1809 Changes in GAP-43-IR in Ruffini Endings during Experimental Tooth Movement. H. KOBAYASHI*, I. SAITO, K. HANADA and T. MAEDA (Niigata University, Niigata, JAPAN)

Growth-associated protein-43 (GAP-43) is an axonal growth marker in neural development and regeneration. However, our recent study has shown that GAP-43-IR was localized in Schwann sheaths in the periodontal Ruffini endings in mature rats. The present study reports on changes in GAP-43-immunoreactivity (IR) in the periodontal Ruffini endings during experimental tooth movement. According to Waldo's method, experimental tooth movement was performed by inserting a piece of elastic band between the upper first and second molars on the right-hand side in the rat. The animals were perfused at 1,3,5,7,9,11,14 days. Then, the tissues were decalcified, frozen sectioned, and reacted for GAP-43-IR by LM and EM. In the untreated molars, the Schwann sheaths surrounding the axon terminals contained GAP-43-IR in the mechanoreceptive Ruffini endings, lacking in the axon terminals. In the experimental groups, the expression pattern of GAP-43-IR was variable in covering Schwann sheaths, in the cell bodies of terminal Schwann cells or the axoplasm after 5 days of tooth movement. Altered expression patterns of GAP-43 in the Ruffini endings show that the orthodontic forces influence the physiological activities of Ruffini endings, and further suggest that GAP-43 is involved in the remodeling of these periodontal mechanoreceptors. Supported by a Grant (No 07672215), Japan.

1810 Distribution of GAP-43 in the PDL following peripheral nerve injury. S. H. YOUN*, S. WAKISAKA, K. KURISU. (Depts. of Orthodontics* and Oral Anatomy & Developmental Biol., Osaka Univ., Faculty of Dentistry, Osaka, JAPAN.)

Previously, we reported that regenerating periodontal primary afferents exhibited neuropeptide Y (NPY)-like immunoreactivity (-LI) following nerve injury to the inferior alveolar nerve (IAN), suggesting the possible contribution of NPY to the regeneration of periodontal primary afferents. It was also reported that regeneration in the peripheral nervous system following nerve injury is controled by the upregulation of growth-associated protein 43 (GAP-43). In the present study, we report the temporal changes in the levels of GAP-43-like immunoreactive (-IR) nerve fibers in the periodontal ligament (PDL) of rat incisors following peripheral resection to the IAN. Three, 5, 7, 10, 14, 21, 28, and 56 days following resection to the IAN, rats were fixed and lower jaws were removed. After decalcification with EDTA, PDL of the incisor was immunostained for GAP-43. Some sections were immunostained for protein gene product 9.5 (PGP 9.5). PGP 9.5-IR nerve fibers showed tree-like appearance in the tooth-related part of normal PDL. GAP-43-IR nerve fibers also had complex ramification and some nodal sprouts in normal animals. Following resection injuries, GAP-43-IR nerve fibers decreased around 3 days following injury and showed dotted appearance. They gradually increased and had peaks around 14 days following injury. Their terminals around 14 days were ramified and had some nodal sprouts resembling as the Schwann cell-like patterns. These nodal sprouting patterns were decreased 28 days following injury. The present results suggest that regeneration of nerve fibers in PDL is associated with the GAP-43 and/or Schwann cell sproutings.

1811 **Biological and Behavioral Characteristics During Chronic TMJ Inflammation.** **R. JOHNSON*, J. SIEBERT, R.P. HARPER, R. SPEARS, R.J. HINTON, L.L. BELLINGER, R.J. GATCHEL, and B. HUTCHINS (Baylor Dental Sch., Texas A&M Univ. Sys., Dallas, TX).**

Although there have been a few studies of acute inflammation of the temporomandibular joint (TMJ), most TMJ disorders are chronic problems. Therefore, in this study we characterized several parameters that might be associated with a chronic inflammation (six weeks) of the rat TMJ: grooming, scratching, meal patterns, body weight (BW), ear temperatures, joint swelling, bony changes, and mechanical stimulation (von Frey filaments). Sixteen adult male rats were randomly assigned to either an experimental (Exp) or control (uninjected) group with baseline data taken prior to the induction of inflammation. Inflammation was produced by placing an injection of Complete Freund's Adjuvant (300 µg *Mycobacterium tuberculosis* [Sigma] in 50 µl paraffin oil) within the superior joint space of one TMJ while the animal was deeply anesthetized with a cocktail of Ketamine and Rompun. All tests were conducted blind to the experimental conditions. The results indicated differences only in meal patterns and BW. During days 4-6, the number of meals taken by the Exp. group decreased significantly (P≤ .001). Weights and total food consumed mimicked this decrease, but were not significantly reduced with all three parameters returning to baseline by day 7. From the 2nd week through the 5th week, the total amount of food consumed and BW for the Exp. group were significantly decreased, with the peak loss occurring between weeks 3 and 4 (P≤ .001). Normal weights and food consumption were regained during week 6. Thus, these data suggest that in this model of chronic inflammation there was an early (4-6 d) and a late effect (2-5 wks) which may mirror an oscillating inflammatory-induced pain.

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1812 **Neuropeptide Levels in Trigeminal Ganglia and Brainstem During Chronic TMJ Inflammation.** **J. SIEBERT*, R. JOHNSON, R.P.HARPER, R.SPEARS, R.J.HINTON, and B.HUTCHINS (Baylor College of Dentistry, Texas A&M Univ. Sys., Dallas, TX).**

Previous studies in this laboratory have examined acute time periods of adjuvant-induced inflammation within the temporomandibular joint (TMJ). Therefore, the aim of this study was to produce an adjuvant-induced chronic inflammation and analyze Substance P (SP) and CGRP levels in the trigeminal ganglion and the trigeminal spinal nucleus. Eighteen adult male rats were deeply anesthetized with a cocktail of Ketamine and Rompun. An injection of CFA (300 µg *Mycobacterium tuberculosis* [Sigma] in 50 µl paraffin oil) was placed within the superior joint space of the left TMJ. Six weeks later, animals were sacrificed with an overdose of Nembutal (50 mg/kg) and perfused with 4% paraformaldehyde. Trigeminal ganglia were dissected from both sides and brainstems were dissected from approximately -5.0 AP to C1/C2. The brainstems were further dissected by making an oblique cut in the sagittal plane to primarily encompass the trigeminal subnucleus caudalis. SP and CGRP levels were measured using routine radioimmunoassay (RIA). So that comparisons could be made with data from earlier time periods, ratios were created by dividing the RIA values from the adjuvant-injected side by those from the contralateral uninjected side and significance was determined by comparing the ratios to "1". Trigeminal ganglion levels for CGRP and SP were not significantly different than the uninjected side, yet brainstem levels for CGRP (1.17 ± 0.22, P≤ .002) and SP (1.22 ± 0.4, P≤ .01) were significantly elevated. Data from earlier time periods have shown elevated neuro-peptides in the ganglia and little change in the brainstem. In this study, data indicate that trigeminal ganglion cells no longer respond to the original inflammation. However, in the brainstem there were chronic changes resulting in increased CGRP and SP levels.

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1813 Rat descending amygdala projections differentially terminate within taste-related brainstem. W. HUANG* and C.B. HALSELL (Dept. Of Oral Biology, The Ohio State University College of Dentistry, Columbus, OH, USA).

Appetite and feeding are regulated by the interaction of internal homeostatic signals and cognitive functions designed to control the drive to replace necessary nutrients and fluids. Higher brain regions associated with these processes project to the sensori-motor circuitry within the brainstem in order to modulate consummatory reflexes. However, little is known about this descending pathway and whether it influences the sensory and/or motor limbs of the brainstem circuitry. Therefore, the current study focuses upon the organization of the descending projection from the amygdala, a forebrain region associated with learning and motivation, and the rostral nucleus of the solitary tract (NST), the brainstem region containing the first central synapse of primary taste information. Five adult male rats were injected with a neuronal anterograde tracer (biotinylated-dextran, 3D; into the central amygdala, based on stereotaxic coordinates. Following the appropriate survival period, the rats were sacrificed and their brains removed, sectioned at 40 µm, and processed to visualize the transported tracer and anatomical topography of the NST. The bulk of the BD-labeled fibers were located within the morphologically-defined medial (M), rostral central (RC) and ventral (V) NST subdivisions, as well in the subjacent reticular formation. Numerous fiber swellings and terminal-like label was evident in each of these regions, suggestive of synaptic endings. Very few labeled fibers were located in the rostral lateral (RL) NST subdivision. Previous studies indicate that RC preferentially contains taste-responsive neurons and the bulk of ascending efferent neurons. Neurons within M and V project to caudal portions of the NST and to the subjacent reticular formation. Neurons within RL, on the other hand, respond preferentially to intraoral somatosensory stimulation. In conclusion, the current results indicate that descending input from the amygdala terminates upon NST subdivisions associated with primary taste afferent input, ascending projections to higher brain regions, and descending projections to oral motor regions via the reticular formation. Thus, the descending amygdalo-bulbar pathway contributes to both the sensory and motor limbs of the taste-related brainstem circuitry. This suggests a circuitry allowing modulation of feeding reflexes by higher brain regions associated with the control of appetitive behaviors. Supported by NIH CD-02016 to C.B.H. and OSU College of Dentistry.

1814 Gustatory Regions of Parabrachial Nucleus Exhibit Differential Projections to Medulla. H. KARIMNAMAZI* and J.B TRAVERS (Dept. Oral Biology, Ohio State University, Columbus, Ohio, USA).

The parabrachial nucleus (PBN) is the main brainstem relay in the transmission of gustatory and visceral information from the medulla to the forebrain. Because decerebrate animals appropriately ingest or reject palatable and unpalatable gustatory stimuli, descending projections from the PBN potentially contribute to these brainstem functions. Little is known, however about the distribution of gustatory PBN projections to the medulla. The objective of this study was to identify the location of terminal fields within the medullary reticular formation (RF) originating from physiologically identified taste and intraoral tactile responsive sites within the PBN. Under electrophysiological guidance, anterograde tracers [biotinylated dextran: BD (n=27) or PHA-L (n=9)] were injected into different PBN subdivisions. In a subset of these animals (n=6) a retrograde tracer (Fluorogold: FG) was also injected into hypoglossal motor nucleus (mXII) to compare the relative distribution of PBN fibers with the lingual premotor neurons in the RF. Following survival times of 7-14 days, animals were perfused and the brains were sectioned and processed to visualize the tracers. Injections centered on anterior tongue responsive sites (n=19) in the PBN 'waist-region', i.e. ventrolateral and centromedial subnuclei, had projections to the parvocellular, intermediate, and dorsal subdivisions of the RF. These projections were distributed mostly laterol to the retrogradely filled lingual premotor neurons in the lRt. Sparse projections were also observed in the nucleus of the solitary tract (NST) and the spinal trigeminal nucleus (pars interpolaris). In contrast, injections centered on posterior oral cavity taste and tactile responsive sites (n=5) in the PBN 'external-region', i.e. external medial and lateral subnuclei, produced few medullary projections. Significantly, both waist and external regions provided dense efferent thalamic and amygdalar projections. Additional experiments (n=2) that combined BD injections into the rostral (gustatory) NST with FG injections into the RF showed that retrogradely labeled RF projection neurons within the waist region were associated with anterograde terminals from the NST injections, further indicating that PBN taste neurons project directly to the medullary RF. In addition, these double tracer experiments confirmed the paucity of connections from EM to RF. These findings suggest that the ascending gustatory projection from the NST becomes segregated at the level of the PBN such that only specific PBN subdivisions sensitive to intraoral stimulation exert a direct influence on medullary substrates coordinating oromotor behavior. This study was supported in part by R01 DC00417 to JBT.

1815 Tenascin and Fibronectin in Oral Squamous Cell Carcinoma after Chemo/Radio-therapy. K KAWANO*, Y. KAKU, K. KAWANO, S. YANAGISAWA and M. SHIMIZU (Department of Oral and Maxillofacial Surgery, Oita Medical University, Japan/Department of Stomatology, University of California San Francisco, USA).

We investigated the change of distribution of tenascin (TN) and fibronectin (FN) induced by preoperative chemotherapy and/or radiotherapy in oral squamous cell carcinoma (SCC) immunohistochemically using 40 sets of biopsy and surgical tissue samples. In biopsy specimens of oral SCC, all samples were positive for TN in the tumor nest-stroma junction and/or tumor stroma. FN immunoreactivity was found to be distributed throughout the tumor stroma in 39 of 40 cases. After therapy, once tumor cells in the periphery of a tumor nest underwent necrotic change and the continuity of the tumor-stroma junction was disrupted, immunoreactivity of TN weakened or disappeared in the junction and in the stroma adjacent to such areas, although stromal FN did not show such obvious changes. FN reactivity, however, diminished concomitant with chronic inflammatory cell infiltration. In addition, there were strong reactivities of TN and FN in the connective tissue around re-proliferating tumor cells. In the repair processes, TN, which had disappeared concomitantly with damage of the tumor cells, never reappeared in the granulation and fibrous connective tissues. In contrast, enhanced FN reactivity was observed in well-organized granulation tissue. We conclude from these results that TN may be related to activity and progression of oral SCC, and FN appears to contribute significantly to the regeneration of connective tissue component after therapy.

1816 Integrin-mediated adhesion to extracellular matrix in tongue cancer cells. H. XUE, G. L. TIPOE and F. H. WHITE (Department of Anatomy, The University of Hong Kong, Hong Kong).

Oral squamous cell carcinomas (SCC) are characterised by high morbidity and mortality due to their invasive and metastatic capabilities. In order to clarify the role of cell-matrix interactions on the metastasis of oral SCC, we compared integrin-mediated adhesion of cultured human tongue SCC cell line (T_{Ca} 8113) and its highly metastatic subline (brain colonisation, TmB) to different components of extracellular matrix (ECM). A matrigel invasion assay showed that TmB cells had significantly greater invasive ability than T_{Ca} 8113 cells ($p<0.01$). Our data on adhesion assays indicated that the adhesion of TmB cells to vitronectin (VN), collagen IV (CoIV), fibronectin (FN) and laminin (LM) were significantly higher than those for T_{Ca} 8113 cells. The adhesion of TmB cells to the different types of extracellular matrix (VN, CoIV, FN and LM) were significantly increased by pretreatment of $\alpha_2\beta_1$ (collagen receptor; 5µg/ml), $\alpha_3\beta_1$ (laminin receptor; 5µg/ml), $\alpha_5\beta_1$ (fibronectin receptor; 5µg/ml), $\alpha_v\beta_5$ (vitronectin receptor; 5µg/ml), $\alpha_{IIb}\beta_3$ (platelet adhesion receptor; 5µg/ml) and $\alpha_6\beta_4$ (laminin receptor; 5µg/ml) antibodies when compared with the control group except for $\alpha_5\beta_1$ in FN and $\alpha_2\beta_1$ in LM. These data suggest that binding of $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_4$, $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_5$ integrins can increase the adhesive capability of highly metastatic TmB subline of human tongue squamous cell carcinoma.