G.E. Gerstner^{1,2*}, R.H. Gracely^{9,10},
A. Deebajah³, E. Ichesco^{4,5},
A. Quintero¹, D.J. Clauw^{5,6},
and P.C. Sundgren^{7,8}

¹Department of Biologic and Materials Sciences, School of Dentistry, 1011 N. University Ave., University of Michigan, Ann Arbor, MI 48109-1078, USA; ²Department of Psychology, University of Michigan, Ann Arbor, MI; ³Department of Restorative Dentistry, School of Dentistry, University of Detroit Mercy, Detroit, MI; ⁴Department of Biologic and Materials Sciences, School of Dentistry, 1011 N. University Ave., University of Michigan, Ann Arbor, MI; ⁵Chronic Pain and Fatigue Research Center, University of Michigan, Ann Arbor, MI; ⁶Department of Anesthesiology, University of Michigan, Ann Arbor, MI; ⁷Department of Diagnostic Radiology, Clinical Sciences/Lund, University of Lund, Lund, Sweden; ⁸Department of Radiology, University of Michigan, Ann Arbor, MI; ⁹Center for Neurosensory Disorders, University of North Carolina, Chapel Hill, NC; and ¹⁰Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI; *corresponding author, geger@umich.edu

J Dent Res 91(5):485-490, 2012

ABSTRACT

Temporomandibular disorders (TMD) include craniocervical pain conditions with unclear etiologies. Central changes are suspected; however, few neuroimaging studies of TMD exist. Single-voxel proton magnetic resonance spectroscopy (¹H-MRS) was used before and after pressure-pain testing to assess glutamate (Glu), glutamine (Gln), N-acetylaspartate (NAA), and choline (Cho) levels in the right and left posterior insulae of 11 individuals with myofascial TMD and 11 matched control individuals. Glu levels were significantly lower in all individuals after pain testing. Among those with TMD, left-insular Gln levels were related to reported pain, left posterior insular NAA and Cho levels were significantly higher at baseline than in control individuals, and NAA levels were significantly correlated with pain-symptom duration, suggesting adaptive changes. The results suggest that significant central cellular and molecular changes can occur in individuals with TMD.

KEY WORDS: magnetic resonance spectroscopy, temporomandibular disorders, N-acetylaspartate, choline, glutamate, glutamine.

DOI: 10.1177/0022034512443366

Received December 7, 2011; Last revision February 6, 2012; Accepted March 3, 2012

© International & American Associations for Dental Research

Posterior Insular Molecular Changes in Myofascial Pain

INTRODUCTION

Temporomandibular disorders (TMD) are musculoskeletal conditions often characterized by pain in the temporomandibular joints and/or masticatory muscles. The prevalence of the myofascial subtype of TMD, as defined by the Research Diagnostic Criteria (RDC), is ~10.5% (Janal *et al.*, 2008). Prevalence is higher among younger women; white and non-white ethnicities suffer similar prevalence rates (Janal *et al.*, 2008).

TMD etiologies and pathophysiologies are poorly understood. Current models have been described as inadequate and lacking an appreciation of the neural basis of chronic pain (Greene, 2001). Only recently have investigators begun using neuroimaging to study the central processing of trigeminal myogenous pain (*e.g.*, Nash *et al.*, 2010a,b).

The insula is involved with central pain processing (reviewed in Peyron *et al.*, 2000). The posterior insula is one of the few cortical sites where electrical stimulation can elicit disabling muscle-pain or headache sensations (Ostrowsky *et al.*, 2002; Mazzola *et al.*, 2009). A somatotopic representation exists in the posterior insula, with the face being represented rostrosuperiorly to the body's representation (Ostrowsky *et al.*, 2002; Brooks *et al.*, 2005). Facial pain may be represented in the contralateral (Coghill *et al.*, 2001), bilateral (Jantsch *et al.*, 2005), or combinations of contralateral, ipsilateral, and bilateral insulae (Ostrowsky *et al.*, 2002; Mazzola *et al.*, 2009). Patients with posterior insular infarcts manifest faciobrachiorucral impairments of elementary sensations, including pain (Cereda *et al.*, 2002).

Recent work has demonstrated insular neurochemical changes in fibromyalgia (FM) (Gracely *et al.*, 2002; Harris *et al.*, 2008, 2009), which is why we focused on this brain region. We used proton magnetic resonance spectroscopy (¹H-MRS) to investigate cellular and molecular activity within the posterior insulae of individuals with TMD and healthy control (HC) individuals. ¹H-MRS, a magnetic resonance imaging (MRI) procedure, measures metabolite

Figure 1. (A) Scanning session protocol. Pre = Pre-pain test ^1H -MRS runs; Post = post-pain test ^1H -MRS runs of left (LPI) followed by right (RPI) posterior insula. Each run lasted ~ 8 min. The pain-testing study (fMRI) lasted ~ 20 min. (B) Voxel placement in RPI. (C) Spectrum and fitted LC-model for one participant.

levels within pre-defined voxels (Ross and Bluml, 2001; Novotny *et al.*, 2003).

We evaluated glutamate (Glu), glutamine (Gln), their combination (Glx = Glu + Gln), N-acetylaspartate (NAA), and choline (Cho) levels. Glu is a major cortical neurotransmitter implicated in negative affect related to pain (Mullins *et al.*, 2005). Gln is a metabolite of Glu; together, Glu and Gln participate in complex metabolic activity cycles and intercellular communication involving neurons and astrocytes (Yang *et al.*, 2008). NAA is a biomarker of neuronal health and of neuronal and axonal numbers (Ross and Bluml, 2001; Gujar *et al.*, 2005). Cho is associated with increased cell numbers, membrane synthesis, and/or membrane breakdown, *e.g.*, demyelination, malignancies (Gujar *et al.*, 2005). These biomarkers provide important information about the health and condition of brain activity.

MATERIALS & METHODS

Study Population

Twenty-two right-handed individuals were screened and recruited, including 11 with TMD and 11 healthy individuals (HC) carefully pair-wise-matched for gender (one male, 10 females *per* group), age (TMD mean $\pm$ SD = 25.8 ± 2.33 vs. HC 24.8 ± 1.20), and ethnicity (seven White, three Asian-American, one African-American *per* group). Because symptoms are not ethnicity-specific (Janal *et al.*, 2008), we recruited according to local ethnic demographics of Washtenaw County, Michigan. All participants gave written informed consent; protocols were approved by the University of Michigan Medical Institutional Review Board.

TMD-group inclusion criteria were based on RDC-Group 1 criteria (Dworkin and LeResche, 1992), *i.e.*, presence of ongoing pain involving ≥ 3 tender muscle sites ipsilateral to palpation pain. HC-group inclusion criteria included < 3 tender muscle sites and

no history of chronic pain. Selected TMD individuals had bilateral symptoms in the masseter (N = 7), temporalis (N = 1), masseters and temporalis (N = 2), or right masseter only (N = 1). None had joint involvement (RDC, Groups 2, 3). Exclusion criteria for both groups were pre-existing medical or outstanding dental conditions, history of arthritis, heart conditions, diabetes, migraine, tension headache, back pain, tooth pain, FM, schizophrenia, use of pain medications within the previous 2 wks, medications with central or peripheral neural actions, bite splints, or current TMD treatments.

Because TMD symptoms vary with menstrual phase (LeResche *et al.*, 2003), female participants were scheduled for appointments between days 1 and 3 of menses.

Study Procedure

Participants underwent screening, a physical examination, and an orofacial examination assessing range of motion, occlusion, TMJ noises, orofacial motor strength, facial sensory status, and RDC examination procedures (Dworkin and LeResche, 1992). Psychometric tests were used to screen for psychiatric illness. The McGill pain questionnaire short form (MPQ-SF) and the State-Trait Personality Inventory (STPI) were identified *a priori* for use in analyses.

On a 2nd day, pressure-pain testing was performed on the right thumbnail bed (thumb test) and then on the right anterior temporalis (head test). Pressures, applied with a 1-cm rubber syringe and customized pneumatic system, were ramped to a pre-selected pressure in 1 sec, held for 5 sec, and then released. Pressure applications recurred every 25 sec.

An ascending-staircase followed by a multiple random-staircase technique identified pressures that elicited subjective responses of mild (3/20), moderate (11/20), and severe (15/20) pain ratings on a Box Scale (Gracely *et al.*, 1988). Pressures eliciting mild, moderate, and severe pain responses were used in day-3 tests.

On day 3, T1 anatomical images were used to locate the voxels of interest (VOI) for the ^1H -MRS procedures. Single-voxel ^1H -MRS (3T, GE Signa MRI scanner, Milwaukee, WI, USA) was performed on posterior insular cortices before pain-testing (pre-pain test or “Pre”, Fig. 1A). Next, pressure-pain testing was performed in conjunction with another study; 25-second painful pressures were applied followed by 25-second rest periods. Twelve pressure-rest blocks were given on the left thumb and then on the left anterior temporalis. Immediately thereafter, single-voxel ^1H -MRS was again performed in posterior insular cortices (post-pain test or “Post”, Fig. 1A).

Imaging Protocol

The protocol (Fig. 1A) included standard T1 structural images, and 4 single-voxel ^1H -MRS sequences with point-resolved

Table. Mean ($\pm$ SD) Metabolite Levels

Metabolite	Trial	HC		TMD	
		Left	Right	Left	Right
Glu	Pre	7.99 (0.54)	7.91 (0.66)	8.05 (0.54)	7.76 (0.94)
	Post	7.83 (0.88)	7.70 (0.69)	7.64 (0.57)	7.23 (0.88)
Gln	Pre	3.14 (1.38)	3.16 (1.55)	3.62 (0.86)	4.48 (1.00)
	Post	3.40 (1.11)	3.79 (1.13)	3.40 (0.89)	4.64 (1.31)
Glx	Pre	11.13 (1.55)	11.07 (1.46)	11.67 (0.87)	12.24 (0.91)
	Post	11.23 (1.38)	11.49 (1.28)	11.05 (1.13)	11.87 (1.51)
NAA	Pre	9.81 (0.36)	9.72 (0.40)	10.30 (0.60)	9.88 (0.64)
Cho	Pre	1.35 (0.43)	1.40 (0.37)	1.65 (0.13)	1.55 (0.11)

spectroscopy (PRESS; TR = 3000 ms, TE = 30 ms, 90-degree flip angle, NEX 8, FOV 16 cm, VOI = $2 \times 2 \times 3$ cm) placed in the left and then right posterior insula (Fig. 1B) pre- and post-pain testing. ^1H -MRS data were analyzed by a neuroradiologist blinded to participant group membership and using a linear combination of individual spectra obtained from pure molecular species to fit the experimental spectra (LCModel; Oakville, ON, Canada; Fig. 1C). Metabolite levels were calculated as absolute concentrations, normalized to the water signal and reported in arbitrary institutional units.

Statistical Analysis

A mixed-effect repeated-measures design analysis of variance (ANOVA) (SAS, ver. 9.2) was used to analyze group differences in Glu, Gln, and Glx metabolite concentrations. Side (left vs. right), time (pre- vs. post-pain testing), group (HC vs. TMD), and participant pairs (11 pairs) were treated as fixed effects; each participant was treated as a random effect. The model also evaluated group $\times$ time and group $\times$ side interactions. Because NAA and Cho probably represent long-term effects, only pre-pain-testing concentrations of these metabolites were tested (paired t test using matched TMD-HC participants for pairing). Each TMD participant was matched to a specific HC participant for age, gender, and ethnicity, to control for demographic heterogeneities, thus allowing for a broader demographic to be used within a small study design.

Correlation analyses were performed to assess the degree of association between TMD-group metabolite levels and both MPQ-SF and STPI psychometric scores. Pearson's product moment correlation coefficient, r , was used for continuous, normally distributed variables. All other correlations used Spearman's rank correlation method, ρ . A $p < 0.05$ defined statistical significance.

RESULTS

TMD mean pain severity scores = 3.8/10 (SD = 2.2) on screening day. Symptom histories ranged from 6 mos to 7 yrs. HC pain severity scores = 0/10 (SD = 0), with no history of TMD symptoms.

The Table shows individual-group mean (1 SD) pre- and post-pain test concentrations in left and right insulae for Glu, Gln, and

Glx. Only pre-test levels appear for NAA and Cho (see Materials & Methods). Tabled values do not reflect within-individual or paired-individual differences, which are detailed below.

Controlling for participant group, Glu levels significantly decreased pre- to post-pain testing within-participants ($F[1,61] = 6.04$, $p = 0.0168$, Fig. 2). Glu levels were higher in the left vs. right insulae within participants ($F[1,61] = 4.14$, $p = 0.0462$). No significant Glu-level differences existed between groups.

A significant interaction between side and group occurred for Gln ($F[1,61] = 4.64$, $p = 0.0353$, Fig. 2). TMD-group Gln levels were significantly higher in the right vs. left insula ($t = -3.83$, $p = 0.0003$). HC-group Gln levels did not differ by side ($t = -0.76$, $p = 0.45$). Right insular Gln levels were higher in TMD vs. HC ($t = -3.16$, $df = 61$, $p = 0.0025$). Between-group differences in left insular Gln levels were not significant ($t = -0.62$, $p = 0.54$). No significant between-group differences in Gln existed pre- vs. post-pain testing, nor were any differences observed involving Glx.

Left insular Gln levels of TMD participants in the pre-test condition were negatively correlated with visual analog scale (VAS) scores of present pain level ($r = -0.78$; $df = 9$, $p = 0.005$), MPQ sensory scale ($\rho = -0.81$, $df = 9$, $p = 0.001$), affective scale ($\rho = -0.67$, $df = 9$, $p = 0.02$), and total pain scores ($\rho = -0.88$, $df = 9$, $p < 0.001$). Left insular Glx levels, pre-test, were significantly correlated with VAS pain ($r = -0.79$; $p = 0.004$), MPQ sensory ($\rho = -0.714$; $p = 0.014$), and MPQ total pain ($\rho = -0.662$; $p = 0.026$) scores, but not with MPQ affective pain ($\rho = -0.469$; $p = 0.146$).

Left insular NAA ($t = 2.33$, $df = 21$, $p = 0.030$; Fig. 3, top) and Cho levels ($t = 2.25$, $df = 21$, $p = 0.036$; Fig. 3, middle) were significantly higher in TMD vs. HC participants. Left insular NAA levels were positively correlated with pain symptom duration in participants with TMD ($r = 0.761$, $df = 9$, $p = 0.0066$; Fig. 3). No significant between-group differences or significant correlations with psychometric scores were observed involving right insular NAA or Cho.

DISCUSSION

This study provides the first evidence of central cellular and molecular alterations in myofascial-type TMD. Recent work has demonstrated cellular and molecular alterations in the posterior

Figure 2. (top) Mean ($\pm$ SD) levels for Glu (pooled across participants and brain side). (bottom) Interaction between side and group for Glu; solid line = TMD, dotted line = HC.

insula of persons with FM (Harris *et al.*, 2009). However, distinctions exist between our results and those from the FM study.

For example, right insular Gln levels were elevated in TMD vs. HC individuals; no such differences were reported in FM vs. HC individuals (Harris *et al.*, 2009). Conversely, we found no differences in Glu levels between TMD and HC participants, whereas insular Glu levels were elevated in FM vs. HC individuals (Harris *et al.*, 2009). Nevertheless, Gln levels tended to be higher in FM vs. HC individuals, albeit not significantly ($p = 0.13$) (Harris *et al.*, 2009).

Insular Glx levels were significantly higher in FM vs. HC individuals (Harris *et al.*, 2009). Although Glx levels were not significantly different between our TMD and HC participants, a tendency existed for Glx to be higher in those with TMD during the pre-test condition (Table). Finally, we reported significant elevations involving NAA and Cho in participants with TMD. No such elevations occurred in persons with FM (Harris *et al.*, 2009); however, insular NAA levels tended to be higher in those with FM ($p = 0.06$) (Harris *et al.*, 2009). Conversely, Cho levels tended to be lower in those with FM ($p = 0.15$) (Harris *et al.*, 2009).

Several potential reasons for the differences between FM and TMD exist. First, our head-based pressure-pain testing was not

Figure 3. Mean left posterior insular levels of NAA (top) and choline (middle), pre-test condition. Correlation between NAA in the LPI and symptom duration in TMD (bottom).

used on participants with FM. Also, TMD symptoms were restricted to the head and neck, whereas FM symptoms occur in numerous limb and trunk sites. Such symptomatic differences would likely reflect central differences.

Also, although the LCModel is considered capable of resolving Glu and Gln at 3T, limitations and caveats remain (for review, see Ross and Bluml, 2001; Panigrahy *et al.*, 2010). Glu and Gln are involved in complex transport, storage, and metabolic cycles among neurons, astrocytes, and glial cells (Shen *et al.*, 1999; Gujar *et al.*, 2005; Yang *et al.*, 2008; Panigrahy *et al.*, 2010). ^1H -MRS cannot distinguish among metabolite concentrations related to different molecular pathways or cell types. Nevertheless, both individuals with FM and those with

TMD apparently manifest individual-group specific changes in posterior insular Glx cycle constituents.

Finally, our TMD participants' ages averaged 25.8 ± 2.33 yrs, whereas the FM study's participants averaged 45.2 ± 15.0 . NAA levels are significantly lower and Cho levels higher in over-40 populations compared with young adults, possibly reflecting reduced neuronal viability or function, accelerated membrane degradation, or increased glial cell numbers in older individuals (Angelie *et al.*, 2001).

Our head and thumb pressure-pain testing procedures may have depleted Glu levels, leading to the post-testing reduction in Glu manifest by both groups (Fig. 2). Future studies should determine whether pain-application duration affects Glu metabolic pathways, which could suggest potential pain 'chronification' mechanisms.

Because NAA is high in neurons and axons (Panigrahy *et al.*, 2010), increased left insular NAA levels in our TMD participants may reflect neuronal or axonal proliferation in response to pain symptoms. Four lines of evidence support this possibility. First, trigeminal sensory processing, including experimental dental pain, occurs in a region contiguous with the posterior insula (Becerra *et al.*, 2006; Ettlin *et al.*, 2009), suggesting that persistent pain could affect this region. Second, individuals with TMD manifest increased white matter volume in a region near the left posterior insula (Gerstner *et al.*, 2009). Third, the significant positive correlation between left insular NAA levels and symptom duration (Fig. 3) suggests a time-dependent increase in neuronal processes. Finally, Cho levels were elevated in this region (Fig. 3), and Cho is a marker of active membrane metabolism in conditions involving increased cell and membrane synthesis and membrane degradation, *e.g.*, demyelination, malignancies (Gujar *et al.*, 2005). Together, these results suggest that our TMD participants' symptoms may have led to neuronal or axonal proliferative changes in the left posterior insula.

Pre-pain, left-insular Gln in TMD participants was negatively correlated with VAS ($r = -0.777$; $p = 0.005$), MPQ sensory ($\rho = -0.811$; $p = 0.002$), affective ($\rho = -0.673$; $p = 0.023$), and total ($\rho = -0.876$; $p < 0.001$) pain scores. Conversely, Glu and Glx levels correlated negatively with pressure pain thresholds in participants with FM and HC participants (Harris *et al.*, 2009). Hence, Gln appears to have been involved with the experience of *clinical* pain in our study, and with the experience of *evoked* pain in the FM study.

The reason for the left-right insular differences reported in our study are unclear. Studies indicate that pain testing affects mainly the contralateral posterior insula (Brooks *et al.*, 2005), suggesting that our asymmetric pressure-pain tests performed on day 2 or during scanning may have played a role. Acute pain studies demonstrate that central effects from painful stimuli may outlast pain reports (Nash *et al.*, 2011b); hence, our pain testing may have caused residual asymmetric central changes that affected our ¹H-MRS results without affecting pain reports. Alternatively, given that left and right insular cortices manifest some asymmetric functions (Cereda *et al.*, 2002), underappreciated neurochemical asymmetries may exist with respect to pain processing.

Our sample size was limited (however, *cf.* Newberg *et al.*, 2011), although great care was taken to match participant pairs between groups. Larger studies must confirm these results,

determine whether our pain tests affected the results, and determine whether the results are clinically significant. Metabolite levels in other pain-processing regions will need to be studied as well.

Finally, our TMD participants had relatively "normal" pain thresholds and relatively minor clinical pain ($\sim 3/10$ average), suggesting that they represented a pain-tolerant TMD phenotype (Pfau *et al.*, 2009). Future investigations will need to study the relationship between pain phenotype and insular metabolite levels.

ACKNOWLEDGMENTS

A preliminary report of this research was presented at the American Association for Dental Research meeting in Washington, DC, March, 2010. We thank Heidi Reichert and the staff at the Center for Statistical Consultation and Research for help with statistical design and analysis, Keith Newnham for MRI technical assistance, and Scott Peltier, Tobias Schmidt-Wilcke, and Richard Harris for study design and analyses discussions. This project was supported by a USPHS Research Grant from the NIDCR (DE-018528) to GEG. The author(s) declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

- Angelie E, Bonmartin A, Boudraa A, Gonnaud PM, Mallet JJ, Sappey-Marini D (2001). Regional differences and metabolic changes in normal aging of the human brain: proton MR spectroscopic imaging study. *AJNR Am J Neuroradiol* 22:119-127.
- Becerra L, Morris S, Bazes S, Gostic R, Sherman S, Gostic J, *et al.* (2006). Trigeminal neuropathic pain alters responses in CNS circuits to mechanical (brush) and thermal (cold and heat) stimuli. *J Neurosci* 26:10646-10657.
- Brooks JC, Zambreau L, Godinez A, Craig AD, Tracey I (2005). Somatotopic organisation of the human insula to painful heat studied with high resolution functional imaging. *Neuroimage* 27:201-209.
- Cereda C, Ghika J, Maeder P, Bogousslavsky J (2002). Strokes restricted to the insular cortex. *Neurology* 59:1950-1955.
- Coghil RC, Gilron I, Iadarola MJ (2001). Hemispheric lateralization of somatosensory processing. *J Neurophysiol* 85:2602-2612.
- Dworkin SF, LeResche L (1992). Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 6:301-355.
- Ettlin D, Brugger M, Keller T, Luechinger R, Jancke L, Palla S, *et al.* (2009). Interindividual differences in the perception of dental stimulation and related brain activity. *Eur J Oral Sci* 117:27-33.
- Gerstner GE, Gracely RH, Harris RE, Clauw DJ (2009). Evidence of central pain augmentation in temporomandibular disorders [abstract]. *J Dent Res* 88(Spec Iss A): #121033. URL accessed on 2/6/2012 at: <http://iadr.confex.com/iadr/2009miami/webprogram/Paper121033.html>.
- Gracely RH, Lota L, Walter D, Dubner R (1988). A multiple random staircase method of psychophysical pain assessment. *Pain* 32:55-63.
- Gracely RH, Petzke F, Wolf JM, Clauw DJ (2002). Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis Rheum* 46:1333-1343.
- Greene CS (2001). The etiology of temporomandibular disorders: implications for treatment. *J Orofac Pain* 15:93-105.
- Gujar SK, Maheshwari S, Bjorkman-Burtscher I, Sundgren PC (2005). Magnetic resonance spectroscopy. *J Neuro-Ophthalmol* 25:217-226.
- Harris R, Sundgren P, Pang Y (2008). Dynamic levels of glutamate within the insula are associated with improvements in multiple pain domains in fibromyalgia. *Arthritis Rheum* 58:903-907.

- Harris R, Sundgren P, Craig A, Kirshenbaum E, Sen A, Napadow V, *et al.* (2009). Elevated insular glutamate in fibromyalgia is associated with experimental pain. *Arthritis Rheum* 60:3146-3152.
- Janal MN, Raphael KG, Nayak S, Klausner J (2008). Prevalence of myofascial temporomandibular disorder in US community women. *J Oral Rehabil* 35:801-809.
- Jantsch HH, Kempainen P, Ringler R, Handwerker HO, Forster C (2005). Cortical representation of experimental tooth pain in humans. *Pain* 118:390-399.
- LeResche L, Mancl L, Sherman JJ, Gandara B, Dworkin SF (2003). Changes in temporomandibular pain and other symptoms across the menstrual cycle. *Pain* 106:253-261.
- Mazzola L, Isnard J, Peyron R, Guenot M, Mauguiere F (2009). Somatotopic organization of pain responses to direct electrical stimulation of the human insular cortex. *Pain* 146:99-104.
- Mullins PG, Rowland LM, Jung RE, Sibbitt WL Jr (2005). A novel technique to study the brain's response to pain: proton magnetic resonance spectroscopy. *NeuroImage* 26:642-646.
- Nash PG, Macefield VG, Klineberg IJ, Gustin SM, Murray GM, Henderson LA (2010a). Bilateral activation of the trigeminothalamic tract by acute orofacial cutaneous and muscle pain in humans. *Pain* 151:384-393.
- Nash PG, Macefield VG, Klineberg IJ, Gustin SM, Murray GM, Henderson LA (2010b). Changes in human primary motor cortex activity during acute cutaneous and muscle orofacial pain. *J Orofac Pain* 24:379-390.
- Newberg AB, Hersch EV, Levin LM, Giannakopoulos H, Secreto SA, Wintering NA, *et al.* (2011). Double-blind, placebo-controlled, randomized pilot study of cerebral blood flow patterns employing SPECT imaging in dental postsurgical pain patients with and without pain relief. *Clin Ther* 33:1894-1903.
- Novotny EJ Jr, Fulbright RK, Pearl PL, Gibson KM, Rothman DL (2003). Magnetic resonance spectroscopy of neurotransmitters in human brain. *Ann Neurol* 54(Suppl 6):25-31.
- Ostrowsky K, Magnin M, Ryvlin P, Isnard J, Guenot M, Mauguiere F (2002). Representation of pain and somatic sensation in the human insula: a study of responses to direct electrical cortical stimulation. *Cereb Cortex* 12:376-385.
- Panigrahy A, Nelson MD Jr, Bluml S (2010). Magnetic resonance spectroscopy in pediatric neuroradiology: clinical and research applications. *Pediatr Radiol* 40:3-30.
- Peyron R, Laurent B, Garcia-Larrea L (2000). Functional imaging of brain responses to pain. A review and meta-analysis. *Neurophysiol Clin* 30:263-288.
- Pfau DB, Rolke R, Nickel R, Treede R-D, Daublaender M (2009). Somatosensory profiles in subgroups of patients with myogenic temporomandibular disorders and fibromyalgia syndrome. *Pain* 147:72-83.
- Ross B, Bluml S (2001). Magnetic resonance spectroscopy of the human brain. *Anat Rec* 265:54-84.
- Shen J, Petersen KF, Behar KL, Brown P, Nixon TW, Mason GF, *et al.* (1999). Determination of the rate of the glutamate/glutamine cycle in the human brain by *in vivo* ¹³C NMR. *Proc Natl Acad Sci USA* 96:8235-8240.
- Yang CZ, Zhao R, Dong Y, Chen XQ, Yu AC (2008). Astrocyte and neuron interplay through glutamate. *Neurochem Res* 33:2480-2486.