JOINT SYMPOSIUM

The Promotion of Multi-disciplinary Research Projects

“Translational Research Network on Orofacial Neurological Disorders (TRON projects)”

&

Japan-Canada Joint Health Research Program – U. Toronto/Nihon U. Grant

“A Collaborative Approach to Clarify Mechanisms of Orofacial Pain and Motor Disorders”

Nihon University School of Dentistry, February 29, 2008
PROGRAM

13:00 Opening Remarks  
Kichibe Otsuka (Nihon Univ.)

13:02 Overview of TRON projects  
Masayuki Kobayashi (Nihon Univ.)

Session I

Basic and Clinical Research Targeting on Neural Mechanisms of Orofacial Neurological Disorder  
Moderator: Noriaki Koshikawa (Nihon Univ.)

13:10 “The behavioural pharmacology of orofacial dyskinesia: validity of rodent models”  
Noriaki Koshikawa (Nihon Univ.)

13:35 “Modulation of primary afferent and trigeminal spinal subnucleus caudalis neuronal activities following regeneration of transected inferior alveolar nerve in rats”  
Koichi Iwata (Nihon Univ.)

14:00 “A clinical approach to burning mouth syndrome”  
Yoshiki Imamura (Nihon Univ.)

14:25 “Evaluation of image-guided puncture technique for the superior temporomandibular joint space with aid of cone beam computed tomography (CBCT)”  
Kazuya Honda (Nihon Univ.)

14:50 Break

Session II

Pain Research in Patients and Animal Models –Collaborative projects of TRON with U. Toronto–  
Moderator: Barry Sessle (Univ. Toronto)

15:00 “Peripheral processes and sensorimotor cortical mechanisms related to orofacial pain and motor control”  
Barry Sessle (Univ. Toronto)

15:45 “Glial cells are involved in central sensitization in the rat medullary dorsal horn”  
Chen-Yu Chiang (Univ. Toronto)

16:30 Break
16:40 “Involvement of P2X receptors in central sensitization in the medullary dorsal horn”
Jonathan Dostrovsky (Univ. Toronto)

17:25 “Src in NMDA receptor-mediated plasticity in pathological pain hypersensitivity”
Michael Solter (Univ. Toronto)

18:10 Closing Remarks
Noriaki Koshikawa (Nihon Univ.)
The behavioural pharmacology of orofacial dyskinesia: validity of rodent models

Noriaki Koshikawa

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Orofacial dyskinesia has characteristic feature of uncontrollable orofacial movements, namely involuntary, purposeless and repetitive movements. Such abnormal movements occur in schizophrenic patients after long-term neuroleptic treatment, in Parkinson’s disease patients after L-DOPA treatment and occasionally in elderly people. Since dopamine D₂ receptor up-regulation and over-stimulation of dopamine receptors are generally considered to lead to orofacial dyskinesia, the present talk focuses on dopaminergic systems.

It is well known that chronically given typical antipsychotics, such as haloperidol, often elicit tardive dyskinesia, while atypical antipsychotics, such as clozapine, elicit considerably less or no tardive dyskinesia. In rats, spontaneous vacuous chewing is often considered as a valid animal model of tardive dyskinesia. In fact, a very long-time treatment, usually more than 6 months, haloperidol, but not clozapine, has been reported to increase spontaneous vacuous jaw movements. In order to improve the efficiency of the model, attempt has been made to reduce the duration of the treatment, usually 4 weeks. However, results of these studies are rather inconsistent. In our hands, even using rather large number of animals, 4 weeks treatment with clozapine and haloperidole similarly increased vacuous chewing, indicating that this paradigm is insufficient for predicting the liability of antipsychotics to produce tardive dyskinesia. However jaw movements elicited by dopaminergic challenge clearly distinguish these two drugs. Dopamine D₁/D₂-like receptor stimulation made by apomorphine or mixture of their selective agonists, SKF 38393 and quinpirole, elicits essentially similar jaw movements accompanied by occasional tongue protrusions. Thus, the jaw movement response is increased in rats treated with haloperidol but not clozapine. Therefore, the first part of this talk concentrates on the rat dopaminergic jaw movements and discuss about possible neuronal connections from the basal ganglia involved in eliciting rat involuntary rhythmical jaw movements.

In the second part, the results of our recent studies on sub-divisions of D₁-like receptors involved in D₁/D₂-like receptor-mediated jaw movements will be presented. D₁-like receptor is recently subdivided into two subtypes. Addition to the defining linkage to adenylyl cyclase (AC), putative subtype that coupled to phosphoinositide (PI) hydrolysis is the most widely entertained candidate. Available compounds specific to the subtypes are still limited, but recently available drugs, i.e. SKF 83822 is suggested to be a selective AC-coupled agonist and SKF 83959 is a PI-coupled agonist, provide us an opportunity to examine their functional roles. In this connection, our recent approach using these drugs in rodents, including mice with dopamine receptor subtypes (D₁, D₂, D₃, D₄ and D₅) knockout, will be discussed.
Modulation of primary afferent and trigeminal spinal subnucleus caudalis neuronal activities following regeneration of transected inferior alveolar nerve in rats

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Trigeminal neuropathic pain conditions are well known to be difficult to diagnose and treat, and for most their etiology and pathogenesis are still unclear. Some trigeminal neuropathic pain behavioral models have been developed in rats to clarify the neural mechanisms underlying these neuropathic pain conditions, but little is known about the peripheral and central neuronal changes that occur after trigeminal nerve injury. In order to clarify the neuronal mechanisms of abnormal pain in the face innervated by the regenerated inferior alveolar nerve (IAN), nocifensive behavior, trigeminal ganglion neuronal labeling following Fluorogold (FG) injection into the mental skin, single neuronal recording from TG neurons, and trigeminal spinal subnucleus caudalis (Vc) neuronal response properties and the phosphorylated extracellular signal-regulated kinase (pERK) immunohistochemistry were conducted in the rats with IAN transection.

We observed significant decrease in escape threshold to mechanical stimulation of the face in these two models. Myelinated fibers were strongly affected in their activities in IAN transected rats. The excitability of TG neurons was also increased in these models. WDR neurons in the Vc were significantly increased in their activities in IAN transected rats. The ERK phosphorylation was also increased in Vc neurons in the rats with IAN transection.

These findings suggest that myelinated primary afferent fibers may have an increased ability to generate action potentials following sensory nerve injury, and that this is reflected behaviorally in a lowering of the escape threshold to mechanical stimulation of the regenerated IAN innervated area, reflecting an increase in the excitability of Vc neurons. These changes in the excitability of TG and Vc WDR neurons could be involved in the mechanical allodynia that can be developed in the cutaneous region innervated by the regenerated IAN.
A clinical approach to burning mouth syndrome

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Although trigeminal neuralgia (TN), complex regional pain syndrome, herpes zoster and burning mouth syndrome (BMS) are classified in a category of neuropathic pain, characteristic features of these conditions are typically different each other and clinical approach to these conditions should be unique in each condition. In general, pain conditions consist of physical and psychological components. TN patients rarely complain of affective problems and they are sufficiently treated by medicines and neurosurgeries in most cases. BMS, however, often requires psycho-therapy that does not always ensure satisfaction of patients. The aim of this study is to clarify the pathophysiology of BMS to establish a treatment strategy. In the first year, we would like to set the goal of the study in comparing the brain activity after specific interventions between BMS patients and healthy volunteers. Brain activation in both BMS patients and healthy volunteers after application of standardized stimuli will be observed with functional MRI. Noxious heat stimulation with a Peltie device to activate C fibers, electrical stimulation with a specific electrode for A-delta fibers and pictures that make people imagine painful sensation will be applied in both groups. Neuroimaging patterns in both groups will be discussed.
Evaluation of image-guided puncture technique for the superior temporomandibular joint space with aid of cone beam computed tomography (CBCT)

Kazuya Honda

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Although perforation and adhesion of temporomandibular joint (TMJ) disk require arthrographic examination for their treatments, puncturing procedure of the TMJ space has always a risk to penetration or damaging the middle cranial fossa. We therefore sought to investigate safer image-guided puncture technique (IGPT) to the superior TMJ space by using the cone beam computed tomography (CBCT).

Fifty-two patients underwent the CBCT examination, and their optimum angles and distances, from the puncture sites to the thinnest points of the glenoid fossa, to penetrate the superior TMJ space were measured using three-directional images.

By using this technique, 50 out of 52 cases were successfully punctured the superior TMJ space with relatively short examination time (average time spent is approximately 20 min). This method gave an average horizontal angle of 8.0° (standard deviation (SD): 9.2), an average coronal angle of 16° (SD: 11.3), and an average distance of 27 mm (SD: 2.8).

Large variation in puncture angle among individuals found in the present study suggests that the CBCT examination is highly recommended to precede the TMJ space puncture. Thus, the present clinical application of IGPT with CBCT provides safeness and high successful rate to the TMJ space penetration technique.
Peripheral processes and sensorimotor cortical mechanisms related to orofacial pain and motor control

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We have carried out series of experiments to investigate (1) peripheral glutamatergic mechanisms of orofacial pain and (2) the effects of glutamate or other changes in orofacial tissues on sensorimotor cortical mechanisms influencing orofacial motor function.

In experimental series (1), effects of glutamate applied to TMJ or masticatory muscle were tested on nociceptive afferents and jaw muscle EMG activity in rats and on perceptual and motor behaviours in humans. Glutamate application activated and sensitized rat nociceptive afferents and reflexly evoked EMG activity in a sex-dependent manner. Sex-dependent effects in glutamate-induced pain and changes in jaw motor function were also demonstrated in human subjects. Glutamate-induced effects could be attenuated by NMDA antagonists and mimicked by NMDA application. These findings reveal that glutamate has NMDA-dependent nociceptive actions in peripheral orofacial tissues and that peripheral, physiologically based sex differences exist in these nociceptive mechanisms.

In experimental series (2), the effects of peripherally applied glutamate or intraoral manipulations (incisor trimming or extraction; lingual nerve transection) were tested on the excitability of face primary motor cortex (MI) identified by intracortical microstimulation in rats. The effects of pain were also tested on face MI excitability (determined with transcranial magnetic stimulation) and on the learning of motor skills in humans. Glutamate application to rat masticatory muscle induced decreased excitability of face MI regions controlling the injected muscle, suggesting that pain can modify the cortical control of motor function. Intraoral pain in humans also interfered with face MI excitability reflecting cortical neuroplasticity associated with their learning of a novel orofacial motor task. Face MI neuroplasticity was also apparent several days after trimming or extraction of the rat incisor or transecting the lingual nerve. These findings reveal the remarkable neuroplasticity of face MI and suggest that face MI excitability and motor control are sensitive to orofacial nociceptive inputs and other changes in the orofacial environment.

Selected References


Glial cells are involved in central sensitization in the rat medullary dorsal horn

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Aim of investigation: In the trigeminal system, we have demonstrated that application of the small-fiber excitant and inflammatory irritant mustard oil (MO) to the rat tooth pulp produces central sensitization in brainstem nociceptive neurons of trigeminal subnucleus caudalis (the medullary dorsal horn, MDH) that is glutamatergic and purinergic receptor-dependent (Chiang et al., 1998, 2005). Recent studies provide evidence that glial (microglia in particular) activation is involved in chronic inflammatory pain and neuropathic pain. We have recently tested if there is astroglial involvement in central sensitization induced in MDH in our acute tooth pulp inflammatory pain model (Chiang et al., 2007; Xie et al., 2007).

Methods: Extracellular activity was recorded from functionally identified single nociceptive neurons in deep MDH of chloralose/urethane-anesthetized rats. Assessments of neuronal mechanoreceptive field (RF) size, mechanical activation threshold and responses to noxious stimuli were performed before and 25 min after continuous i.t. superfusion of glial-modulating drugs or saline (as control) over MDH. Then, 3 min after MO application to the tooth pulp, the standard assessment was repeatedly performed at 10-min intervals for 50 min under continuous superfusion.

Results: MO application during saline superfusion of MDH produced pronounced central sensitization reflected as significant increases in spontaneous activity, RF size, and responses to noxious stimuli, and a decrease in activation threshold. In contrast, superfusion of fluoroacetate (an inhibitor of astroglial metabolic enzyme aconitase) or SB203580 (an inhibitor of p38MAPK) significantly attenuated the MO-induced central sensitization. Similarly, superfusion of methionine sulfoximine (MSO; an inhibitor of astroglial glutamine synthetase) significantly attenuated the MO-induced central sensitization in MDH, whereas simultaneous superfusion of MSO and glutamine (which can be taken up into neurons and transformed into glutamate) could rescue the MO-induced central sensitization in MDH. Superfusion of methylamino-isobutyric acid (MeAIB; a competitive inhibitor of the neuronal system A transporter which is responsible for uptake of astroglial-released glutamine into neurons) also significantly attenuated the MO-induced threshold and response changes and partially attenuated the increase in RF size.

Conclusions: In all the above experiments, superfusion of glial modulator itself did not affect the normal nociceptive transmission in the neurons. Collectively, these findings provide the first documentation of a key role for glial cells in central sensitization of functionally identified nociceptive neurons in trigeminal nociceptive pathways, and indicate that the integrity of astroglial function, including the glutamate-glutamine shuttle, is essential for the development of central sensitization in the acute dental inflammatory pain.

Selected References:
Involvement of P2X receptors in central sensitization in the medullary dorsal horn

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Application of mustard oil (MO) to the rat tooth pulp results in hypersensitivity of nociceptive neurons in the trigeminal subnucleus caudalis (the medullary dorsal horn - MDH) and oralis. Our previous studies demonstrated the involvement of NMDA receptor mechanisms in this MO-induced central sensitization in both regions and showed also that that the central sensitization in oralis can be mimicked by P2X receptor agonist and blocked by P2X receptor antagonist applications onto the MDH. In view of these findings and reports of the involvement of P2X receptors in spinal dorsal horn central sensitization, the aim of the present study was to investigate whether endogenous ATP acting on P2X receptors is involved in the development of MO-induced central sensitization of MDH nociceptive neurons. The medulla and the coronal pulp of the maxillary first molar tooth were surgically exposed in urethane/α-chloralose-anesthetized adult rats. Extracellular recordings of single neuronal activity were made from nociceptive-specific neurons in the deep MDH laminae. The mechanoreceptive field, mechanical activation threshold and responses to graded pinch or pressure of each neuron were assessed before and after MO application to the pulp as measures of sensitization. During continuous saline superfusion over the caudal medulla, MDH neuronal central sensitization was readily induced by MO application to the tooth pulp. However, this MO-induced central sensitization could be completely blocked by continuous i.t. superfusion of the wide-spectrum P2X receptor antagonist pyridoxal-phosphate-6-azophenyl-2, 4-disulphonic acid tetra-sodium (PPADS, 33-100 μM) and by apyrase (an ectonucleotidase enzyme, 30 units/ml). Superfusion of the selective P2X1, P2X3 and P2X2/3 receptor antagonist 2′,3′-O-(2,4,6-trinitrophenyl) adenosine 5′-triphosphate (TNP-ATP, 6-600 μM) partially blocked the MDH central sensitization. However, the two P2X receptor antagonists did not significantly affect the baseline nociceptive properties of the MDH neurons. These findings implicate endogenous ATP acting at P2X receptors as an important mediator contributing to the development of central sensitization in nociceptive neurons of the deep laminae of the MDH.
Regulation of postsynaptic glutamate receptors is one of the principal mechanisms for producing alterations of synaptic efficacy in the CNS. A growing body of evidence indicates that at glutamatergic synapses NMDA receptors are upregulated by Src family tyrosine kinases which are opposed by the action of tyrosine phosphatases, one of which has been identified as STEP. Src itself is expressed nearly ubiquitously in higher organisms with the highest levels of expression found in the CNS. Src represents a point through which multiple signaling cascades from, for example G-protein-coupled receptors, Eph receptors and integrins, converge to upregulate NMDA receptor activity. The upregulation of NMDARs by activation of Src participates in the induction of long-term potentiation of synaptic transmission in the hippocampus and in the spinal cord dorsal horn. We have determined that Src is anchored within the NMDA receptor complex by the protein ND2. Recently, we have found that interfering with the ND2-Src interaction in vivo reverses behavioural pain hypersensitivity in rodent models of inflammatory and neuropathic pain. Also, interfering with this interaction suppressed the increase in tyrosine phosphorylation of the NMDAR subunit NR2B in the spinal dorsal horn. Thus, multiple mechanisms control Src in the NMDA receptor complex and disrupting Src-mediated enhancement of NMDA receptor function affects pathological pain neuroplasticity in the CNS.

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