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A manually controlled new device for punctuate mechanical stimulation of teeth during functional magnetic resonance imaging studies

Pascale Habre-Hallage[1,2], Laurent Hermoye[3], Wojciech Gradkowski[3,4], Reinhilde Jacobs[2], Hervé Reychler[5] and Cécile B. Grandin[4,6]

[1]Department of Prosthodontics, Faculty of Dentistry, Saint-Joseph University, Beirut, Lebanon; [2]Oral Imaging Center, Faculty of Medicine, Catholic University of Leuven, Leuven, Belgium; [3]Imagilys SPRL, Brussels, Belgium; [4]Institute of Neurosciences, Université Catholique de Louvain, Brussels, Belgium; [5]Department of Oral and Maxillo-facial Surgery, St Luc University Hospital, Université Catholique de Louvain, Brussels, Belgium; [6]Medical Imaging Department, St Luc University Hospital, Brussels, Belgium

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Abstract

Aim: To design a simple and affordable device that could apply standardized mechanical punctuate stimuli to trigger the periodontal mechanoreceptors during functional magnetic resonance imaging (fMRI).

Material and Methods: A new manually controlled device using von Frey monofilaments was tested on a phantom and on eight volunteers. Four block design paradigms with different timing were compared. Teeth 11, 12, 13, 21, 22, 23 and the thumb were stimulated.

Results: The device did not induce any artefacts in MR images. The most efficient protocol included an epoch duration of 24 s and stimuli delivered at 1 Hz. When stimulating the teeth, activations of the primary (S1) and secondary (S2) somatosensory areas were consistently obtained, either on the ipsilateral, contra-lateral or both sides. Stimulation of the thumb led to activations of the contra-lateral S1 area and either ipsilateral or contra-lateral S2 area.

Conclusion: The use of this innovative tool should allow to perform fMRI studies aimed to unveil the neural correlates of periodontal neural receptors, and to understand their plasticity induced by tooth loss and their eventual replacement by endosseous oral implants.

Key words: dental; fMRI; MR-compatible; periodontal mechanoreceptor; somatosensory cortex; stimulation device

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In the last decades, knowledge about the cortical organization of the human brain has boomed, but even if the human face contains important sensory organs and is essential for verbal and non-verbal communications in daily life, only a few studies have described its somatotopy (Nguyen et al. 2004, 2005).

Dental and periodontal somatotopy have been hardly addressed, which might be surprising considering the crucial role of the periodontal ligament receptors in adapting daily sensing, chewing, biting and other oral functions (Trulsson et al. 2005). In the past, some studies were performed using trigeminal evoked potentials to analyse somatosensory signals triggered by tooth stimulation (van Loven et al. 2001). Yet, considering that this technique is quite cumbersome and especially complex for trigeminal stimulations, nowadays, functional magnetic resonance imaging (fMRI) has become the preferred approach to non-invasively map the human cortex but its specific environment imposes some constraints in the experimental design. The subject lies in a large tunnel where he must stay still and any device introduced into the magnet room must be specifically designed to avoid electromagnetic interferences with the scanner. In this context, the exploration of the face and the oral area with fMRI remains challenging because of the poor accessibility to the

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head surrounded by a narrow coil and located in the middle of the magnet bore. Moreover, as stimulation and signal recording devices are in the same area, the sensitivity to any distortion of the magnetic field homogeneity is dramatically increased.

A limited number of studies have been performed so far while stimulating the face, lips or tongue using various manually or automatically applied stimuli, and even fewer studies concerned the teeth and other intra-oral structures. The stimuli applied to the teeth included a torque force delivered by a manually controlled rotating stick (Miyamoto et al. 2006), painless vibrotactile stimulation (Ettlin et al. 2004) and unpleasant or even painful electrical stimulation (Ettlin et al. 2004, Jantsch et al. 2005).

The diversity of applied stimuli and the unnatural stimulation mode led to contradictory results. Therefore, there is clearly a need for additional studies using a calibrated physiologic stimulation to unveil the cortical representation of the teeth in the human cortex.

The aim of the present study was to design and evaluate a new device dedicated to tactile teeth stimulation to trigger periodontal mechanoreceptors in the magnetic resonance environment. The concept was based on two strategies. First, it was attempted to deliver physiologic stimuli taking into account that the periodontal mechanoreceptors exhibit a higher sensitivity to low forces (Trulsson & Johansson, 1996a, b). Secondly, it was aimed to design a simple and affordable device enabling standardized stimulation of teeth with a well-controlled force load. As a third obvious requirement, the device should not disturb the fMRI acquisition and lead to consistent activation of the brain.

In the present paper, a new manually controlled intra-oral device is described and its efficacy is demonstrated by showing the absence of interference with the fMRI signal and by reporting the activation maps obtained in volunteers under several experimental protocols. Different teeth with various timing schemes were tried, using stimulation of the thumb as reference.

Materials and Methods

Stimulation device

We used von Frey filaments (VFF, Bioseb™, Chaville, France) to deliver point-like tactile stimuli to the labial side of the teeth. They consist of a set of 20 monofilaments all of constant length but having a stepwise progression of diameters (Fruhstorfer et al. 2001). Each monofilament is labelled with a number (1.65–6.65) that represents the log 10 of the force (mg) required to bend the filament (0.008–300 g according to the manufacturer). VFF are commonly used for quantitative sensory testing in the clinical setting and in neurophysiological experiments (Yarnitsky 1997, Park et al. 2001). Manually applied VFF stimuli have already been used in neuroimaging studies to map the somatosensory cortex of different body areas using positron emission tomography (Hagen & Pardo 2002) and fMRI (Moore et al. 2000). More recently, they have been used in a new computer-controlled MR-compatible stimulation device to deliver punctuate tactile stimuli to the skin (Dresel et al. 2008) but they have never been used for stimulating teeth.

The VFF was removed from the main handle, and only the small handle and the filament itself remained. It could be non-permanently fixed to the stimulation device allowing to use any chosen filament number. The device was built on an arch parallel to the magnet bore and was set on both edges of the scanner bed to avoid touching the subject's body while providing stability to the entire system. Rotation and translation of the VFF support allowed adjusting the position of the VFF in the three axes (horizontally, vertically and towards the teeth). It was possible to stimulate all the anterior teeth (incisors and canines) regardless of the specific morphology of the subject. The VFF supports were positioned near the anterior border of the head coil, allowing to reach the teeth without touching the coil. Two sticks that could be manipulated by an experimenter outside the magnet were connected to the VFF supports through notched stems. The rotation of the sticks around their long axis controlled the displacement (up and down) of the VFF and provided the stimulation of the teeth at the force scaled by the VFF Nos. (Fig. 1a–c).

To guide the course of the VFF towards the tested teeth and avoid touching the lips or other peri-oral structures, rigid removable customized

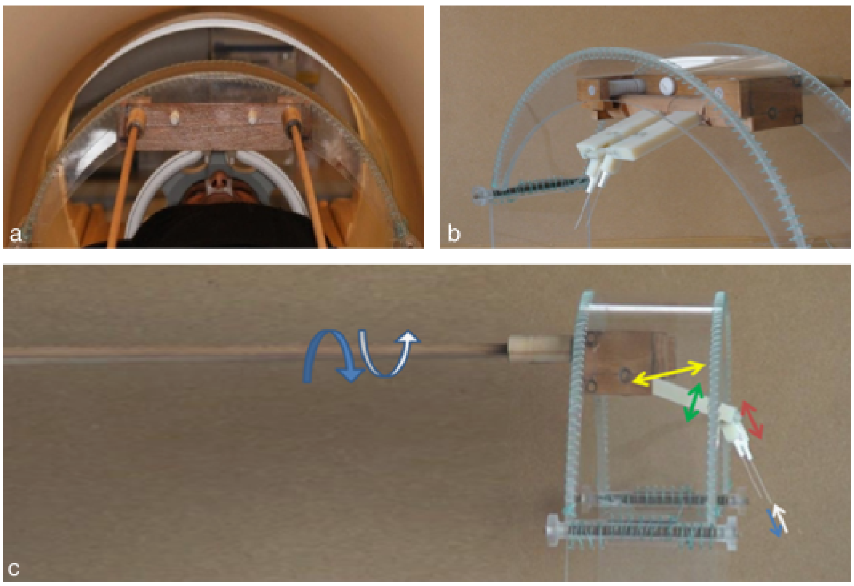

Fig. 1. The stimulation device (a, b). The device was built on an arch parallel to the magnet bore and was set on both edges of the scanner bed to avoid it touching the subject's body and provide stability to the entire system. The patient is lying in the magnet. The von Frey filament (VFF) supports were positioned near the anterior border of the head coil, allowing reaching the teeth without touching the coil. Two sticks that could be manipulated by an experimenter outside the magnet were connected to the VFF supports through notched stems. Note that all materials in the vicinity of the subject and the head coil are non-magnetic. (c) Rotation and translation of the VFF support allowed adjusting the position of the VFF in the three axes: horizontally (yellow arrow), up and down (green arrow) and towards the teeth (red arrow). The rotation of the sticks around their long axis controlled the displacement (up and down) of the VFF and provided the standardized stimulation of the teeth at the force scaled by the VFF. A clockwise rotation induces a movement of the VFF to stimulate the tooth (blue arrow) and then a counter clockwise rotation pulls the filament backward (white arrow).

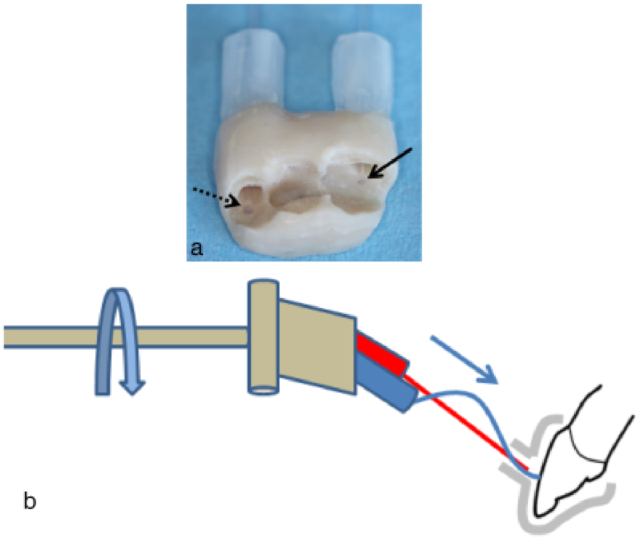

Fig 2. The customized splint (a). The customized dental splint is made of clear acrylic resin with two clear plastic tubes attached to its labial side. The tip of the von Frey filaments (solid arrow) is inserted in the tubes fixed in the splint allowing guiding the filament to the labial aspect of the two teeth chosen for stimulation (dashed arrow). (b) This figure shows how the VFF are bent while its tip is guided by the splint (tube). The VFF support is adjusted 3D so that the tip of the VFF is inside the tube (in grey), which is fixed in the splint but not touching the tooth. A minor clockwise rotation of the wooden stick induces a movement of the VFF to contact the tooth and to stimulate it by bending the filament (blue arrow).

bite splints made of clear acrylic resin (TAB 2000, Kerr Sybron dental specialties, Bioggio, Switzerland) were fabricated for each subject. Plastic tubes (4 mm) were fixed into the splint on the labial aspect of the two teeth chosen for stimulation. The tips of the VFF were inserted into tubes fixed in the splint to ensure that they remained in the right position during the whole experiment without interfering with their bending during the stimulation (Fig. 2a and b). The splint allowed the subject to keep a moderate opening of the mouth while gently biting on it. This approach prevents the use of a cheek retractor, which is much more uncomfortable. All materials used in the stimulation device were non-magnetic, consisting of plexiglass, acrylic, plastic and wood.

Scanning

MRI examinations were performed using a 3-T Achieva system (Philips Healthcare, Best, the Netherlands) equipped with an eight-channel phased array head coil.

In the human subjects, all images were acquired in the bicommissural (AC-PC) orientation (Talairach & Tournoux 1988).

Structural brain images were obtained in all subjects using a 3D fast T1-weighted gradient echo sequence with an inversion pre-pulse [Turbo field echo (TFE), TR (repetition time) = 9 ms, TE (echo time) = 4.6 ms, flip angle (FA) = 8°, 150 slices with a thickness = 1 mm, field of view (FOV) = 220 × 197 mm^2] giving an in plane resolution = 0.81 × 0.95 mm^2 and reconstruction matrix = 398^2. The SENSE factor (parallel imaging) was set to 1.5.

Functional images were obtained with the blood oxygenation level-dependent (BOLD) contrast method, using a 2D gradient-echo single-shot echo-planar imaging (EPI) sequence with the following parameters: TR = 3000 ms, TE = 32 ms, FA = 90°, 44 slices with a thickness = 2.3 mm and no gap, FOV = 220 mm^2 giving a plane resolution of 2.2 mm^2 and reconstruction matrix = 112^2. The SENSE factor was 2.5. To test the potential device-related imaging artefacts, the same EPI sequence was also applied on an MR phantom consisting of a sphere filled with a water solution of $CuSO_4$ that was provided by Philips Healthcare.

Human subjects

Eight healthy right-handed subjects according to the Edinburgh Handedness Inventory (Oldfield 1971), (age 23–51 years, mean 32, SD 10; six females) were recruited for the experiment, which was approved by the local Biomedical Ethical committee.

Inclusion into the study required a full dentition with vital teeth, no periodontal breakdown and no increased tooth mobility. Pregnancy and the usual MRI contra-indications led to exclusion from the study. Subjects were thoroughly briefed about the experimental procedure and they signed an informed consent note before the scan. They were instructed to remain still, to avoid swallowing if possible, to keep their eyes closed and to stay passive without paying any special attention to the stimuli. Tight, but comfortable, foam padding was placed around each subject's head to minimize any movement.

Sensory stimulation

The VFF was chosen to provide stimulation well above the mechanical detection threshold but below the mechanical unpleasantness and definitely pain thresholds. The filament No. 5.88 (60 g), 6.10 (100 g) and 6.45 (180 g) were used for the lateral incisors, the central incisors and the canines, respectively. These choices are based on our experience with tactile threshold level determination of periodontal mechanoreceptors around several types of teeth (vanSteenberghe & deVries 1978). Before the experiment, each stimulus was tested in the scanner to confirm that the stimulation was clear and constant, and that the VFF only touched the intended target. The stimulation was provided by the same well-trained experimenter (P. H. H.) to minimize the variability of stimuli across the subjects. Repetitive punctuate stimulation was delivered by rotating the sticks at a constant frequency of 1 Hz that was acoustically cued to the experimenter. A tactile stimulation of the thumb was also delivered to some subjects to serve as a reference task. In this case, the subject's hand lay comfortably on a foam cushion and the punctuate stimuli were delivered to the lateral side of the thumb's extremity at the same frequency of 1 Hz with a VFF 5.07 (10 g) manually held by the experimenter. Contradictory to other somatosensory experiments, subjects had to stay passive without paying any special attention to the stimuli to avoid unspecific activation of the attention network or coactivation of the motor network that are typically observed when a subject has to push a button.

Experimental paradigm

The device allowed the stimulation of two different teeth in the same experiment. The synchronization with the MR scanner and the programming of the paradigm delivering the cue to the experimenter were provided by the software ParadigmMagix (Imagilys, Brussels, Belgium). Only block design experiments were tested. Into each epoch, the stimuli were administrated to the same area and each active epoch was separated by a period of rest. To test the sensitivity of this repetitive stimulation as a function of the epoch timing, four different protocols were applied (Table 1).

In total, teeth 12, 13, 23 and the right thumb were stimulated in two subjects, teeth 11 and 22 were stimulated in three subjects, while tooth 21 and the left thumb were stimulated in four subjects.

The protocol 2 was also applied on the MR phantom, once without the stimulation device in the magnet bore, once with the device but without using it and once with the device and a sham stimulation to consider the influence of the movement of the mechanical parts.

Behavioural questionnaire

After the fMRI experiment, the participants had to answer the following questionnaire in order to find out how the stimulus was perceived.

1 Was the stimulation on the tooth perceived as a touch, a pressure or something else?
2 Was the subject able to discriminate whether the incisor or the canine was stimulated?
3 Did the subject hear the sound of the filament contacting the surface of the tooth? If yes, did he hear it all the time or sometimes?
4 Did the subject feel any head movement while being tested and any other sensation unrelated to the target stimulation?
5 Was there any unpleasant sensation, pain or anything bothering the subject while being tested inside the machine?

Data analysis

To test if the stimulation device did not affect EPI images, we calculated the pooled standard deviations of the signal across all voxels of the phantom's images acquired either without or with the device in place were calculated (http://www.iupac.org/goldbook/P04758.pdf).

Volunteers data were processed and analysed using Statistical Parametric Mapping (SPM 5, The Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/), implemented in Matlab (Mathworks Inc., Sherborn, MA, USA). The individual structural (TFE) brain volume of each participant was coregistered to the first fMRI volume and spatially normalized into the referential defined by the atlas of Talairach & Tournoux (1988) and the MRI template supplied by the Montreal Neurological Institute (MNI). The fMRI data were then spatially re-aligned and further spatially normalized using the parameters derived from the 3D TFE normalization after the skull had been removed. This resulted in normalized fMRI scans with a cubic voxel size of $2\,\mathrm{mm}^3$ that were spatially smoothed with a Gaussian kernel of 5 mm (full-width at half-maximum, FWHM) to improve the signal-to-noise ratio and to accommodate inter-subject variability of brain anatomy. Condition-related changes in regional brain activity were estimated for each participant by a general linear model in which the responses evoked by each condition of interest were modelled by a standard haemodynamic response function. The contrasts of interest were computed at the individual level to identify the cerebral regions significantly activated by each condition using a t-map. Only the contrasts comparing the stimulation period to the rest period (stimulation minus rest) were considered.

As single subjects were investigated, the statistical threshold was individually adapted by using pre-defined uncorrected p values. This approach is frequently used in individual clinical fMRI examination to take into account for the variable sensitivity of each subject to the BOLD response. We started the analysis with the threshold set at $p<0.00005$ uncorrected for multiple comparisons and combined with an extent threshold of 20 contiguous voxels to reduce the number of isolated false-positive voxels. If the total number of activated voxels was <150, we lowered the threshold by steps ($p<0.0001$, $p<0.0005$, $p<0.001$, and $p<0.005$) until we obtained at least 150 activated voxels. If the total number of activated voxels was >600, we increased the threshold by steps ($p<1\times10^{-5}$, $p<1\times10^{-6}$, $p<1\times10^{-7}$, $p<1\times10^{-8}$, $p<1\times10^{-9}$ ...) until the number of activated voxels dropped under 600. The thresholded activation maps were superimposed on each individual's normalized anatomical image to define the location of the local activation maxima. In all subjects, every activated cluster was then tabulated for each contrast with their MNI coordinates and the corresponding anatomic and Brodmann areas. The local activation maxima belonging to the same gyri and Brodmann areas were averaged.

Numerical data were presented with their median and semi-interquartile deviation (SID) as they were not normally distributed. Non-parametric tests performed in Matlab were used to compare the results (the Wilcoxon signed rank test for paired data or the rank sum test for unpaired data).

Results

Phantom study

The pooled standard deviation of the signal in the phantom without the device, with the device not in use and with the device

Table 1. Description of the four tested paradigms

	First rest period duration (s)	Other rest periods duration (s)	Active periods duration (s)	Number of active epochs/run	Number of volumes/run	Number of runs	Number of subjects
Protocol 1	12	12	12	12	96	1/site (three sites tested)	2
Protocol 2	24	24	24	6	96	1/site (three sites tested)	4
Protocol 3	12	12	24	9	112	4 (three sites/run)	2
Protocol 4	12	24	24	6	100	3 (two sites/run)	2

The site is either a tooth or the thumb. In protocol 3 and 4, the sites were stimulated in an interleaved and counterbalanced order in each run. Two subjects underwent both protocols 1 and 2.

when performing a sham stimulation were not significantly different (46.18, 46.82 and 47.52, respectively).

Behavioural questionnaire

All eight subjects felt the stimulation. Three reported a pressure sensation while two could not discriminate whether the stimulation was touch or pressure. The remaining two subjects reported tactile sensation on the incisor while pressure was reported on the canine.

Out of the eight subjects, four were able to discriminate the incisor from the canine. One volunteer reported that he was able to discriminate most of the times while another reported discriminating with difficulty. Two subjects were unable to discriminate the stimulated tooth.

Only one subject reported hearing constantly the sound of the filament contacting the teeth. Others either heard it sometimes or were not sure or did not hear it at all.

Out of the eight subjects, six reported not moving their head while two subjects were not sure whether they moved or not, with one having the impression that his head was pushed by the stimuli.

Only two subjects reported to be stressed by the recording environment. Most of the reported unpleasant sensations included itching in the throat and the feeling of a need to swallow. Only one volunteer reported pressure on the ears from the headphones, and one subject complained about staying still throughout the experiment.

Influence of epoch timing

When comparing the activations obtained in the same volunteers with the protocols 1 and 2 (epoch duration of, respectively, 12 and 24 s), there were more activated voxels with 24 s. The latter allowed us to use significantly higher statistical thresholds even if the number of volumes acquired during rest and activation periods was the same in the two protocols (Table 2). Moreover, the targeted areas of interest, namely the primary and secondary somatosensory cortex, were more often activated with epochs of 24 s (Table 2).

We also compared the three protocols using activation epochs of 24 s (protocols 2, 3 and 4). It demonstrated that the more powerful activations were obtained with protocol 4 when epochs of 24 s were used for both the rest and activation periods, even if the differences were not statistically significant due to the small sample size and the large variance (Table 3). Indeed, in protocol 3 using rest periods of 12 s, the statistical threshold that led to the targeted number of activated voxels was not different as compared with protocol 2, despite a double number of activated volumes acquired for each site (96 *versus* 48). On the other hand, increasing the number of activated volumes per site while keeping activation and rest periods of 24 s (72 volumes in protocol 4 *versus* 48 volumes in protocol 2) led to a higher statistical power.

Cortical activations

The data obtained in the eight subjects were all exploitable and none of them disclosed head movement superior to 2 mm as measured by the re-alignment algorithm. In this group, the median of the individually adjusted statistical thresholds was $p < 0.000005$ (SID = 0.000024). All activated areas disclosed on individual maps at the chosen threshold are presented in Table 4. An activation of the postcentral gyrus representing the primary somatosensory cortex (S1, mainly Brodmann area 1, but sometimes 2 or 3b) was observed on 8/16 of the stimulated teeth on the contra-lateral side and in 7/16 teeth on the ipsilateral side. The contra-lateral S1 area was activated in 4/6 stimulated thumbs. The parietal operculum corresponding to the secondary somatosensory cortex (S2, OP1-4) was activated in 16/16 stimulated teeth on the contra-lateral side and in 11/16 teeth on the ipsilateral side. The stimulation of the thumb also yielded an activation of SII area on the contra-lateral (3/6) or ipsilateral side (2/6). An activation of the superior temporal gyrus (Brodmann areas 22, 42 and one time 38) was found bilaterally in about half of the stimulated teeth (7/16 on the contra-lateral side and 9/16 on the ipsilateral side). Such activation was rarely found after stimulating the thumb with a contra-lateral focus for 1/2 right thumbs and an ipsilateral focus for 1/4 left thumbs. The middle temporal gyrus (Brodmann areas 21 or 39) was activated in 3/16 stimulated teeth on the contra-lateral side and in 6/16 teeth on the ipsilateral side. The ipsilateral side was activated only by 2/6 stimulated thumbs, all left. An activation of the pre-central gyrus (Brodmann areas 4 or 6) was also found for 6/16 teeth on the contra-lateral side and for 2/16 teeth on the ipsilateral side. Only the stimulation of the left thumb yielded activation of the contra-lateral pre-central gyrus in 3/4 cases. Other areas were only occasionally activated. A typical activation map obtained in a

Table 2. Statistical thresholds and activated foci found in somatosensory areas with epoch duration of 12 or 24 s

Subject no.	Stimulated site	Protocol 1: epochs of 12 s			Protocol 2: epochs of 24 s		
		p value	S1	S2	p value	S1	S2
3	tooth 21	0.000001	–	ipsi	0.0000001	ipsi	ipsi, contra
3	tooth 22	0.0001	–	ipsi, contra	0.000001	contra	ipsi, contra
3	L hand	0.001	ipsi, contra	ipsi	0.000005	contra	ipsi, contra
8	tooth 12	0.01	–	ipsi	0.00005	contra	ipsi, contra
8	tooth 22	0.005	–	–	0.00005	contra	ipsi, contra
8	R hand	0.001	–	–	0.000005	contra	contra
Median		0.001*			0.000005*		
SID		0.0037			0.000025		

*Significant difference between the 2 protocols ($p = 0.03$).

Protocol 1 and 2 included both 48 active and rest volumes, the only difference being the duration of the rest and active periods.

The statistical thresholds (p value) were defined to obtain a total number of activated voxels between 150 and 600 (see details in the text).

L hand, left hand; R hand, right hand; S1, activation in the primary somatosensory area; S2, activation in the secondary somatosensory area; ipsi, ipisilateral regarding the stimulated site; contra, contra-lateral regarding the stimulated site; SID, semi-interquartile deviation.

Table 3. Comparison of the three protocols using active epochs of 24 s

Protocol 2 (48 active volumes/site)			Protocol 3 (96 active volumes/site)			Protocol 4 (72 active volumes/site)		
subject no.	stimulated site	*p* value	subject no.	stimulated site	*p* value	subject no.	stimulated site	*p* value
1	tooth 11	0.0005	2	tooth 21	0.000001	6	tooth 11	0.000005
3	tooth 21	0.0000001	5	tooth 21	0.0005	7	tooth 11	0.000005
4	tooth 21	0.000005	5	tooth 23	0.00005	6	tooth 13	0.00000001
4	tooth 23	0.00000001	2	L hand	0.000005	7	tooth 13	0.000000001
3	L hand	0.000005	5	L hand	0.000005			
4	L hand	0.000005	2	tooth 22	0.0001			
1	R hand	0.000005						
8	R hand	0.000005						
1	tooth 12	0.001						
8	tooth 12	0.00005						
3	tooth 22	0.000001						
8	tooth 22	0.00005						
Median		0.00005	Median		0.0000275	Median		0.0000025
SID		0.00014	SID		0.0000475	SID		0.0000025

The statistical thresholds (*p* value) were defined to obtain a total number of activated voxels between 150 and 600 (see details in the text).
None of the pairwise comparison between the 3 protocols was statistically significant (corrected $p > 0.05$).
L hand, left hand; R hand, right hand; SID, semi-interquartile distribution

Table 4. Location of all activation foci found for the different stimulated sites

Anatomic location	Brodmann area	Contralateral hemisphere Stimulated site (nb+/tot nb)	Ipsilateral hemisphere Stimulated site (nb+/tot nb)
Postcentral S1	1	T11 (2/3), T12 (1/2), T13 (2/2), T22 (2/3) LH (2/4), RH (1/2)	T13 (1/2), T21(2/4), T22 (1/3), T23 (1/2)
	1–2	T12 (1/2), RH (1/2)	T22 (1/3), T21 (1/4)
	2	LH (1/4), RH (1/2)	T22 (1/3)
	3b	LH (1/4)	T21 (1/4)
Parietal operculum S2	OP1	T11 (2/3), T12 (2/2), T21, (2/4), T22 (2/3), T23 (1/2), RH (1/2), LH (1/4)	T11 (2/3), T12 (1/2), T13 (1/2), T21 (4/4), T22 (3/3), T23 (1/2), LH (2/4)
	OP2	T11 (1/3), T13 (2/2), T21 (1/4), T22 (1/3), LH (1/4)	T22 (1/3), T23 (1/2), LH (1/4)
	OP3	LH (1/4)	T21 (2/4)
	OP4	T11 (1/3), T13 (1/2), T21 (2/4), T22 (1/3), T23 (1/2), LH (2/4), RH (1/2)	T21 (2/4), T22 (1/3), LH (1/4)
Superior temporal	22	T11 (1/3), T21 (1/4), RH (1/2)	T11 (1/3), T21 (1/4), T22 (1/3), T23 (2/4), LH (1/4)
	38	T23 (1/2)	
	42	T11 (2/3), T12 (1/2), T13 (1/2)	T11 (3/3), T13 (2/2), T23 (1/2)
Middle temporal	21	T22 (1/3)	T21 (1/4), T22 (1/3), T23 (1/2)
	39	T11 (2/3), T23 (1/2)	T21 (1/4), T22 (1/3), T23 (1/2), LH (2/4)
Precentral	4	T12 (1/2), T22 (1/3), LH (3/4)	T12 (1/2), T13 (1/2)
	4–6	T11 (1/3), LH (1/4)	
	6	T11 (1/3), T12 (1/2), LH (1/4)	
Superior frontal	8		T11 (1/3), T21 (1/4)
	8–9		LH (1/4)
Middle frontal	10		T11 (1/3)
	46		RH (1/2)
Inferior frontal	44		RH (1/2)
	45		LH (1/4
	47		LH (2/4)
Orbital	10	T22 (1/3)	
Cingulate	23–31		T22 (1/3)
Inferior parietal	40	T12 (1/2), LH (2/3)	T12 (1/2), RH (1/2)
Supramarginal	40		RH (1/2)
Cerebellum		T13 (1/2), T22 (1/3)	T13 (1/2), T22 (1/3)
Caudate			T11 (1/3)
Frontal white matter			T11 (1/3)

Txx, tooth number; LH, left hand; RH, right hand; S1, primary somatosensory area; S2, secondary somatosensory area; (nb+/tot nb), number of subjects showing this activation/total number of subjects.

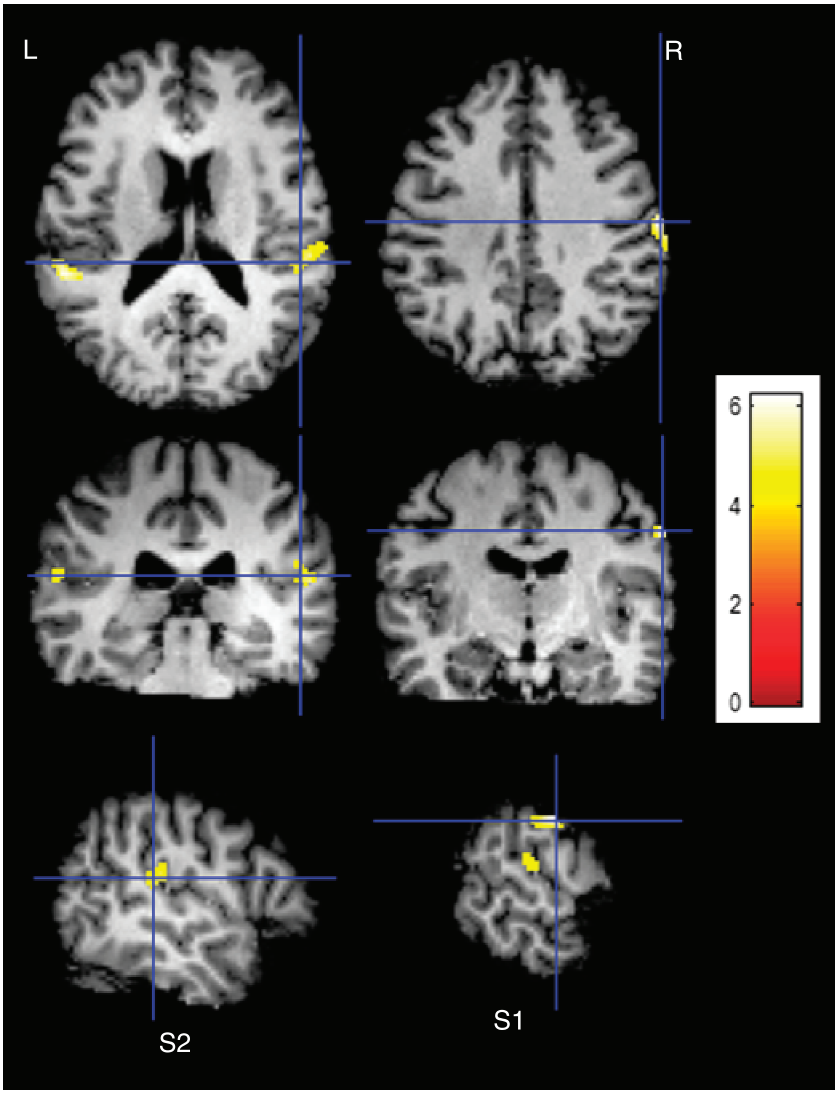

Fig. 3. Cortical activation obtained during sensory stimulation of the teeth 22 in subject 8 with protocol 2 (active and rest epochs of 24 s, 48 activated volumes). The statistical parametric maps are overlaid on the axial, coronal and sagittal sections of the individual normalized anatomical T1-weighted MR images. Images are shown in neurological convention (R =right, L = left) and only pixels exceeding a threshold of $p < 0.00005$ are displayed according the colour scale that codes the T-values. We observe activation in the contra-lateral primary somatosensory cortex (S1) and in the secondary somatosensory cortex (S2) bilaterally.

single subject while stimulating a tooth is showed in Fig. 3.

Discussion

In this study, a new manually controlled stimulation device able to deliver calibrated tactile stimuli to the teeth in an MR environment is described. Consistent activations of the primary and secondary somatosensory areas were obtained with a block design, and an epoch duration of 24 s appeared more efficient as compared with shorter ones.

Stimulation device

Our aim was to design a device able to mimic physiologic oral tactile stimuli on the teeth to trigger periodontal ligament mechanoreceptors.

Although the somatosensory function of teeth is complex and is engaged not only for biting or chewing (Trulsson & Johansson 1996a, b) but also in the reflexes of the masticatory muscles (van Steenberghe 1979, Linden 1990) and oral stereognosis (Jacobs et al. 1997), we wanted to use a pure tactile stimuli without interference with pain, temperature or motor-related tasks. This precludes the use of electrical stimulation, which are painful or unpleasant (Ettlin et al. 2004, Jantsch et al. 2005), and motor task involving clenching (Yan et al. 2008, Byrd et al. 2009). Passive tactile stimulation is the preferred solution but is challenging because of the difficulty to reach the area surrounded by the head coil. Such stimuli have been manually delivered to the face (Iannetti et al. 2003) or to the teeth (Miyamoto et al. 2006) but the intensity of the stimulation was not controlled. Only one study was based on vibrotactile stimulation of the teeth with an automatic device powered by compressed air (Ettlin et al. 2004). Vibrotactile stimuli have been successfully used for somatotropic mapping of the face (Huang & Sereno 2007) but this kind of stimuli might not be physiologic for the teeth and/or periodontium, and did not yield any activation in the somatosensory areas (Ettlin et al. 2004).

The use of VFF appeared as a good alternative to deliver a physiologic and standardized punctuate mechanical stimuli to the teeth. Indeed, the intensity of the stimulation can be controlled by choosing the appropriate VFF among the set of logarithmically scaled filaments to deliver a force between 0.25 and 728 mN.

Most periodontal ligament neural receptors exhibit a high sensitivity to changes in tooth load at very low forces: below 1 N for anterior and 4 N for posterior teeth; at higher forces, the sensitivity gradually decreases. This is the reason why the VFF, which exert 1–2 N have been used to trigger the incisors and canine teeth, respectively. The behavioural questionnaire revealed that the device consistently elicited a touch or pressure sensation on the teeth without any pain. Most volunteers reported a slightly different sensation between the incisive and the canine, probably related to the difference in the direction of the force applied to the tooth (vertical for the incisive but partly tangential for the canine). The main confounding factor might be a sound heard by a few volunteers while the filament contacted the tooth, mainly for the incisor (vertical incidence), but this was not constant (only reported all the time by one volunteer). Two subjects were unable to discriminate the stimulated tooth. This phenomenon is regularly observed. The receptive field of human periodontal mechanoreceptors often extends beyond a single tooth typically two to four adjacent teeth (Trulsson 1993, Johnsen & Trulsson 2003), which may explain these findings.

Contrary to some other authors (Xu et al. 2007), who reported that movements into the magnetic field were able to generate artefacts, none were detected with the present experimental set-up. Although the displacement of the VFF occurred very close to the region of interest, they remained very limited (< 0.5 cm) as well as the movements of the other mechanical components of the device that are mainly rotational.

In this study, only the six anterior teeth of the maxilla were stimulated. The stimulation of the anterior teeth of the mandible should also be possible but the design of the device does not allow the stimulation of the more posterior teeth. The device was conceived to stimulate two teeth during the same experiment. Although it is technically possible to add more sticks and more filaments to stimulate more sites, we anticipate that a manual control of these sticks would not be possible with enough accuracy. The main limitation of this device is the manual control but it was our purpose to keep it simple and affordable to build. Even by using calibrated VFF, the speed of the VFF reaching the tooth might influence the force load and induce some variation in the stimuli. These variations can be minimized when the same well-trained experimenter manipulates the device and can also visually monitor the bending of the filaments while touching the teeth.

The manual delivery of the stimuli was acoustically cued to the experimenter at a frequency of 1 Hz. This provides enough temporal precision for block-designed paradigms but not for event-related experiments. The device could be improved by adding a motorization to automatically rotate the sticks with a higher precision but this would increase its cost and complexity. An elegant solution has been recently presented by Dresel et al. (2008) who have designed a new computer-controlled MR-compatible stimulation device for mapping somatosensory-evoked brain activation during fMRI. This device also uses VFF powered by pressurized air and was successfully used to apply tactile stimuli to the face and the hands. It was never used for stimulating the teeth but it appears well adapted for such stimulation if used with the splint and the tube guides as explained in our set-up. Our device was designed for fMRI only and the manual control of VFF precludes its use for magneto-encephalography (MEG). Indeed MEG requires an averaging of the small magnetic fields generated by the neuronal sources, and therefore a very precise measure of the stimulus onset. To record this information, Jousmäki et al. (2007) have designed a brush stimulator consisting in an optic fibre bundle that is manually held to apply gentle tapping on the skin. The timing relies on the reflectance of the emitted light from the skin. This kind of device should also allow stimulating periodontal mechanoreceptors during MEG or event-related fMRI experiments.

Experimental protocol

The experiment was limited to block design paradigms but cortical activations may be influenced by the stimulation frequency within each epoch and by the epoch duration.

We only used a stimulation frequency of 1 Hz and we are not sure that this rate is optimal to stimulate the periodontal mechanoreceptors, although our choice was guided by previous studies. For stimulus intervals of 0.25 s (4 Hz) or less, a summation effect occurs, which means that the stimuli are experienced as a unique stimulus with a progressively increased amplitude. High frequencies such as this used by Ettlin et al. (2004) for vibrotactile stimuli (80 Hz) did not activate the somatosensory cortex. Low-frequency stimulation (generally a 5 s stimulus interval) is therefore proposed by many authors as a 3-s interval may be too short for the tooth to recover from displacement (Picton 1989). However, Miyamoto et al. (2006) reported clear activations in the somatosensory cortex using a mechanical tactile stimulus at a constant frequency of 1 Hz, and this guided our choice. Regarding the teeth, repetitive stimulations at a sufficient frequency might be particularly physiologic because chewing always involves a series of stimuli. Moreover, many volunteers reported to detect better the repetitive stimuli as compared with single stimuli applied during the pre-testing trials. We do not know if a single stimulation of periodontal mechanoreceptors would be able to elicit cortical activation as an event-related design has never been used for the teeth. For the face or the hand, Dresel et al. (2008) have demonstrated that an event-related paradigm with VFF tactile stimulation was at least as effective to elicit activation in the primary somatosensitive cortex (S1) as compared with a block-wise stimulation.

With longstanding stimulations of periodontal mechanoreceptors, an habituation may occur and decrease the activation in the somatosensory areas (vanSteenberghe & deVries 1978). However, and even if the sample size was too small to definitely conclude, we have shown that the protocols with the longest epoch duration (24 s) for both the activation and rest periods were the most efficient. A longer epoch duration allows a better stabilization of the BOLD signal with a complete recovery of the baseline between the activations. The optimal epoch duration for a classical block-wise paradigm (16–30 s) (Worsley & Friston 1995, Friston et al. 1999) is therefore applicable for the periodontal mechanoreceptors.

Cortical activations

The stimuli delivered by our device yielded significant brain activation in the somatosensory cortex in all volunteers, indicating that the response in this cortical area was dominant and robust. Indeed, the primary somatosensory area (S1) was activated for 81% of the stimulated teeth, while the secondary somatosensory cortex (S2) was activated for all stimulated teeth. These results are remarkable with regard to previous studies that have reported controversial results while trying to map the cortical representation of intra-oral sensations. Conflicting results emerged when painful and non-painful dental stimulations were compared (Hari & Kaukoranta 1985) or when non-physiologic stimuli were applied like vibrotactile stimuli (Ettlin et al. 2004). The latter identified activations primarily and bilaterally in the insular cortex and in the supplementary motor cortex but not in the somatosensory cortex. Using a manually applied torque force, Miyamoto et al. (2006) were able to map the S1 representation of the stimulated tooth. In our study, we show that a stimulation of the periodontal mechanoreceptors led to an activation of S1 and S2 areas as it has been demonstrated for such punctuate tactile stimulation in other areas of the body (Davis et al. 1998, Hagen & Pardo 2002, Iannetti et al. 2003).

In comparison with the thumb, the repetitive stimulation of the teeth with VFF might even be more efficient for activating the somatosensory areas, but the limited number of tested subjects does not allow us to draw definitive conclusions. In this experiment, the absence of task to maintain the attention during the stimulation did not preclude the activation of the somatosensory system even if an increased activation in S2 may be expected when modulating the activation by some attentional processing (Porro et al. 2004). Beside the somatosensory system, other cortical areas were also activated (mainly temporal areas and the precentral gyrus) but less consistently. A description of the entire cortical network involved in the tactile teeth stimulation would require more subjects and a more uniform protocol to perform group analysis but it is out of the scope of this methodological report.

Clinical perspectives

This new device should allow a detailed and systematic cortical mapping of periodontal mechanoreceptors projections from the anterior teeth of the four quadrants. This will provide a reference for further investigating any disturbance in the sensibility of the oral area. This could include an objective testing of sensory loss in periodontal diseases, in polyneuropathy, or after sectioning of a branch of the trigeminal nerve, as well as in abnormal pain sensation elicited by a light touch. Another large field of investigation is the evaluation of cortical plasticity after the loss of one or more teeth and their eventual replacement by endosseous implants. Such cortical plasticity is known to occur after amputation of body parts (Jones 2000). Tooth extractions should be considered as an amputation and the occurrence of such cortical plasticity can be expected. These studies could open the doors to a better understanding of the underlying processes leading to the recuperation of a near-to-normal sensory function after the placement of osseointegrated implants They might provide a neurophysiologic base for optimizing the timing and the technique of implant surgery.

Conclusion

In the present study, we demonstrate that a mechanical stimulation of the teeth with a simple and affordable device delivering calibrated stimuli with von Frey filaments leads to a consistent activation of the somatosensory areas. Although the manual control of the device limits its use to block-wise paradigms on two sites during each scan, we believe that this tool can be used to map the cortical representation of the orofacial sphere and especially to stimulate periodontal mechanoreceptors.

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Address:
Cécile B. Grandin
Department of Medical Imaging
St Luc University Hospital
10, Av Hippocrate
1200 Brussels
Belgium
E-mail: cecile.grandin@uclouvain.be

Clinical relevance

Scientific rationale for the study: Intra-oral somatotopy has been hardly addressed. The few available results are dissenting because of a disparity in methodology. There was a need for an innovative tool designed to deliver calibrated tactile stimuli on the teeth to trigger the periodontal mechanoreceptors during fMRI studies.

Principal findings: When stimulating the teeth, brain activations in the primary and secondary somatosensitive were consistently obtained.

Practical implications: The use of this device may boost the understanding of the cortical projections of periodontal mechanoreceptors before and after tooth loss. It will also allow to investigate the plasticity of their cortical mapping after installation of endosseous oral implants.