection, rats were stimulated by capsaicin (300 μM, 10 μl) in the left upper lip and perfused within 5 minutes. The lower brain stems were removed and cut, and sections were processed for pERK and NK1 immunohistochemistries.
VPM or MTN projection neurons were observed in the Vi/Vc and mid-Vc contralateral to the FG injection side, while PBN projection neurons were observed bilaterally. The number of NK1-IR and pERK-IR neurons was larger in PBN projection neurons than VPM and MTN. pERK- and NK1-IR VPM projection neurons were mainly distributed in the middle Vc, few MTN projection neurons showed pERK- and NK1-IR, and pERK- and NK1-IR PBN projection neurons were observed from mid-Vc to Vc-C1. The percentage of pERK-NK1-IR FG-labeled neurons was as follows: middle-Vc to VPM: 8.6%; caudal-Vc-C1 to MTN: 25.0%; middle-Vc to PBN: 15.3% and caudal-Vc-C1 to PBN: 21.1%.
The present findings suggest that the majority of nociceptive neurons receiving C-fiber afferents might be classified as interneurons and the rostro-caudal distribution differences of pERK-NK1-IR FG-labeled neurons in Vc-C1 may reflect functional differences among these projection areas regarding orofacial nociception.

Key Words: Projection neurons, trigeminal nerve, NK1

GABAergic neuronal membrane potential responses to dental pulp stimulation in the rat insular cortex: An *in vivo* targeted whole-cell patch-clamp recording study

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The insular cortex (IC) processes various sensory information including gustation, visceral sensation, thermal sensation, and nociception. We have demonstrated that IC neurons receive nociceptive inputs directly from the thalamic nuclei and code the intensity and location of nociceptive inputs to the dental pulps (Nakamura et al., 2015, 2016). Our recent *in vivo* study of somatic Ca^{2+} imaging by two-photon-excited fluorescence laser-scanning microscopy (2PLSM) have revealed that the number of IC neurons responding to electrical dental pulp stimulation becomes larger in the model of the trigeminal nerve injury. The IC consists of glutamatergic excitatory neurons (~80%) and GABAergic inhibitory neurons (~20%), however, the profiles of their neural responses to dental pulp stimuli have been unknown. Their profiles are critical to understand the mechanisms of abnormal trigeminal pain induced by nerve injury and inflammation.

In order to understand the spike firing profiles of each neuron subtype in response to noxious stimuli, we tried to record from the rat IC GABAergic interneurons that are visualized by a fluorescent protein, Venus, targeting to the vesicular GABA transporter (VGAT). We combined 2PLSM, which allows us to image GABAergic neurons in the deep cortical layer, to an *in vivo* whole-cell patch-clamp recording, and recorded the membrane fluctuation and responses to electrical stimulation of the dental pulp.

We observed the oscillation of the membrane potential, i.e. Up-state and Down-state, in most of neurons, and the responses to the dental pulp stimulation varied depending on the membrane states. In addition, several GABAergic neurons showed unique membrane potential dynamics and responses to the stimulation, suggesting the diversity of the IC GABAergic neurons and specialized functions of each neuron subtype in regulating the activity of the IC local circuit.

Key Words: Nociception, Insular cortex, GABAergic neuron

Nicotine suppresses synaptic potentiation in layer V pyramidal neurons of the insular cortex

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The insular cortex is a critical brain region involved in nicotine addiction. However, its specific cellular and synaptic mechanisms underlying nicotine addiction remains largely unknown. In the present study, we examined how nicotine modulates synaptic transmission and plasticity in layer V pyramidal neurons of the mouse insular cortex. We also examined which type of neurons express functional nicotinic acetylcholine receptors (nAChRs) in layer V of the insular cortex. We found that nicotine suppresses synaptic potentiation induced by combination of presynaptic stimulation with postsynaptic depolarization (paired training). An application of nicotine significantly enhanced both spontaneous excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs): the former effect was mediated by activation of β2-containing nAChRs while the latter one was mediated largely by activation of β2-containing nAChRs and to a minor extent by activation of α7-containing nAChRs. The application of nicotine significantly enhanced evoked IPSCs but had no effect on evoked EPSCs. We also found that in layer V of the mouse insular cortex, majority of non-fast-spiking (non-FS) interneurons have β2-containing nAChRs while about half of pyramidal neurons and FS interneurons have functional nAChRs. Blockade of GABAA receptors or β2-containing nAChRs prevented the effects of nicotine on synaptic potentiation. Taken together, these results suggest that in layer V pyramidal neurons of the insular cortex, activation of β2-containing nAChRs expressed in non-FS interneurons suppresses synaptic potentiation through enhancing GABAergic synaptic transmission. These findings provide important insights into the cellular and synaptic mechanisms of insular cortical changes in nicotine addiction.

Key Words : nicotine, insular cortex, synaptic plasticity

Differential gene expression patterns in iPSC-derived neurons from monozygotic twin cases with treatment-resistant schizophrenia and discordant for clozapine responses

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Schizophrenia is a severe neuropsychiatric disease with an approximate worldwide prevalence of 1%. Common symptoms include delusions, hallucinations, impaired cognitive function, emotional blunting and incoherent behavior. Although many typical and atypical antipsychotic drugs have been developed and demonstrated to be effective in the treatment of schizophrenia, 20–30% of patients remain partially or fully unresponsive to two or more adequate trials with antipsychotic drugs and are therefore classified as treatment resistant. Clozapine is the most effective antipsychotic drug for treatment resistant schizophrenia; however, clozapine has rare but serious side effects. Furthermore, there is inter-individual variability in the drug response to clozapine treatment. Therefore, the identification of the molecular mechanisms underlying the action of clozapine and drug response predictors is imperative. In the present study, we focused on a pair of monozygotic twin cases with treatment-resistant schizophrenia, in which one twin responded well to clozapine treatment and the other twin did not. Using induced pluripotent stem (iPS) cell-based technology, we generated neurons from iPS cells derived from these patients and subsequently performed RNA-sequencing to compare the transcriptome profiles of the mock or clozapine-treated neurons. Although, these iPS cells similarly differentiated into neurons, several genes encoding homophilic cell adhesion molecules, such as protocadherin genes, showed differential expression patterns between these two patients. These results, which contribute to the current understanding of the molecular mechanisms of clozapine action, establish a new strategy for the use of monozygotic twin studies in schizophrenia research.

Key Words : schizophrenia, monozygotic twin, iPS-cell technology