FUNCTIONAL IMAGING OF INTRAORAL SOMATOSENSORY STIMULI:
DEVELOPMENT AND TESTING OF A NOVEL DEVICE

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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In making this project I humbly stood on the shoulder of giants, getting to achieve this professional milestone with the help and support of several people. To name all of them and express my gratitude accordingly would need a thesis on its own; nonetheless I feel like a few words of appreciation are necessary:

First to my adviser Donald Nixdorf, who were not only a teacher but also a friend who provided support and guidance so that I could thrive in my academic career;

To my fellow residents in the TMD and orofacial pain division, for the laughs and joy shared during this arduous period. I would like to namely thank Dr. Rodrigo Lima and Dr. Vladimir Leon Salazar for their help in shooting several photographs used here;

To our faculty and staff for the patience with the clueless residents that arrive every year;

To Young Cheul Heo, for his invaluable help for the laboratory measurements;

and

To those that in one way or another helped me during this whole process, making me a better professional as well as a better person.
DEDICATION

I dedicate this thesis to:

My loving wife Karla, who stood by my side during my peaks and valleys giving me strength and courage to continue on. My tenacity arises from the foundation you provide, and without you none of this would have happened;

My mother, who could not see my achievements in the last 4 years in person but I know she was watching it all from Heaven;

To my father and sisters, for their support and belief bestow upon me;

To all my friends, for all those shared precious moments we all live for;

I am what you all made me be.

“Always remember that the future comes one day at a time” – Dean Acheson
ABSTRACT

Introduction: Few methods exist to study central nervous system processes following intraoral somatosensory stimulation using functional magnetic resonance imaging (fMRI), due to inherent technical difficulties associated with this imaging tool. Our goal was to develop and perform feasibility testing of a novel device capable of delivering valid and reliable intraoral somatosensory stimuli at dental chair-side and during MRI.

Methods: Details of a device designed to deliver intraoral dynamic pressure stimuli are described. Device testing took place in three settings: a) laboratory testing to assess range of stimulus force intensities, b) dental chair-side to assess reliability, validity and discriminant ability in force-pain relationship; and c) MRI to evaluate magnetic compatibility and ability to evoke brain activation in painfree subjects similar to those described in the literature.

Results: A novel device capable of delivering valid and reliable intraoral somatosensory stimulation was developed (ICC=0.89, 0.78-1 [95% CI]). Psychophysical data analysis showed high discriminant ability in differentiating painfree controls from AO cases (sensitivity=100%, specificity=86.7%, area under ROC curve=0.99). fMRI results of intraoral dynamic pressure pain in painfree subjects revealed activation of brain areas typically associated with
acute pain processing including thalamus, primary and secondary somatosensory, insular, and prefrontal cortices.

**Conclusions:** A novel psychophysical method to deliver dynamic intraoral pressure stimulation was developed and validated, allowing non-invasive exploration of cortical and subcortical mechanisms of intraoral somatosensation.
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INTRODUCTION

The organization of the trigeminal system is unique as it provides somatosensory innervation to the face, masticatory and oral structures, the majority of the intracranial contents (Go et al., 2001) and to specialized structures (tongue, nasal mucosa, auricle, tympanic membrane, cornea and part of the conjunctiva) (Moore et al., 2006, pp 1139-42). Somatic sensory information transmitted by the trigeminal nerve is crucial for normal orofacial function; however, the mechanisms of many chronic pain conditions affecting areas innervated by this sensory system are not well understood (Woda et al., 1999; Woda et al., 2000; Lavigne et al., 2005). Atypical odontalgia (AO), defined as tooth pain or pain in a site formally occupied by a tooth with no clinical or radiological signs of pathology (Melis et al., 2003; Baad-Hansen, 2008), is a chronic dento-alveolar pain condition of particular interest to dentists that is difficult to diagnose and manage. Recent research provided evidence for both peripheral and central nervous system mechanisms being involved in AO pathophysiology (List et al., 2006; Baad-Hansen et al., 2007; List et al., 2008).

Up until now, the majority of mechanism-based research of patients with AO has focused on the “peripheral aspect”, and no investigation using structural or functional neuroimaging has been done in this population thus far (Baad-Hansen, 2008). Recently, functional magnetic resonance imaging (fMRI) has become a more established research technique to study the central aspects of
pain (Kupers et al., 2006). fMRI provides great spatial resolution of cortical and subcortical structures critical in the processing of nociception, acceptable temporal resolution, does not involve ionizing radiation, and can be performed using most MRI systems that already exist in research centers and the community. For these reasons, we sought to explore the use of this emerging tool to investigate the central mechanisms involved in the processes of intraoral pain arising from the dento-alveolar region, with the long-term objective of improving understanding of the underlying mechanisms that is thought to lead to more tailored treatments and hence better patient care.

In the past few years several studies used fMRI to investigate the human trigeminal system (Borsook et al., 2004; Borsook et al., 2006), with a limited subset focusing on intraoral stimulation. In the latter group there are reports of lip, tongue and teeth stimulation (Miyamoto et al., 2006) or only the latter (Ettlin et al., 2004; Jantsch et al., 2005; Ettlin et al., 2009). Some reasons for scarce literature on this topic may be the technical challenges involved in delivering facial/intraoral stimulation inside a MR scanner (Dresel et al., 2008; Ettlin et al., 2009): possibility of magnetic interference, detriment of image quality, subject discomfort and reduced working space between the subject's head and the radiofrequency coil. As a consequence a MR-compatible device would need to not only overcome these challenges but also be capable of delivering a controlled and reproducible stimuli (Graham et al., 2001), as
reliability/reproducibility is a necessary feature of sensory testing (Pigg et al., 2010).

Existing MR-compatible methods of intraoral stimulation are limited and do not adequately deliver intraoral stimuli across a range of non-painful to painful intensities and cannot be adjusted to reach posterior aspects of the dentoalveolar region, all which are important attributes of a device needed for use in exploring central mechanisms of AO. Therefore our goal was to develop and test the feasibility of a device able to: 1) be reliable in providing intraoral stimuli while being able to demonstrate validity, 2) deliver such intraoral stimulation within the restricted space of an MR head coil, 3) be compatible for use within an MR environment, and 4) produce brain activation maps using painfree controls that are consistent to those observed by other researchers using fMRI.
MATERIALS AND METHODS

Intraoral stimulus device

The intraoral stimulus device was developed to deliver dynamic pressure over the subject’s gingiva and/or oral mucosa. The device would be within the radiofrequency (RF) head coil during MRI data acquisition; therefore its components should meet the most stringent MR-compatibility classification, so no detectable magnetic forces or torque is imposed on the device and there is negligible or no image distortion (Schenck, 1996). The device constituent and ancillary parts are: a) supporting frame; b) bite fork; c) montage tools; d) intraoral probe and cam; e) knob and pivoting joint; and f) lips and cheeks retractor.

Supporting frame

A plastic supporting frame shaped to fit the geometry of the circular polarized RF coil used for MR imaging was developed to hold in place other device’s parts (Figure 1A). Montage of the components over the frame was done in part very close to the MR scanner bore, hence the need for custom-made brass tools (Figure 1C). The supporting frame gives flexibility for future device development, and can be modified to hold components for different modalities of psychophysical stimulation.
Bite fork

Proper positioning of subject’s dental arch for intraoral probe placement and prevention of head movement during MRI data acquisition was a two-fold goal obtained with bite fork use (Figure 1B). Bite impression for each subject can be done with materials such as polyvinyl siloxane, which provides a stable bite registration for several weeks.

Intraoral probe, cam device, knob and pivoting joint

The intraoral component in contact with the oral tissues is a plastic probe, which design evolved from a straight-axis bullet-shaped to a curved-axis, reduced head area one (Figure 2). The curved-axis helped avoid touching the subject’s labial commissure during stimulation while the reduced head size propitiated a contact area of approximately $2 \text{ mm}^2$.

A cam device connects the supporting frame and the intraoral probe (Figure 1D and 3). It accomplishes two goals: pressure regulation by holding in place $1/8''$ elastic band(s) between the probe and cam posts (Figure 3), and conversion of the rotational movement of the knob and pivoting joint (Figure 1E) into a reciprocal movement of the intraoral probe. A set of three $3/8''$ elastic bands was used to maintain the cam arms in rest position after stimulation (Figure 3).
The intraoral probe stimulation over the subject’s gingiva/oral mucosa was defined as dynamic pressure, since its tip presses the oral tissues while moving back and forth about 5-7 mm. Lastly, stimulus location was determined by moving the cam device along the rails present in the supporting frame – this way all dental quadrants could be reached if needed.

**Lips and cheeks retractor**

Although different commercially available retractors can adequately pull away lips and cheeks, none proved suitable to our needs due to the space constraints between the subject’s head and the RF head coil. A full-plastic retractor was developed that permitted satisfactory oral tissue exposure while avoiding interference with other stimulus device components (Figure 4).

**Laboratory force measurements**

The relationship between the force delivered by the intraoral probe in grams and the number of elastic bands was measured with a strain gauge attached to the probe (Figure 5). A voltmeter, specialized software for resistance readings (LJstreams, LabJack Corporation, Lakewood, CO) and a set of standardized weights tied to the intraoral probe tip were used to estimate the force delivered by the intraoral probe according to the number of 1/8” elastic bands used (Figure 6A,B). After assembling the elastic band(s), the voltmeter was reset and weights increments were added until there was a noticeable movement of the probe and
the resistance readings reached a plateau (Figure 6C,D), which meant that weight addition would provoke probe translation but minimal voltmeter reading changes. The weight that first elicited probe translation was interpreted as the minimal force the intraoral probe tip exerted when using a particular number of 1/8" elastic band(s). Two trials for each amount of elastic band, from 1 to 8, were performed.

Subjects

Atypical odontalgia patients were recruited from those seeking care from Dr. Donald Nixdorf at the University of Minnesota Temporomandibular Disorders, Orofacial Pain and Oral Medicine clinic. Inclusion criteria for these AO patients were:

• Presence of intraoral pain with the following characteristics:
  o Localized in a endodontically treated tooth or in a place formerly occupied by a tooth (gingiva, oral mucosa, alveolar bone);
  o Present for more than 6 months;
  o Non-paroxysmal in character, and present for eight hours or more within a 24-hour period;
  o Can be provoked/increased by applying pressure to the intraoral site;
• No signs of gross pathology present during clinical examination or in available radiographic imaging.
These criteria are in accordance with recent studies involving AO subjects (Baad-Hansen et al., 2007; List et al., 2007; List et al., 2008). Control subjects were recruited from the University of Minnesota staff and acquaintances of the investigators. Inclusion criterion for age- and gender-matched controls was absence of intraoral pain in the previous six months. Exclusion criteria for both groups were presence of the following conditions, as determined by history and physical exam:

- Tooth pathology, sinus infection, trigeminal neuralgia, herpes zoster;
- History of destructive trigeminal nerve procedures or trauma-associated facial bone fractures within the trigeminal nerve distribution;
- Migraine headache, cluster headache or paroxysmal hemicrania at the screening time;
- Pregnancy, planning pregnancy or the potential of being pregnant; and
- Claustrophobia.

Telephone or in-person screening was performed to assess subject eligibility criteria fit. The Institutional Review Board of the University of Minnesota approved the study protocol, and all subjects participating in the study received and signed an informed consent.

Study protocol

Subjects were scheduled for three experimental visits, two at dental chair-side for all subjects and the last in the MR scanner for controls only (Figure 10). Two
data points were collected during the first visit (initial and 1-hour pressure pain thresholds), while in the second visit we measured the 1-week pressure pain threshold and collected pain ratings during blocked-design stimulation. This psychophysical data was used to evaluate the reliability/reproducibility, sensitivity, specificity, discriminant ability and validity of the device. In the third visit we tested its MR-compatibility by visual inspection of the images produced and the stimulus evoked-brain activation through statistical analysis of functional brain images.

First visit – Initial and 1-hour dynamic pressure pain threshold

Initially subjects received explanation about the study protocol and gave their informed consent. Bite impression was taken using an elastic material (Express™ bite, 3M ESPE®, St Paul, MN) over the bite fork (Figure 1B). Use of a Computadorized Visual Analog Scale (COVAS, Medoc® Ltd, Ramat Yishai, Israel) for pain scoring was explained, where 0 meant no pain and 10 the worst pain imaginable. After stimulus device fitting, the subject was asked to record specific pain scores without stimulation to ensure that pain scoring was understood (Figure 7).

The operator delivered intraoral stimuli at a frequency of approximately 1 Hz. The initial stimulus was a light force using one 1/8” elastic band. According to the subject’s pain rating, the pressure was increased by adding more elastic
bands until one of the two possible endpoints occurred: a) consistent pain rating within 3-5 out of 10 using the COVAS or b) maximum pressure was reached (= 8 elastic bands). At anytime the test could be stopped per subject’s request or if there were any signs of unbearable pain.

After a 1-hour rest period, the stimulus device was re-positioned and adjusted to a pressure one level below the pain threshold. After confirmation or readjustment of the pressure to reach either endpoint, one run with four 30 s ON/OFF blocks preceded by a 30 s baseline was done, totaling 270 s (Figure 8A). Finishing this, the stimulus device was unassembled and the subject’s gingiva and oral mucosa were inspected for any tissue injury.

Second visit – 1-week dynamic pressure pain threshold and full stimulus protocol

Seven to ten days after the initial visit, the subject was accommodated in the dental chair with the stimulus device assembled. The pressure was also initiated one level below the last threshold found, and it was adjusted as necessary to reach one of the two outcomes described for the first visit.

Once the threshold was re-established, four runs using the same block design protocol as the first visit were performed. This procedure had three goals: assess if the intraoral stimuli could consistently elicit pain ratings within the
target range (3-5 out of 10), acquaint the subject with the stimulus protocol that would take place during fMRI data acquisition, and also to determine if the subject was able to endure a full session of experimental intraoral pain. The stimulus device was then unassembled, intraoral examination was carried out and the fMRI session was scheduled within approximately two weeks from this visit.

Third Visit - Functional magnetic resonance imaging

Only controls participated in this visit. The subject was received at the Center for Magnetic Resonance Research (CMRR) at the University of Minnesota, and an explanation of the MRI session was given and potential risks were discussed. A 3 Tesla Siemens Trio scanner with a circular polarized RF head coil was used. After placing the subject in the MR scanner bed, stimulus device fitting followed. The initial step was to position the lips and cheeks retractor in the subject (Figure 9A). The superior part of the RF head coil was carefully fitted since it had the supporting frame and intraoral components attached to it (Figure 9B-E), and the number of 1/8” elastic bands used was that established as 1-week threshold. An arch with a knob was fitted over the scanner bed at the level of the subject’s knees and this knob was then attached to a long stick connected to the cam device, (Figure 9F) in a way that a 90° knob rotation elicited full intraoral prove movement. The final adjustment was to position the intraoral probe to barely touch the subject’s gingiva over the stimulation site. Before imaging
started, the subject underwent 5-10 seconds of intraoral stimulation as to confirm pressure pain threshold and if needed, device fitting and/or number of elastic bands adjustment would take place.

After establishing communication with the subject via intercom, the imaging sequence protocol started. We first acquired T1-weighted magnetization prepared rapid gradient echo (MPRAGE) anatomical images (TR=2530 ms, TE=3.68 ms, flip angle=7°, 224 axial slices, matrix 256 x 256, field of view=245.76 mm², voxel size= 0.96 x 0.96 x 1 mm³). Then four to six functional runs were performed using T2*-weighted echo-planar imaging (TR=3000 ms, TE=30 ms, flip angle = 90°, 36 axial slices, 70 volumes, matrix 64 x 64, field of view=192 mm², voxel size=3 x 3 x 5.625 mm³) while intraoral stimulation was triggered by an operator inside the magnet room using a similar blocked design as during the dental chair visits but with only 3 ON/OFF-blocks, totaling 210 s (Figure 8B). The beginning of the functional run was visually signaled by the operator in the control room to the second operator inside the magnet room, while the timing of stimulus delivery for the remainder of the functional run was controlled by the latter using a digital stopwatch to mark block on/offset. At the end of the imaging session the subject was removed from the magnet bore and a brief inspection of intraoral tissue took place. We asked the subject to do an overall pain rating for the imaging session in a 0 to 10 numerical scale. This completed the subject participation in the study.
**Imaging data processing and analysis**

fMRI data processing was carried out using FMRIB’s Expert Analysis Tool (FEAT) 5.98, part of FSL 4.1.4 (FMRIB’s Software Library, www.fmrib.ox.ac.uk/fsl). Anatomical and functional images were skull-stripped using FSL’s brain extraction tool (Smith, 2002). The first three volumes were discarded from each functional run due to elevated MR signal prior to reaching a longitudinal magnetization steady state. Visual inspection of functional images in cine mode was used to detect gross head movements (>3 mm in any direction). Pre-analysis processing steps included motion correction using FMRIB’s Linear Image Registration Tool (MCFLIRT) (Jenkinson et al., 2002), interleaved slice timing correction, spatial smoothing using a 5 mm full-width-at-half-maximum (FWHM) kernel, grand-mean intensity normalization, and temporal highpass filtering (cutoff 60 s) to remove low-frequency noise. Co-registration of functional to structural images was done with FLIRT, and they were then normalized to the Montreal Neurological Institute (MNI) 152 brain template at 1 mm\(^3\) resolution using FLIRT. Registration of anatomical images to the MNI template was further refined using FMRIB’s Nonlinear Image Registration (FNIRT) tool with a 10 mm warp resolution.

Following preprocessing, each functional run analysis was carried out using FMRIB’s Improved Linear Model (FILM) with local autocorrelation correction (Woolrich et al., 2001). Explanatory variable (EV) for intraoral dynamic pressure pain stimulation was modeled with a boxcar function, and an EV for the temporal
derivative of stimulation timing was generated. The EVs were then convolved with a double-gamma hemodynamic response function. Correction for multiple comparisons was done using cluster-based thresholding of Z statistic images using a cluster significance threshold of $Z \geq 2.3$ and $p=0.05$. Functional runs for each subject were averaged using FEAT fixed-effects model. Group-level analysis also used FEAT fixed-effects model with a more stringent cluster thresholding ($Z \geq 5$, $p=0.001$). Threshold activation maps were then overlaid on the group structural mean image to define anatomical location of activations by using five atlases available in FSLview display tool: Harvard-Oxford cortical and subcortical, Juelich histological, MNI structural and Talairach.

Additionally, three-dimensional digital models of each subject’s brain were created using Freesurfer image analysis suite 4.0.5 (http://surfer.nmr.mgh.harvard.edu/) (Dale et al., 1999; Fischl et al., 1999). FEAT output of each non-spatially smoothed functional run was registered to the respective subject’s brain model, and this registration allowed the subject-level FEAT output to be resampled to the MNI 305 template cortical surface using a surface-based spatial smoothing of 5 mm FWHM. Surface-based group analysis was done using fixed-effects, one-sample group mean model similar to the group-level analysis done with FSL. False discovery rate at 0.05 threshold was used for multiple comparisons correction (Genovese et al., 2002).
FIGURES FOR MATERIAL AND METHODS

Figure 1. Stimulus device components.
(A) Supporting frame. (B) Bite fork and screw. (C) Non-magnetic hexagonal and flat head screw drivers. (D) Intraoral probe attached to cam device. (E) Knob and pivoting joint

Figure 2. Intraoral probe design evolution.
(A) Initial format. (B) Second attempt. (C) Final design
Figure 3. Cam device. Arrows point to gingival pressure (white) and cam arms (yellow) elastic bands.

Figure 4. Lips and cheeks retractor final design.
Figure 5. Laboratorial forces measurement – strain gauge.
(A) Strain gauge diagram. (B) strain gauge attached to the intraoral probe

Figure 6. Laboratorial forces measurement - equipment.
(A) Weights set used. (B) Software interface during forces reading. (C) and (D) Voltmeter readings
Figure 7. Covas pain rating.
Left: subject being stimulated at the dental chair; observe the mirror (white arrow) providing indirect view to the COVAS. Right: Training run without stimulation.
Figure 8. Blocked design stimulus.
(A) Dental chair-side. (B) during fMRI data acquisition.
Figure 9. Stimulus device assembly sequence for an fMRI session. See text for description.
Figure 10. Subjects flow chart.

AO subjects recruited from the TMD & OP clinic
Painfree controls recruited from U of M staff and
investigators acquaintances

Telephone/in-person screening

First Visit – Dental Chair:
  Informed consent signature
  Bite impression
  Initial pain threshold
  One-hour pain threshold
  One run of intraoral stimulus

Second Visit – Dental Chair:
  One-week pain threshold
  Four runs of intraoral stimulus

Third Visit – CMRR:
  Structural imaging
  Functional imaging – 4 to 6 runs

End of subject participation in the study
RESULTS

Subjects

Five female AO patients and five painfree age- and gender-matched control subjects were enrolled and completed the study protocol. Their summary data is presented in Table 1.

Intraoral stimulus device

The intraoral fMRI-compatible stimulation device proved to be suitable to be used at dental chair-side and within a MR scanner (Figure 11). Laboratory force measurements, psychophysical and fMRI data results are described below.

Laboratory force measurements

Resistance readings from the strain gauge attached to the intraoral probe and the amount of weight placed at the probe tip showed a linear relationship up to a "failure" point, i.e., when the probe translated. For each 1/8" elastic band a weight was needed to elicit such translation (1=70g; 2=150g; 3=170g; 4=220g; 5=290g; 6=370g; 7=400g; 8=440g). This laboratory test gave an approximate estimate of the force delivered by the intraoral probe tip with different amounts of 1/8" elastic bands. This way use of 2 elastic bands was deemed to deliver "low pressure" stimulation when compared to 6 elastic bands, as these were the
mean number of elastic bands used for AO and painfree groups respectively (Table 1).

**Psychophysical data**

COVAS pain ratings collected during blocked-design stimulation at dental chair-side supported the validity of the stimulus provided by our device, although there was considerable intra- and inter-subjects variation (Figure 12). Visual inspection of pain rating plots showed that two controls did not report pain during stimulation despite use of 8 elastic bands, while the other 3 controls rated pain within the target range (3 to 5 out of 10). Two AO subjects on the other hand reported pain levels above the target range, even though they were stimulated using same forces as the 1-week pressure pain threshold established previously.

Dynamic pressure pain thresholds at the three time points (Table 2) showed strong agreement (intraclass correlation coefficient=0.89 (0.78-1 [95% CI])). One-way random effects ANOVA provided a receiver operating characteristic (ROC) curve, with the area under this curve describing the device discriminant ability (Figure 13). From the cutoff points derived from the forces delivered by the intraoral probe, it can be seen that when using 4 elastic bands (~220g) AO subjects and controls can be differentiated with sensitivity of 100%, specificity of 86.7% and area under the ROC curve=0.99.
**Functional Magnetic Resonance Imaging data**

The final stimulus device design fit within the limited space between the subject’s head and the RF coil during imaging data acquisition (Figure 9). Visual inspection of structural and functional MR images from all subjects detected minimal distortion, despite the presence of the stimulus device within the imaging field of view during the whole session (Figure 14). Head motion during functional imaging was minimized as a result of bite bar use (mean displacement=0.37 mm (0.26-0.49 [95% CI])).

FSL group-level results revealed that intraoral dynamic pressure pain activated several brain regions (Table 3), including those considered part of a network for acute pain (Apkarian et al., 2005). Images of the activation maps from cortical surface-based group analysis using Freesurfer are displayed in Figure 15 and 16, and subcortical activations from FSL group analysis overlaid on the group mean structural image are shown in Figure 17.
## FIGURES FOR RESULTS

<table>
<thead>
<tr>
<th>Subjects group</th>
<th>Age (yrs)</th>
<th>Handedness</th>
<th>Stimulus location (dental quadrant / closest tooth)</th>
<th>Pressure threshold (average 1/8″ elastic bands number)</th>
<th>Pain intensity rating (0-10 VAS)</th>
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<td>1</td>
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1Self-report, 2 Pain rating at the end of fMRI session; 3 Pain rating at 1-week threshold. Parentheses indicate standard deviation.

### Table 1. Study participants characteristics
Figure 11. Stimulus device final design. Superior (A) and inferior (B) views.
Figure 12. COVAS rating plots of 4 blocked-design runs. (A) painfree controls and (B) AO patients

<table>
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<tr>
<td>2</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>8 (±1)</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7 (±0)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6 (±0)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5 (±0)</td>
</tr>
<tr>
<td>AO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2 (±1)</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2 (±1)</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1 (±1)</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4 (±1)</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2 (±1)</td>
</tr>
</tbody>
</table>

Table 2. Subjects dynamic pressure pain thresholds (# elastic bands)
Figure 13. ROC curve and discriminant ability results.

<table>
<thead>
<tr>
<th>Cutpoint</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Correctly Classified</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>( &gt;= 70 )</td>
<td>100.00%</td>
<td>0.00%</td>
<td>50.00%</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>( &gt;= 150 )</td>
<td>100.00%</td>
<td>26.67%</td>
<td>63.33%</td>
<td>1.3636</td>
<td>0.0000</td>
</tr>
<tr>
<td>( &gt;= 170 )</td>
<td>100.00%</td>
<td>60.00%</td>
<td>90.00%</td>
<td>2.5000</td>
<td>0.0000</td>
</tr>
<tr>
<td>( &gt;= 220 )</td>
<td>100.00%</td>
<td>86.67%</td>
<td>93.33%</td>
<td>7.5000</td>
<td>0.0000</td>
</tr>
<tr>
<td>( &gt;= 290 )</td>
<td>86.67%</td>
<td>100.00%</td>
<td>93.33%</td>
<td>0.1333</td>
<td>0.0000</td>
</tr>
<tr>
<td>( &gt;= 370 )</td>
<td>66.67%</td>
<td>100.00%</td>
<td>93.33%</td>
<td>0.3333</td>
<td>0.0000</td>
</tr>
<tr>
<td>( &gt;= 400 )</td>
<td>33.33%</td>
<td>100.00%</td>
<td>66.67%</td>
<td>0.6667</td>
<td>0.0000</td>
</tr>
<tr>
<td>( &gt;= 440 )</td>
<td>13.33%</td>
<td>100.00%</td>
<td>56.67%</td>
<td>0.8667</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

ROC curve and discriminant ability results.
Figure 14. Example anatomical (A) and functional (B) images.

Table 3. Group-level brain activations

<table>
<thead>
<tr>
<th>Brain region</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Maximum Z-value</th>
<th>Cluster size</th>
<th>Cluster P-value</th>
<th>Acute pain network regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary somatosensory cortex (left)</td>
<td>-60</td>
<td>-12</td>
<td>3</td>
<td>10.3</td>
<td>10431</td>
<td>3.20E-42</td>
<td>✓</td>
</tr>
<tr>
<td>Primary somatosensory cortex (left)</td>
<td>-54</td>
<td>-11</td>
<td>37</td>
<td>8.87</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Primary auditory cortex (right)</td>
<td>54</td>
<td>0</td>
<td>-11</td>
<td>9.22</td>
<td>6709</td>
<td>5.31E-32</td>
<td>✓</td>
</tr>
<tr>
<td>Secondary somatosensory cortex (right)</td>
<td>51</td>
<td>-31</td>
<td>18</td>
<td>8.1</td>
<td>-</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Cerebellum posterior lobe (left)</td>
<td>-44</td>
<td>-79</td>
<td>-30</td>
<td>8.12</td>
<td>6346</td>
<td>6.50E-31</td>
<td>✓</td>
</tr>
<tr>
<td>Cerebellum posterior lobe (right)</td>
<td>23</td>
<td>-72</td>
<td>-23</td>
<td>9.63</td>
<td>5745</td>
<td>4.57E-29</td>
<td>✓</td>
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<tr>
<td>Premotor cortex (right)</td>
<td>48</td>
<td>1</td>
<td>44</td>
<td>8.29</td>
<td>2027</td>
<td>1.41E-15</td>
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<tr>
<td>Thalamus (right)</td>
<td>4</td>
<td>-19</td>
<td>5</td>
<td>6.93</td>
<td>1221</td>
<td>1.01E-11</td>
<td>✓</td>
</tr>
<tr>
<td>Thalamus (left)</td>
<td>-6</td>
<td>-23</td>
<td>4</td>
<td>6.82</td>
<td>-</td>
<td>-</td>
<td>✓</td>
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<td>Prefrontal cortex (left)</td>
<td>-6</td>
<td>40</td>
<td>-27</td>
<td>9.87</td>
<td>930</td>
<td>3.96E-10</td>
<td>✓</td>
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<tr>
<td>Visual cortex V3 (left)</td>
<td>-30</td>
<td>-98</td>
<td>-15</td>
<td>7.22</td>
<td>560</td>
<td>5.96E-08</td>
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<tr>
<td>Middle temporal gyrus (right)</td>
<td>61</td>
<td>-34</td>
<td>-2</td>
<td>5.69</td>
<td>366</td>
<td>2.03E-06</td>
<td>✓</td>
</tr>
<tr>
<td>Premotor cortex (left)</td>
<td>-15</td>
<td>13</td>
<td>61</td>
<td>7.16</td>
<td>296</td>
<td>7.39E-06</td>
<td>✓</td>
</tr>
<tr>
<td>Insular cortex (right)</td>
<td>38</td>
<td>11</td>
<td>1</td>
<td>6.97</td>
<td>196</td>
<td>5.84E-05</td>
<td>✓</td>
</tr>
<tr>
<td>Anterior caudate nuclei (left)</td>
<td>-21</td>
<td>25</td>
<td>0</td>
<td>6.49</td>
<td>159</td>
<td>0.00014</td>
<td>✓</td>
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<tr>
<td>Anterior caudate nuclei (right)</td>
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<td>26</td>
<td>-2</td>
<td>6.01</td>
<td>119</td>
<td>0.00037</td>
<td>✓</td>
</tr>
</tbody>
</table>

Group results from FSL analysis. Cluster-threshold of Z=5 and p=0.001. Decreasing cluster size order. MNI, Montreal Neurological Institute

1From Apkarian et al., 2005

1 Local maxima within the above cluster
Figure 15. Inflated cortical surface: superior and inferior views. Arrows pointing to primary and secondary somatosensory (white) and prefrontal (yellow) cortices. Gyri=light grey; sulci=dark grey.
Figure 16. Inflated cortical surface: lateral and medial views. Arrows pointing to primary and secondary somatosensory (white), prefrontal (yellow), anterior cingulate (green) and insular (red) cortices. Gyri=light grey; sulci=dark grey.
Figure 17. Subcortical brain activations. (A) Thalamus and (B) cerebellum.
DISCUSSION

A novel method for intraoral stimulation was developed that discriminated pain thresholds of AO subjects from painfree controls. The advantages of this device include: anatomical and functional MRI data acquisition with minimal image distortion, presence of a customizable bite bar that reduces head motion, MR-compatibility at the most stringent level (Schenck, 1996), modular design that fits within the restricted space between the subject’s head and the RF coil, and ease to reach any dento-alveolar quadrant. Limitations encountered were long assembly time prior to MR imaging (10-15 minutes), which may be of importance given the costs involved for scanner time, and need for previously trained operators for device assembly and fitting.

Few fMRI studies using intraoral stimulation are reported in the literature, likely due to the technical challenges associated (Ettlin et al., 2009). This is illustrated by the approach of Miyamoto and colleagues, where an operator stimulated lower lip and tongue of subjects using a stick with a piece of Velcro at its tip and generated torque forces in the right upper incisor adding a rubber tip with a groove (Miyamoto et al., 2006). Other three fMRI intraoral studies used somewhat more sophisticated stimuli delivery, using vibrotactile (Ettlin et al., 2004) or electrical stimulation of teeth (Jantsch et al., 2005; Ettlin et al., 2009). Common to these stimulation methods were their fixed stimulation site and lack of more rigid head stabilization. The present device avoids these shortcomings.
by allowing reach of buccal oral tissues in all intraoral quadrants, and by providing a customizable bite fork that is comfortable for the subjects and significantly reduces head motion during MRI data acquisition.

Dynamic pressure was the stimulus of choice since a significant number of AO patients describe touch allodynia at the intraoral pain site, mentioning that they avoid chewing around that area – a feature that it is not well described in the literature (Melis et al., 2003; List et al., 2007; Baad-Hansen, 2008; Baad-Hansen et al., 2008) although recently a well-designed study found quantitative evidence for this clinical finding (List et al., 2008). Our psychophysical results provided additional support to this given the sensitivity, specificity and discriminant ability values found for the two groups when using such stimulus.

A second goal was to develop a device suitable for use during fMRI data acquisition and to test its feasibility in evoking brain activation without significant imaging distortions. A simple experimental design with a blocked stimulation paradigm was used that is known to give robust results with high statistical power and relatively large BOLD signal changes (Amaro et al., 2006), but it is more susceptible to anticipation, habituation and attention modulatory effects (Dresel et al., 2008). Another simplification was the use of fixed-effects group-level analysis, which uses a within-subject variance that prevented generalization of our findings to the population level (Woolrich et al., 2004). Taking these caveats into consideration, our fMRI results are in agreement with
those of a meta-analysis that reported a network of brain areas that are activated during acute pain stimulation in painfree subjects (Apkarian et al., 2005). fMRI data collection using the same protocol as described here is ongoing on both painfree controls and AO subjects, and it may provide us a better picture of brain activation following intraoral dynamic pressure in healthy and diseased states, as there is evidence that these may be different (Apkarian et al., 2005).

Further development of our device can broaden its scope. Modifying it to support other auxiliary modules as intraoral thermal probe, Von Frey filaments or to be fit in RF head coils with diverse geometry are a few possibilities to accommodate different research needs. Regarding fMRI research specifically, more reliable stimulus delivery can be achieved by using a computer to trigger stimulus on- and off-set, what has been suggested as a way of reducing BOLD signal changes variability and to increase sensitivity for brain activation (Graham et al., 2001). This putative versatility may help serve a growing need to better measure sensory functions of the human trigeminal system (Jaaskelainen, 2004; Pigg et al., 2010) by adding a way to map intraoral somatosensory representation in the central nervous system.
CONCLUSION

A novel device that delivers intraoral dynamic pressure stimulation was developed and validated. It allows investigators to deliver this and potentially other psychophysical modalities in all quadrants of the oral cavity, and importantly its MR-compatibility provides an opportunity to correlate dental chair-side psychophysical findings with fMRI data.


