Oral Neuroscience 2018

October 7th, 2018

Program & Abstract

Venue
Osaka University
Graduate School of Dentistry
Osaka, Japan

大阪大学 OSAKA UNIVERSITY

Challenge to Intractable Oral Diseases

Remarks

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

Oral Neuroscience 2018

Program & Abstract

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Osaka University Graduate School of Dentistry

Osaka, Japan

Organizing Committee

Takafumi Kato*, Dept. of Oral Physiology, Osaka Univ.

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.

Susumu Tanaka**, First Dept. of Oral and Maxillofacial Surgery, Osaka Univ.

Hiroki Toyoda**, Dept. of Oral Physiology, Osaka Univ.

* Chair

** Secretary

Cosponsor

"Challenge to Intractable Oral diseases" from Osaka University Graduate School of Dentistry

Contact

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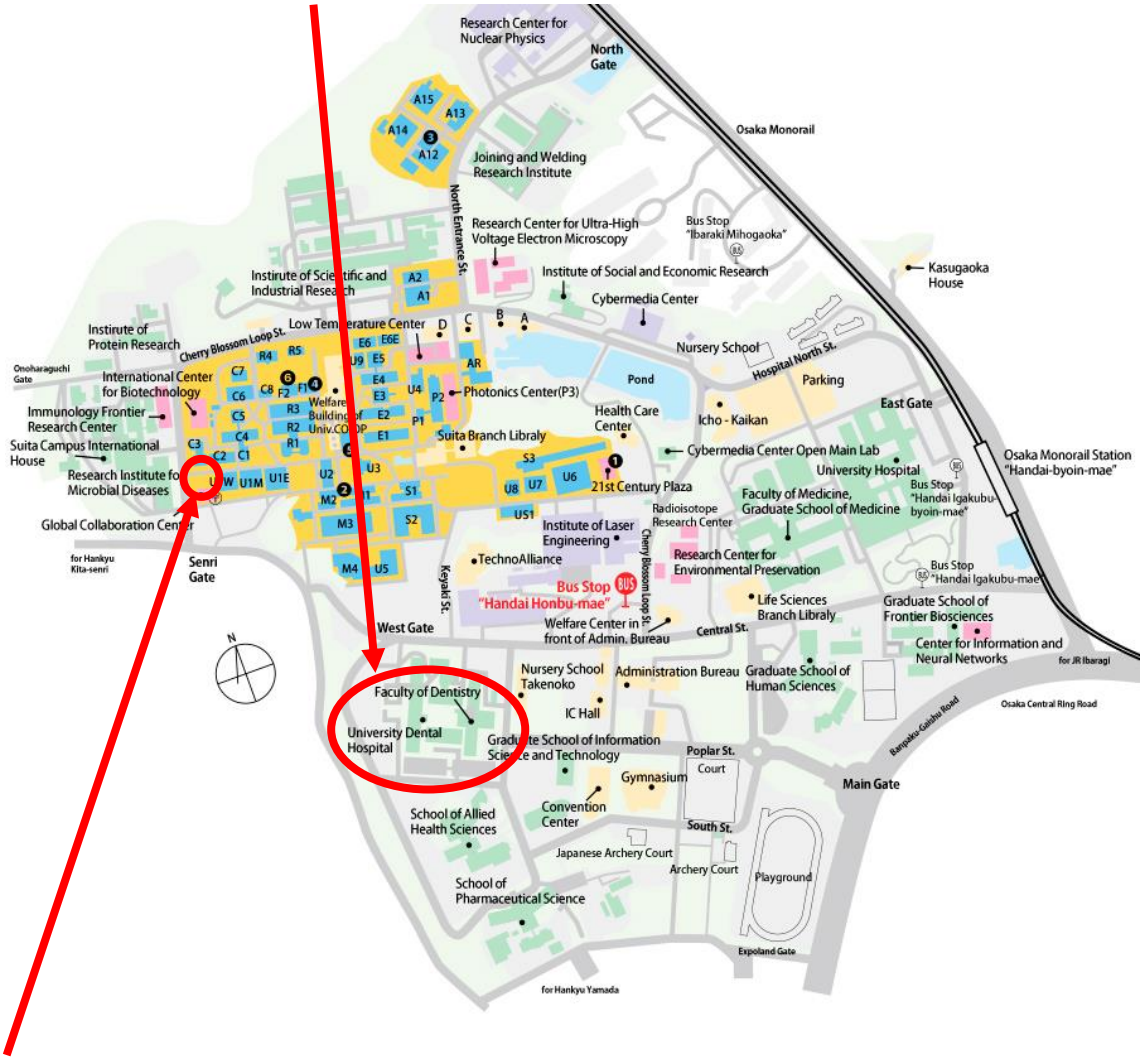
Access

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)

Registration Fees

General JPY 3,000　Students JPY 1,000

Oral presentation information

As you prepare for your oral presentation at Oral Neuroscience 2018, please find important information concerning your oral presentation.

Presentation time

- ✓ The time allowed for the slide presentation of Lectures is 30 minutes including 5 minutes for discussion.
- ✓ The time allowed for the slide presentation of Plenary Lecture is 60 minutes including 10 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 2016 (Windows) 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: 12:15-12:45

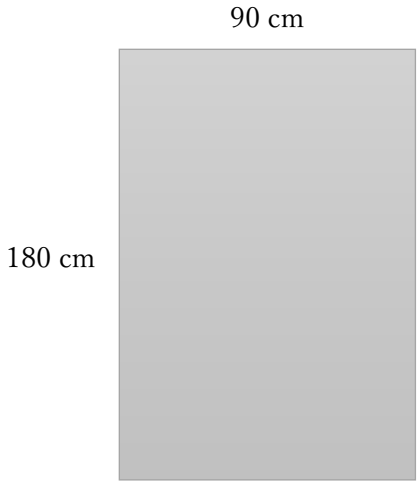
- ✓ We appreciate if you bring your data (USB drive) before the onset of morning session.

Poster presentation information

As you prepare for your poster presentation at Oral Neuroscience 2018, please find important information concerning your poster presentation.

Presentation time: 11:40-13:00, 14:30-15:00, 16:00-16:30

- ✓ Area of poster presentation: Height 180 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 2018

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

10:00- Opening Remarks Takafumi Kato

Session 1 (10:10-11:40)	Chair	Hiroki Toyoda

10:10- [Lecture-1] Behavioral and physiological consequences of disorderly-layered motor cortex in *reeler* mutants
Mariko Nishibe (Graduate School of Dentistry, Osaka University)

10:40- [Lecture-2] Developmental alteration of synaptic properties of jaw-closing and jaw-opening motoneurons
Shiro Nakamura (Showa University School of Dentistry)

11:10- [Lecture-3] Central action of substance P on swallowing activity in working heart brainstem preparation
Hironobu Kobashi (Graduate School of Dentistry, Osaka University)

Lunch and Poster Session (11:40-13:00)

Session 2 (13:00-14:30)	Chair	Takahiro Furuta

13:00- [Lecture-4] Enhancement of responses to upper molar pulp stimulation in somatosensory and insular cortices in an ectopic pain model of the rat
Satoshi Fujita (Nihon University School of Dentistry)

13:30- [Lecture-5] Modulatory effects of psychological stress on descending pain controls
Keiichiro Okamoto (Niigata University Graduate School of Medical and Dental Sciences)

14:00- [Lecture-6] Role of the VPAC2 receptor overactivation implicated in schizophrenia susceptibility

Yukio Ago (Graduate School of Pharmaceutical Sciences, Osaka University)

Coffee Break and Poster Session (14:30-15:00)

Session 3 (15:00-16:00)	Chair	Hiroshi Yoshimura

15:00- [Lecture-7] Degradation of parotid gland AQP5 protein by isoproterenol via calpain system

Chenjuan Yao (Institute of Health Biosciences, University of Tokushima Graduate School)

15:30- [Lecture-8] Wnt5a, released from mechanically stimulated rat periodontal ligament cells enhances the neurite elongation

Takashi Yoshida (Tohoku University Graduate School of Dentistry)

Coffee Break and Poster Session (16:00-16:30)

Plenary Lecture (16:30-17:30)	Chair	Takafumi Kato

16:30- Sleep bruxism-rhythmic masticatory muscle activity (RMMA): continuum from a physiological activity to a behavior to a movement disorder

Gilles Lavigne (University of Montreal)

17:30- Closing Remarks

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

Poster presentations

[Poster-1]

A functional connectivity analysis of the neural circuit associated with jaw tapping in the elderly people

Hideyuki Fukami[1,2] and Yoshinori Sahara[2]

[1]Department of Oral Health Sciences, Faculty of Nursing and Health Care, Baika Woman's University; [2]Department of Physiology, Iwate Medical University, School of Dentistry

[Poster-2]

Sleep-related respiratory variables in sleep bruxism: a controlled study

Chizuko Yokoe[1], Akiko Tsujisaka[2], Shingo Haraki[2], Mutsumi Okura[1], Takafumi Kato[1]

[1]Department of Oral Physiology, Osaka University Graduate School of Dentistry; [2]Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry

[Poster-3]

Efficacy of low and high contingent electric stimulation on temporalis EMG activity and clinical muscle symptoms in patients with sleep bruxism

Akiko Shimada[1], E.E. Castrillon[2,3], P. Svensson[2,3,4]

[1]Osaka Dental University Hospital; [2]Section of orofacial pain and jaw function, Department of Dentistry, Aarhus University, Denmark; [3]Scandinavian Center for Orofacial Neuroscience (SCON); [4]Orofacial pain and Jaw function, Department of Dental Medicine, Karolinska Institutet, Sweden

[Poster-4]

Temporal changes in occlusal vertical dimension after bite-raising in bite-reducing model animals

Ryosuke Shimono[1], Yuji Masuda[2], Takafumi Kato[3]

[1]Dept of Prosthodont, [2]Dept of Oral and Maxilofacial Biol, Grad Sch of Oral Med, Matsumoto Dental Univ, [3]Dept Oral Physiol, Grad Sch of Dent, Osaka Univ

[Poster-5]

Time-course changes in cortical, cardiac and respiratory changes before spontaneous rhythmic jaw movements after repeated ketamine injections in anesthetized guinea pigs

Yutaka Matsuura[1], Hiroshi Yano[3], Hiroki Toyoda[1], Ayano Katagiri[1], Makoto Higashiyama[1], Hajime Sato[1], Narikazu Uzawa[3], Atsushi Yoshida[2], Takafumi Kato[1]

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

[Poster-6]

A descending circuit derived from the superior colliculus modulates vibrissal movements

Takahiro Furuta[1], Miki Kaneshige[2], Atsushi Yoshida[1]

[1]Osaka Univ, [2]Kyoto Univ

[Poster-7]

Velopharyngeal function following levator veli palatini muscle reconstruction

Kiyoko Nakagawa[1,2], Emiko T. Isomura[1,2], Makoto Matsukawa[1], Ryou Mitsui[1], Yousuke Shogen[1], Mikihiko Kogo[1,2]

[1]First Department of Oral and Maxillofacial Surgery, Osaka University, Graduate School of Dentistry, Osaka, Japan; [2]Unit of Dentistry, Osaka University Hospital, Osaka, Japan

[Poster-8]

Membrane potential responses of the excitatory and inhibitory neurons in layer II/III of the insular cortex by dental pulp stimulation: In vivo whole-cell targeting patch-clamp recording

Shota Murayama[1,2] and Masayuki Kobayashi[2,3]

[1]Department of Endodontics, School of Dentistry, Nihon University; [2]Department of Pharmacology, School of Dentistry, Nihon University; [3]Division of Oral and Craniomaxillofacial Research, Nihon University

[Poster-9]

Presymptomatic abnormalities and associated channelopathies in spindle afferent trigeminal Mesencephalic V neurons in the SOD1G93A mouse model for Amyotrophic Lateral Sclerosis

Soju Seki[1,2], Antonios Pantazis[3], Riccardo Olcese[3,4], Mikihiko Kogo[2], Susumu Tanaka[2], Scott H Chandler[1,5], Sharmila Venugopal[1]

[1]Department of Integrative Biology & Physiology UCLA; [2]The 1st Department of Oral & Maxillofacial Surgery Osaka University; [3]Department of Anesthesiology & Perioperative Medicine UCLA; [4]Department of Ophthalmology UCLA; [5]Brain Research Institute UCLA

[Poster-10]

Corticofugal pathways from granular insular cortex conveying orofacial proprioception

Etsuko Ikenoue[1], Yumi Tsutsumi[1], Fumihiko Sato[1], Haruka Ohara[1], Katsuro Uchino[2], Takahiro Furuta[1], Yume Uemura[1], Akiko Tomita[1], Yoshihisa Tachibana[3], Atsushi Yoshida[1]

[1]Department of Oral Anatomy and Neurobiology, Graduate School of Dentistry, Osaka University; [2]Department of Acupuncture, Takarazuka University of Medical and Health Care; [3]Division of System Neuroscience, Kobe University Graduate School of Medicine

[Poster-11]

Functional alteration of ascending noxious pathways associated with trigeminal nerve injury

Shinji Okada[1,2], Ayano Katagiri[2,3], Koichi Iwata[2]

[1]Department of Complete Denture Prosthodontics, Nihon University School of Dentistry; [2]Department of Physiology, Nihon University School of Dentistry; [3]Department of Oral Physiology, Osaka University Graduate School of Dentistry

[Poster-12]

Prevention of Dry-Eye Pain by $P2Y_2$ receptor agonist Diquafosal Sodium Administration

Ayano Katagiri[1,2], Lou Mikuzuki[2,3], Shinji Okada[2,4], Koichi Iwata[2]

[1]Department of Oral Physiology, Osaka University Graduate School of Dentistry; [2]Department of Physiology, Nihon University School of Dentistry; [3]Department of Psychosomatic Dentistry, Tokyo Medical and Dental University (TMDU) Graduate School; [4]Department of Complete Denture Prosthodontics, Nihon University School of Dentistry

[Poster-13]

Basolateral amygdala inactivation suppresses the taste but not olfactory neophobia in rats

Keisuke Shinohara and Yasunobu Yasoshima

Behavioral Physiology, Human Sciences, Osaka University

[Poster-14]

The *de novo* Q1042R *POGZ* mutation in sporadic ASD patients impairs the neural development

Takanobu Nakazawa and Kazuhiro Takuma

Department of Pharmacology, Graduate School of Dentistry, Osaka University

[Poster-15]

Role of intracellular Ca^{2+} stores in layer 2/3 pyramidal cells of the somatosensory cortex during oxygen and glucose deprivation

Hiroki Toyoda, Tsutomu Kawano, Mao Shimoda, Hajime Sato, Dong Xu Yin, Ayano Katagiri, Takafumi Kato

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

Plenary Lecture

Sleep bruxism-rhythmic masticatory muscle activity (RMMA): continuum from a physiological activity to a behavior to a movement disorder

Gilles Lavigne, DMD, PhD, hc (U Zurich), CM (Order of Canada)
Fellows of Royal Coll Dentists (Canada), Am Coll Dentists and Can Acad Health Sci.
Canada Research Chair in Pain, sleep and trauma
Faculties of dental medicine and medicine, Universite de Montreal, Canada
Research Center, CIUSSS Nord Ile Montreal (Formerly Sacre Coeur Hospital)

Sleep bruxism (SB), characterized by tooth grinding and/or sustained jaw muscle activity, is a repetitive motor activity. During sleep, it is estimated by recording a biomarker, the rhythmic masticatory muscle activity (RMMA). Incidence and prevalence of SB-RMMA activities are highly prevalent in child and young healthy subjects and drop with age.

Bruxism can be observed during sleep and wake; the last is most likely reactive to life events. Both can overlap. In most persons, SB is a 'normal' oral behavior. Presence of RMMA can be classified as a disorder if it interferes with quality of life and/or if it is concomitant to other conditions (e.g., insomnia, headache, GERD, apnea, Temporomandibular pain and epilepsy or REM Behavior Disorder).

The role/function of such natural activity is debated: it can be a physiological reactivity and/or an autonomic cardiac and respiratory adjustment. During sleep of young and healthy subjects, most RMMA are associated with sleep arousals, with the A3 arousal phase of the Cyclic Alternating Pattern (CAP). Clonidine, an alpha 2 adrenergic agonist, reduces the autonomic activation and the frequency of RMMA. The occurrence of SB is dominant in light sleep and in the minutes before REM sleep.

In some individuals, RMMA may be associated with some breathing events, either preceding or following RMMA. Then the putative association of RMMA to apnea is debate, i.e., probably coincidental and not a cause of SB. Then we should assess the presence of concomitant conditions (bruxism and sleep disordered breathing) in subgroup of patients presenting risk factors (retrognathia, large tonsils/adenoids or tongue, obesity, etc).

The role of doctor in dental and oral medicine, if co-morbidities are suspected, is to collaborate with physicians for diagnosis and management planning. Few approaches are available to reduce the consequence(s) of bruxism: cognitive and behavioral treatment, oral splint, mandibular advancement appliance, medication, emerging *E* type biofeedback.

NB: Arigato to all my Japanese colleagues and friends, without you my research will not have been as stimulating, you are a source of inspiration.

Abstract
Oral Session

Lecture-1

Behavioral and physiological consequences of disorderly-layered motor cortex in *reeler* mutants

Mariko Nishibe[1], Yu Katusyama[2], Toshihide Yamashita[3]

[1]Department of Oral Physiology, Osaka University Graduate School of Dentistry; [2]Department of Anatomy, Shiga University of Medical Science; [3]Department of Molecular Neuroscience, Osaka University Graduate School of Medicine

The homozygous *reeler* mutants exhibit developmental abnormality in the laminal formation of the cortex and cerebellum. The motor deficit of the mutants (e.g. ataxia) has largely been considered cerebellum-related, and the developmental consequence of the cortex on *reeler* motor behavior was not clear. We herein showed that there are behavioral consequences to *reeler* mutation in models examined at cortex-dependent bimanual tasks that require forepaw dexterity. Using intracortical microstimulation (ICMS), we found a consequence of the disordered lamina in *reeler*, requiring a higher current to evoke skeletal muscle movements. No cortical layer-dependency of the threshold alternation may be reported statistically; though there was a propensity to require a higher current stimulation in the upper layers in *reeler*. Upon using different ICMS stimulation upper-limits for the wild type and *reeler*, mapped body representation organization and areal measures were similar. No statistical significance was found in the forelimb representation area of the *reeler* compared to that of the wild type.

To elucidate the influence of cerebellum atrophy and ataxia on the obtained results, we used Disabled-1 (Dab1) cKO mice, in which the Reelin-Dab1 signal deficiency was confined to the cerebral cortex. The behavioral and neurophysiological findings in *reeler* mice were reproduced in the Dab1 cKO mice. The Dab1 cKO mice were further assessed at the single-pellet reach and retrieval task, displaying a lower number of successfully retrieved pellets. It suggests the abnormality confined to the cortex affects the level of motor performance.

Although possible muscular dysfunction was reported in *REELIN*-deficient humans, the function of the *reeler* forelimb muscle examined by electromyography, morphology of neuromuscular junction and the expression level of choline acetyltransferase were normal. Our results suggest that the mammalian laminar structure is necessary for the cortical control in dexterous movements and for trans-synaptic efficacy in the cortical output.

Key Words: Motor Control, Reelin, Developmental Abnormality

Lecture-2

Developmental alteration of synaptic properties of jaw-closing and jaw-opening motoneurons

Shiro Nakamura, Kiyomi Nakayama, Ayako Mochizuki, Masanori Dantsuji, Tomio Inoue
Department of Oral Physiology, Showa University School of Dentistry

One of the most important behavioral characteristics of mammals is to change their feeding from suckling to chewing during early postnatal life. The properties of the central neural circuits responsible for such behaviors could alter in this period, which is accompanied by the formation of the oral and facial musculoskeletal system. However, the details of developmental changes of these neural circuits remain unknown. In this study, we examined synaptic properties of jaw-closing and jaw-opening motoneurons using optical and electrophysiological techniques. We found that jaw-closing and jaw-opening motoneurons receive glutamatergic, glycinergic and GABAergic inputs from the reticular formation surrounding the trigeminal motor nucleus such as supratrigeminal regions (SupV) and the reticular formation dorsal to the facial nucleus (RdVII), and the properties of these inputs change in postnatal development. Neonatal masseter and digastric motoneurons aged postnatal 1-4 days old rats showed excitatory effects via the activation of glutamate, glycine, and putative $GABA_A$ receptors, while juvenile motoneurons aged postnatal 9-12 days old exhibited inhibitory effects after glycinergic and GABAergic input. For glutamatergic inputs, there were little differences in the properties of non-NMDA receptor-mediated inputs to masseter and digastric motoneurons during postnatal periods; however, the characteristics of the NMDA receptor-mediated inputs to the masseter motoneurons changed from postnatal 2-5 to 14-17 days old, and those at digastric motoneurons remained constant throughout postnatal ages. These results suggest that the postnatal development of synaptic characteristics of jaw-closing and jaw-opening motoneurons are possibly related to the developmental changes of feeding behavior from suckling to chewing.

Key Words: postnatal development, jaw-closing and jaw-opening motoneurons, premotor neurons

Lecture-3

Central action of substance P on swallowing activity in working heart brainstem preparation

Hironobu Kobashi, Tadashi Yamanishi, Tetsuya Seikai, Takeshi Togawa, Takahiro Nishio, Takeshi Harada, Mikihiko Kogo
First Department of Oral and Maxillofacial Surgery, Osaka University Graduate School of Dentistry, Osaka, Japan

Substance P (SP) is a neuropeptide acting on the neurokinin-1 (NK1) receptor. It holds attention as a marker of swallowing disorder. Substance P is considered to play an important role in controlling swallowing. However, its role on generation or modulation of swallowing activity is poorly understood. In the present study, we aimed to study a role of SP playing on swallowing activity in working heart-brainstem preparation (WHBP). Working heart-brainstem preparations is a useful model for studying neuronal mechanisms of centrally controlled functions such as blood pressure control and breathing. We have found that electrical stimulation of the superior laryngeal nerve of WHBP induced robust swallowing activity. Therefore, we applied this preparation in the present study.

Experiments were performed with in situ WHBP obtained from juvenile Sprague-Dawley rats (postnatal day 21(P21)-P30, body weight 60-100g). Electrical stimulation to the unilateral supra laryngeal nerve (SLN) was applied to elicit swallowing activity. Then, we studied changes in swallowing activity before and after application of NK1 receptor agonist (SP) or antagonist (CP-100263) either in the recording chamber or in the nucleus tractus solitaries (NTS) through a glass pipett. Two types of electrical stimulation procedures (interval stimulation and continuous stimulation) were applied into the SLN to induce swallowing activity.

Whole application of SP increased number of swallowing activity elicited by both types of stimulation procedures, whereas whole application of CP-100263 did not change frequency of swallowing activity. Comparable results were seen when those agents were applied directly into the NTS. Previous local application of CP-100263 into the NTS blocked enhancing action of whole application of SP on swallowing activity.

The results suggested that SP decreased the threshold of generation of swallowing activity induced by SLN stimulation.

Lecture-4

Enhancement of responses to upper molar pulp stimulation in somatosensory and insular cortices in an ectopic pain model of the rat

Satoshi Fujita, Kiyofumi Yamamoto, Masayuki Kobayashi
Department of Pharmacology, Nihon University School of Dentistry

The inferior alveolar nerve (IAN) is a mandibular branch of the trigeminal nerve and innervates to orofacial region. IAN transection (IANX) induces allodynia in the maxillary nerve-projecting region. However, it is not fully understood whether and how the neural activity in these cortices is changed by nerve injury which could be causes of ectopic pain. To address this issue, we observed neural activity during nociceptive information processing in IAN transection rats using optical imaging with voltage-sensitive dye and calcium imaging by a two-photon microscopy. We previously elucidated that the area between secondary somatosensory and insular cortices (S2/IOR) play a role in nociceptive information processing of teeth. Since the dental pulps principally consist of Aδ and C fibers that transmit nociceptive information, we considered that S2/IOR responding to upper molar pulp stimulation is a suitable target to investigate the cortical changes in IANX rats. Optical imaging at macroscopic level revealed that excitatory propagation induced by the upper molar pulp stimulation was expanded in 1-2 weeks after IANX. The peak amplitude of excitation in S2/IOR was also increased in 1 week after the IANX. To examine changes in neural activity at cellular level, calcium imaging using a two-photon microscopy was performed in 1 week after IANX. The number of both excitatory and inhibitory neurons responding to upper molar pulp stimulation increased in IANX rats. The calcium responses increased in both types of neurons, and the duration of calcium responses increased in excitatory neurons. Whole-cell patch-clamp recording in slice preparation revealed that inhibitory postsynaptic inputs to pyramidal neurons were decreased in IANX rats. These results suggest that IANX induces enhancement of neural activity during nociceptive information processing in S2/IOR, and plastic changes of the local circuits of S2/IOR might contribute to the enhancement.

Key Words: insular cortex, nociception, imaging

Lecture-5

Modulatory effects of psychological stress on descending pain controls

Keiichiro Okamoto, Masayuki Kurose, Kensuke Yamamura
Niigata University Graduate School of Medical and Dental Sciences Division of Oral Physiology

Emotional stress is a risk factor for chronic pain. Evidence indicated that several orofacial pain conditions such as temporomandibular joint disorders could be induced by dysfunction of central nervous systems including descending pain controls. We previously showed that repeated psychophysical stress enhanced nociceptive neural activity at the upper cervical spinal cord (C2) evoked by noxious stimulation to the temporomandibular joint or masseter muscle. These findings clearly supported the idea that enhanced deep craniofacial nociception in response to psychological stress could be attributed to the enhanced neural activity in the C2 region; however, it remains unknown by which neural activity in the C2 could be regulated.

We have recently investigated the roles of Nucleus Raphe Magnus (NRM), the main area for descending pain control, to understand the effect of psychological stress on deep craniofacial nociception. To assess repeated stress effects on neural function in the NRM, Fos protein expression evoked by masseter muscle injury was quantified in the NRM in the rats in the presence or absence of repeated forced swim stress (FS) conditionings for 3 days. Results revealed that 1) increased neural activity in the NRM in FS rats compared to sham rats, 2) systemic administration of selective 5HT-reuptake inhibitor, antidepressant drug, decreased neural activity in the NRM in FS, but not in sham rats, and 3) FS did not affect the number of serotonin-positive neurons, whereas several serotonergic neurons appeared to be sensitive to masseter muscle injury.

These results supported the hypothesis that enhanced masseter muscle nociception under psychological stress conditions could be due to the changes in serotonergic functions in the NRM that could reflect the dysfunction of descending pain controls.

Key Words: orofacial pain, psychological stress, descending pain control

Lecture-6

Role of the VPAC2 receptor overactivation implicated in schizophrenia susceptibility

Yukio Ago[1], Atsuko Hayata-Takano[2], Takanobu Nakazawa[3], Kazuhiro Takuma[3], Hitoshi Hashimoto[1,4,5]

[1]Graduate School of Pharmaceutical Sciences, Osaka University; [2]United Graduate School of Child Development, Osaka University; [3]Graduate School of Dentistry, Osaka University; [4]Institute for Datability Science, Osaka University; [5]Institute for Open and Transdisciplinary Research Initiatives, Osaka University

The advent of the genomic era has led to the discovery of linkages of several genes and pathways to psychiatric disorders. Two large-scale genetic studies published early in 2011 provided first evidence that functional microduplications at 7q36.3, containing *VIPR2*, are a risk factor for schizophrenia. A *VIPR2* genetic linkage to schizophrenia is also replicated in the recent largest genome-wide analysis of copy number variants. *VIPR2* encodes VPAC2, a seven transmembrane heterotrimeric G protein-coupled receptor that binds two homologous neuropeptides PACAP and VIP with high affinity. Lymphocytes from patients with these microduplications exhibited higher *VIPR2* gene expression and VIP responsiveness, but mechanisms by which overactive VPAC2 signaling may lead to psychiatric disorders are unknown. Here we aimed to determine if the *VIPR2*-linkage to mental health disorders might be due to overactive VPAC2 receptor signaling during early neurodevelopment by daily administration of the selective VPAC2 receptor agonist Ro25-1553 from postnatal day 1 (P1) to P14 in male mice. Western blot analyses on P21 revealed significant reductions of synaptophysin and PSD-95 in the prefrontal cortex of Ro25-1553-treated mice. Golgi staining in adult brain revealed alterations in dendritic morphology of prefrontal and perirhinal cortical neurons in Ro25-1553-treated mice. The same postnatally-restricted treatment resulted in cognitive impairments in the novel object recognition and social recognition tests. Chronic treatment with clozapine, an atypical antipsychotic drug, ameliorated these behavioral and neuroanatomical abnormalities. Additionally, Ro25-1553 caused reductions in total numbers and length of neuronal dendrites and length of axon in primary mouse cortical neurons, and the effects were abolished in VPAC2 receptor-deficient mice. These findings implicate a potential pathological role of VPAC2 receptor overactivation, providing the hypothesis that *VIPR2* duplications increase the risk of schizophrenia partly due to deficits in neuronal maturation by overactivation of VPAC2 signaling at a critical stage of brain development.

Key Words: Schizophrenia, *VIPR2*, Copy number variants

Lecture-7

Degradation of parotid gland AQP5 protein by isoproterenol via calpain system

Chenjuan Yao[1], Gang Chen[1,2], Takahiro Hasegawa[1], Tetsuya Akamatsu[1], Kazuo Hosoi[1], Shali Yu[2], Xinyuan Zhao[2], Hiroshi Yoshimura[1]

[1]Department of Molecular Oral Physiology, Institute of Health Biosciences, University of Tokushima Graduate School, Japan; [2]Department of Occupational Medicine and Environmental Toxicology, School of Public Health, Nantong University, China

The parotid gland (PG) is an exocrine salivary gland that stores a large amount of proteins such as amylase in the secretory granules; their constituents are secreted into saliva by exocytosis. Exocytosis is induced primarily by the activation of β-adrenergic receptors, which leads to the intracellular accumulation of cAMP. In the present study, the molecular mechanism was explored that could regulate the level of aquaporin 5 (AQP5) in the PG of isoproterenol (IPR)-injected mice. By western blotting analysis, in the membrane fraction of PG, the protein level of AQP5, a member of the water channel family, was increased by injection IPR at 1 h, and stayed at high levels until 6 h; this change occurred simultaneously as amylase secretion. The AQP5 level then decreased and returned toward the original level at 12-48 h. On the other hand, the AQP5 mRNA gradually increased and reached a maximum at 24 h, after IPR injection. The facts suggest a rapid appearance of AQP5 at plasma membrane by IPR and subsequent degradation/metabolism by activation of proteolytic systems. On the other hand, after IPR treatment, the decrease of AQP5 protein level was partially or strongly suppressed by two calpain inhibitors, N-Ac-Leu-Leu-methininal (ALLM) and calpeptin, as well as a protein synthesis inhibitor, cycloheximide (CHX) but not by two proteasome inhibitors, MG132 and lactacystin, or the lysosome denaturant chloroquine (CQ). Furthermore, the AQP5 protein was also degraded by μ-calpain and the degradation was inhibited by calpain inhibitors ALLM and calpeptin in vitro. By immunohistochemistry, μ-calpain was colocalized with AQP5 in the acinar cells and its activity in the PG was increased at 6 h after IPR injection. These results suggest that the calpain system was responsible for IPR-induced AQP5 degradation in the PG and that such a system was coupled to the secretory-restoration cycle of amylase in the PG.

Lecture-8

Wnt5a, released from mechanically stimulated rat periodontal ligament cells enhances the neurite elongation

Takashi Yoshida, Kaori Takahashi, Minoru Wakamori
Div. Mol. Pharmacol. Cell Biophys., Tohoku Univ. Grad. Sch. Dent.

The periodontal ligaments (PDLs), located at the interface between the tooth and alveolar bones, play a role not only in the tooth planting and the shock-absorbing actions but also in the pressure sensing. The Ruffini's corpuscles, one of the mechanoreceptors, located in the PDLs play a pivotal role in pressure sensing to identify various food properties and to adjust occlusal force. Recent studies have shown that the loss of mechanical stimulation results in the disappearance of branches of Ruffini's corpuscles in the rat PDL. However, there is no report to evaluate the regulatory mechanisms for the peripheral nerve structure by mechanically stimulated PDL cells.

We have established primary PDL cell lines derived from rat PDLs (rPDL). The RT-PCR analysis confirmed the expression of NGF, BDNF, neurotrophin-4 (NT-4) and Wnt5a in these rPDL cell lines. One of the rPDL cell lines was seeded on a silicon chamber and loaded with periodic mechanical stimulation (0.5 Hz, 15% expansion). The qPCR analysis revealed Wnt5a mRNA levels increased in a time-dependent manner, while that of neurotrophic factors did not. LY294002, a PI3K inhibitor, and U0126, a MEK1/2 inhibitor, diminished the increase of Wnt5a mRNA expression levels in the rPDL cells loaded with mechanical stimulation. In order to confirm whether the released Wnt5a from rPDL cells can elongate the neurite or not, the culture media for the primary mouse trigeminal ganglion (mTG) neurons were replaced with the rPDL cell culture supernatant fluid with or without mechanical stimulation. The supernatant fluid of the mechanically stimulated rPDL cells enhanced the neurite elongation, and anti-Wnt5a antibody suppressed this effect.

These results suggest that the mechanical stimulated PDL cells produce Wnt5a via MEK1/2 and PI3K pathways and the secreted Wnt5a from PDL cells elongates neurites.

Key Words: Mechanical stimulation, periodontal ligament, Wnt5a

Abstract
Poster Session

Poster-1

A functional connectivity analysis of the neural circuit associated with jaw tapping in the elderly people

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The cortical activities during jaw movements have been investigated with various noninvasive brain imaging methods (e.g., PET, MRI, MEG). However, brain circuitry that initiates and executes jaw movement remains poorly unclear. In this study, we examined brain activities during a simple jaw tapping by functional magnetic resonance imaging (fMRI) in elderly subjects. Three groups of healthy subjects were included in this study: an elderly dentulous (ED) group, an elderly edentulous group without denture wearing (EE) and an elderly edentulous group with denture wearing (EEd). Each participant performed 3 cycles of 30 s tapping and 30 s rests. A general linear model analysis revealed that teeth tapping induced activation at various foci (p<0.05, FWE corrected), including the primary sensory cortex (SI), primary motor cortex (MI), supplementary motor cortex (SMC)/premotor cortex (PMA)(=BA6), insula cortex, cerebellum, thalamus, and basal ganglia in each group. To search for how sensory input increase induced by denture wearing affects to motor connectivity (i.e., voluntary movement) using a functional connectivity analysis, Psychophysiological interaction (PPI) was made. The PPI analysis confirmed that sensory input increase modulate connectivity between cortical and subcortical regions implicated in voluntary movements. These results suggest that sensory inputs from oral region are involved in the cortical control coordination and modulation of jaw movement that in the past have been attributed largely to brain stem regulatory mechanisms.

Key Words: fMRI, tapping, sensory

Poster-2

Sleep-related respiratory variables in sleep bruxism: a controlled study

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Recent studies have reported that obstructive sleep apnea (OSA) occasionally coexists with sleep bruxism (SB), and oral appliance for OSA can reduce rhythmic masticatory muscle activity (RMMA) during sleep in SB. Therefore, even though patients with SB did not fulfil clinical diagnosis of OSA, they may have some disadvantages related to respiratory activity during sleep. The aim of this study is to compare respiratory variables between normal subjects and subjects with polysomnographic diagnosis of SB.

The polysomnographic data were analyzed for 23 control (CTL) subjects (F11M12; age 24.4±2.9 years) who had RMMA less than 2 times per hour of sleep and 23 SB subjects (F8M15; age 24.3±2.3 years) who had RMMA equal or more than 4 times per hour of sleep. Respiratory events were scored and apnea-hypopnea index was calculated according to the AASM scoring criteria. Subjects in each two groups were subdivided into AHI<5 (AHI-L) or AHI≧5 (AHI-H). Sleep and respiratory variables were compared between the groups.

AHI did not differ between SB [median (minimum–maximum): 2.6 (0.4-13.8) /hr] and CTL [1.2 (0.1-7.9) /hr] groups. Apnea index and hypopnea index did not differ between the two groups. The number of subjects with AHI-H did not differ between CTL (N=5) and SB (N=7) groups. AHI during NREM sleep was significantly higher in SB [1.4 (0.2-11.0) /hr] than in CTL [0.9 (0.0-7.2) /hr] while AHI during REM sleep was not.

The results suggest that general respiratory variables did not differ between SB and CTL subjects. Further study needs to investigate whether minor but significant difference in AHI during NREM sleep characterizes respiration during sleep in SB subjects.

Key Words: sleep bruxism, respiratory activity, apnea-hypopnea index

Poster-3

Efficacy of low and high contingent electric stimulation on temporalis EMG activity and clinical muscle symptoms in patients with sleep bruxism

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Objectives: Sleep bruxism (SB), characterized by repetitive jaw-muscle activity that could be present as clenching or grinding of the teeth and/or by bracing or thrusting of the mandible that could happen during sleep period, is often suggested as a cause of temporomandibular disorders (TMD), orofacial pain and headache. The aim of this study was to demonstrate the efficacy of contingent electrical stimulation (CES) using a portable single-channel EMG device and to compare inhibitory effect of different stimulus intensities of CES over masticatory muscle EMG activity in pain intensity and mechanical muscle sensitivity.

Methods: Sixty possible sleep bruxers, screened and confirmed by a 2-week use of Grind Care (GC3+), out of 93 probable bruxers, were allocated in one of the 3 groups (High/Low/Placebo) in a randomized order. The participants were not informed which level of CES they would receive. In baseline and after the CES intervention, the participants were asked to score pain intensity, as well as unpleasantness, tiredness, tension, soreness and stiffness, on a 0-10 NRS scale. Mechanical muscle sensitivity was also measured in the masseter muscle and the temporalis muscle on a 0-100 NRS scale bilaterally.

Results: The number of EMG grinds/hour was significantly decreased in High-CES group after the intervention ($P = 0.024$). Interestingly, the NRS scores of unpleasantness ($P = 0.037$), tension ($P < 0.001$) and soreness ($P = 0.004$) in High-CES group and tiredness ($P = 0.002$) and soreness ($P = 0.006$) in Low-CES group were significantly decreased after the CES intervention compared to baseline.

Conclusion: This study indicated that the High-intensity CES showed inhibitory effect on masticatory muscle EMG activity, associated with significant decreases in jaw muscle symptoms such as unpleasantness/tiredness/soreness) but not pain responses.

Key Words: Sleep bruxism, contingent electrical stimulation, EMG

Poster-4

Temporal changes in occlusal vertical dimension after bite-raising in bite-reducing model animals

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Occlusal vertical dimension (OVD) in the guinea pig is maintained by tooth eruption and grinding. It has been reported that the experimentally raised OVD is recovered to the innate OVD within a few days in the guinea pig. However, in the bite-reduced model animals the experimentally-reduced OVD could not be fully recovered. In this study, we aimed to clarify the mechanism of regulation after bite-reducing which is different from that after bite-raising in guinea pigs.

First, inflammatory reaction was histologically examined around the root apex of bite-reduced model animals. Bite-reduced animals were developed in guinea pigs by applying bilateral intermaxillary elastics for 10 days. No inflammatory reaction was observed in the bite-reduced animals. It suggests that there is no deficit of eruption in the bite-reduced animals.

Second, the temporal change in OVD after bite-raising in bite-reduced model animals was examined. In the experimental group, the intermaxillary elastics were removed 10 days after application. Seven days after that, the bite-raising device was mounted for 10 days and then the device was removed. In the bite-reduced group, the intermaxillary elastics were only applied for 10 days. Guinea pigs without any OVD treatment were used as a control group. After the application of elastics for 10 days the OVDs were significantly reduced in both experimental and bite-reduced groups. In the experimental group bite-raising made the OVD nearly the same as that in the control group. After removal of the bite-raising device, the OVD was reduced to the same as that in the bite-reduced group.

These results suggest that once the OVD has been reduced and maintained, the innate OVD can be recognized to be high, and the amount of grinding can exceed the amount of eruption until it reaches the reduced OVD.

Key Words: Occlusal vertical dimension, Oral behavior, Guinea pig

Poster-5

Time-course changes in cortical, cardiac and respiratory changes before spontaneous rhythmic jaw movements after repeated ketamine injections in anesthetized guinea pigs

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[Aim] Rhythmic jaw movements (RJMs) under unconscious sleep states were found to occur in association with the transition or the shift within sleep states or to the arousal states. Previous studies showed that spontaneous RJMs occurred after repeated injections of ketamine. Spontaneous RJMs under ketamine anesthesia can be an interesting venue to understand the physiological processes in generating involuntary rhythmic movements during unconscious states. This study aimed to investigate physiological processes in cortical, cardiac and respiratory activities in the occurrence of RJMs in anesthetized guinea pigs.
[Methods] Male Hartley guinea pigs (600-800g) were prepared for the recordings of electroencephalogram (EEG), electrocardiogram, electromyograms of the jaw muscles, jaw movements, and nasal expiratory airflow under ketamine-xylazine anesthesia. After RJMs initially occurred on the stereotaxic frame, small boluses of supplemental ketamine (12.5 mg/kg and 25.0 mg/kg) were repeatedly injected through external jugular vein every time after RJMs occurred. Cortical EEG power, heart rate and respiratory rate were quantified and the time-course changes after ketamine injections before the onset of RJMs were analyzed.
[Results] RJMs occurred approximately 20 to 30 min after supplemental ketamine injections. Cortical delta power significantly decreased during a few minutes after a bolus injection and then gradually increased until the RJMs occurred. Heart rate and respiratory rate also decreased after injections and gradually increased towards the onset of RJMs. The minimal values of these variables after injection were significantly lower when higher dose of ketamine was injected.
[Conclusion] Spontaneous RJMs under ketamine anesthesia were preceded by a stereotypical change in cortical, cardiac and respiratory activities.

Poster-6

A descending circuit derived from the superior colliculus modulates vibrissal movements

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The superior colliculus (SC) is an essential structure for the control of eye movements. In rodents, the SC is also considered to play an important role in whisking behavior, in which animals actively move their vibrissae (mechanosensors) to gather tactile information about the space around them during exploration. We investigated how the SC contributes to vibrissal movement control. We found that when the SC was unilaterally lesioned, the resting position of the vibrissae shifted backward on the side contralateral to the lesion. The unilateral SC lesion also induced an increase in the whisking amplitude on the contralateral side. To explore the anatomical basis for SC involvement in vibrissal movement control, we then quantitatively evaluated axonal projections from the SC to the brainstem using neuronal labeling with a virus vector. Neurons of the SC mainly sent axons to the contralateral side in the lower brainstem. We found that the facial nucleus received input directly from the SC, and that the descending projections from the SC also reached the intermediate reticular formation and pre-Bötzinger complex, which are both considered to contain neural oscillators generating rhythmic movements of the vibrissae. Together, these results indicate the existence of a neural circuit in which the SC modulates vibrissal movements mainly on the contralateral side, via direct connections to motoneurons, and via indirect connections including the central pattern generators.

Key Words: premotor neurons, rat, anterograde tracing

Poster-7

Velopharyngeal function following levator veli palatini muscle reconstruction

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Velopharyngeal function plays an important role in swallowing, blowing, and phonation. Velopharyngeal function is regulated by the movement of the levator veli palatini (LVP) muscles. Thus, when performing palatoplasty, it is necessary to reconstruct the LVP muscles such that the function remains intact. There are 2 ways to reconstruct LVP muscles: suturing the left and right LVP muscles end-to-end (the push-back method) or suturing them such that they overlap (Furlow's method). The resulting velopharyngeal function has not been compared for these 2 LVP muscle reconstruction methods to date. We hypothesized that the manner of reconstructing LVP muscles would influence velopharyngeal function. We studied 18 beagle dogs, assigned to 4 groups according to the manner of LVP muscle reconstruction: normal LVP, cut LVP, end-to-end sutured LVP, and overlapped-sutured LVP. We induced velopharyngeal closure under rebreathing conditions and stimulation of the LVP muscles. We recorded the electromyographic (EMG) activity of the LVP muscles in the different velopharyngeal closure pattern groups to analyze velopharyngeal function. Under rebreathing conditions, the velopharyngeal function did not differ significantly among the 4 groups in terms of velopharyngeal pressure ($p = 0.055$). Under stimulation with 2–10 mA current, the 4 groups differed statistically significantly ($p = 0.007$–0.038), and the overlapped-sutured LVP group demonstrated the best velopharyngeal function. We thus concluded that Furlow's method for LVP muscle reconstruction is superior than the push-back method for obtaining stronger velopharyngeal function.

Key Words: cleft palate; muscle strength; velopharyngeal insufficiency

Poster-8

Membrane potential responses of the excitatory and inhibitory neurons in layer II/III of the insular cortex by dental pulp stimulation: In vivo whole-cell targeting patch-clamp recording

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The insular cortex (IC) receives multimodal sensation including dental nociception. Recent optical imaging studies reported electrical stimulation of dental pulp evokes excitatory propagation in the IC surrounded by the rhinal fissure and the middle cerebral artery. However, it is unknown how the membrane potential (Mp) of the excitatory pyramidal neurons (Pyr) and GABAergic inhibitory interneurons (IN) in the IC are driven by nociceptive inputs. To address this question, we performed in vivo whole-cell patch-clamp recording combined with two-photon microscopy to record from genetically labeled neurons. Here, we show Mp fluctuation in the IC driven by electrical dental pulp stimulation. The resting Mp of IN was more depolarized than Pyr (-45.1 ± 1.8 mV, n = 10 vs. -62.3 ± 1.0 mV, n = 11), and most IN showed spontaneous spike firing. Dental pulp stimulation elicited larger amplitude of excitatory postsynaptic potential (EPSP) in IN than that in Pyr (17.3 ± 3.6 mV, n = 10 vs. 6.5 ± 2.5 mV, n = 11). EPSPs of IN often excided the spike threshold and evoked action potentials. Subsequently, the compatible size of inhibitory postsynaptic potentials was observed in both IN and Pyr (-5.3 ± 1.0 mV, n = 10 vs. -7.2 ± 0.7 mV, n = 11). These results suggest the higher activities of IN contributes to suppressing Pyr activities in the IC.

Key Words: *In vivo* whole-cell patch-clamp recording, membrane potential, nociception

Poster-9

Presymptomatic abnormalities and associated channelopathies in spindle afferent trigeminal Mesencephalic V neurons in the SOD1G93A mouse model for Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease wherein upper and lower motor neurons (MNs) progressively degenerate leading to muscle atrophy, paralysis and death. Recent evidence has suggested involvement of the fusimotor system in contributing to motor neuron vulnerability (Lalancette-Hebert et al., 2016, PNAS.). Here we propose involvement of spindle afferent neurons in early disease development. In the present experiments, we used in vitro patch-clamp methods to record from primary sensory Mesencephalic V (Mes V) neurons that relay reflex and proprioceptive inputs from jaw muscle spindles to trigeminal MNs. We demonstrate early (P8-P14) abnormalities in membrane properties of these neurons in the well-characterized SOD1G93A mouse model (mSOD1) for ALS when there are no phenotypic behavioral deficits. Such neuronal changes included hypo-excitability and irregularity in spike discharge patterns of Mes V neurons displaying both burst and tonic discharge. Mean spike frequencies were significantly lower in mSOD1 animals. Further examination of ionic mechanisms mediating excitability changes in Mes V neurons using voltage-clamp experiments revealed reduction of persistent and resurgent sodium conductances. Ongoing experiments are examining restoration of normal levels of sodium conductances using realistic computational models and dynamic-clamp technique to control membrane excitability and reverse abnormal changes in mSOD1 Mes V neurons. For example, we are able to reverse irregular spike patterns in mSOD1 Mes V cells in vitro via injection of biophysically based resurgent sodium current. Together these experiments offer the first evidence of circuit-level dysfunction and spindle afferent sensory changes due to multiple channelopathies. This result is transformative in our understanding of disease development in ALS, and suggest novel directions for biomarker search and design of multifaceted strategies for disease management.

Key Words: ALS, Mes V, Patch-clamp

Poster-10

Corticofugal pathways from granular insular cortex conveying orofacial proprioception

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Our motor behavior can be affected by proprioceptive information. However, little is known about which brain circuits contribute to this process. We have recently revealed that the proprioceptive information arising from jaw-closing muscle spindles (JCMSs) is conveyed to the supratrigeminal nucleus (Su5) by neurons in the trigeminal mesencephalic nucleus (Me5), then to the caudo-ventromedial edge of ventral posteromedial thalamic nucleus (VPMcvm), and finally to the dorsal part of granular insular cortex rostroventrally adjacent to the rostralmost part of secondary somatosensory cortex (dGIrvs2).

Our next question is which brain areas receive the information from the dGIrvs2 for the jaw-movements. To test this issue, we injected an anterograde tracer, biotinylated dextranamine, into the dGIrvs2, and analyzed the resultant distribution profiles of the labeled axon terminals. Anterogradely labeled axons were distributed in the pontomedullary areas (including the Su5) which are known to receive JCMS proprioceptive inputs conveyed directly by the Me5 neurons and to contain premotoneurons projecting to the jaw-closing motoneurons in the trigeminal motor nucleus (Mo5). They were also found in and around the VPMcvm. In contrast, no labeled axonal terminals were detected on the cell bodies of Me5 neurons and motoneurons in the Mo5. These data suggest that jaw-movements, which are evoked by the classically defined jaw-reflex arc originating from the peripheral JCMS proprioceptive information, could also be modulated by the transcortical feedback connections from the dGIrvs2 to the VPMcvm and Su5.

Key Words: Proprioception, Insula, Corticofugal

Poster-11

Functional alteration of ascending noxious pathways associated with trigeminal nerve injury

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Second-order neurons in trigeminal subnucleus caudalis and upper cervical spinal cord (Vc-C1) are critical for craniofacial pain processing and project to the ventral posteromedial thalamic nucleus (VPM) and parabrachial nuclei (PBN). We hypothesized that VPM and PBN projection Vc-C1 neurons responding to noxious stimuli might alter their functional features following trigeminal nerve injury, and these changes may be involved in orofacial persistent pain mechanisms. In this study, neuroanatomical approaches were used to elucidate functional modifications in VPM or PBN projection Vc-C1 neurons following chronic constriction injury of the left infraorbital nerve (ION-CCI) in rats.

Heat hyperalgesia and mechanical allodynia in the left upper lip were induced by ION-CCI on day 7. These rats were injected retrograde tracer fluorogold (FG) into the right VPM or PBN. Capsaicin injection or noxious mechanical stimuli was applied to the left upper lip on day 3 after FG-injection. Many phosphorylated extracellular signal-regulated kinase-immunoreactive (pERK-IR) neurons were found in the superficial laminae of Vc-C1 ipsilateral to the stimulation sites, and the number of them was significantly greater in ION-CCI than sham rats. VPM FG-labeled neurons in Vc-C1 were found in the side contralateral to FG-injection site, whereas PBN FG-labeled neurons were bilateral. The percentage of pERK-IR VPM and PBN projection neurons vs. FG-labeled neurons following capsaicin injection was significantly larger in ION-CCI than sham rats. However, the percentage of pERK-IR PBN projection neurons was larger in noxious mechanical-stimulated ION-CCI rats compared with sham rats.

The present findings suggest that these functional changes in Vc-C1 VPM and PBN projection neurons following ION-CCI may reflect differential involvement of ascending pathways receiving C-fiber and mechanical afferents in orofacial persistent pain associated with trigeminal nerve injury.

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Key Words: ventral posteromedial thalamic nucleus (VPM), parabrachial nuclei (PBN), neuropathic pain

Poster-12

Prevention of Dry-Eye Pain by $P2Y_2$ receptor agonist Diquafosal Sodium Administration

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Dry eye (DE) is known to cause ocular pain with unpleasantness in the eye. The cornea is innervated by the first branch of the trigeminal nerve, and the density of C-fibers innervating cornea is very high. Recently, $P2Y_2$ receptor agonist diquafosol sodium is used for the treatment of DE. Diquafosol accelerates moisture and mucin secretion from conjunctival epithelium and goblet cells. However, it's still unknown whether the tear secretion following diquafosol treatment attenuates ocular pain and neuronal hyperactivity in brainstem neurons associated with dry eye.

DE rats were developed by the removal of unilateral exorbital grand. We studied the tear volume, the number of eyeblinks evoked by hypertonic saline application to the eye, and the expression of phosphorylation of extracellular signal-regulated kinase (pERK) and c-Fos in trigeminal subnucleus caudalis-upper cervical spinal cord (Vc-C1). Three percent diquafosol solution (10 μl 6 times/day) was applied to the eye for 4 weeks in DE rats.

Tear volume was significantly reduced, and the number of eye blinks elicited by the hypertonic saline application was substantially higher in DE rats. The reduction of tear volume and the number of eyeblinks in DE rats were recovered by diquafosol treatment. Many pERK-immunoreactive (IR) cells were restricted in the superficial laminae of the ventral portion of Vc-C1 after hypertonic saline application to the eye, whereas c-Fos-IR cells were detected in the deep as well as superficial laminae of the Vc-C1 in DE rats. The number of pERK-IR cells and c-Fos-IR cells was also significantly suppressed by diquafosol treatment. The suppression of neural activity in Vc-C1 was observed in diquafosol administrated rats.

The present findings suggest that diquafosol treatment causes the enhancement of the tear secretion, and then the hyperactivity of Vc-C1 neurons in DE rats is attenuated, resulting in the prevention of DE pain.

Acknowledgement: Santen Pharmaceutical Co.

Key Words: Dry eye, diquafosol sodium, trigeminal subnucleus caudalis

Poster-13

Basolateral amygdala inactivation suppresses the taste but not olfactory neophobia in rats

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Animals tend to hesitate to consume a new food (food neophobia). In the absence of negative post-ingestive consequences, the intake increases over exposures (attenuation of neophobia). Here, we examined whether basolateral amygdala (BLA) inactivation influences the taste and olfactory neophobia. Wistar rats were exposed to the sweet taste solution (0.5% saccharin) for 20 min for four consecutive days (the taste tests). Before the first exposure, they received a microinjection of a gamma aminobutyric acid type A ($GABA_A$) agonist muscimol (50 ng/0.25 μl) or saline into the bilateral BLA. After the end of taste tests, the same procedure was repeated with exposures to the almond odor solution (0.15% benzaldehyde) instead of the saccharin for the odor tests. At the first taste test, the saccharin consumption in both groups were lower than their water baseline, suggesting that they expressed the taste neophobia. However, the muscimol-injected rats showed significantly higher consumption of saccharin than controls did. At the subsequent three taste tests, there were no differences between groups on saccharin consumption. On the other hand, muscimol injection did not influence the benzaldehyde consumption through the four odor tests. These results suggest that the BLA inactivation suppresses, but not eliminates, the neophobic reaction to a novel taste, but not odor, solution.

Key Words: taste neophobia, amygdala, rats

Poster-14

The *de novo* Q1042R *POGZ* mutation in sporadic ASD patients impairs the neural development

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interactions. Although genetic studies have identified numerous candidate genetic variants, the molecular etiology of ASD remains poorly understood. Recent studies suggest that *de novo* mutations contribute to the risk of ASD. In particular, genes with highly recurrent *de novo* possible loss-of-function mutations, which are likely to play key roles in the etiology of ASD, have been identified in multiple unrelated patients. Among these high-confidence ASD risk genes, *POGZ* is one of the most recurrently mutated genes in ASD patients; we and other groups have recently identified at least 20 independent *de novo* possible loss-of-function mutations in *POGZ*. However, the precise role of POGZ remains unknown. Here we present the role of POGZ in the neuronal differentiation and the functional characterization of *de novo POGZ* mutations.

We found that *Pogz* was highly expressed in the developing mouse cerebral cortex during embryonic neurogenesis and that POGZ knockdown impaired the neuronal differentiation. Since POGZ contains a centromere protein-B-like DNA-binding (CENPB-DB) domain and is likely to function as a chromatin regulator and a transcription factor, we examined the effect of the *de novo* mutations on the DNA-binding activity of POGZ and found that the *de novo* Q1042R mutation within the CENPB-DB domain of POGZ, which we recently identified in a sporadic ASD case, resulted in a reduction of approximately 60% in the DNA-binding activity of POGZ. Then, we established iPS cells from the patient who has the Q1042R mutation and found that the patient's neural stem cells showed impaired neuronal differentiation.
Our results suggest that ASD-associated *de novo* mutations impair the DNA-binding activity of POGZ, an effect likely to result in the perturbation of chromatin function and the neuronal differentiation. These findings provide important insights into the cellular basis of ASD.

Key Words: autism spectrum disorder, de novo mutation, iPS cells

Poster-15

Role of intracellular Ca^{2+} stores in layer 2/3 pyramidal cells of the somatosensory cortex during oxygen and glucose deprivation

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Increasing evidence suggests that release of Ca^{2+} from endoplasmic reticulum (ER) occurs through ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP_3Rs) during cerebral ischemia and contributes to cell death. However, its underlying mechanisms remain largely unknown. In the present study, we examined how RyRs and IP_3Rs are involved in cell death caused by oxygen-glucose deprivation (OGD) in layer 2/3 pyramidal neurons of the mouse somatosensory cortex using whole-cell patch-clamp recordings. We also examined the possible involvement of mitochondria and the role of calcineurin in the neuronal cell death. Bath application of OGD solution produced a rapid depolarization after approximately 8 min of exposure. When OGD solution was bath-applied in the presence of 100 μM ruthenium red (RR, a RyR inhibitor), 10 μM xestospongin C (Xest C, a IP_3R inhibitor) or RR + Xest C in the patch-pipette solution, the onsets of the rapid depolarization were significantly delayed (RR, 16 min; Xest C, 17 min; RR+Xest C, 23 min). Next, when OGD solution was bath-applied in the presence of ketone bodies [1 mM β-hydroxybutyrate (BHB) and 1 mM acetoacetate (ACA)], the onset of the rapid depolarization was significantly delayed (18 min). Furthermore, when OGD solution was bath-applied in the presence of a calcineurin inhibitor (1 μM FK506 or 20 μM fenvalerate) in the patch-pipette solution, the onsets of the rapid depolarization were significantly delayed (FK506, 19 min; fenvalerate, 20 min). These results suggest that release of Ca^{2+} from ER is followed by mitochondrial deenergization and calcineurin activation, which may lead to failure of Na^+-K^+-pump.

Key Words: intracellular Ca^{2+} store, pyramidal cell, ischemia