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LABORATORY OF ORAL PHYSIOLOGY

ASSESSMENT OF CHANGES IN ORAL TACTILE FUNCTION AND OSSEOPERCEPTION BY ORAL ENDOSSEOUS IMPLANT PLACEMENT

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PREFACE

This thesis is based on the following papers:

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Habre-Hallage P*., Tanaka. M., van Steenberghe D., Reychler H., Jacobs R., Grandin C.*
fMRI as a tool to unravel the sensory function in the oral area. Invited review by The Journal of Oral Rehabilitation. In preparation

Chapter 2:
Habre-*Hallage P**., Bou Abboud-Naaman N., Reychler H., van Steenberghe D., Jacobs R.* (2009) Assessment of changes in the oral tactile function of the soft tissues by implant placement in the anterior maxilla: a prospective study. Clin Oral Investig. 2010; 14: 161-8. Epub 2009 Apr 16

Chapter 3:
Habre-Hallage P*., Bou Abboud-Naaman N., Reychler H., van Steenberghe D., Jacobs R* (2009) Perceptual Changes in the Peri-Implant Soft Tissues Assessed by Directional Cutaneous Kinaesthesia and Graphaesthesia: A Prospective Study. Clin Implant Dent Relat Res. 2009. [Epub ahead of print]

Chapter 4:
Habre-Hallage P*., Hermoye L., Gradkowski W., Reychler H. , Jacobs R., Grandin C.* A manually-controlled new device for punctuate mechanical stimulation of teeth designed for fMRI studies. J Clin Periodontol. 2010; 37: 863-72.

Chapter 5:
Habre-Hallage P., *Dricot L., Hermoye L., Reychler H., van Steenberghe D., Jacobs R., Grandin* C. Cortical activation resulting from stimulation of periodontal mechanoreceptors measured by functional magnetic resonance imaging (fMRI). Submitted

Chapter 6:
Habre-Hallage P*., Dricot L., Reychler H., van Steenberghe D., Jacobs R., Grandin* C. Brain plasticity and cortical correlates of osseoperception revealed by punctuate mechanical stimulation of osseointegrated oral implants during fMRI. Submitted

Chapter 1

This chapter is partially based on an invited review for the Journal of Oral Rehabilitation :

Habre-Hallage P., Tanaka. M., van Steenberghe D., Reychler H., Jacobs R., Grandin C.

fMRI as a tool to unravel the sensory function in the oral area. In preparation

1.1 General introduction:

Teeth are surrounded by a periodontal ligament that attaches the root of the tooth to the alveolar bone. The periodontal ligament contains many mechanoreceptors that encode temporal, spatial and intensive aspects of force development on the dentition (Trulsson and Johansson, 2002). These mechanoreceptors efficiently encode tooth load when subjects contact and gently manipulate food by the teeth. They are also involved in jaw muscle motor control (Trulsson and Johansson, 2002).

The refined mechanoreceptive properties are due to an intimate contact between collagen fibres and Ruffini-like endings (Lambrichts et al., 1992). Tooth loss will remove these receptors and reduce the input to the brain (Klineberg and Murray, 1999).

A tooth extraction may be considered as some kind of amputation. It leads to loss of both intradental nociceptors and periodontal mechanoreceptors. After tooth loss, the myelinated fibre content of the inferior alveolar nerve is reduced by 20% (Heasman, 1984). Remaining nervous tissue may no longer be stimulated and thus lead to nerve degeneration (Hansen, 1980) or nervous branches may start sprouting and simply provide innervation to some more distant structures, like the overlying healed soft tissues (Desjardins et al., 1971; Yamamoto and Sakada, 1986). The surviving mechanoreceptive neurons maintain their functionality only to a certain extent (Linden and Scott, 1989). On a clinical level it is observed that tooth loss may decrease masticatory efficacy, hamper muscle function, alter speech and even induce neurosensory disorders and/or pain. It is estimated that 20% of edentulous adults suffer major oral functional problems (Sessle et al., 2005).

Replacement of teeth to restore oral function is a routine clinical procedure. It includes the use of removable dentures or fixed prostheses, the latter either tooth-supported or bone-anchored by means of oral osseointegrated implants.

In 1965, Professor P-I Brånemark, discovered that a titanium implant can predictably integrate into the bone if the surgery is properly handled to avoid heating of the bone and microbial contamination. Once osseointegrated, it cannot be removed without fracturing the bone (Branemark et al., 1970). Since, Millions of patients have been treated by means of implant-supported prostheses to restore the edentulous jaw bone.

Patients with a lower limb prosthesis anchored to percutaneous osseointegrated implant reported that this allows them to feel the kind of soil they walked on, while with

socket prosthesis they only detected the contact with the floor (Brånemark, 1997). It has been assumed that by anchoring prosthetic limbs directly to the bone via osseointegrated implants, a partial sensory substitution can be achieved (Jacobs, 1998; Jacobs et al., 2000; Jacobs et al., 2001). These psychophysical studies confirmed that patients may perceive mechanical stimuli exerted on osseointegrated implants in the bone.

Histological, neurophysiological and psychophysical evidence of osseoperception suggests that the peripheral feedback pathway can be (partly) restored by means of prostheses anchored to osseointegrated implants (Feine et al., 2006).

Although the surgical trauma of implant placement may induce the degeneration of environing neural fibres, histology indicates some reinnervation occurs around osseointegrated implants (Lambrichts, 1998; Wang et al., 1998). Nerve fiber endings are even abundantly present at the implant-bone interface (Wada et al., 2001; Weiner et al., 1995). Sprouting nerves close to the bone-to implant interface gradually increase during the first weeks of healing (Wada et al., 2001). These findings have been the basis for a further and long-lasting debate on the presence and the potential function of sensory nerves fibres in the bone and peri-implant environment.

The rehabilitation of tooth loss with an endosseous implant showed an improvement in the sensory and motor capabilities but do not appear to match those of dentate individuals (Jacobs and van Steenberghe, 1993, 2006; Jacobs et al., 1993). This sensory function has been coined ‘osseoperception’. It was defined as a perception of external stimuli transmitted via the implant through the bone by activation of receptors located in the peri-implant bony environment, the periosteum, the skin, the muscles and/or the joints (Brånemark, 1997). This phenomenon has raised questions. Would this special tactile feeling result from a changed force impact through the rigid implant–bone interface, in contrast to the cushioning effect of the skin or mucosa under a socket prosthesis? Or would intra-osseous or periosteal neural endings really be involved? It remains uncertain whether it is attributed to neural endings in the implant-bone interface itself or to neural endings ('osseoreceptors') located at some distance such as the periosteum?

Existing mechanoreceptors in the periosteum may also play a role in tactile function upon implant loading. It is evident that oral implants offer another type of force transfer than teeth, considering the intimate bone-to-implant contact. The elastic bone properties

contrast with the viscoelasticity of the periodontal ligament. Thus, forces applied to osseointegrated implants are directly transferred to the surrounding bone. The resulting bone deformation may lead to receptor activation in the peri-implant bone but also in the neighbouring periosteum which is known to be richly innervated by mechanoreceptors such as Pacini endings (Jacobs et al., 2002a; Sakada, 1974).

Another issue is that oral implants are fixed into the jaw bone but emerge in the oral cavity piercing the soft tissues. Histological findings report that regenerated nerve fiber endings containing substance P invade the superficial layer of the peri-implant epithelium (Tanaka et al., 1996; Trulsson and Johansson, 2002). Their function may be related to pain, touch and pressure (Jacobs and van Steenberghe, 1991).

Histological findings report an increased innervation in the peri-implant epithelium after implant placement (Garzino et al., 1996).

The cortex of the brain reveals a somatotopically ordered representational map for movements that resembles a distorted cartoon of the body (Gray et al., 1993). After limb amputation, the regions of the cortex deprived of a target acquire new targets as a projection area. Remodeling takes place at both a cortical or subcortical level (Braune and Schady, 1993). The potential cortical adaptation and/or plasticity that might occur after tooth extraction and implant placement has not yet been fully explored (Calford, 2005).

An interesting study on mole-rats (Henry et al., 2005) revealed that after lower tooth extractions the oro-facial representation in the primary somatosensory cortex (S1) of the brain located in the lateral post-central gyrus was considerably reorganized. Neurons in the cortical lower tooth representation became responsive to tactile inputs from surrounding oro-facial structures. These data parallel findings observed after deafferentation in the somatosensory hand area of primates where tactile inputs from the chin and upper arm may activate the hand cortical area.

The abovementioned findings have triggered our interest on which receptor groups are responsible for this so-called “osseoperception” phenomenon. Several methodologies are available to explore the issue from a functional viewpoint.

1.1.1. Psychophysics:

Psychophysics dates back to the 19th century. Its central enquiry has remained the quantitative relation between stimulus intensity and sensation. The oral sensory

function has been investigated intensively by psychophysical methods (Jacobs et al., 2002a). These studies are simple and non-invasive and can be even used in a clinical environment. Psychophysics includes a series of well-defined methodologies used to determine for example the absolute threshold level of sensory receptors in man. When performed meticulously and under standardized conditions, this approach may reveal as precise information as neurophysiological setups (Jacobs et al., 2002a; Spiegel et al., 1999).

There are 2 types of psychophysical tests.

Modality tests evaluate the presence of one or more of the four cutaneous sensory functions: pain, heat, cold and touch/pressure. However having a sensory modality does not prove its functionality.

Functionality tests assess the quality of a sensibility modality by either detection or discrimination or more complicated manipulations. Although some of these tests may not identify the specific receptor groups involved, they can properly reflect the oral tactile function. Threshold levels can be determined by active and passive detection or by discrimination tasks (Jacobs et al., 1992a; Jacobs and van Steenberghe, 1991, 1993, 1994; Mericske-Stern, 1994; Mericske-Stern et al., 1995).

In a detection task, the subject has to indicate the presence or absence of a stimulus ("yes" or "no" strategy) while in a discrimination task, the subject has to compare two stimuli ("smaller" or "larger" strategy). In a passive threshold level determination forces are applied to a tooth or to the soft tissues (**fig. 1A**). In an active threshold level determination, an object mostly a foil of a certain thickness, is placed in between two antagonistic teeth (**fig. 1B**). The active tactile function of teeth may thus not only involve periodontal mechanoreceptors but also muscle receptors, joint and even inner ear receptors. The passive test exclusively involves periodontal ligament mechanoreceptors (Jacobs et al., 1992a). An interocclusal discrimination task determines the differential threshold level. For size discrimination with a mouth opening of less than 5 mm, periodontal mechanoreceptive input plays the primary role. For larger mouth openings muscle spindles become predominant.

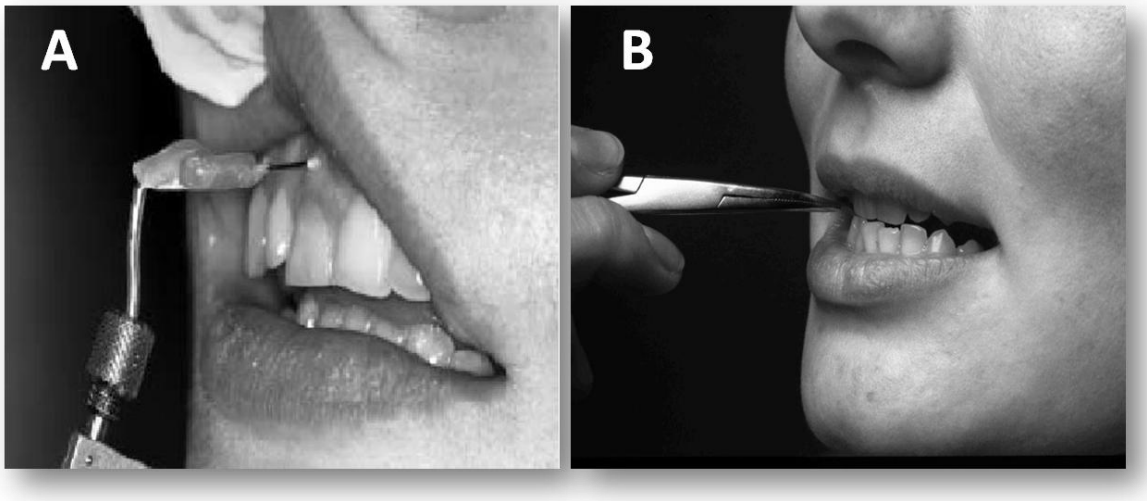


Figure 1. Passive detection test (A) and an active discrimination test (B) of intra-oral testing sites.
In A, passive detection test: two point discrimination with a pressure probe.
In B, active discrimination test: interocclusal discrimination of steel foils.

Other psychophysical functional tests are:

==> Graphesthesia : the perception of figures, ranging from simple lines to complex symbols, such as numbers and letters, drawn on the skin or the oral mucosa (Bender et al., 1982).

==> Two-point discrimination: the ability to differentiate between two points during simultaneous contact. Two points placed at standard distances, usually between 2 to 25 mm (Mackinnon and Dellon, 1985). They can be applied on the skin (Callahan, 1984) or the oral mucosa (Wu, 2000).

==> Size discrimination consists in holding a stick between two fingers or antagonistic teeth or implants. The size discrimination ability is better for antagonistic teeth than for fingers (Morimoto, 1990).

==> Directional cutaneous kinaesthesia is the ability to recognise the direction in which a cutaneous stimulus is moving (Ahmed et al., 2006; Keyson and Houtsma, 1995).

==> Stereognostic sense is the ability to recognise well-defined forms (Ahmed et al., 2006).

Psychophysical sensory testing remains subjective and many variables may intervene with the subject's response (Lundborg and Rosen, 2004). Among the interfering factors are environmental noise, and patient-related factors (Jacobs and van Steenberghe, 1994; Jacobs et al., 1992c; 2002a). When properly performed it can become a valid instrument

of investigation as illustrated by the fact that in monkeys the cutaneous sensory threshold level determined psychophysically coincides with the one determined neurophysiologically (Werner and Mountcastle, 1965).

Any psychophysical task implies cognitive factors such as response bias, guessing strategy and motivation. Many psychophysical procedures have been used to assess the threshold level for various detection or discrimination tasks. A threshold value is usually defined as the level at which a positive response is elicited in 50-75 % of the cases (Gescheider, 1997). In the **method of limits**, the stimulus value is changed at each trial in ascending (**ascending method of limits**) or descending steps (**descending method of limits**), until the subject's response shifts from one answer to another. In detection tasks, the shift implies a change from "yes" to "no" or the reverse. In discrimination tasks the shift may occur between the answers "smaller than" and "larger than". In the **method of constant stimuli**, values are presented in a random order. These approaches have the drawback that a large amount of data is required to avoid response bias or guessing strategies. Adaptive methods have therefore been proposed which means that the subsequent stimulus value depends on the subject's response in the preceding trials. In the up-down or **staircase method**, the stimulus value is changed by a constant amount. When the answer shifts from yes to no or vice-versa, the stimulus intensity is reversed. The threshold is then determined by averaging the peaks and valleys in all runs. When psychophysical methods are accurately applied under standardized conditions their outcome can be compared (Jacobs et al., 1992a; Jacobs et al., 2002b).

Environmental noise To avoid interfering effects, testing should be done in a quiet room with a stable illumination (Jacobs et al., 1992c).

Patient-related variables

Patient-related variables may be psychological or physical. This may lead to an inter- and intra-subject variability. Psychological factors include motivation, level of concentration and anxiety. Some patients may imagine a stimulus when there is none. Others admit experiencing a sensation only if they are absolutely positive that it was felt. The inclusion of false alarms (implying that no stimulus is presented in the specified time interval) may exclude such response bias and the guessing strategy of the subject. A

thorough and standardized instruction to all subjects prior to the actual experiment is of the utmost importance in this perspective.

Physical factors include age, gender, and dexterity:

- Elderly subjects may suffer from deterioration of most sensory modalities (Masoro, 2001). A decline in oral sensory function has also been documented. After the age of 80, the tactile, vibratory function and two-point discrimination of the lip and cheek decreases , but not of the tongue and the palate (Calhoun et al., 1992). The stereognostic ability also declines with age (Muller et al., 1995).
- Gender effect has been established. Women have a lower reflex threshold and pain threshold to cutaneous electrical stimulation than men (Komiyama et al., 2005).Moreover, females were better able to discern lip, cheek and chin position than males (Chen et al., 1995).
- Anxiety: habituation or decreased fear from the experimental set-up may affect the quantitative sensory testing (Komiyama et al., 2008).
- Dexterity may also affect the psychophysical response. No relation was found between dexterity and either oral tactile function or Stereognosis (Jacobs et al., 1992c).

1.1.1.1 Oral tactile function assessment:

To measure light touch sensation, the device most commonly used in clinical neurology, is a set of Semmes-Weinstein monofilaments (Semmes-Weinstein Aesthesiometer®, Stoelting, Illinois, USA]. The original idea dates back from the 19th century when von Frey suggested testing cutaneous light touch by using calibrated hairs of different stiffness by changing their length and hardness. Later on, the so-called von Frey hairs were replaced by nylon monofilaments mounted into a plastic handle (Levin et al., 1978; Poort et al., 2009). This technique has also been applied intra-orally for assessment of light touch thresholds for teeth, implants or oral mucosa (Jacobs et al., 1992b; Jacobs and van Steenberghe, 1993; Wu, 2000). The drawback of this technique is the application of stimuli by means of hand-held instruments. They became more elaborate providing standardized force stimulating conditions for both cutaneous and oral light touch (Dellon et al., 1992; Jacobs and van Steenberghe, 1993; Lundborg and Rosen, 2004).

1.1.1.2 Oral active threshold determination

Periodontal tactile inputs are influenced by tooth position and periodontal status (Jacobs, 1998; Jacobs et al., 1992a; Jacobs and van Steenberghe, 1994; Jacobs et al., 2002a). While vital and non-vital teeth show a similar tactile function (Jacobs et al., 2002a), the latter is impaired by periodontitis, anaesthesia, extraction etc. (Jacobs et al., 2002a). Chewing that involves progressive intrusion of the tooth after each chewing cycle may also affect tactile function. Thus chewing or bruxism may lead to an increase in tactile threshold levels by a factor 60 (Kiliaridis et al., 1990). Moreover, foil materials may have different thermal and mechanical properties, resulting in conflicting results for the interocclusal threshold (Jacobs et al., 1992a). Foil materials with high thermal conductivity (e.g. steel, aluminium) may lower the threshold level by activation of thermal receptors. Foil materials with a low compressibility (e.g. steel) may induce a steep pressure build-up in the periodontal ligament, thus further decreasing the threshold level.

Edentulism reduces the tactile function significantly (Jacobs and van Steenberghe, 1991, 1993; Jacobs et al., 2002a; Jacobs et al., 2001). Even after rehabilitation with a bone-anchored prosthesis, the tactile function remains impaired (**Fig. 2**). The reduced exteroceptive feedback could even lead to an overloading of the prosthesis (Hammerle et al., 1995; Jacobs, 1998; Jacobs et al., 2000; Jacobs and van Steenberghe, 1991). In comparison with the tactile function of natural dentitions, the active threshold is seven to eight times higher for dentures and three to five times higher for implant- supported prostheses (Jacobs and van Steenberghe, 1991).

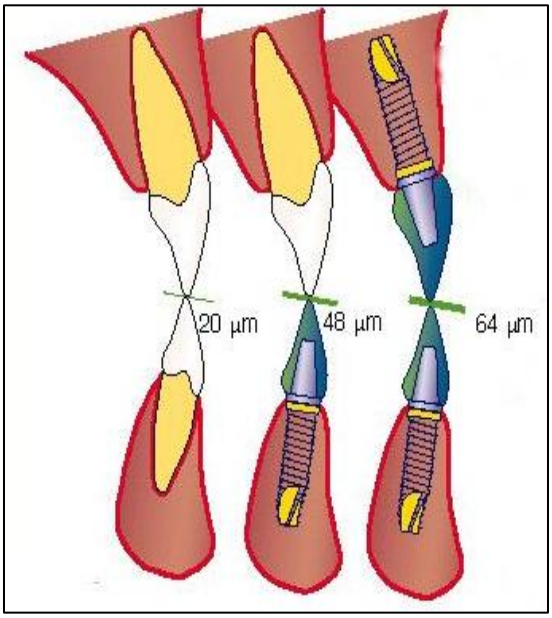


Figure 2. The oral tactile function as evaluated by means of teeth or implant-supported prostheses. The 50% interocclusal thickness detection threshold of steel foils is: 20 µm for tooth/tooth, 48 µm for implant/ tooth and 64 µm for implant/ implant. From Jacobs.R & van Steenberghe D. Clin Oral Implants Res. 1991, 2:75-80

1.1.1.3 Passive threshold determination

During passive threshold level assessment the passive patient has to detect the applied external force. The threshold depends on the frequency and the intensity of the applied force. At low forces, passive threshold levels are much lower for teeth than implants while at suprathreshold force levels, both become equally sensitive. For the passive detection of forces applied to a tooth, different stimulating devices have been developed. In order to avoid the stimulation of more distant receptors, such as the inner ear receptors, progressive force build-up is preferred to tapping forces. To avoid vibrations which through bone conduction may trigger distant receptors a constant contact is secured between the stimulating rod and the investigated tissue (Jacobs and van Steenberghe, 1993) (**Table 1**). The passive detection thresholds are higher 75 times for dentures and 50 times for implants when compared to teeth (Jacobs and van Steenberghe, 1993) (**Table 1**).

Table 1. Factors influencing the tactile function of teeth

Dental status	Active Detection Threshold	Passive Detection Threshold
Vital tooth	20 μm	2 g
Non-vital tooth	20 μm	2 g
Removable prosthesis	150 μm	150 g
Implant–supported prosthesis	50 μm	100 g

Thresholds based on datapooling and averaging from (Jacobs et al., 1992a; Jacobs and van Steenberghe, 1991, 1993)

After rehabilitation with a bone-anchored prosthesis however, edentulous patients seem to function quite well. They become conscious of the mechanical stimuli exerted on osseointegrated implants in the jaw bone (Jacobs and van Steenberghe, 1993). There is a relevant improvement in tactile function with oral implants following a 3-months healing period (El-Sheikh et al., 2003). Some people rehabilitated with osseointegrated implants even note a special sensory awareness with the bone-anchored prosthesis, coined 'osseoperception' (Jacobs, 1998; Jacobs et al., 2000). The existence of this phenomenon could imply that the feedback pathway to the sensory cortex is partly restored with a hypothetical representation of the bone-anchored prosthesis in the sensory cortex. This can allow a more appropriate modulation of the motoneuron pool, leading to a more natural oral function and avoiding overload.

1.1.2. Neurophysiological approaches

Numerous methods can be used to study the neural activity. Some record directly the neural activity while others record the metabolic response induced by the neural activity. These methods differ also by their temporal and spatial resolution (**Fig. 3**).

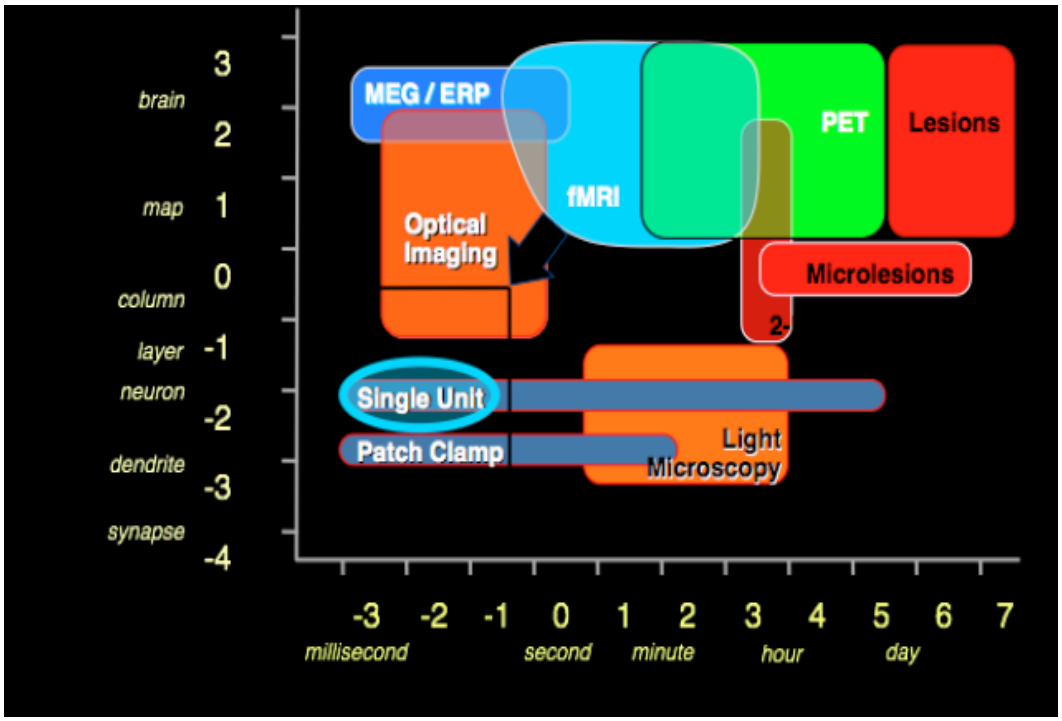


Figure 3. Among the available techniques in humans, fMRI offers a good compromise between temporal and spatial resolution while being non invasive. However combining different techniques is the ideal approach to study in details a functional process. Re illustrated from Gazzaniga, Ivry & Mangun. The new cognitive neurosciences, 2nd ed. 2000

1.1.2.1. Direct measure of the neural activity

1.1.2.1.1. **Electroencephalography** (**EEG**) is used to measure the brain's spontaneous electrical activity over a short period of time via electrodes placed on the scalp of a human. Its temporal resolution is very high but its spatial resolution is low.

1.1.2.1.2. **Evoked Related Potentials** (**ERP**) consist in recording the electrical activity of neurons on the scalp after a specific stimulus. They require a very precise synchronization between the stimulus and the recording, and an averaging of the signal. Recording trigeminal somatosensory evoked potentials (TSEP) in humans is cumbersome and requires particular expertise which explains the few studies that have been performed (Johansson et al., 1988; Trulsson, 2006). This technique offers information on the cortical response of the trigeminal afferent system upon stimulation of oral receptors (Swinnen et al., 2000; Van Loven et al., 2001) (**Fig. 4**). TSEPs can be detected upon intra-oral implant stimulation. It has been shown that endosseous and/or periosteal receptors around the implants are the origin which conveys this sensation (Swinnen et al., 2000; Van Loven et al., 2000).

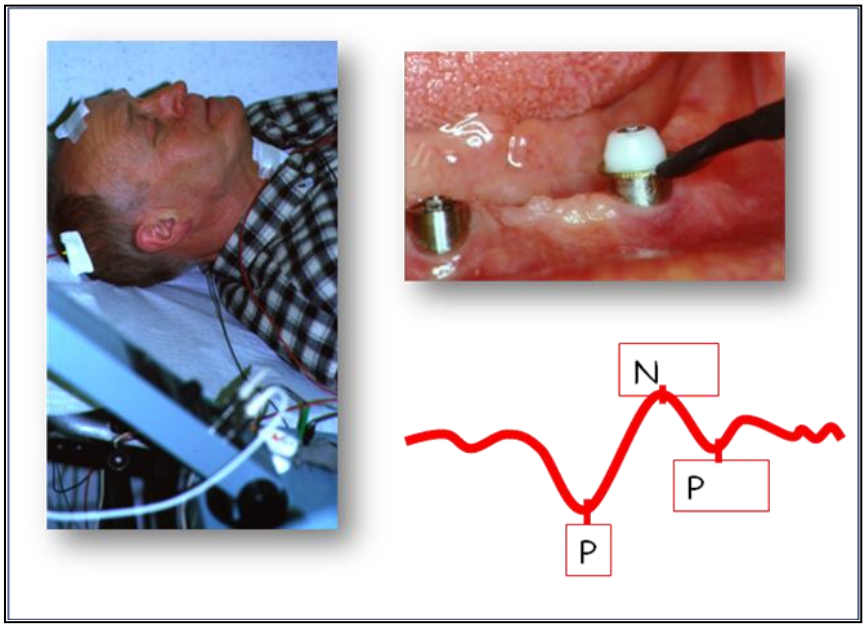


Figure 4. TSEPs recorded from a voluntary subject who is edentulous in the lower jaw, rehabilitated with an overdenture supported by 2 osseointegrated implants.
A ring-shaped stimulation electrode is placed on one of the implants. Example of TSEP traces recorded from the channel Fpz–C'6 or C'5. Negativity is deflected upward. From Van Loven et al.2000

1.1.2.1.3. **Magnetoencephalography (MEG)** measures the magnetic fields produced by the electrical activity of the brain via extremely sensitive devices such as superconducting quantum interference devices (SQUIDs). This technique offers a direct measurement of neural electrical activity with a very high temporal resolution but a relatively low spatial resolution (although better than EEG). Moreover, it is very expensive.

1.1.2.1.4. **Neuronal recordings** is an invasive technique only used in animals and in humans to record the electrical activity in single neurons (single unit recording) or in the immediate vicinity of a group of neurons through the introduction of microelectrodes in the brain. This is the technique of reference in neurophysiology due to its excessively high temporal and spatial resolution (Toda and Taoka, 2004).

1.1.2.1.5. **Electrocorticography and electrocortical stimulation (ESC)** consist in recording the electrical activity neurons directly at the surface of the brain during neurosurgery. It was the basis of the pioneering works of Penfield et al. *(Penfield et al., 1938).* This is the most precise measure of neural activity in humans but its application is obviously limited.

1.1.2.2. Indirect measure of the neural activity

1.1.2.2.1 Relationship between neural activity and local metabolism

More than 100 years ago, experiments conducted on laboratory animals demonstrated the evidence of a macroscopic coupling between regional cerebral activity and blood flow (Roy and Sherrington, 1890).–When neural cells are active, they increase their consumption of energy from glucose and switch to less energetically effective, but more rapid anaerobic glycolysis (Raichle and Mintun, 2006). The local response to this energy utilization is to increase cerebral blood flow (CBF) to regions of increased neural activity, which occurs after a delay of approximately 1–5 seconds. This hemodynamic response rises to a peak over 4–5 seconds, before falling back to baseline (and typically undershooting slightly).

The exact coupling between CBF increase and the local consumption in glucose (CMRglc) and oxygen (CMRO2) has been subject to debate.

The introduction of the deoxyglucose autoradiographic technique enabled spatially resolved measurements of glucose metabolism in laboratory animals and revealed a clear relationship between local cerebral activation and glucose consumption (Sokoloff et al., 1977).

On the other hand, the first quantitative measurements of regional brain blood flow and oxygen consumption in humans were performed using the radiotracer techniques developed by Raichle et al. (Raichle et al., 1976; Ter-Pogossian et al., 1969).

Optical imaging, using either voltage sensitive dyes or intrinsic signals, was used to construct detailed maps of cortical microvasculature in both the anaesthetized and the alert animal (Bonhoeffer and Grinvald, 1991). Near infrared spectroscopy (NIRS) was also used in humans through the scalp (but with a very low spatial resolution). These techniques demonstrated the dynamics changes in cerebral blood flow and volume as well as the variation in oxyhemoglobin (oxyHb) and deoxyhemoglobin (deoxyHb) concentration (**fig. 5**). Based on these studies, it is admitted that during neural activation, regional CBF increases more than CMRO2, leading to local changes in the relative increase in concentration of oxyHb and decrease in deoxyHb.

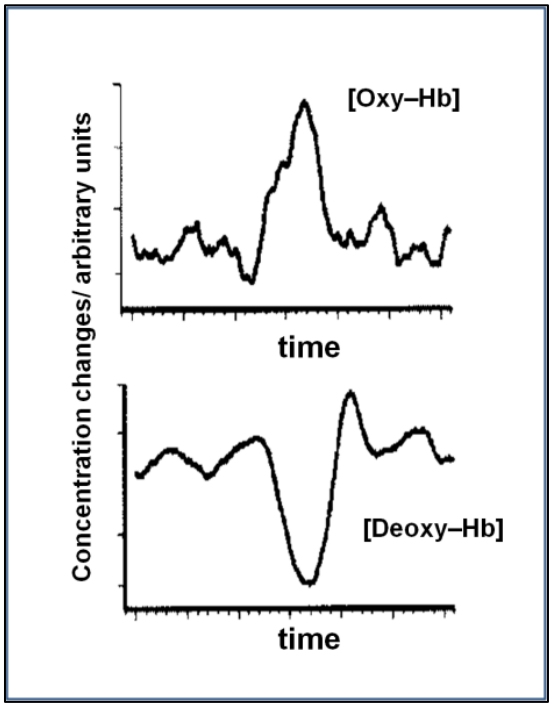


Figure 5. Cerebral blood oxygenation changes induced by visual stimulation in humans.
Local changes of cerebral oxygenation observed in response to visual stimuli by means of near infrared spectroscopy; [Oxy-Hb] rises to its maximum during the stimulation period. The increase in [oxy-Hb] is mirrored by a decrease in [deoxy-Hb] with an almost symmetrical time course. Re drawn from Wenzel R. et al. 1996.

1.1.2.2.2 Imaging techniques in humans

These techniques allow to measure local variations of cerebral metabolism or blood flow related to neural activity and to show in it on 3D maps of the brain.

1.1.2.2.2.1. Positron emission tomography (PET) is a nuclear medicine imaging technique which produces images of functional processes in the body. The system detects pairs of gamma rays emitted from radioactively labelled metabolically active chemicals. The labelled compound, called a *radiotracer*, is injected into the bloodstream and eventually makes its way to the brain. Sensors in the PET scanner detect the radioactivity as the compound accumulates in various regions of the brain. Different ligands are used to map different aspects of neurotransmitter activity. The most commonly used PET tracer is a labelled form of glucose (Fludeoxyglucose (^{18}F-FDG) which measure CMRglc. However, its low temporal resolution makes it not practical for functional studies (Ogawa et al., 1995). CMR02 can also be measured by PET using $^{15}O_2$ (Iida et al., 2000). It is the measure of regional CBF by the injection of $H_2{}^{15}O$ labelled water that opened the doors to widespread functional studies of the working human brain (Law, 2007). However, the technique is semi-invasive and

expensive, and its spatial and temporal resolution remains limited. Therefore, it has been supplanted by magnetic resonance imaging.

1.1.2.2.2.3. Magnetic resonance imaging (MRI) is a totally non invasive technique that uses magnetic fields and radio waves to produce high quality two- or three-dimensional images of brain structures without use of ionizing radiation (X-rays) or radioactive tracers. During an MRI, a large cylindrical magnet creates a magnetic field around the head of the patient through which radio waves are sent. When the magnetic field is imposed, each point in space has a unique radio frequency at which the signal is received and transmitted (Grossman et al., 1994). Dedicated coils read the frequencies and a computer uses the information to construct an image.

Depending on the chosen technique, images covering the whole brain are generally acquired in a few minutes but fast imaging like echo-planar-imaging (EPI) produce an image in less than 100 ms. MRI provides us with high resolution images and offers a high contrast between tissues. Indeed, five different tissue variables — spin density, T_1 and T_2 relaxation times and flow and spectral shifts can be used to construct images. By changing the parameters on the scanner, one can create different contrast between body tissue, and that makes this technique extremely powerful for numerous applications. Moreover, contrast agent may also be injected intravenously to enhance some contrasts and highlight some physiological properties. The first successful use of MRI for a functional study in human was reported by John W. Belliveau and colleagues in 1991 using an intravenously administered paramagnetic contrast agent (Gadolinium) (Belliveau et al., 1991b). It demonstrated the local increase in cerebral blood flow and volume in the occipital cortex during visual stimulation.

Later, the measure of CBF by arterial spin labelling were also used (Melzer et al., 2011) but the huge development of functional MRI (fMRI) was based on the use of blood-oxygen-level dependence (BOLD) contrast.

1.1.2.2.2.3. Blood-oxygen-level dependence (BOLD) fMRI

Blood-oxygen-level dependence (BOLD) is the MRI contrast of blood deoxyHb, first discovered in 1990 by Ogawa (Ogawa et al., 1990).

During neuronal activation the CBF increases but the increase is not commensurate in CMRO2 (Fox and Raichle, 1986). This results in an increase in the relative oxyHb

concentration and in a decrease in the relative deoxyHb concentration, therefore creating the BOLD signal (Belliveau et al., 1991a; Belliveau et al., 1990) (**Fig. 6**). OxyHb is diamagnetic and does not influence the magnetic field. DeoxyHb is paramagnetic and, when compartmentalized in blood vessels, it creates intravoxels inhomogeneities in the magnetic field and phase shifts called the "susceptibility" effect (mainly dependent on the T2* effect). The BOLD contrast consists in a local increase of the signal due to the relative decrease in deoxyHb concentration. It is better seen on sequences sensitive to the T2* effect and at high magnetic fields.

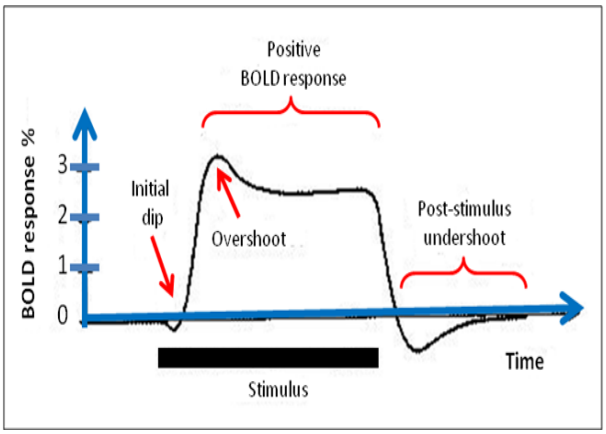


Figure 6. BOLD time course.

***% signal change**= (point – baseline)/ baseline usually 0.5- 3%; **initial dip**= more focal and potentially a better measure; **time to rise** = signal begins to rise soon after stimulus begins; **time to peak** = signal peaks 4-6 sec after stimulus begins; **post stimulus undershoot** = signal suppressed after stimulation ends. Redrawn from Jody Culham tutorials.*
http://psychology.uwo.ca/fMRI4Newbies/Tutorials.html

As the method is based on a signal difference, it requires the comparison between images taken during the task of interest and images taken during rest (or a task of reference). The signal difference is very small, but T2*- EPI sequences allow to obtain images very quickly and to accumulate a large number of images during the task. From this huge amount of data, statistical methods must be used to determine which pixels reliably show a signal difference between the activated periods and the rest periods, and therefore which areas of the brain are active during the task of interest.

Two different experimental paradigms can be used: a blocked and an event-related design. During runs with a blocked design, stimuli are applied continuously for a period of typically 15 to 30 seconds. Active blocks alternate with rest blocks. During each run, active blocks per condition are completed in a randomized or pseudorandomized order. This design is very robust because it allows a stabilization of the BOLD signal during each block. However, it is not adapted to some neurocognitive experiments which cannot accommodate repetitive stimuli. On the other hand, event-related design consists in a series of consecutive isolated stimuli applied in a pseudorandomized fashion. The

BOLD response to a single event can be recorded but is much more subtle than with block designs. To distinguish each event, a slow design can be applied to allow the recovery of BOLD signal to baseline between two stimulations, but this makes the experiment very long. A rapid design does not wait for signal recovery between events but the onset of stimulation is jittered by an interstimulus interval to allow the use of deconvolution methods. This fastens the experiment but the method is less robust.

The statistical methods used in fMRI are complex. First of all, the data must be pre-processed to correct for motion during the acquisition and for other artefactual effects. This includes 3D data realignment and filtering for removing unwanted low or high frequencies signal variation. Spatial and/or temporal smoothing may also be performed to increase the signal-to-noise ratio. At this point the data provides a time series of samples for each voxel in the scanned volume. A variety of methods are used to correlate these voxel time series with the task in order to produce maps of task-dependent activation. There are many software packages available for analyzing fMRI data. Many of them are based on the general linear model (GLM) providing a modelization of the signal time course through different predictors (Friston et al., 1995). Regressions and t-tests are then applied but the need and the way to correct for multiple comparisons is a matter of debate. As the comparisons are made on a large amount of voxels, some correction of the level of significancy is necessary to avoid false positive results (activation only by chance). However, the signal in adjacent voxels is not independent and a correction based on the Bonferroni method is too severe. Therefore, other models have been developed like a threshold based on a cluster of activated voxels or a qFDR correction. Working by regions of interest may also be appropriate, therefore reducing the number of compared voxels. When looking at individual results, we can admit a more liberal threshold because of the lack of statistical power and because the level of activation is variable from one subject to another. The thresholded activation maps are then superimposed on individual's anatomical image to visualize the activated areas.

When the results from several subjects must be grouped and compared to previous studies, normalization of the images to a “standard” brain must be done. The most commonly used template is based on the work of Talairach and Tournoux (1988) who described the brain of an old woman in a three dimensions (3D) coordinates system (Talairach and Tournoux, 1988). The Montreal Neurological Institute (MNI) template is based on the MRI of more than 100 young subjects, and uses the same kind of 3D

coordinates. When performing multisubject studies, the brain of each individual must be deformed to correspond at the best to this template. This allows grouping the subjects and describing the localization of the activated foci with their x, y and z coordinates. Fixed effect (FFX) group analysis simply adds the data of several subjects but the final result is dependant of the level of activation of each subject and it cannot be extrapolated to the entire population. On the other hand, random effect analysis (RFX) is a statistical method which generalizes the results to the entire population and is by far the most preferred method.

1.1.2.3 Use of fMRI in the oral area:

The study of the tactile function of teeth and implants by fMRI has so far received hardly any attention. The oral area is characterized by an exquisite tactile sensitivity. Moreover, the oral motor behaviors and the control of the jaw muscles are controlled by sensory signals from a variety of sensory organs that includes mucosal and periodontal innervations. If complete tooth loss occurs, dentures only partially restore oral function. When a dental prosthesis is anchored or supported by oral endosseous implants, the sensory motor function improves. The cortical changes, associated with tooth loss and their eventual replacement by implants, needs investigation since data in this field are scarce. To elucidate this matter, invasive and non invasive techniques have been applied. Thus fMRI seemed to offer a tremendous potential to better understand the changes in cortical behavior that may occur after loss of teeth and their replacement by osseointegrated implants.

A Pubmed research has shown that studies concerning the tactile function of teeth investigated with fMRI are limited (**Tables 2 and 3**).

Most of these studies were carried out on healthy volunteers, and sample sizes remain small. The limitation of the number of patients does not allow group comparisons. This means that generalization of results should be performed with caution. Some papers do mention a relatively large number of subjects, up to 20 (Yan et al., 2008). However, this sample of 20 subjects consists of a heterogeneous group of subjects, with different rehabilitations who are further subdivided into 2 different categories, with varying stimulations preventing any group comparison (**Tables 2 and 3**).

The use of fMRI to explore the tactile function of the oral area requires MRI compatible stimulation devices, i.e. non ferro magnetic. Different kinds of stimuli and stimulation

devices have been used; mechanical (cotton-wool tips, pneumatic, vibratory…) electrical, thermal… (**Table 2**).

These devices can be divided in two categories:

- Manual stimulation, in which ➔ group comparison is problematic
- Automatic stimulation which are surely ➔ more standardized offering possibilities for more sensitive registration, small variance of bold signal and an increased brain activity

While an electrical stimulation device provides a standardized stimulus, the latter rather triggers pain than tactile units. It may also produce imaging artifacts by electro-magnetic pulses. Electrical stimuli show a higher inter-subject threshold level variance when compared to tactile or thermal stimulation. The vibrotactile stimulation devices are difficult to customize and the stimulation intensity can vary during the experimentation. Several researchers used von Frey filaments which are used as a standard physiologic mechanical testing. Von Frey testing can be normalized on an individual basis. If we consider fMRI regarding tactile tooth stimulation (**table 2**): 7 articles were found. In 3 of these articles, the stimulation of teeth during fMRI was done electrically (Ettlin et al., 2009; Jantsch et al., 2005; Weigelt et al., 2010), while in the 4 other articles the teeth were stimulated mechanically (tooth brush, von Frey monofilaments or vibratory) (Ettlin et al., 2004; Habre-Hallage et al., 2010; Miyamoto et al., 2006; Trulsson et al., 2010). While electrical stimulation is capable of giving S1, S2 activations, vibratory stimulation does not (table 2). Accordingly, the type of stimulation may affect the results. Because of insufficiency of available studies and results and considering the variety of stimulation techniques, more systematic research in the field of oral sensory mechanisms and their brain projections is needed.

Table 2. Summary of the literature on the tactile function of the oral area investigated with fMRI

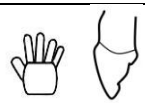
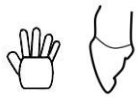
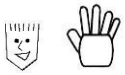
		Methodology				Imaging results	
Authors	**Year**	**N**	**subjects**	**Situation**		**stimuli**	**Activation**
Trulsson M et al.	2010	10	Healthy	**Oral area** : teeth		**Vibrotactile stimulation :** piezoelectric stimulator	S1, S2, insular, inferior frontal gyrus, inferior parietal lobe and supplementary motor area as well as middle frontal gyrus and cerebellum. S1 and S2 at 20 Hz, no significant activity at 100 Hz.
Habre-Hallage P et al.	2010	8	Healthy	**Oral area** : teeth		**Tactile stimulation:** von Frey monofilaments	Teeth : S1 and S2 either on the ipsilateral, contra-lateral or both sides Thumb : contralateral S1 and either ipsilateral or contra-lateral S2
Weigelt A et al.	2010	13	Healthy	**Oral area** : Upper and lower canines		**Nociceptive electrical stimulation :**	SpV ipsi, pons contra, thalamus (ventral) ipsi, Medial dorsal nuclei bilat., bilaterally in S1, cingulate, insula. No difference between upper and lower teeth.
Said Yekta S et al.	2009	20	Healthy	**Virtual dental treatment**		**Video clips**	pain-related brain areas : cingulate cortex, insula, S1, S2
Ettlin DA et al.	2009	14	Healthy	**Oral area** : teeth		**Electrical** of increasing intensity➔ canine	superior parietal lobule, superior temporal gyrus/ anterior insula, inferior and middle temporal gyrus, lingual gyrus, anterior cingulate, and caudate nucleus.
Kopietz R et al.	2009	30	Healthy	**Face**: skin (V1, V2, and V3)		**Tactile stimulation:** Pneumatic device	S1 contralaterally and S2 bilaterally. Somatotopic organization not detectable
Moulton EA et al.	2009	12	Healthy	**Face**: skin (V1, V2, and V3)		**Tactile stimulation:** Brush (1Hz, 15 s)	somatotopic activation in the post-central gyrus
Dresel C et al.	2008	8	Healthy	**Face**: skin (V1, V2, and V3) and thumb		**Tactile stimulation:** von Frey monofilaments	S1, S2, PMC and th
Sörös P et al.	2008	6	Healthy	oropharynx		**Tactile stimulation:** Brief air pulses	S1 and th, PMC, SMA, cingulate motor areas, insula and frontal cortex.

Table 2. Continued

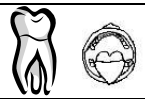
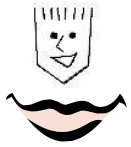
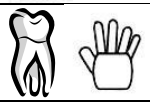
		Methodology				Imaging results	
Authors	**Year**	**N**	**subjects**	**Situation**		**stimuli**	**Activation**
Guest S et al.	2007	5	Healthy	**Oral area: mouth**		**Thermal stimulation:** Liquid in the mouth at 5, 20 and 50 °C	IC, ACC, a part of the somatosensory cortex, the orbitofrontal cortex and the ventral striatum.
Huang RS et al.	2007	6	Healthy	**Face, lip and finger**		**Tactile** "Dodecapus" computer-controlled pneumatic system air puffs	M1, PMv, PZ, S1, S2, PV, AIP and VIP
Miyamoto JJ et al.	2006	5	Healthy	**Oral area** : tooth, tongue, lip		**Tactile stimulation:** brush on the tooth, tongue, lip	In the the GPoC, teeth representation was located superior to that of the tongue and inferior to that of the lip
Ettlin DA et al.	2004	5	Healthy	**Oral area** : teeth		**Mechanical vibratory:** pneumatically driven piston	IC bilaterally and the supplementary motor cortex No activation in S1 or S2
Iannetti GD et al.	2003	14	Healthy	**Oral area**: Skin left side forehead (V1) and left lower lip (V3)		**Mechanical stimulation:** cotton swab /2Hz same operator	**S1**and **S2**; V3 ➔S1and S2 ipsilaterally V1 ➔ S1 and S2 bilaterally
Binkofski F et al.	1998	5	Healthy	**Oral area**: esophagus		**Mechanical stimulatation:** Repetitive distension 0.5 Hz; 1 Hz	< 0.5 Hz ➔ S2 0.5 Hz ➔ S2, S1 and premotor cortex 1 Hz ➔ S2, S1, premotor cortex and insula Painful ➔ anterior cingulate cortex
de Leeuw R et al.	2006	9	Healthy	**Face**: skin \ the masseter muscle		**Thermal stimulation:** painful hot stimulation	IC, cingulate gyrus, Th, GpoC, right middle and inferior frontal gyri, cuneus, precuneus, and precentral gyrus
Chen Y et al.	2006	8	Healthy	**Oral area :** maxillary bicuspid		**Anticipation of dental pain** : dentinalgia	S1, SMA and CB
Jantsch HH et al.	2005	8	Healthy	**Oral area** tooth and hand		**Electrical ➔** tooth **mechanical painful stimulation ➔** hand	S2 and IC bilaterally activated by both stimuli hand painful stimulation ➔ S1 tooth pain ➔ S1 bilaterally

Table 2. Continued

Methodology						***Imaging results***
Authors	***Year***	***N***	***subjects***	***situation***	***stimuli***	***Activation***
Borsook D et al.	2003	9	Healthy	facial skin (V1, V2, and V3) 	**Mechanical : brush** **Thermal: 46^{o}C** To the 3 divisions of the trigeminal nerve	Ipsilateral trigeminal nucleus somatotopically
DaSilva et al.	2002	9	Healthy	facial skin (V1, V2, and V3) and thumb 	**Noxious thermal: 46^{o} C**	ipsilateral spinal trigeminal nucleus in the contralateral thalamus

Table 3. Summary of the literature on the motor function of the oral area investigated with fMRI

Methodology						***Imaging results***
Authors	***Year***	***N***	***subjects***	***situation***	***stimuli***	***Activation***
Yan C et al.	2008	12 8	Impl supported full dentures Complete dentures	**Mouth** 	Motor: **Clenching task**	S1, prefrontal cortex, Broca's area, premotor cortex, supplementary motor area, superior temporal gyrus, insular, basal ganglion and hippocampus with implant supported fixed dentures
Kordass B et al.	2007	13	Healthy	**Mouth** 	**Chewing** and centric **Occlusion** on natural teeth or on occlusal splints	Chewing : sensorimotor cortex. Mainly ipsilaterality, tap-tap movements on natural teeth and splint occlusion : only one activation foci.
Guest S et al.	2007	12	Healthy	**Mouth** 	**Thermal stimulation :** cool 5, 20 and warm 50	Insula, somatosensory cortex, the orbitofrontal cortex, the anterior cingulate cortex, and the ventral striatum, orbitofrontal cortex and pregenual cingulate cortex
Byrd KE et al.	2009	10 10	Healthy Bruxers	**Mouth** 	**Clenching and grinding**	motor cortical (supplementary motor area, sensorimotor cortex and rolandic operculum) and subcortical (caudate)
Havel P et al.	2006	15	Healthy	**Hand, Foot, mouth & Tongue** 	simple **Motor**-paradigms	pre- and postcentral gyri, paracentral lobule and the supplementary motor area
Soltysik DA et al.	2006	5	Healthy	**Jaw and tongue** 	**Chewing**	an appropriate task duration, three motion-sensitive postprocessing methods allow the use of block design in an fMRI study of a jaw motion task.
Fang M et al.	2005	21	Healthy	**Hand and mouth** 	**Chewing, opening and closing of mouth**	the prefrontal cortex, insula and cingulate gyrus

Table 3. Continued

Methodology						***Imaging results***
Authors	***Year***	***N***	***subjects***	***situation***	***stimuli***	***Activation***
Sakreida K et al.	2005	19	healthy	**Fingers and mouth; knee, ankle, elbow, and wrist; trunk and shoulder** 	cycles of **Intransitive Motion**	extended PM activation for each motion condition
Lotze M et al.	2000	7	healthy	**Lip and tongue** 	**Repetitive** Lip and vertical tongue **Movements: Speech**	in M1 and S1 revealed /pa/- adjacent to lip and /ta/- to tongue, /pataka/ a combination of M1/S1 and in SMA
Lotze M et al.	2001	14 upper limb amputees 7 healthy		**Hand and lip** 	Hand and **Lip Movements** and imagined movements of the phantom limb	Displacement of the lip representation in the primary motor and somatosensory cortex was positively correlated to the amount of phantom limb pain
Dresel C et al.	2005	15	healthy	**Mouth** 	**Whistling**, coordinated orofacial movement with auditory input	ventral PMC, CB and somatosensory areas cingulate cortex, basal ganglia, amyg. and Th
Grodd W et al.	2001	46	healthy	**lips, tongue, hands, and feet** 	**Motor tasks**	superior and inferior cerebellum
Watanabe J et al.	2004	24	healthy	**tongue** 	three blocks; **Resting** of the tongue, **Tongue Movement** and tongue **retraction**	primary sensorimotor area and supplementary motor area bilaterally, and in the left inferior parietal lobule
He AG et al.	2003	18	healthy	**tongue** 	**Tongue Movement** and **reading** Chinese pinyins and logographic characters	M1, SMA, Broca's area, and Wernicke's area.
Shinagawa H et al.	2004	6	healthy	**tongue** 	**Chewing**: before and after gum-chewing	bilateral S1/M1➔ signal intensity and the area of activation significantly increased after 10 min of chewing

Legend of tables 2 and 3:
S1 , primary somatosensory cortex; S2 , secondary somatosensory cortex; IC, insular cortex; ACC, anterior cingulated cortex; Th, thalamus; CB, cerebellum; PMC, premotor cortex; M1or MI, primary motor cortex; PMv, ventral premotor cortex; PV, parietal ventral area; PZ, polysensory zone ; AIP, anterior intraparietal area; VIP, ventral intraparietal area ; SMA, supplementary motor area; GpoC, postcentral gyrus; V1–3, trigeminal divisions; Ipsi, ipsilateral; Contra, contralateral; Bilat, bilateral

Motor tasks applied in the oral area involve different parts such as the muscles, the skin and the TMJs… Consequently, the images of the activated areas obtained with fMRI are more difficult to isolate. On the other hand, although it is more intricate to use tactile stimulation of the teeth, yet these can generate more precise results where the activations are precisely linked to the teeth. However, with all the performed studies, there are still unanswered questions (Feine et al., 2006; Klineberg et al., 2005) concerning:

1. The types, locations and properties of receptors that are activated by stimulation of implant-supported prostheses require elucidation.
2. The central neural pathways and properties of neurons in these pathways are largely unexplored.
3. Detailed investigations of the possible sensorimotor cortical adaptive processes (neuroplasticity) that may be associated with the loss of teeth in the first place or with their replacement are lacking.

1.2 Aims of the thesis

The overall aim of this thesis is to offer proper (partial) answers to these questions by:

*** The assessment of the sensory tactile function in peri-implant soft tissue**. Longitudinal studies are needed to monitor changes that occur after tooth loss and implant placement and their loading. To elucidate this question, psychophysical methods were used to investigate if changes in the sensory tactile function of the peri-implant soft tissues occur over time. A differentiation between the effect of surgery as such, and the presence of load bearing implants was also performed. The methodologies used were

- von Frey monofilaments and two point discrimination (Chapter 2).
- Graphaesthesia and Kinaesthesia (Chapter 3).

Hypothesis*:* The sensory tactile function of the peri-implant soft tissues may recover over time and could be identified by psychophysical tests.

*** The exploration of the mechanisms of osseoperception using functional magnetic resonance imaging (fMRI)**. The fMRI has a tremendous potential to understand the possible sensorimotor cortical adaptive processes that may be associated with the loss of teeth or with their replacement by endosseous implants.

The ultimate objective would be to understand how humans adapt (or not) to an altered oral environment and how clinical approaches aiming at restoring orofacial function may produce their rehabilitative effect. This part of the investigations was done in an orderly step-by-step manner.

- The development of a new stimulation device MRI compatible and capable of applying standardized physiologic tactile stimuli to the teeth and other parts of the face (Chapter 4).

Hypothesis : a new calibrated physiologic stimulation is able to trigger the periodontal mechanoreceptors during fMRI.

- To establish a three-dimensional human cortical map of periodontal mechanoreceptos based on painless tactile stimulation of the upper incisors and canines (Chapter 5).

Hypothesis : fMRI is able to unveil the cortical representation of teeth in the human cortex.

- To identify the possible sensorimotor cortical adaptive processes that may be associated with the loss of teeth and their replacement by endosseous implants (Chapter 6).

Hypothesis : the use of fMRI associated to a punctuate mechanical stimulation of osseointegrated oral implants can activate cortical somatosensory areas.

Sensory Tactile Function Of Peri-Implant Soft Tissues Identified By Von Frey Monofilaments And Two Point Discrimination

Chapter 2

This chapter has been published as:

Habre-*Hallage P., Bou Abboud-Naaman N., Reychler H., van Steenberghe D., Jacobs R.* (2009) Assessment of changes in the oral tactile function of the soft tissues by implant placement in the anterior maxilla: a prospective study. Clin Oral Investig. 2010 Apr;14(2):161-8. Epub 2009 Apr 16

Abstract

The aim of the present study was to assess the somatosensory function in the peri-implant soft tissues in the anterior jaw bone by means of two psychophysical tests.

Light-touch sensation (LTS) and two-point discrimination (2PD) were performed before, and at planned intervals until 18 months after the placement of one or two implants in the anterior maxilla. The same tests were used on the contralateral control sites. The psychophysical threshold was determined by performing the staircase method. The mean values and standard deviation of LTS and 2PD, pooled over the four sessions at each test area, were calculated.

Despite a large intersubject variation in both the LTS and 2PD, significantly high intra-individual correlations were found ($P<0.005$). For LTS, the thresholds were not significantly affected over time ($P>0.05$) on both implant and control sites. The 2PD increased significantly after surgery and maintained the higher discriminatory sense for 1 year (P-value 0.005). The control sites remained stable over time. However, no correlation was revealed between LTS and 2PD perception (Pearson correlation test).

In this prospective study, no major differences between the different sites and testing sessions were reported; except for the 2PD thresholds which were lowered after implant surgery. These findings suggest that the regenerated nerves may be responsible for the increased 2PD sensitivity in the peri-implant soft tissue. The unchanged LTS thresholds did not allow to confirm this hypothesis.

2.1. Introduction

The control of oral motor behavior relies on a variety of receptors such as the periodontal mechanoreceptors and the intradental nociceptors (Jacobs and van Steenberghe, 1994). Tooth loss will remove these receptors and reduce the inputs to the brain. The feedback pathway is considerably damaged (Klineberg and Murray, 1999). After tooth loss, the socket becomes filled by bone. Nerve endings and mechanoreceptors are damaged and remaining nervous tissue may no longer be stimulated and thus lead to nerve degeneration (Hansen, 1980a) or nervous branches may start sprouting and simply provide innervation to some more distant structures, like the overlying healed soft tissues (Desjardins et al., 1971). The remaining receptors in the gingiva, alveolar mucosa and periosteum may partly take over the exteroceptive function (Jacobs et al., 2001). Different types of mechanoreceptors were identified in the oral mucosa. They include Meissner's corpuscules, glomerular endings, Merkel cells, Ruffini-like endings, and free nerve endings.

The mechanoreceptors in the denture-bearing gingiva play a predominant role in trigeminal motor control (Garzino et al., 1996; Mericske-Stern, 1994); however dentures may only partially restore jaw function (Jacobs et al., 2002a). When Tübingen implants were used, teeth were more sensitive than implants at low forces application but were equally sensitive at higher forces up to 1400cN (Muhlbradt et al., 1989; Mühlbradt et al., 1990). With oral implants, the sensory and motor functions seem to improve but fail to reach the same level of sensitivity as dentate subjects (El-Sheikh et al., 2003; Jacobs and van Steenberghe, 1993, 2006; Lundqvist, 1993; Lundqvist and Haraldson, 1992). Hence, it remains uncertain whether this improvement can be ascribed to 'osseoreceptors' located in the periosteum or within the bone marrow itself (Rowe et al., 2005).

Oral implants are fixed into the jaw bone, but emerge through the keratinized or alveolar mucosa. The peri-implant junctional epithelium, including its neural components, is similar to that of natural teeth (Marchetti et al., 2002).

Regenerative nerve fibers, invaded the superficial layer of the peri-implant epithelium. These nerve fibers contain substance P and possess free nerve endings. Their functions might be a sensory system for pain, touch and pressure (Tanaka et al., 1996; Weinstein, 1962). Merkel cells are important in tactile function and they are normally found in the oral mucosa and in the gingiva. They seem to be absent in the

hamster's peri-implant epithelium mucosa (Tanaka et al., 1996) but were found in the peri-implant mucosa in humans (Marchetti et al., 2002). Indeed, histological findings report an increased innervation in the peri-implant epithelium after implant placement (Weinstein, 1962). Their presence in the periosteum has not been described in the literature (Macefield, 2005).

From the current evidence it remains unclear whether an altered innervation (from periodontal to peri-implant) may have changed the tactile function of implant-rehabilitated sites. To elucidate this question, psychophysical methods can be used. These are non-invasive, and well defined techniques. They allow to relate the physiological functions of the receptors to the subjective experience of the subject (Jacobs et al., 2002a).

The objectives of this prospective study were to assess the sensory tactile function in peri-implant soft tissue and to investigate if changes in the sensory tactile function occurred over time.

To reach this goal, tactile thresholds of the keratinized and/or alveolar mucosa surrounding oral implants in patients were determined and compared to the controlateral dentate site: (i) before implant placement ; (ii) after implant placement but before implant loading; (iii) after prosthetic rehabilitation to detect if any change occurs over time.

2.2. Materials and Methods

2.2.1. Subjects:

9 dentate adults (ages 19–32 yr), three males (5 implants) and six females (7 implants) were selected based on their dental status. Subjects included had a complete natural dentition with the exception of one or two teeth missing in the anterior region of the maxilla (incisor teeth). These patients had to be rehabilitated with osseointegrated implants. (table1) The implant insertion was made by the same operator at modum Brånemark. The implants healed under the closed mucosa during a period of 3 to 5 months. The abutments were mounted on the implants one month after the second surgery time.

None of the subjects had a history of any neurologic disorder or periodontitis or dysesthesia in the oral cavity. Informed consent was obtained from each participant prior to investigation. Two sensory tests, including light-touch sensation and two point

discrimination were used to determine the passive threshold (without any physical action of the subject). The subjects were asked to report the presence of the object, as soon as it was perceived.

The four consecutive measurements were performed over a period of 18 months: In each subject, one implant was stimulated while the tissues surrounding the contralateral natural tooth served as a control.

The patients were tested in a quiet room with stable illumination while seated comfortably in a dental chair. A protocol form of all testing procedures was presented and explained to the subject before the actual test, the probes were shown to the subject to alleviate his or her apprehension regarding the testing procedure. All the tests were performed at the buccal site of the keratinized or alveolar mucosa in the anterior maxilla for both right and left sides. The subjects were instructed to close their eyes during the whole testing procedure.

2.2.2. Tactile detection threshold or light touch sensation

The tests were performed by using von Frey filaments (Bioseb™, Chaville, France). This device consists of 20 monofilaments, all of constant length but having a stepwise progression of diameters. Each monofilament is labeled with a number that represents the log10 of the force (mg) required to cause the filament to bend **(Fig 1)**.

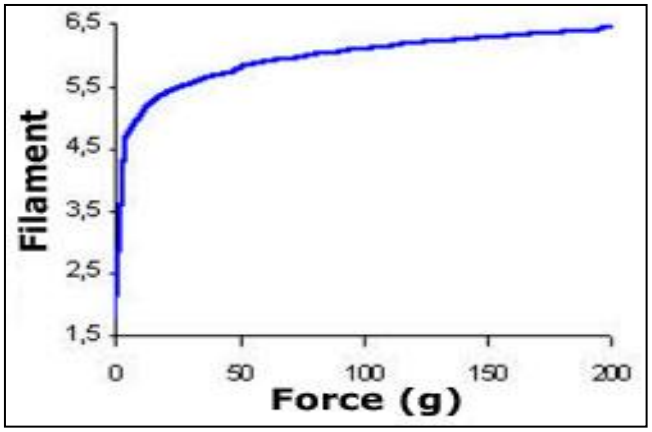
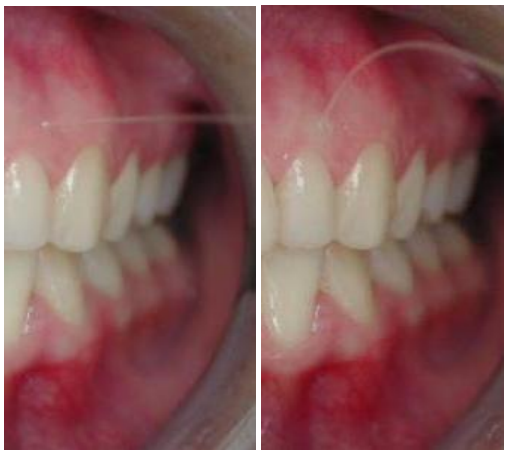


Figure 1. The relation between the filament number and the force (g) developed by the filament bending is reported in this graph provided by the manufacturer (Bioseb™, Chaville, France)
The filament number represents log10 of the force (mg) required to cause the filament to bend.

The number of the filaments (1.65–6.65) corresponds to a logarithmic function of the equivalent forces of 0.008–300g, according to the manufacturer.

When the tip of a fiber of given length and diameter was pressed against the tested area at right angles, the force of application increased as long as the researcher continued to advance the probe, until the fiber bent. After bending, continuous probe advancing may induce more bending, but not more force of application which made it possible to apply a reproducible force, within a wide tolerance, to the tested surface.

The filament should not be allowed to slip but must remain pinned to the gingiva at the point of initial contact. The force is continuously applied for one second and then removed (**Fig 2)**.



a b

Figure 2. The use of the Light touch sensation instrument (Von Frey Filament, Bioseb™, Chaville, France):
(a) the filament was applied on the tested area at right angles
(b) the filament was pressed until the fiber bent

The subjects were instructed to respond“yes” (i.e., contact was felt during the stimulation) or “no” (i.e., contact was not felt during the stimulation).

The threshold calculation was determined by performing the psychophysical staircase method [10].. A first filament is applied. If the subject reports a negative answer (does not detect a pressure) a filament with a larger diameter is used and appliedwith increasing intensity until the subject reacts then the pressure is immediately highered by using a larger filament. This procedure continued until eight minimum values were recorded and the threshold was calculated as the average of these values. Fake stimuli – which means approaching the subject with a probe but turning the probe slightly so that no contact with the tissue was achieved - were applied after peaks 5 and 11 as false positives. These may have occurred when the subject detected the movement of the examiner’s hand as it approached with the probe. If the subject did not report a

sensation during the blank stimuli, the test was continued. If he did, the test was discontinued and the subject was questioned about what kind of stimulus he had perceived. The whole procedure was explained again and the test was restarted later. The stimulation sessions were interleaved with periods of rest of 5 minutes to avoid fatigue.

2.2.3. The tactile spatial acuity thresholds or two point discrimination thresholds:

Two-point discrimination thresholds can be done with ordinary dividers. When the closed dividers touched the skin, the perception is of being touched with only a single point. As the dividers are opened more and more on successive applications to the skin, at a certain distance, the perception is of being touched at two points. This test was performed using a dedicated custom-made device [24]. A tip made by a 1.5mm diameter wire was connected to a hinge handle of a constant force periodontal probe (Brodontic™, Prima, Byfleet, UK), which can apply a constant 25 N force to oral tissue surfaces. 15 plastic disks were made by self curing acrylic resin in which two wires were embedded at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20mm). Each wire was sphere-shaped at the end. This test was performed very carefully to make sure that these two points simultaneously contacted the tissue surface about 2s, although one of these two points might touch prior to another **(Fig 3)**.

Figure 3. The two-point discrimination test
The two-point discrimination instrument was applied on the tested area for two seconds then removed.

In order to convince the subjects that the sensation of one or two points was possible they were demonstrated that either one or two points would be in contact with

the alveolar mucosa. In fact, only the blank stimuli were tested with one probe. All other stimuli involved two simultaneous contacts. The staircase method was also used to evaluate the two point discrimination. When the subjects answered 'two points', it was marked as '+'. The next application was a disk with a narrower interprobe distance. This procedure was repeated until subjects answered 'one point', which was marked as '-'. Subsequently, a series with increasing distance was applied. When eight maximum and eight minimum values were recorded, the average threshold was calculated. Two extra blank stimuli were applied after peaks 5 and 11. This means that only one point of the probes made contact with the mucosa. If the subject answered correctly ('one point') the test could be continued. Otherwise, the test was stopped and the subject would be thoroughly reinformed about the experimental procedure. The average of these values was calculated.

2.2.4. Data and statistical analysis

Descriptive statistics were used to summarize all measurements. Pressure sensitivity thresholds, did not require log transformation, since monofilament numbers already correspond to log-force in milligrams. The mean values and standard deviation of LTS and 2PD pooled over the four sessions, at each test area, were calculated. Since the same subjects were submitted to repeated measures, Friedman's ANOVA with two independent variables (time and person) was performed. It assessed their independent effect concurrently, and determined whether they interact with respect to their effect on the threshold. After obtaining a significant ANOVA test, the multiple comparison Scheffe's test was performed. It was used for all possible paired comparisons (e.g., 2PD before implant placement and 2PD at abutment connection) to determine which time periods were significantly different from each other. The evaluation of the threshold as a function of time at implant and control sites, at the four testing sessions, was performed using the Wilcoxon signed-rank test. This test was used to compare thresholds between the implant and control at matching sites, and to test their stability between sites. Its use is limited to the comparison of two groups at a time. Finally, the Pearson's correlation coefficient was used to evaluate the relationship between the LTS and the 2PD tests among the different sites, for each subject. It measured the tendency of the variables to increase or decrease together. A level of significance of 0.05 was chosen for all the statistical tests.

2.3. Results

2.3.1. Characteristics of subjects

Out of the 9 enrolled patients, 8 were seen at all control visits. One patient (female, 2 implants) was lost to follow-up because she moved abroad after the prosthetic rehabilitation. All subjects reported perceived sensitivity to tactile stimuli. None of the subjects reported areas in which very light tactile stimuli produced pain.

2.3.2. Tactile detection threshold

The measurement of the threshold of the light touch sensation on both implant and control sites, before implant placement, at abutment connection, six and twelve months after prosthetic rehabilitation are shown in **table 1. (Fig 4)**

Table 1. Threshold changes for LTS from initial to follow up examination at the implant and control sites for all subjects.

Test type		Implant site				Control site			
		Before implant	Abutment placement	6 months	12 months	Before implant	Abutment placement	6 months	12 months
Light touch sensation	Filament number	4,18	4,48	4,07	4,22	4,47	4,35	4,45	4,30
		4,27	4,14	4,30	4,13	4,18	4,15	4,17	4,16
		3,70	4,10	4,31	4,09	3,65	4,48	4,41	4,17
		3,76	3,36	3,31	3,28	3,40	3,41	3,45	3,09
		3,87	3,80	3,89	3,79	3,52	3,50	3,68	3,73
		4,26	4,44	4,31	4,02	3,52	3,50	3,68	3,73
		3,47	3,48	3,75	3,45	3,20	3,17	3,32	3,35
		3,32	3,12	3,72	3,76	3,69	3,60	3,71	3,72
		4,09	4,06	4,05	3,87	3,86	3,85	3,71	3,80
		3,72	3,81	3,80	3,69	3,21	3,56	3,29	3,53
	mean	3,86	3,88	3,95	3,83	3,67	3,76	3,79	3,76
	std	0,33	0,45	0,32	0,30	0,41	0,44	0,42	0,38
	min	3,32	3,12	3,31	3,28	3,20	3,17	3,29	3,09
	max	4,27	4,48	4,31	4,22	4,47	4,48	4,45	4,30

**LTS light touch sensation*

** mean : mean value of von Frey hair*
** std : standard deviation value*
** min : minimum value of von Frey hair*
** max: maximum value of von Frey hair*

The results were reported using the logarithmic value of the LTS thresholds; but for the statistical analysis, the LTS threshold values in (g) were used. The thresholds were not significantly affected by time (P-value 0.26) on the implant site and (P-value 0.41) at the control site. But these were significantly affected by subjects at both sites (P-value 0.005). Tactile detection thresholds were not significantly different (Wilcoxon

signed-rank test) between the implant and control sites at the four testing periods. At the individual level, one patient exhibited large variations in LTS thresholds (implant 3)

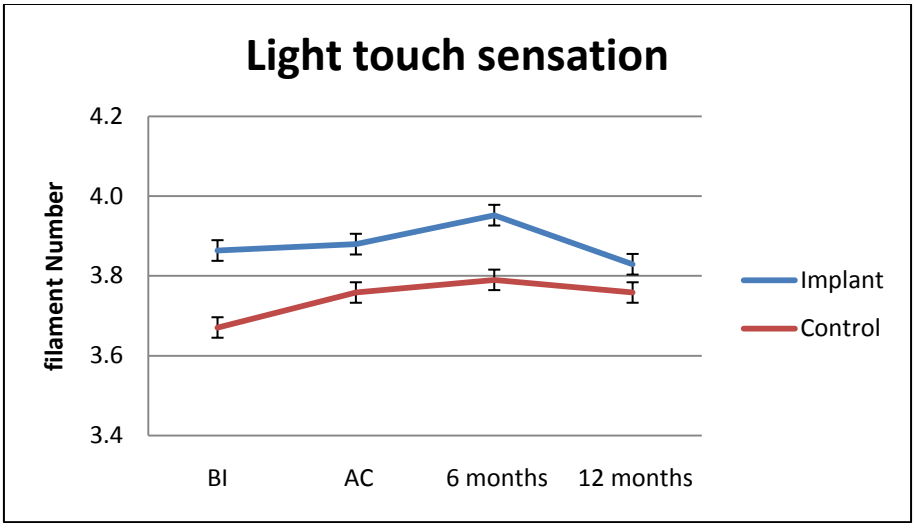


Fig 4 *Graph showing the average threshold for LTS from initial to follow up examination for all subjects.*
BI *von Frey test before implant placement*
AC *von Frey test at abutment placement*
6 months *von Frey test at 6 months*
12 months *von Frey test at 12 months*

2.3.3. The tactile spatial acuity thresholds :

The measurement of the tactile spatial acuity thresholds on both implant and control sites, before implant placement, at abutment connection, six and twelve months after prosthetic rehabilitation are shown in **table 2.**

At the implant sites, five sites (implants n°3, 4, 6, 7, and 9) showed lowered 2PD thresholds at 12 months while the 5 remaining sites (implants n°1, 2, 5, 8 and 10), showed no or very small differences. The differences in threshold values did not exceed 2 to 3 mm. At the control sites, three of the 10 sites (1, 5, and 8) showed more important 2PD thresholds values compared to the threshold distance on the homologous area at the implant site. **(Fig 5)**

Table 2. Threshold changes for 2PD, from initial to follow up examination – implant-side and control-side for all subjects

Test type		Implant site				Control site			
		Before implant	Abutment placement	6 months	12 months	Before implant	Abutment placement	6 months	12 months
Two point discrimination	Threshold (mm)	3,25	4,44	3,56	4,31	6,19	4,56	5,00	4,94
		4,50	3,63	6,13	5,06	3,75	4,25	4,63	4,69
		8,88	9,25	6,94	5,69	5,94	6,25	5,31	4,44
		10,00	8,81	8,06	7,31	4,94	8,44	7,69	7,50
		5,25	5,94	5,25	4,50	7,75	6,63	5,63	6,25
		10,06	10,25	10,25	8,38	7,94	7,94	8,88	8,25
		7,25	7,44	5,63	4,69	7,13	6,25	5,88	6,00
		5,81	5,06	5,00	4,50	7,81	7,63	7,69	7,31
		8,38	8,75	8,13	6,31	6,44	6,75	6,50	6,31
		7,38	8,13	6,69	7,38	5,50	5,75	6,13	4,81
	mean	7,08	7,17	6,56	5,81	6,34	6,44	6,33	6,05
	std	2,33	2,26	1,91	1,45	1,37	1,36	1,36	1,33
	min	3,25	3,63	3,56	4,31	3,75	4,25	4,63	4,44
	max	10,06	10,25	10,25	8,38	7,94	8,44	8,88	8,25

**2PD Two point discrimination*

** mean : mean value*
** std : standard deviation value*
** min : minimum value*
** max: maximum value*

The 2PD thresholds, were significantly affected, per subject at both sites (P-value 0.00) for implant and control sites; They were also significantly affected by time (P-value 0.005) on the implant site but not significant (P-value 0.68) at the control site (Two-Way Anova). Since a significant Anova test was obtained at the implant side, the multiple comparison Scheffe's test was performed, for all possible paired comparisons (e.g., 2PD before implant placement and 2PD at abutment connection) to determine which time periods were significantly different from each other. A significant difference was found at two periods, before implantation and twelve months after the prosthetic rehabilitation (0.13; 2.33), and at abutment connection and twelve months after the prosthetic rehabilitation (0.23; 2.48).

The 2PD thresholds were not significantly different (Wilcoxon signed-rank test) between the implant and control sites at the four testing periods.

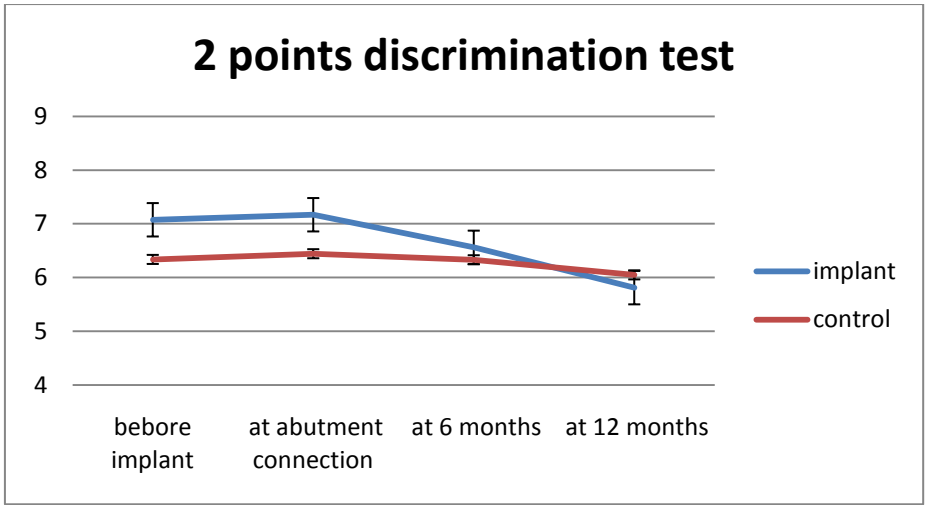


Figure 5. Graph showing the average threshold for 2PD from initial to follow up examination for all subjects

2PD bef. I	*two point discrimination test before implant placement*
2PD at abutment	*two point discrimination test at abutment placement*
2PD 6 months	*two point discrimination test at 6 months*
2PD 12 months	*two point discrimination test at 12 months*

2.4. Discussion

The gingiva contains round and oval lamellar corpuscles (Lambrichts et al., 1992). These receptors respond to mechanical stimuli and are involved in the co-ordination of lip and buccal muscles during mastication (Johansson et al., 1988; Johansson and Vallbo, 1979). The same receptors are found in the gingiva and in the oral mucosa (Jacobs et al., 2002a). They are sensitive to mechanical stimuli, requiring displacements of only a few to tens of micrometers to be activated. The sensory receptors are more frequently found in the anterior part of the mouth with a lower sensitivity in the ridge crest compared to the vestibular areas, suggesting that receptor density was more important in the latter (Ogawa et al., 2003; Rapp et al., 1957).

After tooth extraction, the formation of keratinized, scarless tissue occurs (Muller et al., 2000; Muller and Schroeder, 1980). The thickness of the healed mucosa is not related to the original gingival thickness (Kydd et al., 1971; Muhlbradt et al., 1989) and could affect its mechanical properties, such as elasticity (Bale and White, 1982). Loss of teeth should be considered an amputation and could thus result in a neurophysiological deficit comparable to the loss of a limb. Tooth extraction damages a large number of sensory nerve fibers of the inferior alveolar nerves, and alters projection to the sensorimotor cortex (Mason and Holland, 1993). Consequently, the nerve trunks may

degenerate in response to the loss of stimulation (Hansen, 1980a). Adjacent tissues may also respond, with afferent projections, to presumably reprogrammed sensorimotor representation in an attempt to restore sensory function (Jacobs and Van Steenberghe, 2006). Linden and Scott were able to stimulate nerves of periodontal origin in healed extractions sockets, which implies that the nerve endings were still present in the alveolar bone (Linden and Scott, 1989). Various branches originating from the trigeminal ganglion may reinnervate other structures such as the overlying oral mucosa (Desjardins et al., 1971). The reinnervation was less dense toward the superficial mucosa in comparison to the buccal and lingual.

Animal studies have demonstrated that regenerated nerve fibers in the peri-implant gingiva showed the same neural characteristics as those in the junctional epithelium surrounding teeth (Fujii et al., 2003). Garzino et al. compared the density of mucosal innervation between edentulous and dentate subjects (Garzino et al., 1996). They reported a decreased number of sensory receptors in the edentulous mucosa but a minor increase in the number of nerves in the peri-implant mucosa and a significant increase of innervation in the distal peri-implant mucosa. These changes could partially explain the clinically observed differences in sensory skills before and after implant placement (Garzino et al., 1996).

Both LTS and 2PD are simple but reliable oral sensory tests (Jacobs et al., 2001). Despite a large intersubject variation in the LTS and 2PD, the thresholds were significantly affected in the present investigation at the subject level for both sites. (The observed session-to-session threshold variability could be either due to variation in psychological factors (i.e., “response bias”) or to individual differences in the tactile sensitivity of the oral mucosa (Sessle et al., 2005).

With regard to tactile detection threshold and in accordance with previous studies, the LTS thresholds were not significantly affected by time both at the implant and dentate control sides (Aviv et al., 1992; Cordeiro et al., 1997; Komiyama and De Laat, 2005). The lack of differences observed at the four testing sessions, illustrate the variability of the tested afferents, their density, and/or variations in the processing within the central nervous system of tactile information (Johansson and Vallbo, 1980). A decrease of the light touch sensation would indicate a deterioration of the large myelinated fibre function (Dyck et al., 1974). This lack of difference contrasts with other studies reporting an increased sensitivity after tooth extraction, attributed to the

regeneration of nerve fibers into the soft tissues (Linden and Scott, 1989). Sometimes a loss of tactile sensitivity was reported after surgery, but this is not always reflected in psychophysical testing (Essick et al., 2002).The presence (Marchetti et al., 2002) or absence of Merkel cells (Tanaka et al., 1996) in the peri-implant soft tissue did not seem to affect the LTS threshold values.

The size of a receptive field varies over the body surface, with those located on the extremities being the smallest, growing in size along the leg or arm, and reaching a maximum size on the trunk (Sukotjo et al., 2002). Thus for the 2PD one should consider that two sensations need to be evoked and thus the stimuli must activate at least two primary afferent fibers. In the present study the measurement of the 2PD thresholds were significantly affected over time on the implant but not at the control site. The increased sensitivity to 2PD at the implant site which may reflect the origin of the regenerating nerves, i.e., the larger myelinated Aα afferent nerve fibers. These results are in agreement with the hypothesis proposed by Linden and Scott who attributed the increased sensitivity to nerve fibers regenerating into adjacent soft tissues (Linden and Scott, 1989).

The 2PD threshold levels showed an increased sensitivity at twelve months after the prosthetic rehabilitation. These findings suggest that the surgery had no effect on the soft tissues sensitivity but the regenerated nerve fibers may have increased the sensitivity in the peri-implant soft tissues twelve months after implant loading. These findings are in accordance with the results of Essick who assessed the borders of decreased sensitivity to pinprick in patients with mandibular nerve injuries (Essick et al., 2002). The magnitudes of loss of light touch sensitivity were greatest while they were the least in 2-point discrimination tests.

2.5. Conclusion

The present study revealed no major changes in the tactile sensitivity of the gingiva over time and after surgery except for decreased 2PD thresholds after months at the side of implantation. These findings suggest that the regenerated nerves increased the 2PD sensitivity in the peri-implant soft tissue. The lack of changes in LTS thresholds did not confirm or infirm this hypothesis. Thus, more research on larger patient samples will be needed.

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Sensory Tactile Function of Peri-Implant Soft Tissues identified by Graphaesthesia and Kinaesthesia

Chapter 3

This chapter has been published as:

Habre-Hallage P., Bou Abboud-Naaman N., Reychler H., van Steenberghe D., Jacobs R (2009) Perceptual Changes in the Peri-Implant Soft Tissues Assessed by Directional Cutaneous Kinaesthesia and Graphaesthesia: A Prospective Study. Clin Implant Dent Relat Res. 2009 Aug 5. [Epub ahead of print]

Abstract

Background: The innervation of skin and oral mucosa plays a major physiological role in exteroception. This innervations is also clinically relevant as sensory changes occur after neurosurgical procedures.

Purpose: The goal of this study was to compare the perception of mechanical stimuli applied to the buccal mucosa in the vicinity of osseointegrated oral implants with that in the controlateral dentate side. The role of the previously reported increased innervation in the peri-implant soft tissues in the oral sensorimotor function was thus examined.

Materials and Methods: Seventeen subjects with 20 implants were tested. Directional cutaneous kinaesthesia (DCK) and graphesthesia (G) were performed on the buccal side of the alveolar mucosa before and at planned intervals after implant placement. The observation was pursued until 6months after the prosthetic rehabilitation. In each subject, the contralateral mucosa served as a control to the implant sites. Average percentages of correct responses in a four-choice task for DCK and a three-choice task for G were calculated.

Results: Despite an intersubject variation in both the DCK and G, high intra individual correlations were found ($p < .005$).The implant sites showed a significant difference toward the control sites at the four interval test for both tests. For DCK and G, the average of correct responses decreased after abutment connection (i.e., after the implant uncovering surgery) to increase afterwards to reach a level close to, but still lower than, the control sites 3 to 6 months after the prosthetic rehabilitation.

Conclusion: The DCK and G are simple but reliable sensory tests that can be easily applied in the oral region. This prospective study indicates that tooth loss reduces tactile function compared with implant-supported prostheses. The peri-implant soft tissues could be partially involved in the osseoperception function.

1.1. Introduction:

To evaluate oral sensorimotor function of a patient, psychophysical studies can be carried out determining tactile threshold levels (Jacobs and van Steenberghe, 1994), as well as Oral Stereognostic Ability (OSA) and Oral Motor Ability (OMA)(Berry and Mahood, 1966; Landt and Fransson, 1975). Other functional tests such as the Directional Cutaneous Kinaesthesia (DCK) and Graphaesthesia (G) have been used as early as 1858 (Aubert and Kammler, 1975) but not intra-orally. DCK is the ability to recognise the direction of movement of a cutaneous stimulus. G is the perception of figures, drawn on the skin. Both DCK and G assess the kinesthetic functions implying orientation in cutaneous sensory space and are thus considered as valuable adjuncts to the clinical sensory examination (Bender et al., 1982).

Clinical neurological examinations involving the latter functions have been found to be more sensitive to disturbances of the nervous system than two point discrimination, or point localization tests (Bender et al., 1982; Norrsell, 1973; Wall and Noordenbos, 1977). The friction between the moving object and the skin might also activate receptors which are sensitive to lateral stretching, and probably essential for directional sensibility (Aubert and Kammler, 1975).

Edin & Abbs found that slowly adapting skin receptors can reproducibly measure small changes of lateral skin tension (Edin and Abbs, 1991). In addition, the transsection of the dorsal columns of the spinal cord did not affect the ability to detect tactile sensation or tactile movements, but only impaired the ability to determine the direction of movement of the cutaneous stimuli (Makous and Vierck, 1994). Many neurons in the primary somatosensory cortex respond most rapidly to the movement of a cutaneous stimulus in a particular direction (Gilman, 2002).

The afferents in the buccal mucosa are very sensitive; they respond to contact between the lips and to environmental objects, to changes in air pressure generated for speech, sounds, and to facial skin and mucosa deformations that accompany lip and jaw movements associated with chewing and swallowing (Trulsson and Johansson, 2002).

These sensory receptors are more frequently found in the anterior part of the mouth. It has been histologically documented that the number of nerve fibers per unit area is greater in the anterior areas of the oral cavity, making this region the most sensitive part of the oral mucosa (Ogawa et al., 2003). They demonstrate a lower sensitivity when

localized on the ridge (crest) when compared to the vestibular areas, suggesting that receptor density is more important in the former (Ogawa et al., 2003; Rapp et al., 1957). The changes of the dental representation in the primary somatosensory cortex (SI) was investigated after the extraction of a single lower tooth in the naked mole-rats (Henry et al., 2005). Five to eight months after tooth extraction, a dramatic reorganization of the orofacial representation in SI was observed for the zone that lost input from the extracted teeth. Neurons in the cortical lower tooth representation were responsive to tactile inputs from surrounding orofacial structures, including the contralateral upper incisor, ipsilateral lower incisor, tongue, chin, gums, and buccal pad (Henry et al., 2005).
These results suggest that the representation of the dentition in mammals is capable of significant reorganization after the loss of sensory inputs from the teeth (Henry et al., 2005).
Histological findings reported an increased innervation in the peri-implant epithelium after implant placement (Suzuki et al., 2005).
Yet, the functional role of the peri-implant innervation remains unclear and when focused on the kinesthetic function of the oral mucosa, no information is present to enable to differentiate peri-implant from periodontal soft tissues.
The aim of this study was therefore to evaluate the sensory changes that occur in the soft tissues after installation of an oral endosseous implant. Such information might give a better insight to the functional role of the peri-implant soft tissue innervation.
To reach this goal, two simple oral sensory tests (DCK and G) were performed at the implant sites and the responses were compared to the controlateral dentate sites at 4 different intervals: (i) before implant placement; (ii) after implant placement but before implant loading; (iii) 3 months after prosthetic rehabilitation and (iv) 6 months after prosthetic rehabilitation.

3.2 Materials and Methods

3.2.1. Subjects:

Seventeen subjects (ages 19–60 yrs, mean 35.28; SD 11.62), nine males (11 implants) and eight females (9 implants) were selected based on their dental status. Subjects had a complete natural dentition with the exception of one or two missing teeth in the maxilla or the mandible. They had to be rehabilitated with osseointegrated implants. The implant insertion was made at modum Brånemark by the same surgeon.

The implants healed under the closed mucosa during a period of 3 to 5 months. The abutments were mounted on the implants one month after the implant uncovering surgery. None of the subjects had a history of any neurologic disorder such as dysesthesia or periodontitis in the oral cavity. Informed consent was obtained from each participant prior to investigation. The subjects were tested in a quiet room with stable illumination while seated comfortably in a chair with a headrest. They were instructed to close their eyes during the whole testing procedure and were familiarised with the set-up following a standardised instruction sheet including some test trials prior to the actual start of the experiments.

The same operator performed four consecutive measurements; before implant placement, at abutment connection, at 3 and at 6 months after the prosthetic rehabilitation: In each subject, two sensory tests were applied, the directional cutaneous kinaesthesia (DCK) and graphesthesia (G). Both tests were performed at the buccal site of the keratinized or alveolar mucosa (at 1 mm from gingival or soft tissue margin) in the maxilla or the mandible for both implant and control sites. Cheek retractors were used to avoid stimulation of any other oral structures.

3.2.2. The testing procedure:

Directional Cutaneous Kinaesthesia (DCK) is the ability to recognise the direction in which a cutaneous stimulus is moving. This technique was described in details by Norsell and Olausson (Norrsell and Olausson, 1994; Olausson and Norrsell, 1993). The examiner of this study drew a line of 5mm with a rubber tip gum stimulator (Oral B®) at the buccal side of the alveolar mucosa. This device was chosen because it is gentle and flexible yet firm enough to stimulate the soft tissue.The subject was asked to report the direction corresponding to the line: up, down, left or right.

Graphaesthesia (G) has been described as the perception of figures, ranging from simple lines to complex symbols, such as numbers and letters, drawn on the skin or the mucosa.[5] In this study, a circle, triangle or a square shape was drawn 5 X 5 mm in size at the buccal side of the alveolar mucosa with the rubber tip gum stimulator, at the speed of ~1-2 s per shape.

The patients were asked to recognise the shape that was drawn in the testing area.

For both tests, the number of experimental runs was limited to 4 in DCK and 3 in G. The sessions were interleaved by resting periods of 3 minutes to maintain the perceptual acuity of the patients throughout the experiments and to avoid fatigue.

3.2.3. Data and statistical analysis

The method used was the method of constant stimuli, with four-alternative forced-choice for Kinaesthesia (Sekuler et al., 1973) and three for Graphaesthesia. This approach has the drawback that a large amount of data is required to avoid response bias or guessing strategies.

The order of presentation of the forms or lines was randomized and the percentage of correct answers was calculated. Data are expressed as percentages (%), calculated by multiplying the mean number (n) of correct responses out of the number of trials (t) within each group by 100: %=(n/t) X100. In each testing session, the number of trials (t) is 30 for graphaesthesia (10 for square, 10 for circle and 10 for triangle) and 28 for DCK (7 for up, 7 for down, 7 for left and 7 for right).

The SPSS software for windows version 15.0 (SPSS Inc. Chicago, IL, USA) was applied for statistical analysis. A 5% level of significance was chosen.

Despite an intersubject variation in both the DCK and G, significantly high intra-individual correlations were found ($P < 0.005$). Repeated measures analysis of variance (ANOVA), with 3 within-units factor (4 measurement times, 3 measurement forms, 2 measurements groups) for graphaesthesia and (4 measurement times, 4 measurement directions, 2 measurement groups) for kinaesthesia were conducted to simultaneously explore the effect of each of the independent variable: time, groups, and forms or directions on the percentage of right answers and to also identify any interaction effect.

Because of interaction, further analysis was carried out at particular time, for particular groups and forms or directions.

For each form and each direction, one way repeated measure ANOVA and Bonferroni post test analysis were applied to test if the percentage of right answers varied significantly over time for implant or control sites.

For each form and each direction, paired t test was used to examine if the percentage of right answers are significantly different at each time between implant and control sites.

At each time and for each group, other one way repeated measure ANOVA and Bonferroni post test analysis were applied to explore if significant difference in

"Percentage of right answers" occurred, among forms (graphaesthesia) or among directions (kinaesthesia), or between forms and directions.

3.3. Results

The results of the tests with regard to the number of correct responses on both implant and control sites, before implant placement, at abutment connection, and at three and six months after prosthetic rehabilitation are listed in **tables 1** and **2** and **fig. 1** and **2.**

Data are expressed as percentages (%), calculated by multiplying the mean number (*n*) of correct responses out of the number of trials (t) within each group by 100: **%=(*n*/t) X 100** (The number of trials is 30 for G and 28 for DCK).

Table 1
Average of correct responses for Graphaesthesia, from initial to follow up examination – implant-site and control-site for all subjects

			BI		AC		3 months		6 months	
			Implant	Control	Implant	Control	Implant	Control	Implant	Control
Graphaesthesia	**triangle**	Mean	66.5%	73.0%	62.5%	73.5%	68.0%	74.5%	69.0%	74.0%
		SD	6.7	6.6	6.4	7.4	7.0	8.9	7.2	9.4
	square	Mean	58.5%	72.0%	56.0%	72.0%	65.0%	70.5%	67.0%	75.0%
		SD	7.45	7.7	8.2	7.7	6.9	7.6	7.3	5.1
	circle	Mean	71.5%	80.5%	71.0%	79.5%	76.5%	84.0%	79.5%	84.0%
		SD	12.3	8.9	10.7	9.4	10.4	8.8	10.0	7.5

BI = Before implant placement; AC = at the abutment connection; 3 months = 3 months after the prosthetic rehabilitation; 6 months = 6 months after the prosthetic rehabilitation; SD = standard deviation. $P \leq .005$ compared with natural dentition

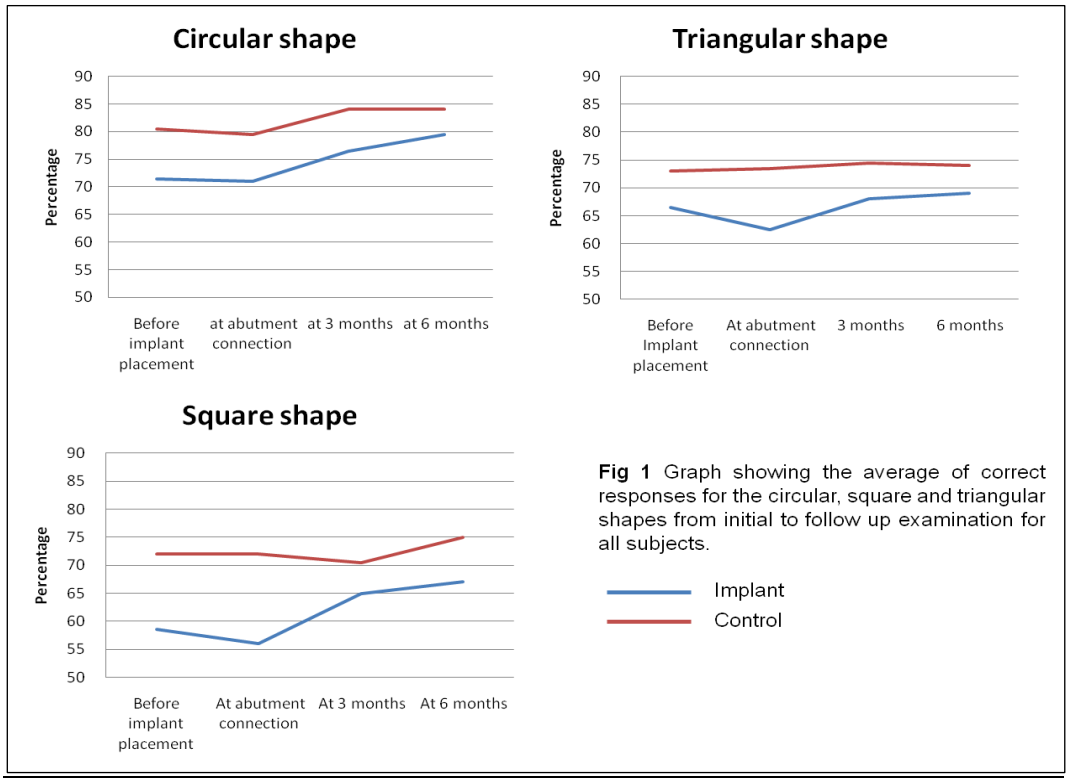

Fig 1 Graph showing the average of correct responses for the circular, square and triangular shapes from initial to follow up examination for all subjects.

Table 2
Average of correct responses for Kinaesthesia, from initial to follow up examination – implant-site and control-site for all subjects

			BI		AC		3 months		6 months	
			Implant	Control	Implant	Control	Implant	Control	Implant	Control
kinaesthesia	**Up**	Mean	76.4%	88.6%	76.4%	90.0%	80.7%	91.4%	85.0%	90.0%
		SD	9.6	11.9	7.0	9.4	9.6	9.7	5.6	9.4
	right	Mean	77.9%	82.8%	73.6%	85.8%	80.0%	87.8%	82.7%	89.3%
		SD	11.5	8.8	8.4	14.3	11.7	13.0	7.5	7.9
	down	Mean	79.3%	90.0%	78.6%	87.8%	84.3%	89.3%	83.6%	88.6%
		SD	7.3	8.2	8.7	8.4	9.1	7.9	9.6	10.9
	left	Mean	74.3%	82.2%	75.0%	85.0%	80.1%	85.1%	82.9%	87.1%
		SD	7.5	12.7	6.3	11.8	13.1	12.3	8.8	11.3

BI = Before implant placement; AC = at the abutment connection; 3 months = 3 months after the prosthetic rehabilitation; 6 months = 6 months after the prosthetic rehabilitation; SD = standard deviation. $P \leq .005$ compared with natural dentition

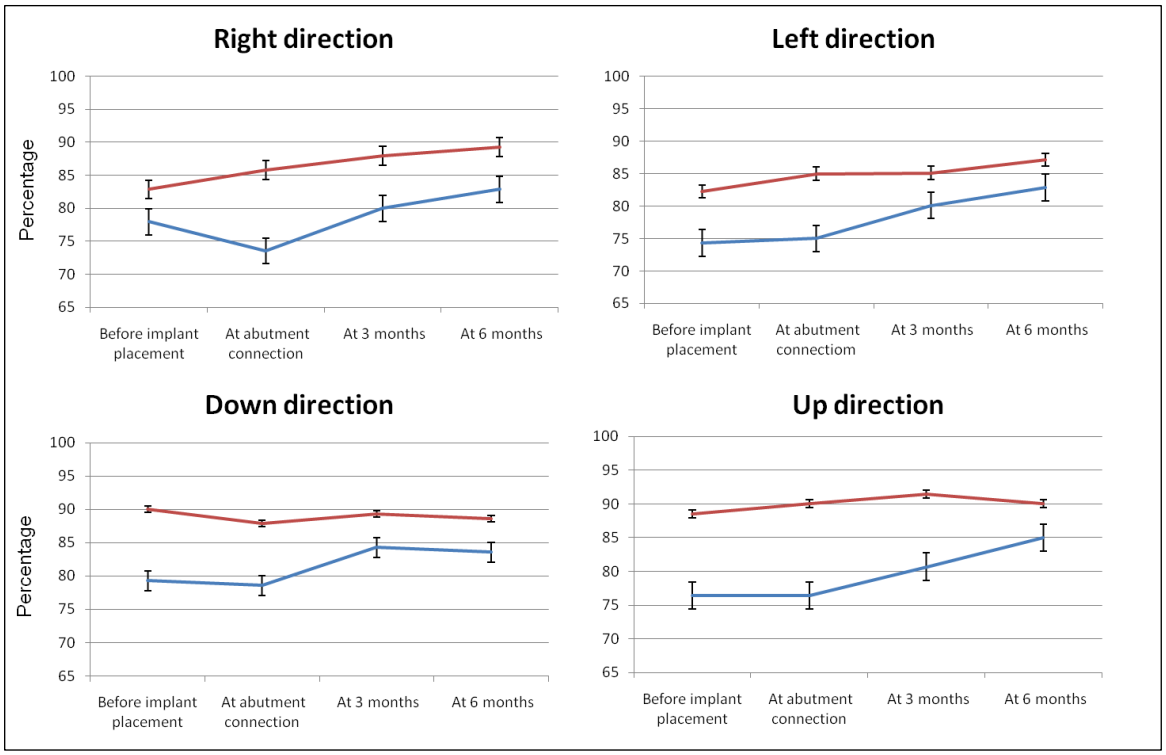

Figure 2. Graph showing the average of correct responses for the four directions from initial to follow up examination for all subjects.

3.3.1. At the control site

The percentage of correct responses was not significantly different at the four test periods. The control sites remained stable over time; this is illustrated in the figures 1 to 7 and confirmed by statistic analysis (one way repeated measure ANOVA P > 0.05). The control site achieved a significantly higher level of correct responses than the implant site for both graphaesthesia (Paired t test $P<0.05$) and kinaesthesia (Paired t test $P<0.05$).

3.3.2. At the implant site

A significant difference was found for both tests among the four observation periods. A reduced level of perception was revealed before implant installation in comparison to the dentate control site. The average of correct responses decreased at the time of abutment connection (after the implant uncovering surgery). Then it started to increase to reach a level near but still lower than the control site after 3 to 6 months of function. These results are statistically significant for both tests: Graphaesthesia (one way repeated measure ANOVA followed by Bonferroni post test comparisons P<0.05) and kinaesthesia (One way repeated measure ANOVA followed by Bonferroni post test comparisons P<0.05).

The recognition of the circle shape was more significant than the two other shapes at the four test periods (One way repeated measure ANOVA followed by Bonferroni post test comparisons P<0.05). However, no difference in perception for different directions was found (One way repeated measure ANOVA P>0.05).

3.4. Discussion

After tooth loss, the alveolar socket fills up with bone and the periodontal ligament innervation degenerates partially (Hansen, 1980a) or starts innervating other structures like overlying scarless healed tissues (Desjardins et al., 1971; Linden and Scott, 1989).

In the present experiment for the control sites, the percentage of correct responses was not significantly different over time at the four test periods, and achieved systematically a higher level of correct responses than the edentulous site even before the implant placement for both tests. This seems to be in accordance with previous findings on the skin

that the soft tissue sensitivity decreases for light-touch sensation, two-point discrimination and vibrotactile function following amputation (Braune and Schady, 1993).

This reinnervation along with the receptor density was less dense toward the superficial mucosa in comparison with the innervation of the buccal and lingual vestibules (Garzino et al., 1996). In fully edentulous patients, the mucosa-borne denture can only partly restore sensory function (Mericske-Stern, 1994).

Yet, the number of Merkel cells in the gingiva was found to be significantly higher in edentulous areas when compared to dentate ones (Kingsmill et al., 2005). This increase in the Merkel cell population might compensate for the loss of the teeth.

The directional sensitivity is most responsive for small distances than the two-point discrimination and point localization (Hamburger, 1980; Weinstein, 1962); because the moving stimulus causes a continuous afferent flow during the period of motion and may be more efficient. The friction between the moving stimulus and the underlying skin is critical for the determination of the direction of motion (Olausson and Norssell, 1991, 1992). It induces a chronological activation of adjacent receptors and a friction-induced activation of stretch-sensitive receptors (Backlund et al., 2005; Norrsell and Olausson, 1992, 1994; Olausson and Norrsell, 1993).

These 'friction' receptors are activated to the relative lateral tensions of the skin. The moving object seems to reorient, elongate or shorten the friction receptors. The transmission of lateral forces may depend on the skin’s elasticity and resistance. These factors are determined by the mechanical properties of the skin, and consequently vary with the skin's thickness as well as the subject's age and sex (Olausson and Norrsell, 1993; Piérard, 1989). The thickness of the healed mucosa is not related to the original gingival thickness (Kydd et al., 1971; Muller et al., 2000) and could affect its mechanical properties, such as elasticity (Bale and White, 1982). This remains undocumented so far in literature. Olausson and Norssel were able to demonstrate that the mechanical properties of the skin are critical for the friction-induced activation of stretch-sensitive receptors (Olausson and Norrsell, 1993). This is in agreement with our findings which suggest that the scarless healed oral tissues may lower the sensitivity to the frictional stimulus.

At the implant site, a significant difference was found for graphaesthesia and kinaesthesia over time, for the four test period. A reduced level of perception was already revealed

before implant installation in comparison to the dentate control site revealing the impact of the tooth extraction. After 3 to 6 months of implant function, tactile responses increased and approached but were still significantly less than the control.

At the abutment connection, after the implant uncovering surgery, the tactile response decreased. This could be easily explained by the trauma caused by 2 surgical interventions (flap surgery for implant placement and implant uncovering surgery), with periosteal elevation. Considering the rich periosteal innervation with Pacinian corpuscules and free nerve endings (Macefield, 2005), which are both sensitive to stretching, the present observation of reduced sensory function might be partly attributed to a disrupted or damaged periosteal innervation.

It is interesting to note that animal studies have demonstrated that regenerated nerve fibres in the peri-implant gingiva show the same neural characteristics as those in the normal, dental junctional epithelium (Fujii et al., 2003; Marchetti et al., 2002). Regenerative nerve fibers invade the superficial layer of the peri-implant epithelium. These nerve fibers contain substance P and possess free nerve endings. They may respond to pain, touch and pressure (Suzuki et al., 2005; Tanaka et al., 1996). Unfortunately, none of the reports was able to characterize the function of the detected fibers. Merkel cells are important in tactile function and they are normally found in both the oral mucosa and in the gingiva. They seem to be absent in the hamster's peri-implant epithelium mucosa (Suzuki et al., 2005) but were found in the peri-implant mucosa in humans (Marchetti et al., 2002). However, their presence in the periosteum has not been described in the literature (Macefield, 2005). Histological findings report an increased innervation in the peri-implant epithelium after implant placement (Suzuki et al., 2005).

When applying forces to osseointegrated implants in the jaw bone, the pressure build-up in the bone is sometimes large enough to allow deformation of the bone and its surrounding periosteum (Sakada, 1974). It is already established that Pacinian corpuscles have an exquisite sensitivity to brisk mechanical events and could respond to such stimuli transmitted through the bone to a remote receptor (Macefield, 2005).

Consequently, the presence of a functional implant may induce the improvement of the ability to detect a moving stimulus on the peri-implant soft tissues, shown in our findings.

Moreover, the presence of the implant restores the orofacial functions and stimulates the surrounding tissues which may lead to changes in the cortical reorganisation. After amputation of a limb, the regions of the cortex deprived of a target acquire new targets. It has been demonstrated that several changes take place at the cortical or subcortical level (Hansen, 1980a). But even if a reorganization of these regions occurs very fast (within hours) (Miles, 2005; Sanes and Donoghue, 2000), what happens in the intrinsic connections of the cortical areas is still unrevealed (Kaas and Qi, 2004; Sessle et al., 2005). In humans, the possible cortical adaptive processes (cortical plasticity) that can be associated with the loss of teeth, or with their replacement by means of oral implants has not been explored extensively (Calford, 2005; Klineberg and Murray, 1999). This hypothesis may also explain the improvement of the ability to detect a moving stimulus on the peri-implant soft tissues.

3.5. Conclusions

Postsurgical sensory changes may be bothersome to patients, even though the main goal of the surgery has been completely accomplished. To assess this observation, one cannot simply rely on the patient's subjective report. The directional cutaneous/mucosal kinaesthesia and the graphaesthesia are simple but reliable sensory tests that can be easily applied in the oral region and thus allow to evaluate sensorimotor function during oral rehabilitation by means of implants. Both tests correlate the physiologic function of the receptors to the subjective response of the patient (Jacobs et al., 2002a).

The present study reveals that tooth loss decreases the sensory function of the oral mucosa, while this function seems partially restored after implant-installation. Whether this peri-implant soft tissue innervation may contribute to the osseoperception phenomenon remains to be unraveled. Brånemark in 1997, defined osseoperception as “the perception of external stimuli transmitted via the implant through the bone by activation of receptors located in the peri-implant environment, the periostium, the skin, the muscles and /or the joints” (Brånemark et al., 1997).

Since this study confirmed that mucoperiosteal-flap procedures reduce the directional sensitivity, further investigation may demonstrate the merit of the flapless approach during implant surgery.

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A Manually-Controlled New Device for Punctuate Mechanical Stimulation of Teeth Designed for fMRI Studies

Chapter 4

This chapter has been published as:

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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 8 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 artifacts in MR images. The most efficient protocol included epoch duration of 24s 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, controlateral or both sides. Stimulation of the thumb led to activations of the contralateral S1 area and either ipsilateral or contralateral 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.

Clinical relevance

Scientific rationale: 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 finding: 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 prior to and after tooth loss. It will also allow to investigate the plasticity of their cortical mapping after installation of endosseous oral implants.

4.1. Introduction

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

Dental and periodontal somatotopy has 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 analyze 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 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 and 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 manually-controlled intra-oral new 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.

4.2. Materials and Methods

4.2.1. 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 labeled with a number (1.65–6.65) that represents the log10 of the force (mg) required to bend the filament (0.008–300g according to the manufacturer). VFF are commonly used for quantitative sensory testing in the clinical setting and in neurophysiological experiments (Park et al., 2001; Rolke et al., 2006a; Rolke et al., 2006b; Yarnitsky, 1997). 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 and Pardo, 2002; 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 N° (**figure 1a, 1b & 1c**).

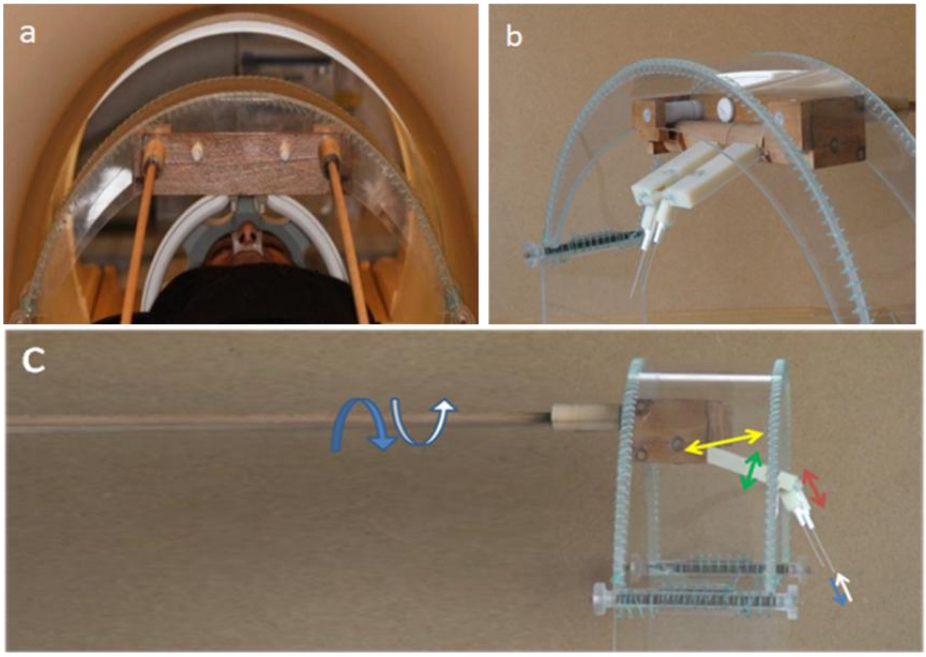

Figure 1. The stimulation device

a. *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 coil. 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. 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.*

b. Rotation and translation of the VFF support allowed adjusting the position of the VFF in the 3 axes (horizontally, vertically and toward the teeth). Note that all materials in the vicinity of the subject and the head coil are nonmagnetic.

c. This illustration shows the stimulation device. The VFF support can be adjusted in the three dimensions: horizontally (yellow arrow), up and down (green arrow) and toward the teeth (red arrow). The clockwise rotation of the wooden stick induces a movement of the VFF to stimulate the tooth (blue arrow) and then a counter clockwise rotation pulls the filament backward (white arrow).

To guide the course of the VFF towards the tested teeth and avoid touching the lips or other peri-oral structures, rigid removable customized 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 (figure 2a & 2b). 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.

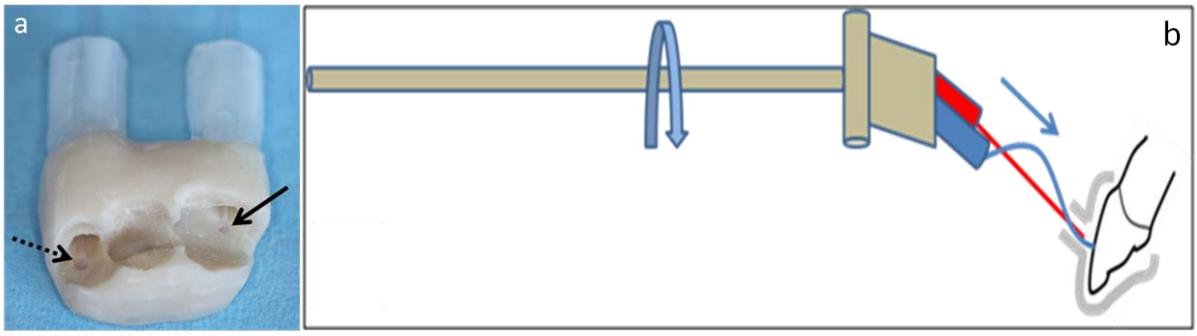

Figure 2: The customized splint

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- b- This figure shows how the VFF is bent while its tip is guided by the splint (tube). The VFF support is adjusted three dimensionally so that the tip of the VFF is inside the tube (in grey) that 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).

4.2.2. Scanning

MRI examinations were performed on a 3-T Achieva system (Philips Healthcare, Best, The Netherlands) equipped with an 8 channels phased array head coil.

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

Structural brain images were obtained in all subjects using a 3D fast T1-weighted gradient echo sequence with an inversion prepulse (Turbo field echo [TFE], TR

[repetition time] = 9 ms, TE [echo time] = 4.6 ms, flip angle [FA] = 8 degree, 150 slices with a thickness = 1mm, field of view [FOV] = 220 x 197 mm^2 giving an in plane resolution = 0.81 x 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 degree, 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 artifacts, the same EPI sequence was also applied on a MR phantom consisting of a sphere filled with a water solution of CuSO4 that was provided by Philips Healthcare.

4.2.3. Human subjects

Eight healthy right-handed subjects according to the Edinburgh Handedness Inventory (Oldfield, 1971), (age 23–51 yrs, mean 32, SD 10; 6 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 prior to 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.

4.2.4. 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 N^o 5.88 (60g), 6.10 (100g) and 6.45 (180g) 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. (van Steenberghe and De Vries, 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 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 that case, the subject' hand lied 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 (10g) 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.

4.2.5. 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 was 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 4 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.

Table 1: *Description of the four tested paradigms*

	First rest period Duration(s)	Other rest periods Duration (s)	Active periods Duration (s)	Num of active epochs/run	Num of vol/ run	Num of runs	Num of subj
Protocol 1	12	12	12	12	96	1/site (3 sites tested)	2
Protocol 2	24	24	24	6	96	1/site (3 sites tested)	4
Protocol 3	12	12	24	9	112	4 (3 sites/run)	2
Protocol 4	12	24	24	6	100	3 (2 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.
Num = number ; subj = subjects ; vol = volumes

4.2.6. Behavioral 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?

4.2.7. Data analysis

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

Volunteers data were processed and analyzed using Statistical Parametric Mapping (SPM 5, The Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ac.uk/spm), implemented in Matlab (Mathworks Inc. Sherborn MA,

USA). The individual structural (TFE) brain volume of each participant was co-registered to the first fMRI volume and spatially normalized into the referential defined by the atlas of Talairach and Tournoux (1988) and the MRI template supplied by the Montreal Neurological Institute (MNI). The fMRI data were then spatially realigned 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 $2mm^3$ 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 (GLM) in which the responses evoked by each condition of interest were modelled by a standard hemodynamic 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 predefined uncorrected p values. This approach is frequently used in individual clinical fMRI examination to 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 ($p<0.0001$, $p<0.0005$, $p<0.001$, $p<0.005$) until we obtained at least 150 activated voxels. If the total number of activated voxels was > 600, we increased the threshold by steps ($p<1.10^{-5}$, $p<1.10^{-6}$, $p<1.10^{-7}$, $p<1.10^{-8}$, $p<1.10^{-9}$ …) 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 (Wilcoxon signed rank test for paired data or rank sum test for unpaired data).

4.3. Results

4.3.1. Phantom study

The pooled standard deviations of the phantom without the device, with the device not in use, and with the device when performing a sham stimulation were not significantly different (46.18, 46.82, and 47.52, respectively).

4.3.2. Behavioral 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.

4.3.3. 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 24s), there were more activated voxels with 24s. 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 24s (**Table 2**).

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

Subject	Stimulated	Protocol 1 : epochs of 12 s			Protocol 2 : epochs of 24 s		
No	site	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		

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 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 = contralateral regarding the stimulated site, SID = semi-interquartile deviation,
** = significant difference between the 2 protocols (p=0.03)*

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

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

Protocol 2 (48 active vol./site)			Protocol 3 (96 active vol./site)			Protocol 4 (72 active vol./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	0.00000001
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 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, vol. = volumes

4.3.4. Cortical activations

The data obtained in the 8 subjects were all exploitable and none of them disclosed head movement superior to 2 mm as measured by the realignment 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 contralateral side and in 7/16 teeth on the ipsilateral side. The contralateral 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 contralateral side and in 11/16 teeth on the ipsilateral side. The stimulation of the thumb also yielded an activation of SII area on the contralateral (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 contralateral side and 9/16 on the ipsilateral side). Such activation was rarely found

after stimulating the thumb with a contralateral 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 contralateral 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 precentral gyrus (Brodmann areas 4 or 6) was also found for 6/16 teeth on the contralateral side and for 2/16 teeth on the ipsilateral side. Only the stimulation of the left thumb yielded activation of the contralateral precentral gyrus in 3/4 cases. Other areas were only occasionally activated. A typical activation map obtained in a single subject while stimulating a tooth is showed in **Figure 3**.

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 araea, (nb+/tot nb) = number of subjects showing this activation / total number of subjects)

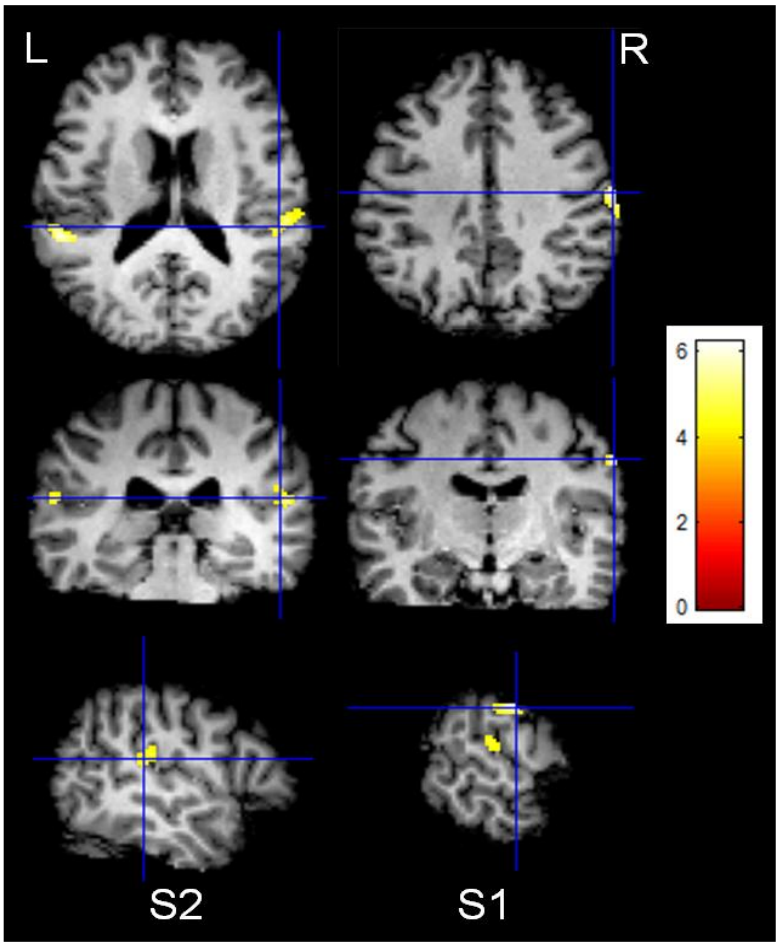

Figure 3 : Activation map obtained during tooth stimulation
Cortical activation obtained during sensory stimulation of the teeth 22 in subject 8 with protocol 2 (active and rest epochs of 24s, 48 activated volumes). The statistical parametric maps are overlaid on the axial, coronal and sagittal sections 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 color scale which code the T-values.
We observe activation in the contralateral primary somatosensory cortex (S1) and in the secondary somatosensory cortex (S2) bilaterally.

4.4. **Discussion**

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

4.4.1. 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 and Johansson, 1996a, b), but also in the reflexes of the masticatory muscles (Linden, 1990; van Steenberghe, 1979) 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 (Byrd et al., 2009; Yan et al., 2008). 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 a somatotopic mapping of the face (Huang and 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 mN 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

to 2 N have been used to trigger the incisors and canine teeth respectively. The behavioral 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 inconstant (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 M. 1993; Johnsen SE 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 artifacts, 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 (less than 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 6 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 employs 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 seems 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 fiber 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.

4.4.2. 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 0.25s (4Hz) or less, a summation effect occurs which means the stimuli are experienced as a unique stimulus with a progressively increased amplitude. High frequencies such as these used by Ettlin et al. (2004) for vibrotactile stimuli (80 Hz) did not activate the somatosensory cortex. Low frequency stimulation (generally a 5-sec stimulus interval) is therefore proposed by many authors as a 3 sec 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 to 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 to a block-wise stimulation.

With longstanding stimulations of periodontal mechanoreceptors, an habituation may occur and decrease the activation in the somatosensory areas (vanSteenberghe& de Vries, 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. 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 (16-30s) (Friston et al., 1999; Worsley and Friston, 1995) is therefore applicable for the periodontal mechanoreceptors.

4.4.3. 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 compared to previous studies which 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 and 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 showed 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 and Pardo, 2002; Iannetti et al., 2003).

In comparison to 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.

4.4.4. Clinical perpectives

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.

4.5 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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Cortical Activation Resulting from Stimulation of Periodontal Mechano-receptors Measured by fMRI

Chapter 5

This chapter is submitted for publication:

Habre-Hallage P., *Dricot L., Hermoye L., Reychler H., van Steenberghe D., Jacobs R., Grandin* C. Cortical activation resulting from stimulation of periodontal mechanoreceptors measured by functional magnetic resonance imaging (fMRI). Submitted

Abstract

Aim: To describe the normal cortical projections of periodontal mechanoreceptors.

Material and methods: A device using von Frey filaments delivered 1 Hz punctuate tactile stimuli to the teeth during fMRI. In a block design paradigm, tooth (T) 11 and T13 were stimulated in 10 volunteers, and T21 and T23 in 10 other subjects. Random effect group analyses were performed for each tooth, and differences between teeth were examined using ANOVA.

Results: The parietal operculum (S2) was activated bilaterally for all teeth, the postcentral gyrus (S1) was activated bilaterally for T21 and T23, and contralaterally for T11 and T13. In the second level analysis including the four teeth, we found 5 clusters: bilateral S1 and S2, and left inferior frontal gyrus, with no difference between teeth in somatosensory areas. However, the ANOVA performed on the S1 clusters found separately in each tooth showed that S1 activation was more contralateral for the canines.

Conclusion: 1 Hz mechanical stimulation activates periodontal mechanoreceptors and elicits bilateral cortical activity in S1 and S2, with a double representation in S2, namely in OP1 and OP4.

Clinical relevance

Scientific rationale: The cortical somatotopy of periodontal mechanoreceptors is poorly known. By using fMRI, we are the first to describe the activation elicited by 1 Hz tactile simulation of 4 teeth pertaining to the left and right side.

Principal finding: We showed bilateral receptive fields in S1 with a slight preponderance of contralateral projections for the canines. Bilateral activation in S2 was also found with a double representation of the teeth in subdivisions OP1 and OP4.

Practical implications: Our data may serve as normal reference to further explore the cortical plasticity induced by periodontal or neurological diseases, or by the placement of endosseous implants.

5.1. Introduction

The periodontal ligament attaching the tooth root to the alveolar bone contains many mechanoreceptors tightly connected with the fiber bundles that very precisely encode the intensity and spatiotemporal aspects of the forces applied to the tooth. These mechanoreceptors are particularly important when biting and chewing because they efficiently encode tooth load during intraoral food manipulation and are involved in jaw motor control (Trulsson and Johansson, 2002). Several studies have been performed on peripheral tooth sensation (Trulsson and Johansson, 1994, 1996a, 2002; Trulsson et al., 1992), but only three research teams have investigated the cortical representation of tactile tooth sensation (Ettlin et al., 2004; Miyamoto et al., 2006; Trulsson et al., 2010).

To process sensory input, the brain is organized hierarchically and topographically with a somatotopy that can be defined in each sensitive area with a variable degree of precision. Anatomic, electrophysiological and functional imaging studies performed in humans led to the identification of the primary (S1) and secondary (S2) sensitive areas with their subdivisions and homologous correspondence in monkeys. However, cortical representation of the hand has been the main focus of research, with far less studies devoted to other parts of the body such as the trigeminal areas.

In his intraoperative studies, Penfield (Penfield, 1950) found that cortical representation of teeth, gingiva and jaws could not be subdivided but was located below that of the lips and above that of the tongue. Since then, the cortical projections of the teeth have hardly been addressed. This might be due to the difficulty of exploring the oro-facial area as the stimulation and signal recording are in the same area which may lead to interactions and distortion of the recorded signal (Van Loven et al., 2001). This is especially true with functional magnetic resonance imaging (fMRI) which is nowadays one of the main techniques to map the human cortex. Indeed, exploring the oral area remains challenging because of its poor accessibility and because it requires a stimulation device that does not interfere with the MR system. Using magnetoencephalography (MEG) and electrical stimulation of the gingiva but not of the teeth, Nakahara et al. (2004) found an activation in the primary somatosensitive cortex located inferior to that of the lips and close to that of the tongue.

In the few studies where the teeth were stimulated during fMRI, the results are dissenting. Using a torque force delivered by a manually controlled rotating stick,

Miyamoto et al. (2006) were able to identify the representation of the teeth in the postcentral gyrus (SI) in a location superior to that of the tongue and inferior to that of the lip. On the other hand, using vibrotactile dental stimulation, Ettlin et al. (2004) found bilateral activation in the insula and the supplementary motor area, but not in the somatosensory areas. Very recently, Trulsson et al. (2010) demonstrated that low frequency vibrotactile stimulation was able to activate both S1 and S2 while higher frequencies did not. Using unpleasant or even painful electrical stimuli, a wide cortical network including the insula and several frontal, parietal, temporal, occipital areas as well as the cerebellum were described (Ettlin et al., 2004; Jantsch et al., 2005). The somatosensory areas (S1 and S2) were activated in only one of these studies and without any description of their precise somatotopy (Jantsch et al., 2005).

The diversity of applied stimuli and the unnatural stimulation mode explain the variability of the 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. To achieve this, we recently developed a non-magnetic manually-controlled device that can apply calibrated mechanical punctuate stimuli to the teeth to trigger the periodontal mechanoreceptors during fMRI studies (Habre-Hallage et al., 2010).

The aim of the present study was to clarify the cortical projections of periodontal mechanoreceptors and to describe their putative somatotopic organization in the primary and secondary somatosensory cortex. Our hypothesis, in line with the work of Trulsson et al (2010), was that very low frequency (1 Hz) was the most appropriate to elicit a pure tactile sensation on the teeth and to activate mainly the somatosensory areas with a somatotopy comparable to the other body parts.

5.2. Materials and methods:

5.2.1. Subjects:

The study was approved by the local Biomedical Ethical committee. Written informed consent were obtained from twenty healthy subjects (mean age 31.5 ± 8.1 years; 14 females) who were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). Inclusion into the study required a full dentition with vital teeth, no periodontal breakdown, and no increased tooth mobility. Subjects were instructed to refrain from moving, to avoid swallowing during the registration period, 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.

5.2.2. Sensory stimulation

Since the methodological aspects have been described in details in a previous paper (Habre-Hallage et al., 2010) the present description will be limited. A manually-controlled device designed for fMRI was used to deliver repetitive punctuate stimulation to the incisors and the canines with von Frey filaments (VFF). The device allowed adjusting the position of the VFF in the three axes (horizontally, vertically and towards the teeth) to accommodate the morphology of any subject and to stimulate all the anterior teeth (incisors and canines). 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. To guide the course of the VFF towards the tested teeth, the tips of the VFF were inserted into 4 mm plastic tubes fixed in a rigid removable customized splint. The tubes did not interfere with the bending of the filaments. The splint allowed the subject to keep a moderate opening of the mouth while gently biting on it.

The VFF No 6.10 (100g) and 6.45 (180g) were used to stimulate the central incisors and the canines respectively since we demonstrated that these forces elicited a clear cortical activation during fMRI (Habre-Hallage et al., 2010). A pretest outside the magnet ensured that for all subjects the stimulation was well above the psychophysical mechanical detection threshold but below the level of mechanical unpleasantness, and

that each stimulus was clear, constant, and touched only the intended target. The stimuli were provided by the same well-trained experimenter (P.H.H.) and the constant frequency of 1 Hz, the rhythm being acoustically cued to the experimenter.

5.2.3. Experimental paradigm

The device allowed the use of block design paradigms and the stimulation of two different teeth in the same experiment. Into each epoch, the stimuli were administrated to the same area and each active epoch was separated by a period of rest.

In 10 subjects the right central upper incisor and canine (teeth 11 and 13) were stimulated while in another group of 10 subjects the contralateral teeth were stimulated (teeth 21 and 23). The paradigm consisted of 3 runs with stimulation and rest periods of 24s, except for the first rest period lasting 12s. There were 6 active epochs per run with a total number of brain volumes/run=100. The two teeth were stimulated 3 times/run in a random and counterbalanced order, the same site being stimulated during each active epoch.

5.2.4. Images acquisition

MRI examinations were performed on a 3-T Achieva system (Philips Healthcare, Best, The Netherlands) equipped with an 8 channels phased array head coil.

All images were acquired in the bicommissural (AC-PC) orientation (Talairach and Tournoux, 1988). Structural brain images were obtained in all subjects using a 3D fast T1-weighted gradient echo sequence with an inversion prepulse (Turbo field echo [TFE]).

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 degree, 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.

5.2.5. Images analysis

The first step of the preprocessing was done with SPM5 (Statistical Parametric Mapping, The Wellcome Department of Imaging Neuroscience, London, UK,

http://www.fil.ion.ucl. ac.uk/spm). It consisted in an optimized spatial realignment of the functional dataset to the first fMRI volume to correct for the small interscan movements so that no run was rejected. The rest of the analysis was performed with BrainVoyager QX (Version 2.1.2, Brain Innovation, Maastricht, The Netherlands). Further preprocessing included a linear trend removal for excluding scanner-related signal and a temporal high-pass filtering applied to remove temporal frequencies lower than 3 cycles per run. Data were not smoothed in the spatial domain. The anatomical 3D T1-weighted scan of each participant was manually coregistered to the first fMRI volume. Both anatomical and functional volumes were spatially normalized (Talairach and Tournoux, 1988) so that the statistical maps could be overlaid to the 3D T1-weighted scans to calculate Talairach coordinates for all activated clusters.

Subsequently, the functional data were analyzed using a multiple regression model (General Linear Model; GLM) consisting of predictors, which corresponded to the particular conditions of each experiment. The predictor time courses used were computed on the basis of a linear model of the relation between neural activity and hemodynamic response (Boynton et al., 1996).

5.2.6. Statistical analyses and contrasts of interest

Since our goal was to define the set of areas responding preferentially or exclusively to the teeth (T) stimulation, we performed 8 contrasts of interests in group analysis (random effect analysis, 10 subjects included in each contrast): T11, T13, T21, T23 versus baseline, the difference between the right incisor and the right canine (T11 - T13) and the equivalent contrast on the left (T21- T23), and the right and left conjunction (T11 ∩ T13, T21 ∩ T23). We defined teeth-sensitive areas using a statistical threshold of $p<0.025$ (not corrected) and a minimum cluster size of 40 mm^3, except for the conjunction (T21 ∩ T23) where a $p<0.05$ was chosen to accommodate for the lower global level of activation in this group of subjects.

A second level random effect group analysis was also performed with all the 20 subjects to compare the left and the right stimulation and compute the contrast: T11 U T13 U T21 U T23. The statistical threshold was set to $p< 0.0005$ (not corrected) and a minimum cluster size = 40 mm^3. For each cluster found in this analysis, we directly compared the right and left stimulations with an ANOVA (with brain voyager). For the

four somatosensitive areas, we plotted the values of the predictor for every single participant. We performed also one additionnal ANOVA with Statistica v 8 (Statsoft, Inc., Tulsa, OK) to evaluate in details the differences observed in S1.

The anatomic location and the cytoarchitectonic correspondence of each activated clusters were defined thanks to the atlas of Talairach and Tournoux and the stereotaxic maps of the parietal operculum provided by (Eickhoff et al., 2006). An ultimate check-up was made by a senior neuroradiologist (CG) on the averaged 3D T1-weighted anatomy of the subjects included in the study. For a cytoarchitectonic based display of the parietal operculum (**Figure 3**), we used the software tool integrated in SPM (The Wellcome Department of Imaging Neurosciences, www.fil.ion.ucl.ac.uk/SPM) that is made freely available by Eickhoff at al. (2005) at www.fz-juelich.de/ime/spm anatomy toobox, and we translated the Talairach coordinates of our activated foci into the MNI space.

5.3. Results

The activated clusters found during the stimulation of T11, T13, T21 and T23 are presented in **Table 1** and displayed on brain anatomy in **Figure 1**. The postcentral gyrus (S1) was activated on the contralateral side for T11 and T13, and bilaterally for T21 and T23. However, we had to lower the threshold until $p<0.05$ to find an activation in the contralateral S1 area for T21. At this lower threshold, the activation in S1 remained only contralateral for T11 and T13. The parietal operculum (S2) was activated bilaterally for all teeth and the foci of activation were located mainly in OP1 (all teeth), but also in OP4 (3 out of 4 teeth) and OP2 (3 out of 4 teeth). Other areas like the inferior parietal lobule, the superior temporal gyrus, the precentral gyrus, the middle or inferior frontal gyri, the insula and the cerebellum were less consistently activated.

Table 1: Location of all activated clusters found for each stimulated site

Stimulated site	**Brain area**	**Side**	**Cytoarchitectonic area #**	**x**	**y**	**z**	**Volume (mm^3)**
Tooth 13	Cluster S1-S2 *	Contra		-53	-24	23	2937
	Parietal operculum (S2)	*Contra*	*OP1*	*-64*	*-27*	*22*	*11*
		Contra	*OP4*	*-64*	*-18*	*24*	*19*
		Contra	*OP2*	*-44*	*-31*	*15*	*29*
	Postcentral gyrus (S1)	*Contra*	*2*	*-50*	*-27*	*33*	*38*

		Contra	*1*	*-57*	*-20*	*35*	*7*
	Cluster S1-S2 *	Ipsi		57	-26	18	2265
	Parietal operculum (S2)	*Ipsi*	*OP1*	*59*	*-22*	*17*	*308*
		Ipsi	*OP1*	*47*	*-32*	*18*	*21*
	Parietal operculum (S2)	Ipsi	OP2	37	-30	16	51
	Inferior parietal lobule	Ipsi	40	53	-43	45	46
		Contra	40	-58	-38	42	46
	Precentral gyrus	Contra	6	-47	-13	49	89
		Contra	6	-52	3	34	540
	Middle frontal gyrus	Contra	6	-24	-5	52	108
	Posterior insula	Contra	13	-40	-14	-5	101
		Contra	13	-38	-6	-1	60
	Cerebellum, posterior declive	Ipsi		17	-68	-18	77
		Ipsi		7	-68	-15	56
	Caudate, corpus	Contra		-15	8	15	59
	White matter, periventricular	Ipsi		23	-33	22	75
Tooth 11	Cluster S1-S2 *	Contra		-54	-23	22	2934
	Parietal operculum (S2)	*Contra*	*OP1*	*-54*	*-26*	*18*	*12*
		Contra	*OP4*	*-55*	*-19*	*18*	*21*
		Contra	*OP4*	*-57*	*-22*	*24*	*13*
		Contra	*OP4*	*-62*	*-20*	*18*	*24*
	Postcentral gyrus (S1)	*Contra*	*1*	*-58*	*-20*	*36*	*30*
	Cluster S1-S2 *	Ipsi		55	-28	18	2828
	Parietal operculum (S2)	*Ipsi*	*OP1*	*54*	*-23*	*22*	*268*
		Ipsi	*OP1*	*47*	*-32*	*18*	*5*
		Ipsi	*OP1*	*54*	*-23*	*18*	*143*
	Superior temporal gyrus	Contra	22	-59	-35	15	52
	Precentral gyrus	Contra	6	-46	-12	48	74
		Contra	6	-52	1	38	106
	Inferior frontal gyrus	Contra	44	-51	5	29	207
		Contra	10	-38	42	-1	87
	Posterior insula	Contra	13	-38	-6	0	89
	Cerebellum, post declive	Ipsi		18	-67	-18	63
	Corpus callosum, splenium	Contra		-5.5	-29	19	50

Tooth 23	Parietal operculum (S2)	Contra	OP2	44	-24	17	42
		Ipsi	OP1	-52	-24	16	240
		Ipsi	OP4	-63	-17	17	43
	Postcentral gyrus (S1)	Contra	2	54	-23	36	689
		Ipsi	1 , 2	-56	-20	37	525
	Middle frontal gyrus	Ipsi	6	-37	3	47	46
Tooth 21	Parietal operculum (S2)	Contra	OP1	51	-25	15	45
		Contra	OP2	34	-30	17	57
		Ipsi	OP1	-53	-22	16	551
		Ipsi	OP1	-48	-36	18	41
	Postcentral gyrus (S1)	Contra **	2	54	-20	37	276
		Ipsi	1	-56	-19	40	280
		Ispi	1	-61	-16	34	97
	Superior temporal gyrus	Contra	22	52	-32	13	184
	Middle frontal gyrus	Ipsi	46	-46	40	14	62
		Ipsi	9	-50	6	41	61

Random effect analysis (10 subjects/tooth); foci of activation found at t=2.8 (p< 0.025, not corrected), minimum cluster size=40 mm³
** If cluster size > 2000 mm³ in S1-S2, it was split in several local maxima by looking at the activated peaks at a more severe statistical threshold (p<0.0025); these local maxima are written in italic.*
*** Activated area found at p<0.05.*
 # The numbers represent the Brodman areas; OP1-4 = subdivisions of the parietal operculum; x, y, z = Talairach stereotaxic coordinates (mm); S1= primary somatosensory area; S2= secondary somatosensory area; ipsi=ipsilateral; contra=contralateral

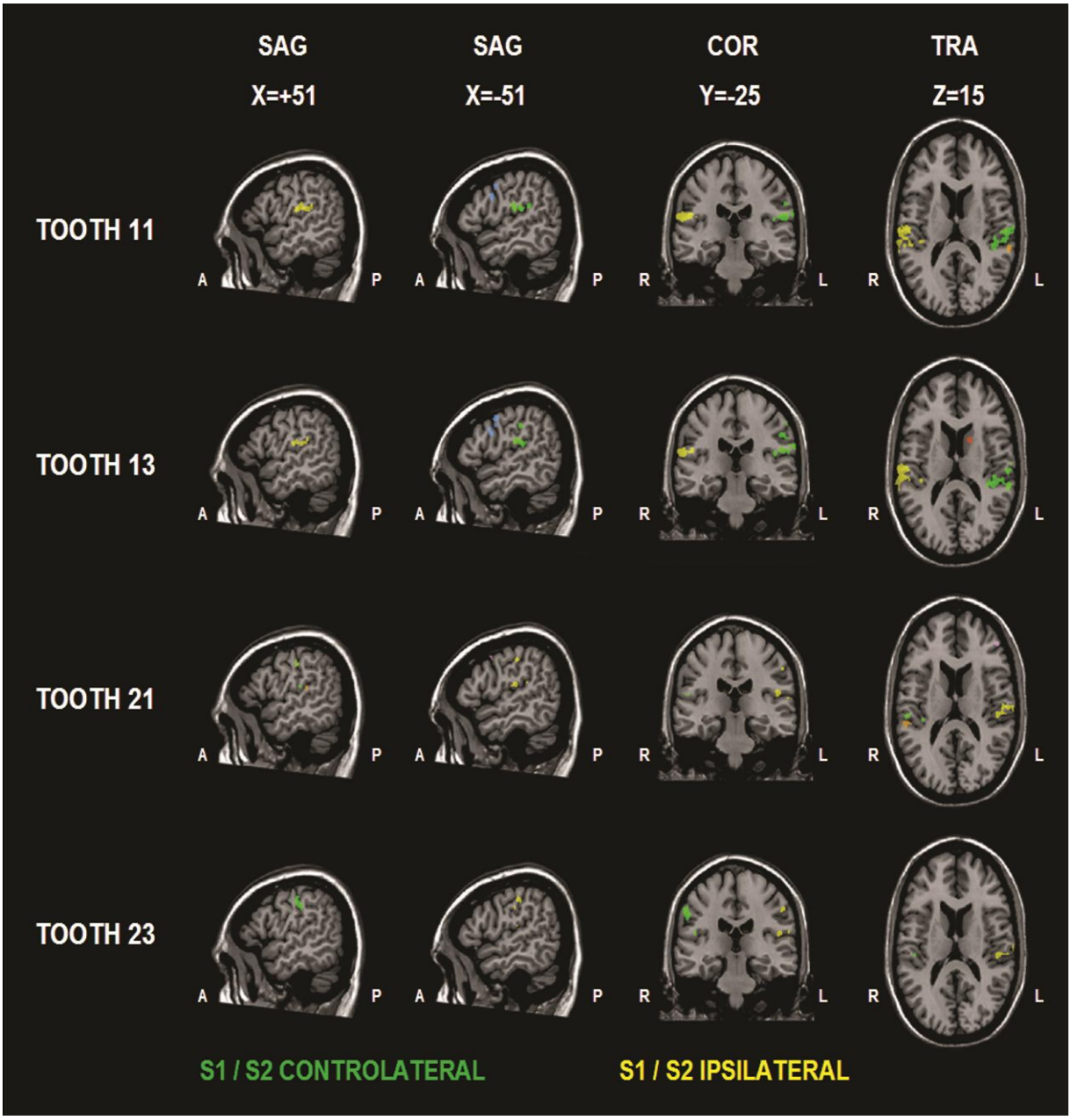

Figure 1. Foci of activation found during punctuate tactile stimulation (1 Hz) of each tooth at p<0.025, minimum 40 mm³ (not corrected, random effect analysis, 10 subjects per tooth).
The primary somatosensory area (postcentral gyrus, S1) is activated bilaterally for tooth (T) 21 and T23 and on the contralateral side for T11 and T13. The secondary somatosensory area (parietal operculum, S2) is activated bilaterally for all teeth. Beside S1 and S2 displayed in green (contralateral) and yellow (ipsilateral), you may see other foci of activation less consistently activated (precentral and inferior frontal gyri in blue, caudate in dark orange and superior temporal gyrus in light orange)

The results of the conjunction analysis are displayed in **Table 2.** They revealed the areas which are commonly activated for T11 and T13 (S2 bilateral, S1 contralateral, precentral gyrus and inferior frontal gyrus) and for T21 and T23 (S2 ipsilateral, S1 bilateral, superior and middle temporal gyri). For the contrast (T21 n T23), we might be

surprised to find only an ipsilateral activation in S2 while this area was activated bilaterally for both T21 and T23. However, the conjunction analysis highlights only the foci which are exactly at the same location and the coordinates of the activated cluster in the contralateral S2 were too different for T21 and T23 to be considered as "common". On the other hand, we observed some activation in the middle and superior temporal gyri because the conjunction may show activations which do not appear for an individual tooth but are present at a low level for both teeth.

The subtraction analysis revealed only scattered activated areas outside the somatosensory network (mainly in temporo-occipital areas, cerebellum, white matter, and occasionally in precentral gyrus, middle frontal gyrus and insula).

Table 2: Location of all activated clusters found in the conjunction analysis

A: Teeth 11 and 13: foci of activation found at t=2.8 (p< 0.025, not corrected), minimum cluster size=40 mm3

Brain area	Side	Cytoarchitectonic area #	x	y	z	Volume (mm^3)
Parietal operculum (S2)	Contra	OP1	-51	-25	18	1058
	Contra	OP1	-64	-26	22	56
	Contra	OP4	-60	-14	21	43
	Ipsi	OP1	56	-27	18	1645
Postcentral gyrus (S1)	Contra	1	-58	20	34	333
Superior temporal gyrus	Contra	22	-59	-34	15	41
Precentral gyrus	Contra	6	-52	1	38	56
	Contra	6	-46	12	48	47
Inferior frontal gyrus	Contra	44	-52	6	28	53

B: Teeth 21 and 23: foci of activation found at t=2.3 (p< 0.05, not corrected), minimum cluster size=40 mm3

Brain area	Side	Cytoarchitectonic area #	x	y	z	Volume (mm^3)
Parietal operculum (S2)	Ipsi	OP1	-55	-22	16	781
	Ipsi	OP2	-38	-32	18	57
Postcentral gyrus (S1)	Contra	2	54	-21	37	215
	Ipsi	1, 2	-57	-19	38	791
Superior temporal gyrus	Contra	22	51	-32	13	120
Middle temporal gyrus	Ipsi	39	-42	-69	25	66
		21	-54	-39	-6	41

 # The numbers represent the Brodman areas; OP1-4 = subdivisions of the parietal operculum; x, y, z = Talairach stereotaxic coordinates (mm); S1= primary somatosensory area; S2= secondary somatosensory area; ipsi=ipsilateral; contra=contralateral

The network globally activated by the four teeth was revealed by the contrast T11 U T13 U T21 U T23 performed in the second level random analysis (**Table 3**). Foci of activation were found in S1 and S2 bilaterally and in the left inferior frontal gyrus (ventral premotor area).

Table 3: Location of all activated clusters found in the second level random effect analysis including all teeth (20 subjects)

Brain area	Side	Cytoarchitectonic area #	x	y	z	Volume (mm3)
Parietal operculum (S2)	Left		-50	-26	18	3444
	Left	*OP1*	*-56*	*-22*	*24*	*38 **
	Left		*-51*	*-26*	*17*	*1706 **
	Left	*OP1*	*-58*	*-30*	*13*	*15 ***
	Left	*OP4*	*-58*	*-14*	*21*	*2 ***
	Left	*OP4*	*-60*	*-15*	*21*	*1 ***
	Left	*OP1*	*-51*	*-25*	*17*	*895 ***
Parietal operculum (S2)	Right		52	-27	17	2600
Superior temporal gyrus	*Right*	*22*	*61*	*-35*	*9*	*10 **
Parietal operculum (S2)	*Right*	*OP4*	*60*	*-20*	*27*	*2 **
	Right	*OP2*	*39*	*-29*	*18*	*66 **
	Right	*OP1*	*54*	*-26*	*16*	*1054 **
Postcentral gyrus (S1)	Left	1, 2	-55	-21	35	918
	Right	2	52	-26	32	82
Inferior frontal gyrus	Left	44	-52	6	32	66

Foci of activation found at t= 3.8 (p< 0.0005, not corrected), minimum cluster size = 40 mm³
*If cluster size >1500 mm³, it was split in several local maxima by looking at the activated peaks at a more severe statistical threshold; these local maxima are written in italic; * foci found at p<0.000045; ** foci found at p<0.00001*
 # The numbers represent the Brodman areas; OP1-4 = subdivisions of the parietal operculum; x, y, z = Talairach stereotaxic coordinates (mm); S1= primary somatosensory area; S2= secondary somatosensory area; ipsi=ipsilateral; contra=contralateral

The detailed topography of the activation in S2 area is provided in **Figure 2**. It shows that the foci of activation were distributed in both OP1 and OP2. The ANOVA ($F_{1,18}$) performed in this second level random analysis to compare the activation among teeth and between the right and left quadrants did not revealed any significant difference (p ranging from 0.134 to 0.578) except in the left ventral premotor area where T11 and T13 were more activated compared to T21 and T23 (p=0.033 between the quadrants, p=0.056 between T11 and T21 and p=0.033 between T13 and T23).

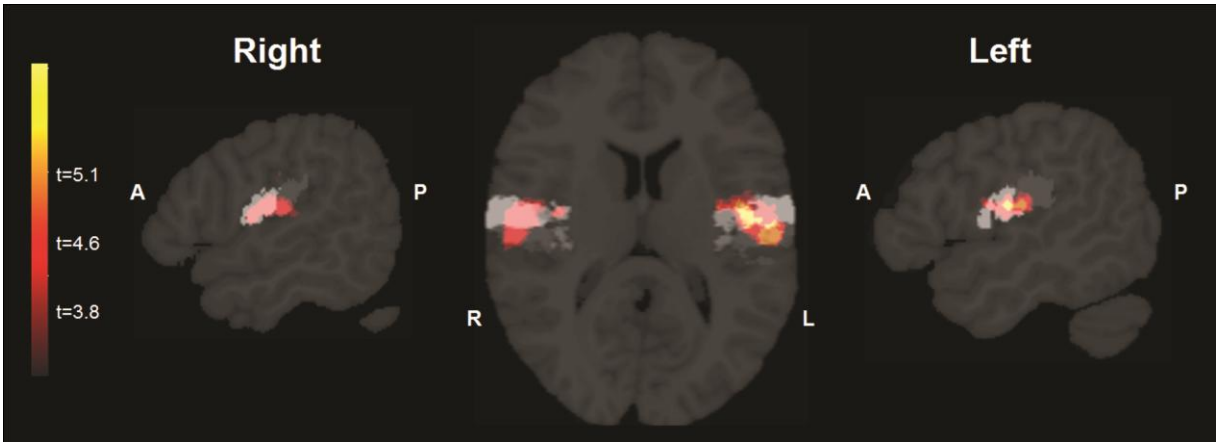

Figure 2. Magnified view of the parietal operculum with its subdivisions based on the probabilistic cytoarchitectonic maps proposed by Eickhoff et al. (2005, 2006).

OP1 is in dark grey, OP2 in middle grey, OP3 in light grey, and OP4 in very light grey. The activated clusters found for the four teeth (11, 13, 21 and 23) in the second level random analysis (20 subjects) are superimposed. The statistical threshold is indicated by the colour scale (t between 3.8 and 5.1, not corrected). Activation is mainly distributed in OP1 and OP4.

For the four somatosensory areas, the values of the predictors of each subject are displayed in **Figure 3**. For S1, a 2 (*Group*: Right stimulation *vs.* Left) x 2 (*S1*: Right *vs*. Left) x 2 (*Tooth:* Incisor *vs.* Canine) ANOVA with the 20 volunteers was performed on these predictor's values. This confirmed that there was no effect of *group* or *tooth.* However an effect of *S1* was found with a higher activation in left S1 than right S1 ($F_{1,18}$= 13.17; p=0.002). This effect was driven by the right stimulation ($F_{1,18}$= 10.60; p=0.004) with a contralateral dominance and not by the left stimulation ($F_{1,18}$= 3.52; p=0.077) where the activations were bilateral with only a trend to an ipsilateral dominance. We may also note that for all teeth, the predictors were lower for the right S1 as compared to the other areas. To further increase the sensitivity of our analysis, we then also performed the right-left comparison in the S1 clusters found separately in each individual tooth. We found consistent differences only in the primary somatosensory area, and the detailed results for the contralateral S1 area activated for each tooth are shown in **table 4**. The right-left differences were always more significant for the canines (T13 and T23) than for the incisors. This may be interpreted as a slight preponderance of contralateral projections in S1 area for the canines while the incisors have more bilateral projections. No such difference was found in the secondary somatosensory area: in the four S2 areas defined contralaterally to the 4 teeth, the left-right difference was significant in only 1 out of 8 contrasts, and consistent differences between canines and incisors were not present.

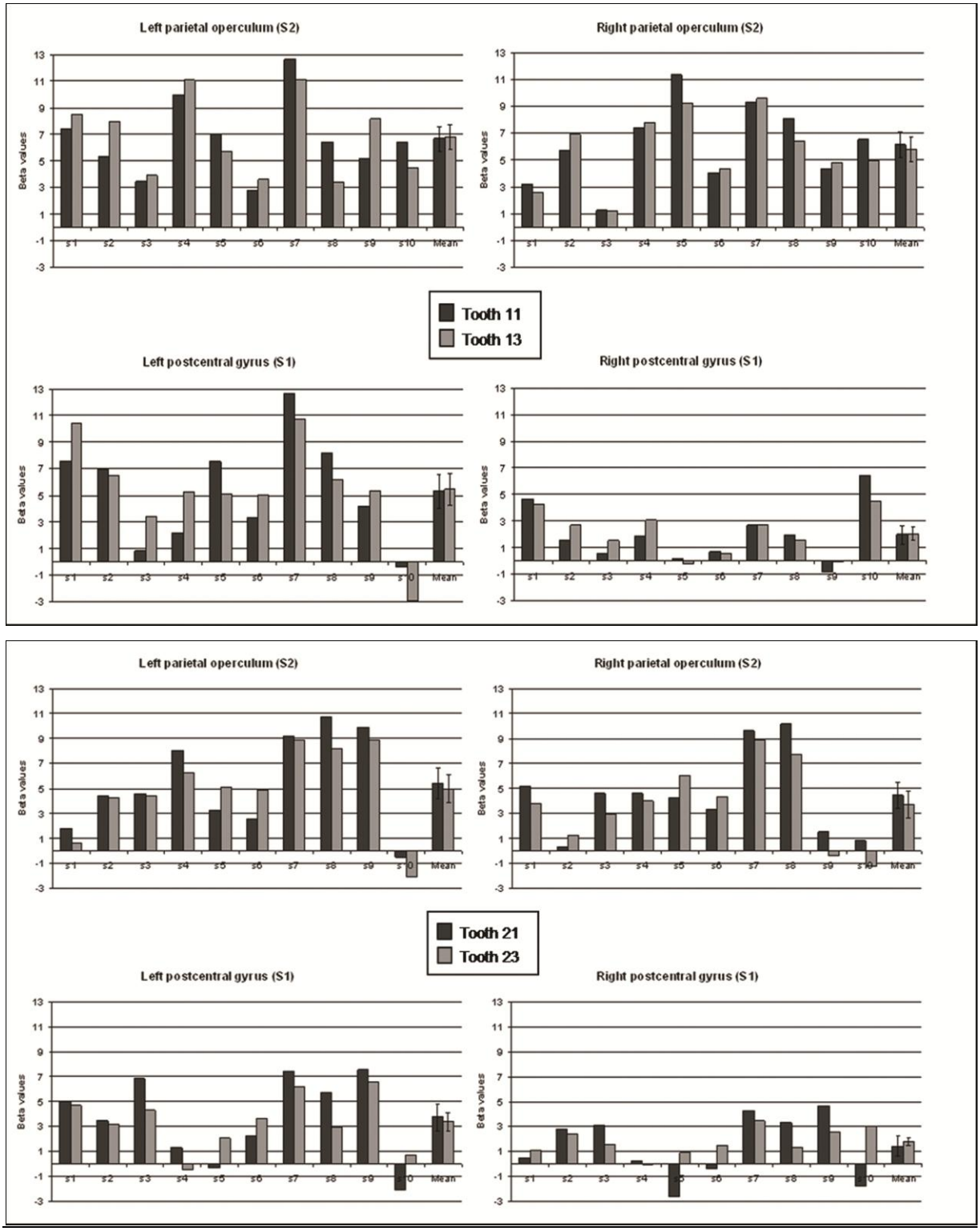

Figure 3:
Values of the predictors (beta) for each subject in the four somatosensory areas found in the second level random analysis including the four teeth.

A: subjects who underwent a stimulation of teeth 11 and 13, and B: subjects who underwent a stimulation of teeth 21 and 23.

Table 4: Second level random effect analysis (20 subjects included).

Area of interest			Comparison	p value
Area	Tooth (T)	Side		
S1	11	Left	T11 > T21	0.289
			T13 > T23	0.134
S1	13	Left	T11 > T21	0.112
			T13 > T23	0.0006
S1	21	Right	T21 > T11	0.089
			T23 > T13	0.008
S1	23	Right	T21 > T11	0.114
			T23 > T13	0.002

ANOVA performed in the somatosensory areas found for each tooth. Only the results for the contralateral S1 area are presented.

5.4. Discussion

The present fMRI study provides a thorough description of the activation pattern elicited by human periodontal mechanoreceptors triggered by 1 Hz tactile stimulation applied on the upper central incisors and canines of the left or right quadrant. We globally observed bilateral activations in the primary and secondary somatosensory cortex, without any significant difference in the cortical projections between the side of stimulation, and between the incisors and canines. However, there was a trend to more contralateral projections in S1 area, mainly for the canines.

5.4.1. Cortical activations

Significant activations in the somatosensory cortex were found for all stimulated teeth indicating that the response in these cortical areas was dominant and robust. In previous studies, conflicting results emerged when painful and non-painful dental stimulations were compared (Hari and Kaukoranta, 1985)or when non physiologic stimuli were applied like vibrotactile stimuli (Ettlin et al., 2004). Vibrotactile stimuli are known to trigger distant receptors through bone conducation. The latter authors identified activations primarily in the insular cortex bilaterally and in the supplementary motor cortex but not in the somatosensory cortex. Using a manually applied torque force, Miyamoto et al. (Miyamoto et al., 2006) were able to map the S1 representation of the stimulated tooth. Very recently, Trulsson et al. (2010) demonstrated that low frequency vibrotactile stumuli (20 Hz) were able to activate somatosensory areas while higher frequencies did not. Our study confirms this finding

by showing that very low frequency stimulation (1 Hz) triggers periodontal mechanoreceptors and activates mainly 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 and Pardo, 2002; Iannetti et al., 2003). Therefore, we believe that these somatosensory areas play a major role in the sensory feedback control of the forces used to hold and manipulate the food between teeth, in a comparable way as for the fingers during the precision grip.

5.4.1.1. Primary somatosensory cortex

5.4.1.1.1. Cortical coordinates:

As expected, the cortical representation of the teeth within S1 was located on the inferior lateral aspect of the post-central gyrus, close but clearly separated from the Sylvian fissure. However, the exact z-coordinates reported in the few studies who have mapped the teeth projections within S1 were dissenting. Miyamoto et al. ((Miyamoto et al., 2006) reported coordinates comprised between 39 and 41 while Jantsch at al. (Jantsch et al., 2005) reported values between 19 and 22. In our study, the z-coordinates of S1 were comprised between 32 and 40, in accordance to the values of 36 to 40 reported recently by Trulsson et al. (2010), and close to those reported for the trigeminal projections by Fox at al. (1987), Iannetti at al. (Iannetti et al., 2003), Schulz at al. (2004), Huang and Sereno (2007), and Dresel at al. (Dresel et al., 2008).

5.4.1.1.2. Laterality of the activations:

Few studies have addressed the laterality of the facial representation in humans and the results are contradictory. Using fMRI, many authors have demonstrated bilateral activation of S1 when mechanically stimulating different areas of the face (Eickhoff et al., 2008; Dresel et al., 2008; Huang and Sereno, 2007). However, Kopietz et al. (Kopietz et al., 2009) reported only contralateral activation in S1, and Iannetti et al. (2003) reported bilateral activation except for the lower lip where the activation was only contralateral. This latter finding was not confirmed by Shulz et al. (2004) and Jousmäki et al. (Jousmaki et al., 2007) who used MEG and showed bilateral activation of S1 when stimulating the lower lip.

Regarding intraoral structures, Disbrow et al. (Disbrow et al., 2003) used MEG to map the cortical representation of lips and tongue in humans, and found a bilateral representation of both regions in S1. One fMRI study on dental pain receptors reported bilateral activation in S1 area (Jantsch et al., 2005). Periondontal mechanoreceptors were specifically stimulated in only one study, and bilateral S1 activation with a contralateral dominance was reported (Trulsson et al., 2010). However, only one tooth (T21) was stimulated.

In our study, we are the first who stimulated the periodontal mechanoreceptors around 4 teeth pertaining to the right and left quadrants. In the second level analysis, we found a bilateral activation of the primary somatosensitive area without any significant difference between the teeth. However, in the individual analysis, S1 was activated bilaterally for T21 and T23 but only contralaterally for T11 and T13. This finding cannot be explained by a masticatory dominance which was not analyzed since we only stimulated anterior teeth. It could be related to the fact that 2 different groups of subjects underwent the right and left stimulation with potential differences in the tactile threshold level (Robertson et al., 2003). Indeed, for the stimulation the same VFF N°s were used for all subjects and we did not try to determine the individual sensory perception threshold by testing several VFF N°s in each subject.

On the other hand, we also demonstrated subtle differences between the right and left quadrants in the regions of interest found for each individual tooth. In the contralateral S1 area, the difference between the right and left side was significant in 3 out of 4 canines and in none of the incisors. We therefore hypothesize that the cortical projections in S1 are bilateral with a slight contralateral preponderance, especially for the teeth located at some distance from the midline.

To verify the present results and make more definitive statements, studies including more subjects should be performed with all teeth tested in each subject using a more precise and individualized calibration of the stimuli relative to the individual sensory perception threshold.

5.4.1.1.3. Neuronal network from periodontal mechanoreceptors to S1:

The brain receives tactile information from the mechanoreceptors via sensory input terminating in the thalamus, which in turn forwards the information mainly to the primary somatosensitive cortex.

In the macaque, it has been demonstrated that the ventral posteromedial nucleus (VPM) of the thalamus contains representation of the contralateral head from the three divisions of the trigeminal nerve and ipsilateral representation of intraoral structures, almost exclusively those innerved by the nerve V3 (Rausell and Jones, 1991a, b). In humans, bilateral projections to the thalamus from both upper and lower teeth have been demonstrated for nociceptive stimuli (Weigelt et al. 2010). However, we were unable to find any study about the thalamic projections of human periodontal mechanoreptors. Therefore, we may only speculate that a bilateral representation of intraoral structures is present as early as in the thalamus.

At the cortical level, Manger et al. (Manger et al., 1996) used microelectrode recording in the macaque to map the trigeminal projections in S1 area. They showed a contralateral representation of the face but a bilateral representation of intra-oral structures. The ipsilateral representation formed 40% of the trigeminal projections, consistent with the amount of the VPM devoted to ipsilateral representation of intraoral structures.

S1 area actually contains four representations of the body: area 3a responding mainly to muscle receptors, and areas 3b, 1 and 2 responding to light touch (Merzenich et al., 1978).

The somatotopy of the oral cavity representation in cortical area 3b has been determined in new world monkeys by Jain at al. (Jain et al., 2001). Cortical sections revealed a division into a series of myelin-dense ovals where the more rostral ovals successively represented the contralateral teeth, tongue, and the ipsilateral teeth and tongue. Similar sequences were also found in areas 3a and 1. Injection of fluorescent tracers into the ovals representing the teeth in area 3b revealed ipsilateral cortico-cortical connections to adjacent areas and to the frontal lobe, callosal connections to contralateral corresponding ovals, and thalamic connections to the VPM (Iyengar et al., 2007). Similar findings have been found by Henry and Catania (2006) in the naked mole-rat. In their study, the lower incisor S1 area had intrahemispheric connections to adjacent areas, to the secondary somatosensory cortex (S2) and parietal ventral (PV) area and to the anterior cortex, together with homotopic callosal projections and thalamocortical connections.

Our findings are in accordance with these data. There is strong evidence for a bilateral representation of the teeth into the primary sensitive cortex coming directly from the

thalamus or via transcallosal projections. The center of gravity of the activation in area 3b might be slightly different for left and right teeth, but we were unable to demonstrate a clear difference. This might be caused by the insufficient spatial resolution of fMRI. From our data, we hypothesize a small preponderance of contralateral representation in S1 which is in accordance with the results reported in the macaque (Manger et al., 1996). It is not surprising that this was found mainly for the canines as compared to the incisors. Indeed, about half of single nerve afferents originating from periodontal mechanoreceptors have receptive fields responding to 3 teeth: the main one and the two adjacent ones in contact with their crown (Trulsson, 1993). The response profiles indicated that this was due to mechanical coupling rather than branching of single afferents to innervate several teeth (Johnsen and Trulsson, 2003). As a consequence, the afferent from incisors (T11 and T21) have bilateral receptive fields and a more bilateral representation in S1 area.

Beside S1 and S2, we also found activations in premotor areas for all teeth (either on the precentral gyrus or in the middle or inferior frontal gyri) that may correspond to the frontal connections described in the aforementioned animal studies. Such premotor activation has also been reported by Dresel et al. (Dresel et al., 2008) when stimulating the face, and Trulsson et al. (2010) described activated foci in various frontal areas when stimulating the periodontal mechanoreceptors. As for the hand, these areas may be part of the sensory-motor network involved in the control of precise movements (Ehrsson et al., 2001).

In old world monkeys and in humans, the primary somatosensory cortex also includes area 2 which is known to have more complex and often bilateral receptive fields.

The representation of the oral structures in area 2 was studied in the macaque by Toda and Taoka (2001). They recorded single-neuron activities and found neurons responding to mechanical tooth stimulation. The majority of them (81%) had receptive fields from several teeth in either jaw and 37% from other oral structures surrounding the teeth, such as gingiva, lip, and tongue mucosa. The authors suggested that area 2 could be the stage of integration of sensory information from the periodontal ligament and from other oral structures representing a combination of the regions stimulated simultaneously during food intake.

This report is in congruence with the bilateral activation of SI observed in our study and with the absence of distinction between the teeth that may be explained by the

necessary integration of the sensory input from several teeth during intra-oral food manipulation.

The hierarchical convergence of sensory input from the oral area across the primary somatosensory cortex has also been demonstrated in humans by Miyamato et al. (Miyamoto et al., 2006). In their fMRI study, they reported some segregation of the cortical representation of the lower lip, tongue and upper central incisor into the anterior part of the postcentral gyrus (areas 3b) but not in the posterior part (area 2). However, the laterality of the activations was not mentioned.

5.4.1.2. Secondary somatosensory area

In contrast to S1, the human parietal operculum is known to show bilateral activation even with unilateral peripheral stimulation of most parts of the body (Eickhoff et al., 2006). The bilateral representation of sensory input in S2 area is thought to be the result of transcallosal projections rather than thalamic input. Indeed, homotopic transcallosal projections between the different S2 areas have been demonstrated in many species and are likely to exist in humans too (Disbrow et al., 2003a; Qi et al., 2002). Moreover, heterotopic callosal connections between S1 and S2 have also been demonstrated in the macaque (Manzoni et al., 1986). To support this view, an fMRI study conducted in a single subject showed that a robust bilateral S2 activation was abolished following a callosotomy (Fabri et al., 2001). Electrophysiological studies also have shown that the contralateral S2 responses precede ipsilateral ones by about 13 ms, favoring a monosynaptic transcallosal connection (Eickhoff et al., 2008; Karhu and Tesche, 1999).

It was therefore expected to find bilateral activation of S2 during the stimulation of every tooth, first because the projections received from the ipsilateral S1 area already integrate information coming from both sides, and second because of the transcallosal projections from the contralateral S1 and S2 areas.

There is converging evidence that areas S2, PV and VS in the monkey correspond to the S2 area defined in humans with a homology with OP1, OP4 and OP3, respectively (Eickhoff et al., 2007). Each of these regions contains a complete somatotopic map (although less precise than in S1), and this has also been demonstrated in human OP1 and OP4 and less clearly in OP3. In our study, the more robust activation was found in OP1. However, when splitting the main activated foci into several local maxima, a

representation of the teeth in both OP1 and OP4 was most often present, in accordance with the somatotopy found for other parts of the body. The center of gravity of the activated foci was never located in OP3 but some maxima were located in OP2. According to Eickhoff et al. (Eickhoff et al., 2006), OP2 is not part of S2 but rather comparable with the parietal-insular-vestibular cortex (PIVC) in non human primates. The significance of this activation is therefore unclear and might correspond to a spillover of the hemodynamic response from the true S2 area. Similarly, the significance of the activation found in the superior temporal gyrus is equivocal. It might also correspond to a spillover of the activation of the parietal operculum or correspond to a distinct activation in this area known as a multi-sensory region that responds to tactile, auditory and visual stimulation (Macaluso and Driver, 2005). This activation might be enhanced by some auditory input reported by some subjects who heard the sound of the VFF contacting the surface of the tooth during the experiment (Habre-Hallage et al. 2010).

5.4.2. Periodontal mechanoreceptors

Microneurographic recordings from single nerve fibers reveal that human periodontal receptors adapt slowly to maintained tooth loads. They exhibit a markedly curved relationship between discharge rate and force amplitude, featuring the highest sensitivity to changes in tooth load at very low force levels (below 1 N for anterior teeth and 4 N for posterior teeth) (Trulsson and Johansson, 1994, 1996a, b). That is why we choose to stimulate the incisor and canine with VFF of 100 g= 1N and 180g = 1.8 N respectively.

Periodontal receptors, reliably encode information about both the teeth stimulated and the direction of forces applied to the individual teeth. Considering the rather sharp decline in both number of activated afferents and response intensities from the receptor bearing tooth to the adjacent teeth, Trulsson and Johansson (1996a) hypothesized that human periodontal afferents accurately encodes the location of the tooth directly loaded. Therefore, when a tooth is stimulated, the brain is most of the time (but not always) able to recognise its location although fMRI was unable to discriminate between the bilateral activated areas corresponding to the stimulated teeth. This must be interpret as both a lack of spatial resolution and a lack of temporal resolution of fMRI

which cannot distinguish the first spike from the following ones leading to the necessary integration of the sensory input in areas 3b, 1 and 2.

On the other hand, based on previous studies (Miyamoto et al., 2006, Habre-Hallage et al., 2010), we used a very low frequency stimulation (1 Hz). This may represent a trade-off between single stimulation (> 3s interval) allowing for the tooth to recover from displacement (Picton, 1989) but leading to presumably poor cortical activation, and high frequency vibrotactile stimuli which do not activate specifically the periondondal mechanoreptors.

Indeed, Dong et al. (1993) demonstrated in the cat that periodontal afferent responses were strongest at low frequencies (<32 Hz). This finding was reproduced in humans by Trulsson et al. (2010) who showed that S1 and S2 areas were activated when using a 20 Hz vibrotactile stimulation, while only S2 was activated at 50 Hz and none of the somatosensory areas at 100 Hz. They postulated that high frequency stimuli are outside the range of activity of periodontal mechanoreceptors and activate other kind of receptors located in adjacent areas. This is in accordance with the findings of Ettlin et al. (2004) who used a 80 Hz stimulation and found bilateral activation in the insula and the supplementary motor area, but not in somatosensory areas. We think that our 1 Hz stimuli triggered very specifically periodontal mechanoreptors, explaining why our activations were found almost exclusively in somatosensory areas and the left ventral premotor area, with only minor and non consistant activations in the inferior parietal lobule, superior temporal gyrus, precentral gyrus, middle or inferior frontal gyri, posterior insula and the cerebellum. Most of these additional areas may be regarded as part of the sensory-motor network and were also reported by Trulsson et al. (2010), but they are less specific and increasingly recruited when high frequency stimuli are applied.

5.5. Conclusion

Punctuate tactile stimulation of the teeth at a constant frequency of 1 Hz were able to trigger the periodontal mechanoreceptors and to elicit consistent bilateral cortical activation in the primary and secondary somatosensory areas during an fMRI study.

For the first time, the cortical representation of teeth pertaining to the right and left quadrants were compared and thoroughly described in human subjects. Our findings

are in accordance with the homunculus sensory somatotopy and with the presence of bilateral receptive fields in area S1 with a slight preponderance of contralateral projections for the canines, as found for periodontal mechanoreceptive neurons in monkeys. This study also provides arguments for a double representation of the teeth in area S2, namely in OP1 and OP4 which is congruent with the somatotopic organization of the parietal operculum for other parts of the body.

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Brain Plasticity and Cortical Correlates of Osseoperception Revealed by Punctuate Mechanical Stimulation of Osseointegrated Oral Implants During fMRI

Chapter 6

This chapter is submitted for publication:

Habre-Hallage P., *Dricot L., Reychler H., van Steenberghe D., Jacobs R., Grandin* C. Functional magnetic resonance imaging shows cortical activation following punctuate mechanical stimulation of osseointegrated oral implants. Submitted

Abstract

Our aim was to unveil the neural correlates of the osseoperception by taking bone-anchored oral implants as a model. During fMRI, we applied 1 Hz punctuate tactile stimuli on teeth and implant to trigger periodontal mechanoreceptors and receptors in peri-implant tissues respectively. A block design paradigm was used to stimulate tooth(T) 21 and T23 in 10 controls, and implant (I21) and T23 in 9 patients. Random effect group analyses were performed for each stimulated site, and differences between teeth and implant were examined using ANOVA.

As a group, patients activated S2 bilaterally for both I21 and T23, while controls activated S1 and S2 bilaterally for T21 and T23. However, at an individual level, S1 was activated by 4/9 implants, mainly on the ipsilateral side. The implant activated a larger bilateral cortical network outside the somatosensory areas, with activations found in parietal, frontal and insular lobes, the main clusters being located in the inferior frontal gyri. This may be viewed as a compensatory mechanism for the lower sensory input provided by the implant. Stimulation of T23 in patients resulted in an activation pattern intermediate between this of the implant and natural teeth.

This study demonstrates that punctuated mechanical stimulation on an osseointegrated oral implant activates cortical somatosensory areas. This activation may represent the underlying mechanism of osseoperception. We also show that tooth loss and its replacement by an osseointegrated implant induce brain plasticity as indicated by the difference between the cortical network activated when stimulating the implant and natural teeth.

6.1. Introduction

Tooth loss represents a major oral disability that should be considered as an amputation. It implies severe impairment of biting and chewing functions and leads to a remodeling of the oro-facial area with implications in language articulation and facial expression. Replacement of teeth to restore oral function includes the use of removable dentures or fixed prostheses, the latter eventually supported by bone-anchored implants. Since the discovery that a titanium implant can be permanently integrated into the bone (Branemark et al., 1970; Branemark et al., 1977), millions of amputees, mostly in the oral cavity, have been rehabilitated by osseointegrated implants. Osseointegration of implants has been extensively studied from a histological, biomechanical and microbiologic point of view, but the physiologic integration of implant-supported prostheses has received less attention. Conventional (socket) prosthetic limbs or dentures do not carry enough potential to restore the sensory input. It has been demonstrated that by anchoring prosthetic limbs or tooth directly to the bone, partial sensory substitution can be realized (Jacobs et al., 2000).

In the oral area, recovery of tactile capabilities and masticatory function to a level approaching the natural situation has been described by many authors (Haraldson et al., 1979; Jacobs and van Steenberghe, 1991 and 1993; Branemark, 1999). Similar findings have been described in patients rehabilitated with bone-anchored limb prosthesis who reported being able to recognize the type of soil they were walking on (Branemark, 1999). This special sensory awareness was referred as “osseoperception”. However, its underlying mechanism remains unclear and is the subject of considerable controversy because the term is ill-defined (Branemark, 1998; Klineberg and Murray, 1999; Trulsson, 2005; Klineberg et al., 2005; Jacobs and van Steenberghe, 2006; Yan et al., 2008). Some of these have used it in a broad sense, referring to any sensory input conveyed through an endosseous implant. Moreover, the type and location of mechanoreceptors that could mediate this sense of “osseoperception” remains debated (Macefield, 2005; Rowe et al., 2005).

After tooth loss, most of periodontal ligament receptors are eliminated and the loss of afferent nerve fibers in the mandibular canal as it has been observed so that the impact on the sensory feedback pathway is expectedly considerable ((Linden and Scott, 1989)Trulsson and Gunne, 1998). This may be compared to nerve degeneration and loss of peripheral sensory feedback occurring after limb amputation or spinal cord injury,

with important consequences in tuning motor control (Kass et al., 2008). In the absence of periodontal mechanoreceptors, the transmission of tactile perception must rely on the spread of the force applied to the implant throughout the bone. In turn, this force should be able to trigger neural ending in the bone, in/or beside the periosteum or in the peri-implant soft tissues (Lambrichts, 1998). Histological evidence indicates that, after the surgical trauma related to implantation, some reinnervation occurs and that a gradually increasing number of free nerve endings are found in the vicinity of the bone-to implant interface (Wang et al., 1998; Wada et al., 2001). Their function might be a sensory system for pain, touch and pressure (Jacobs and van Steenberghe, 1991). To test this hypothesis, Van Loven et al. (2000) were able to elicit trigeminal somatosensory-evoked potentials by electrical stimulation of endosseous oral implants in humans, indicating that it are indeed endosseous and/or periosteal receptors around the implants which convey the sensation. While psychophysical, histological and neurophysiological evidence of osseoperception have been collected, the neural correlates of this phenomenon at the cortical level remain poorly known.

Periodontal mechanoreceptors play a major role in conveying some of the sensory feedbacks necessary for the control of mastication. It has been demonstrated that these receptors have projections on cortical somatosensory areas (Miyamoto et al., 2006; Habre-Hallage et al., 2010; Trulsson et al, 2010). How the somatosensory cortex behaves after tooth loss and its replacement by an osseointegrated implant has been hardly explored (Calford, 2005). Neurophysiologic and neuroimaging studies have provided convincing evidence that the adult cerebral cortex is capable of significant plasticity in response to over or under use, change in environment and sensory input, or following various central nervous system injuries to allow functional recovery (Donoghue, 1995; Kaas and Qi, 2004; Sessle et al., 2005; Dancause, 2006; Kaas et al., 2008; Flor and Diers, 2009). Regarding oral implants, only one functional study was performed to assess the cortical plasticity of edentulous patients with implant-supported full denture but the authors used a clenching task and not a pure sensory stimulation (Yan et al., 2008). Recently, we have developed and validated the use of a device able to deliver standardised punctuate mechanical stimuli on the teeth during functional magnetic resonance imaging (fMRI) recordings (Habre-Hallage et al., 2010). The tactile stimulation applied to the teeth or implants allowed us to study the cortical

activation induced by triggering the periodontal mechanoreceptors and/or the receptors located in periimplant tissues.

The aim of the present study was to use a pure tactile stimulation to identify the cortical adaptive processes that may be associated with the loss of teeth and their subsequent replacement by endosseous implants. To achieve this goal, we compared the cortical projections of periodontal mechanoreceptors with those induced by stimulation of peri-implant tissues in the hope to unveil the neural correlates of the osseoperception phenomenon at the cortical level. The ultimate objective would be to understand how humans adapt (or not) to an altered oral environment due to tooth loss and how restoring orofacial function by bone-anchored prostheses may produce their rehabilitation effect.

6.2. Materials and methods:

6.2.1. Subjects

Right-handed volunteers according to the Edinburgh Handedness Inventory (Oldfield, 1971), were recruited for the experiment, which was approved by the local biomedical ethical committee. We included a patient group of nine subjects (age 39.7 ± 12.6 years; 4 females) with a complete natural dentition excepted for the upper left incisor tooth (21) that was missing and replaced by a single crown on an endosseous two-stage implant (Branemark system®, Nobel Biocare, Gothenburg, Sweden). Three patients (pts 1, 3 and 9) lost their upper incisor because of an infection and the six others because of tooth fracture. Four patients received a bone graft before implant surgery (pts 1, 2, 6, 8). The implant was loaded for at least 2 years before the fMRI study (2.9 ± 1.1 years). Ten subjects without any oral implant (age 34.3 ± 8.9 years; 5 females) served as control group. Vital teeth with no periodontal breakdown, and no increased tooth mobility were required. 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 prior to 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.

6.2.2. Materials

A manually-controlled device designed for fMRI was used to deliver calibrated repetitive punctuate stimulation to the anterior teeth at a constant frequency of 1 Hz. The device has been thoroughly described previously (Habre-Hallage et al., 2010). It allows the use of von Frey filaments (VFF) that can be adjusted to stimulate exclusively a chosen tooth from outside the magnet. Two different teeth can be alternatively stimulated in the same experiment.

The VFF was chosen to provide stimulation well above the mechanical detection threshold but below the mechanical unpleasantness and definitely pain thresholds. The filament N^{o} 6.65 (300g) and 6.45 (180g) were used for the implants and the canines respectively, and the VFF No 6.10 (100g) for the incisors. We have previously demonstrated that these forces applied to incisors and canines are able to elicit good cortical activations in somatosensory areas (Habre-Hallage et al., 2010). For the implant, we chosen a VFF delivering a higher force as the tactile function by implant is reduced as compared to natural teeth (Jacobs and van Steenberghe, 1991 and 1993). 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 repetitive punctuated stimuli were delivered by the same well-trained experimenter (P.H.H.) to minimize the variability of stimuli across the subjects, and the pace of stimulation was acoustically cued to the experimenter. All subjects felt the stimulation as a pressure on both the teeth and the implants

6.2.3. Experimental paradigm

The fMRI experiment was based on a block wise paradigm with stimulation periods of 24s separated by a rest period of 24s, except for the first rest period which lasted 12s. Two sites were stimulated in each subject: either implant 21 (I21, corresponding to the replaced upper left central incisor) and tooth 23 (T23, corresponding to the upper left canine) in the implanted group, or T21 and T23 corresponding to the homologous natural teeth in the control group. The paradigm consisted of 3 runs with 6 active epochs per run, each site being stimulated 3 times/run with a total number of brain volumes/run=100. Into each epoch, the stimuli were

administrated to the same area, the two activated sites being interleaved in a random and counterbalanced order.

6.2.4. Data acquisition

MRI measurements were performed on a 3 Tesla MRI Scanner (Achieva, Philips Healthcare, Best, The Netherlands) equipped with an 8 channels phased array head coil. All images were acquired in the bicommissural (AC-PC) orientation (Talairach and Tournoux, 1988).

A BOLD sensitive single-shot gradient echo echo planar imaging sequence was used for the functional scans. We acquired 44 axial slices with the following parameters: repetition time (TR) = 3000 ms, echo time (TE) = 32 ms, flip angle (FA) = 90 degree, , slice thickness = 2.3 mm and no gap, field of view (FOV) = 220 mm^2, in plane resolution at the acquisition = 2.2 mm^2, and reconstruction matrix = 112^2. The SENSE factor (parallel imaging) was 2.5.

Structural brain images were also obtained in all subjects using a 3D fast T1-weighted gradient echo sequence with an inversion prepulse (Turbo field echo [TFE]) and the following parameters: TR = 9 ms, TE = 4.6 ms, FA = 8 degree, 150 slices with a thickness = 1mm, FOV= 220 x 197 mm^2 giving an in plane resolution = 0.81 x 0.95 mm^2 and reconstruction matrix = 398^2. The SENSE factor was set to 1.5.

6.2.5 Data analysis

We first proceeded to an optimized spatial realignment of the functional dataset to the first fMRI volume to correct for the small interscan movements. This was done with SPM5 (Statistical Parametric Mapping, The Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm). The detected movements did not exceed 1.5 mm so that no run was rejected.

The rest of the analysis was performed with BrainVoyager QX (Version 2.1.2, Brain Innovation, Maastricht, The Netherlands). It included further preprocessing consisting in a linear trend removal for excluding scanner-related signal, and a temporal high-pass filtering applied to remove temporal frequencies lower than 3 cycles per run. Functional data were not smoothed in the spatial or temporal domain. This no-smoothing option was based on the work of Weibull et al. (2008). The anatomical 3D T1-weighted scan of each participant was manually coregistered to the first fMRI volume. Both anatomical

and functional volumes were spatially normalized (Talairach and Tournoux, 1988) so that the statistical maps could be overlaid to the 3D T1-weighted scans to provide Talairach coordinates for all activated clusters.

We then applied a multiple regression model (General Linear Model; GLM) to the functional dataset using predictors which corresponded to the particular conditions of each experiment. The predictor time courses used were computed on the basis of a linear model of the relation between neural activity and hemodynamic response (Boynton et al., 1996).

6.2.6. Contrasts of interest and statistical analyses

Since our goal was to describe the cortical network activated by the implant but also to compare it to that of natural teeth, we first performed a random effect analysis (RFX) with 2 contrasts of interests in each group to compare each tooth (or the implant) versus rest: implant 21 in patients (I21-p), T23 in patients (T23-p), T21 in controls (T21-c) and T23 in controls (T23-c); 9 patients and 10 controls included in each contrast. For the 4 contrasts, we defined all the teeth-sensitive areas using a statistical threshold of $p<0.05$ and a minimum cluster size of 45 mm^3. The anatomic location and the cytoarchitectonic correspondence of each activated clusters were defined thanks to the atlas of Talairach and Tournoux (1988), and the steretotaxic maps of the parietal operculum provided by Eickhoff et al. (2006) after having transformed the Talairach coordinates into the MNI space. An ultimate check-up was made by a senior neuroradiologist (CG) who looked at the clusters projection on the 3D T1-weigted anatomy. A laterality coefficient was calculated by dividing the number of activated voxels in the contralateral side by the total number of activated voxels.

A second level random effect group analysis was then created with all the 19 subjects to compare the two groups. For each regions found in the 4 contrasts, we compared directly the two groups (I21-p vs T21-c, T23-p vs T23-c), and the incisor versus canine (I21-p vs T23-p, T21-c vs T23-c) with an ANOVA.

As the heterogeneity that can be found in patients is not revealed by the group analysis, we also looked at the individual activation pattern. For each region of interest found in the random affect analysis, we plotted the values of the predictor (beta) for every single participant of each group and counted the number of subjects with a significant predictor ($t > 1.97$).

Finally, we try to better understand the implication of the activated areas found in the random effect analysis by looking at the characteristics of the signal-to-time curve. For the two groups, we considered the percent signal change of the I21-T21's and T23's signals using as baseline the average of every value of each pre-period of stimulation over the whole time course. One observer who was blind to the location of the clusters classified the curves into eight categories: S = signal time course linked to the stimulation, s = signal linked to the stimulation but at a low level or with a high variance, D = delayed signal after the stimulation onset, M = maintained signal after the end of the stimulation, N = noisy signal, No = signal not different from the baseline, P = signal showing a peak during the stimulation, U = unclassified signal-to-time curve. An example of each type of curve is given in **Figure 1**. The activated clusters with a signal time course classified as N were excluded from further analysis.

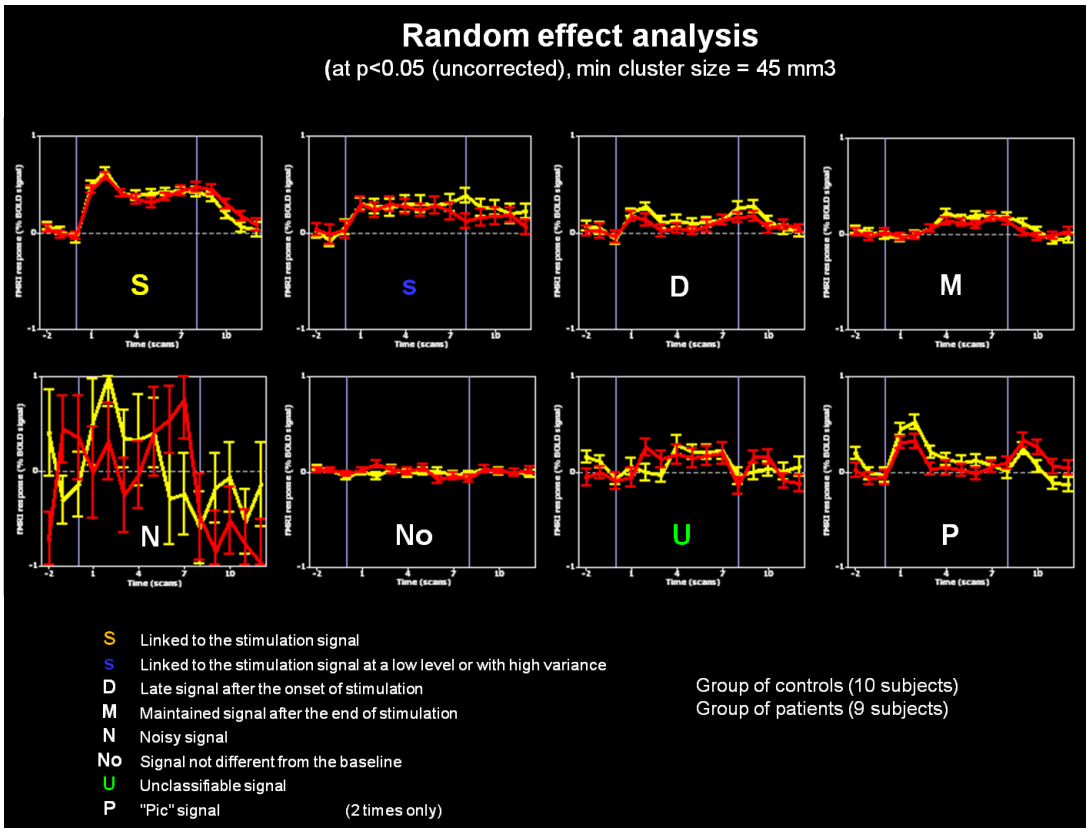

Figure 1: Examples of curves representing the percent change of the signal registered during the stimulation of tooth or implant 21 and teeth 23.
The baseline was calculated as the average of every value of each pre-period of stimulation over the whole time course. The time unit is one volume (3 s). The stimulation began at 0 and ended at 8 (8 volumes/epoch). The curves were classified into eight categories: S = signal time course linked to the stimulation, s = signal linked to the stimulation but at a low level or with a high variance, D = delayed signal after the stimulation onset, M = maintained signal after the end of the stimulation, N = noisy signal, No = signal not different from the baseline, P = signal showing a peak during the stimulation, U = unclassified signal-to-time curve. 132x197mm (600 x 600 DPI)

6.3. Results

6.3.1. Random effect analysis for the 4 contrasts of interest

The activated clusters found during the stimulation of I21 and T23 in patients, and T21 and T23 in controls are presented in **Table 1** and displayed on the brain anatomy in **Figure 2**. In controls, the primary (S1) and secondary (S2) somatosensory areas were activated bilaterally for T21 and T23. In patients, we found a bilateral activation in the parietal operculum (S2) but no activation in the postcentral gyrus (S1) for both I21 and T23.

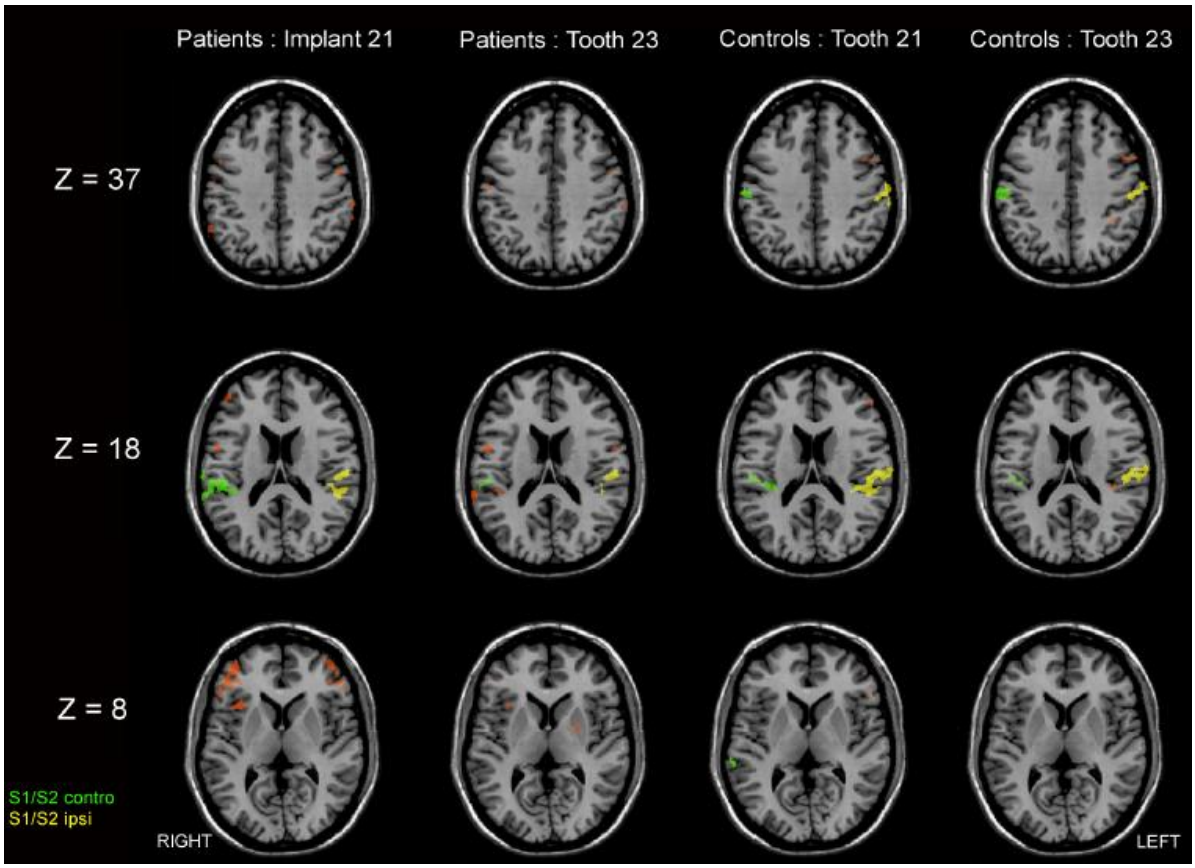

Figure 2: Foci of activation found during punctuate tactile stimulation (1 Hz) of teeth 21 and 23 in controls, and of implant 21 and tooth 23 in patients (random effect analysis, 10 controls and 9 patients, p<0.05 not corrected, minimum cluster size = 45 mm³).
The primary somatosensory area (postcentral gyrus, S1) is displayed in green, the secondary somatosensory area (parietal operculum, S2) is displayed in yellow, and the other foci of activation are in orange. SI and S2 were activated bilaterally for both teeth in controls, while only S2 was activated bilaterally in patients for implant 21 and tooth 23. The implant activated a larger bilateral cortical network outside the somatosensory areas, with activations found in parietal, frontal and insular lobes, the main clusters being located in the inferior frontal gyri. Stimulation of T23 in patients resulted in an activation pattern intermediate between this of the implant and natural teeth.

The ratio between the number of activated voxels in somatosensory areas (S1 + S2) and the total number of activated voxels was lower for I21-p (0.34) as compared to T21-c and T23-c (0.59 and 0.60 respectively), with an intermediate value (0.45) for T23-p. This was due to the large activation of frontal areas elicited by the implant, especially in the inferior frontal gyrus. When all activated areas were considered, the cortical network activated by the implant was bilateral, while it was more ipsilateral for the other teeth (laterality coefficient = 0.47 for I21-p, but 0.11, 0.28 and 0.35 for T23-p, T21-c and T23-c, respectively).

6.3.2. Second level random effect group analysis

The results of the ANOVA comparing the teeth are presented in **Table 1** for each activated cluster. In **Table 2**, the activated clusters found in somatosensory areas for each group are reported. For clusters found in patients, we looked at the individual predictors in controls, and for clusters found in controls, we looked at the individual predictors in patients.

6.3.2.1. Somatosensory areas

6.3.2.1.1. Patients versus controls:

When the implant was stimulated, there was a larger heterogeneity in the activation pattern as compared to controls. However, the activated somatosensory areas were relatively similar to those of controls in 4 on 9 patients. One patient (pt 4) had bilateral activations in S1 and S2, with 6 of the 7 clusters found in controls. Three patients (pts 2, 5 and 7) activated S2 bilaterally and S1 at the ipsilateral side, with 5 of the 7 clusters found in controls. The activated foci found in S2 for patients (I21 and T23) were not different to that of the controls (**Table 1**). However, when looking at the individual predictors (**Table 2**), one of the 3 clusters found in the contralateral S2 for I21-p was present in only 2 controls for T21 and T23, and in 1 patient for T23. This means that this cluster was specific to the implant. We may note that the four patients who exhibited activation in S1 received an implant because of tooth fracture, and only one needed a bone graft before implantation. S1 was not activated in the 3 patients who lost their tooth because of an infection, and in 3 out of 4 patients who received a graft. On the other hand, S1 was activated in all patients who had a tooth fracture and no graft (n=3).

Table I: Location of all activated clusters found for each stimulated site. A: Clusters found in patients (n= 9)

Stimulated site	Brain area	Side	BA	x	y	z	Volume (mm^3)	P value I21-p > T21-c	P value I21-p > T23-p	ST curve Patients	ST curve Controls
Implant 21	Parietal operculum (S2)	Contra-1	OP1	64	-23	17	71	NS	NS	s	s
		Contra-2	OP1	50	-32	18	1137	NS	0.04	S	S
		Contra-3	OP1	62	-28	28	74	NS	NS	s	No
		Ipsi	OP1	-48	-29	17	1437	NS	NS	S	S
	Supramaginal gyrus	Contra	40	53	-48	36	371	0.02	0.005	s	No
		Ipsi	40	-48	-37	31	62	0.05	NS	s	No
	Inferior parietal lobule	Ipsi-1	40	-61	-29	37	84	NS	NS	M	U
		Ipsi-2	40	-57	-42	42	284	NS	NS	M	U
	Precentral gyrus	Contra-1	4	53	-11	39	46	NS	NS	s	No
		Contra-2	6	52	1	17	79	NS	NS	M	No
		Ipsi	6	-50	-3	40	162	0.04	NS	s	No
	Inferior frontal gyrus	Contra-1	45	49	24	8	68	0.003	NS	M	No
		Contra-2	46	41	41	9	1298	0.001	0.03	M	No
		Contra-3	44	45	13	24	77	NS	NS	M	No
		Ipsi	46	-43	44	9	760	0.04	0.03	M	No
	Middle frontal gyrus	Contra	9	48	7	35	68	0.01	NS	D	No
		Ipsi	9	-40	10	41	105	NS	NS	D	No
	Superior frontal gyrus	Contra	8	17	14	46	46	NS	0.03	U	No
	Medial frontal gyrus	Contra	8	2	23	45	69	0.005	0.029	U	No
	Insula	Contra	13	35	16	8	265	0.0003	NS	s	No
Tooth 23	Parietal operculum (S2)	Contra-1	OP1	49	-27	18	47	NS	NS	S	s
		Contra-2	OP1	56	-29	19	59	NS	NS	S	P
		Ipsi	OP1	-48	-25	16	852	NS	NS	S	S
	Superior temporal gyrus	Contra-1	22	62	-39	18	247	0.02	NS	S	P
		Contra-2	22	41	-35	17	53	NS	NS	S	S
	Inferior parietal lobule	Ipsi	40	-59	-29	35	81	NS	NS	M	No
	Precentral gyrus	Contra-1	4	52	-12	38	77	NS	NS	M	M
		Contra-2	6	49	1	16	127	NS	NS	M	No
		Ipsi-1	6	-51	-1	40	82	NS	NS	M	No
		Ipsi-2	6	-55	3	15	112	0.006	NS	M	No
	Inferior frontal gyrus	Contra-1	46	47	34	6	56	0.008	NS	s	No
		Contra-2	46	42	45	11	73	NS	NS	s	No
	Insula	Contra	13	32	17	9	62	0.000004	NS	s	No
	Putamen	Ipsi		-23	-3	8	128	NS	NS	U	No

B: Clusters found in controls (n= 10)

Stimulated Site	Brain area	Side	BA	X	y	z	Volume (mm^3)	P value I21-p > T21-c	P value I21-p > T23-p	ST curve patients	ST curve controls
Tooth 21	Parietal operculum (S2)	Contra-1	OP1	53	-30	13	776	0.02	NS	S	s
		Contra-2	OP2	35	-30	17	219	0.05	NS	S	No
	Postcentral gyrus (S1)	Contra	?	54	-20	37	309	0.008	NS	S	No
	Cluster S1-S2 *	Ipsi		-54	-23	26	3330 *				
	Parietal operculum (S2)	*Ipsi-1*	*OP1*	*-53*	*-22*	*16*	*551*	*NS*	*NS*	*S*	*S*
		Ipsi-2	*OP1*	*-48*	*-36*	*18*	*41*	*NS*	*NS*	*S*	*S*
	Postcentral gyrus (S1)	*Ipsi-1*	*1*	*-56*	*-19*	*40*	*280*	*0.03*	*NS*	*S*	*s*
		Ipsi-2	*1*	*-61*	*-16*	*34*	*97*	*0.02*	*NS*	*S*	*No*
	Middle temporal gyrus	Contra-1	21	64	-38	0	87	0.003	NS	U	No
		Contra-2	37	41	-62	2	75	0.001	0.03	U	No
		Contra-3	37	49	-59	-7	54	0.03	0.01	M	No
		Ipsi-1	39	-37	-68	26	399	0.007	NS	D	No
		Ipsi-2	21	-53	-39	-7	120	0.003	NS	U	No
		Ipsi-3	37	-57	-48	-8	65	0.02	NS	D	No
	Inferior frontal gyrus	Ipsi-1	47	-44	34	-4	235	0.004	NS	D	No
		Ipsi-2	45	-48	26	6	65	NS	NS	D	U
	Middle frontal gyrus	Ipsi-1	9	-50	6	40	227	0.03	NS	D	No
		Ipsi-2	46	-47	40	14	217	NS	NS	D	U
		Ipsi-3	9	-40	7	34	168	0.04	NS	D	No
		Ipsi-4	6	-39	4	54	82	0.04	NS	D	No
	Medial frontal gyrus	Ipsi	8	-4	30	52	166	0.021	NS	U	No
Tooth 23	Parietal operculum (S2)	Contra-1	OP1	51	-31	14	171	NS	NS	S	s
		Contra-2	OP1	47	-24	17	124	NS	NS	S	s
		Contra-3	OP2	39	-28	18	77	0.01	NS	S	No
		Ipsi-1	OP1-4	-55	-20	18	1156	NS	NS	S	S
		Ipsi-2	OP2	-37	-31	19	107	NS	NS	S	S
	Postcentral gyrus (S1)	Contra	1, 2	55	-22	37	1069	0.0004	NS	S	No
		Ipsi	1, 2, 3b	-55	-19	39	1389	0.02	NS	S	No
	Inferior parietal lobule	Ipsi-1	40	-56	-28	25	96	NS	NS	s	s
		Ipsi-2	40	-34	-44	36	70	0.003	NS	U	No
	Middle temporal gyrus	Ipsi-1	39	-51	-62	26	355	NS	NS	U	No
		Ipsi-2	39	-40	-71	26	241	0.01	NS	D	No

	Ipsi-3	39	-33	-70	28	76	0.003	NS	D	No
Inferior temporal gyrus	Ipsi	37	-48	-52	-6	628	0.0003	NS	U	No
Inferior frontal gyrus	Contra	47	44	35	-4	142	0.03	NS	D	No
	Ipsi	47	-38	34	-5	107	0.0002	NS	D	No
Middle frontal gyrus	Ipsi-1	6	-35	4	48	133	NS	NS	D	No
	Ipsi-2	6	-36	6	56	65	NS	NS	D	No
	Ipsi-3	9	-48	8	35	156	NS	NS	D	No
Medial frontal gyrus	Ipsi	6	-7	14	46	59	NS	0.015	D	No

Random effect analysis; foci of activation found at $p<0.05$ (uncorrected), min cluster size = 45 mm3
* If cluster size > 2000 mm^3 in S1-S2, it was split in several local maxima by looking at the activated peaks at a more severe statistical threshold ($p<0.025$); these local maxima are written in italic.
BA = Brodman areas; OP1-4 = subdivisions of the parietal operculum; x, y, z = Talairach stereotaxic coordinates (mm);
S1 = primary somatosensory area; S2 = secondary somatosensory area; Ipsi = ipsilateral; Contra = contralateral
I21-p = implant 21 in patients, T23-p = tooth 23 in patients, T21-c = tooth 21 in controls, T23-c = tooth 23 in controls.
P values = results of the ANOVA in the second-level random effect analysis including all the 19 subjects; NS = $p > 0.05$
ST curves = signal to-time curves; S = signal time course linked to the stimulation; s = signal linked to the stimulation but at a low level or with a high variance; D = delayed signal after the stimulation onset, M = maintained signal after the end of the stimulation; N = noisy signal; No = signal not different from the baseline; P = signal showing a peak during the stimulation; U = unclassified signal-to-time curve.
For I21-p, 7 additional clusters are not described: 2 were in the ventricles, 1 in white matter, 1 outside the brain, and 3 had a noisy ST curve.
For T23-p, 1 additional cluster with a noisy ST curve is not described.
For T21-c, 5 additional clusters are not described: 3 were outside the brain, and 2 had a noisy ST curve.
For T23-c, 7 additional clusters are not described: 1 was in the ventricles, 4 in white matter, and 2 had a noisy ST curve.

6.3.2.1.2. Controls versus patients:

In the primary somatosensory areas, the difference between controls and patients was more important for the contralateral side (see **Table** 1: mean p value when comparing S1 to the homologous teeth = 0.004 for the contralateral side and 0.023 for the ipsilateral side). In **Table 2**, we can see that in patients, S1 was activated in a minority of subjects when the implant was stimulated, but in some more subjects when T23 was, especially at the ipsilateral side.

In the secondary somatosensory area, the contralateral activated clusters found in controls for T21 and T23 were present in only few patients, while all ipsilateral clusters were also found in a majority of patients.

Table 2: Activated clusters found in somatosensory areas: comparison between groups.

For the clusters found in patients, we report the mean value of the predictors (beta) in controls and the number of controls (n) with a significant predictor (t > 1.97). For the clusters found in controls, we report the mean value of the predictors (beta) in patients and the number of patients (n) with a significant predictor (t > 1.97).

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A: Activated clusters found in patients at t= (p< 0.05, not corrected), minimum cluster size=45 mm3

Tooth	Brain area	Side	Mean beta T21-c	Mean beta T23-c
Implant 21	S2	Contra-1	2.67 (n=4)	2.10 (n=4)
		Contra-2	3.49 (n=7)	2.69 (n=7)
		Contra-3	0.52 (n=2)	0.40 (n=2)
		Ipsi	4.54 (n=9)	4.24 (n=8)
Tooth 23	S2	Contra-1	2.65 (n=5)	2.01 (n=4)
		Contra-2	2;62 (n=5)	2.02 (n=5)
		Ipsi	4.83 (n=9)	4.48 (n=8)

B: Activated clusters found in controls at t= (p< 0.05, not corrected), minimum cluster size=45 mm3

Tooth	Brain area	Side	Mean beta I21-p	Mean beta T23-p
Tooth 21	S1	Contra	-0.2 (n=2)	-0.19 (n=3)
		Ipsi-1	1.09 (n=3)	1.21 (n=5)
		Ipsi_2	0.05 (n=2)	-0.21 (n=2)
	S2	Contra-1	0.06 (n=1)	0.01 (n=2)
		Contra-2	1.05 (n=3)	0.81 (n=4)
		Ipsi-1	1.57 (n=4)	1.14 (n=3)
		Ipsi-2	0.82 (n=4)	-0.39 (n=2)
Tooth 23	S1	Contra	3.41 (n=7)	3.63 (n=5)
		Ipsi	3.59 (n=7)	2.80 (n=5)
	S2	Contra-1	1.68 (n=4)	1.47 (n=3)
		Contra-2	1.45 (n=4)	0.63 (n=2)
		Contra-3	1.26 (n=3)	-0.27 (n=1)
		Ipsi-1	3.14 (n=5)	3.37 (n=6)
		Ipsi-2	2.99 (n=6)	2.27 (n=3)

S1= primary somatosensory area; S2= secondary somatosensory area; Ipsi=ipsilateral; Contra= contralateral
I21-p = implant 21 in patients, T23-p = tooth 23 in patients, T21-c = tooth 21 in controls, T23-c = tooth 23 in controls.

6.3.2.1.3. Incisor (or implant) versus canine

There was no difference between I21-p vs T23-p and between T21-c vs T23-c (**Table 1**), with the exception of the above mentioned S2 cluster that was found only in I21-p.

6.3.2.2. Cortical network activated outside S1 and S2

6.3.2.2.1. Patients versus controls

Four activated clusters were found for the implant but not for T21-c or T23-p, therefore being unique to the implant (contralateral supramarginal gyrus, inferior frontal gyrus bilaterally, and contralateral medial frontal gyrus). Five additional clusters found for I21-p were not different from those of T23-p but were not found for T21-c (ispilateral supramarginal gyrus, ipsilateral precentral gyrus, contralateral inferior and middle frontal gyri, and contralateral insula). The stimulation of the implant clearly elicited more activation in the inferior frontal gyrus as compared to the other teeth. Indeed, the ratio between the number of activated voxels in this area and the total number of activated voxels was 0.27 for I21-p, but only 0.06, 0.04, and 0.04 for T23-p, T21-c, and T23-c, respectively.

For T23-p, we also observed 4 activated clusters that were not different from those of I21-p but were not present for T23-c (contralateral superior temporal gyrus, ipsilateral precentral gyrus, contralateral inferior frontal gyrus and contralateral insula). We may see that the activation network elicited by T23-p was somewhat intermediate between this of the implant and of the tooth in controls. Indeed, several activated areas were specific to patients for both I21 and T23. This included the ipsilateral precentral gyrus, contralateral inferior frontal gyrus and contralateral insula.

6.3.2.2.2. Controls versus patients

The activated clusters found for T21-c and not for I21-p included 6 foci (middle temporal gyrus bilaterally, and 5 foci in the ipsilateral inferior, middle and medial frontal gyri). Six activated clusters were found for T23-c and not for T23-p. They were all ipsilateral but one, and located in the inferior parietal lobule, middle and inferior

temporal gyri, and inferior frontal gyrus. For 14 out of the 16 clusters found in controls but not in patients, there was no difference between T21-c and T23-c.

6.3.3. Signal-to-time curve analysis

The type of curve found for each cluster is reported in **Table 1**. A signal time course clearly and strongly linked to the stimuli could only be found in somatosensory areas (S1 and S2) where such curve was always observed in both patients and controls. The only exception was the superior temporal gyrus for T23-p where such signal was also observed. For I21-p, a signal time course linked to the stimulation but at a low level was found in the supramarginal gyrus, the precentral gyrus and the insula. This was also the case for T23-p in the inferior frontal gyrus and in the insula. The signal time course observed in other frontal areas was either delayed or maintained for both patients and controls. An unclassified signal-to-time curve (badly correlated with the stimuli) was observed in the medial and superior frontal gyri, in the putamen and in many middle and inferior temporal foci.

6.4. Discussion

This study demonstrates for the first time that punctuated mechanical stimulation on an osseointegrated tooth implant activates cortical somatosensory areas. This activation may represent the underlying mechanism of osseoperception. We also show that tooth loss and its replacement by an osseointegrated implant induce brain plasticity as indicated by the difference between the cortical network activated when stimulating the implant and natural teeth. As a group, patients activated S2 bilaterally for both I21 and T23, while controls activated S1 and S2 bilaterally for T21 and T23. However, at an individual level, S1 was activated by 4/9 implants, mainly on the ipsilateral side. The implant activated a larger bilateral cortical network outside the somatosensory areas, with activations found in parietal, frontal and insular lobes, the main clusters being located in the inferior frontal gyri. Stimulation of T23 in patients resulted in an activation pattern intermediate between this of the implant and natural teeth.

6.4.1. Cortical plasticity induced by tooth loss and its replacement by an osseointegrated implant

The first question raised by this study was the occurrence of brain reorganization following single tooth loss. Teeth are important for the survival of nearly all mammals and it has been shown in many species that dental and periodontal afferents have a prominent representation in the cortex (Jain et al., 2001, for primates; Catania and Remple, 2002, for naked mole-rats; Remple et al., 2003, for rats). In a study carried out on mole-rats, Henry et al. (2005) extracted the lower right incisor and 5 to 8 months afterwards, he demonstrated a considerable reorganization of the oro-facial representation in S1, with neurons of the missing tooth being responsive to tactile inputs from surrounding oro-facial structures. Other studies performed in rats have shown that following teeth trimming or extraction, rapid and reversible neuroplastic changes occurred within the face-M1 and adjacent face-S1 (Avivi-Arber et al., 2010; Sessle et al., 2005). These findings indicate that tooth loss must be considered as an amputation and that cortical representation of teeth significantly restructure after tooth loss. However translation of these observations to human should be done with caution as the representation of teeth is not as huge as in the rat.

Traumatic amputation of a hand results in large cortical areas being deafferented and followed by extensive reorganisation so that adjacent and contralateral areas take over the function of the vacant area although some remaining inputs may subside to explain the phantom and phantom-pain phenomenon (Elbert et al., 1995, Dettmers et al., 2001, Grüsser et al., 2003). We do not expect such large cortical deafferentiation after single tooth extraction as in normal dentate volunteers, we have demonstrated a considerable macroscopic overlap of the somatosensory areas activated by teeth 21, 23, 11 and 13 (Habre-Hallage et al., submitted for publication). However, a phantom tooth phenomenon has also been described after tooth loss (Jacobs et al., 2002), suggesting that cortical reorganisation similar to that occurring after limb amputation may occur. In the present study, we are the first to demonstrate the occurrence of brain plasticity after single tooth loss and its replacement by an osseointegrated implant, allowing us to compare our findings to that occurring after hand replantation, transplantation or rehabilitation by an osseointegrated implant. Osseointegrated oral implants might

therefore be used as a model to help in the design of many bone-anchored prosthetic appliances and bionic limb.

6.4.2. Cortical network activated by the osseointegrated implant and brain plasticity

6.4.2.1. Somatosensory areas

To our knowledge, we are the first to demonstrate activation in somatosensory areas after mechanical punctuate stimulation of bone-anchored maxillary implants. Yan et al. (2008) have studied the neuroplasticity of edentulous patients with implant-supported full denture but they used a clenching task and not a pure sensory stimulation. They observed bilateral activation of S1 and M1 areas but movements and sensory input provided by masticatory muscles and temporomandibular joints hamper the interpretation of their results.

6.4.2.1.1. Primary somatosensory area

The activation in S1 was far less strong for the implant than for natural teeth. This is in accordance with the findings of many authors who showed that a considerable improvement in the sensory and motor capabilities occurs after rehabilitation with oral implants even though they fail to reach those of dentate individuals (Jacobs and van Steenberghe, 1991 and 1993). Interestingly, when S1 was activated by some implants, it was mainly on the ipsilateral side. In controls, the cortical somatosensory projections of T21 were bilateral in S1 but with a trend to an ipsilateral dominance. Based on these data, we might hypothesize that for the central incisor, ipsilateral connexions are more prominent and can be more easily restored after tooth replacement by an osseointegrated implant. However, in a previous study we also stimulated T11 and we did not found an ipsilateral dominance (Habre-Hallage et al., submitted for publication). Trulsson et al. (2010) who stimulated periondontal mechanoreceptors of T21 reported bilateral S1 activation with a contralateral dominance. From the available data, central incisors appear to have bilateral projections in S1 area without a clear side dominance.

A comparison with upper ou lower limbs should be done with caution as, contrary to the trigeminal system, their sensorimotor projections are cleary contralateral. However, studies performed after limb rehabilitation can put some light on our findings. Neugroschl et al. (2005) studied a hand-grafted patient with exceptionally early recovery. Two weeks after hand transplantation, the clinical findings

could be correlated with bilateral activation in S1 found during passive tactile stimulation. Björkman (2007a) noted an initial ipsilateral activation in S1 as early as four weeks after hand replantation when the patient had no measurable sensitivity. After gradual recovery of sensitivity in the replanted hand, fMRI activation shifted to a bilateral pattern, and then to a more normal activation pattern with a predominantly contralateral activation. What this early ipsilateral activation indicates is not known, but one can speculate that the homologous somatosensory area mainly devoted to the intact hand is initially recruited, and that reappropriation of the deafferented contralateral area takes more time but eventually leads to sensory function recovery. This cortical plasticity includes modifications in transcallosal inhibition and redistribution of the balance between ipsi- and contralateral activation for both the replanted and intact hand as demonstrated by Frey et al. (2008) who reported an increased activation of the ipsilateral S1 area for the intact hand. Cortical reintegration of an osseointegrated implant was reported in only one patient where sensory stimulation of an osseointegrated thumb prosthesis led to bilateral activation in S1 (Lundborg et al., 2006; Björkman et al., 2007b). The authors suggested that this bilateral activation (instead of the predominantly contralateral activation for healthy thumb) may be regarded as a compensatory mechanism in the brain to substitute for the inferior sensory function in the prosthetic thumb. This single case report was the first one to demonstrate that a true tactile function mediated by cortical somatosensory areas can be recovered thanks to a bone anchored implant. Our findings about osseointegrated tooth implants are in accordance with this study even if activation in S1 was seen in only 4/9 patients.

6.4.2.1.2. Secondary somatosensory areas

The activation in S2 was bilateral for both patients and controls and the precise location of the activated clusters were similar for the ipsilateral side and slitghtly different for the contralateral side. This adds arguments for a near-to-normal restoration of the ipsilateral cortical network, at least in some patients, and for more cortical reorganisation on the contralateral side. The activation of S2 proves that sensations elicited by osseointegrated implants can be interpreted by the brain as meaningful sensory imput as they are analyzed by the cortical areas physiologically dedicated to this function. Using MEG, Karhu et al. (1999) have shown a simultaneous

early processing of sensory input in human S1 and S2 somatosensory cortices. This indicates that S2 must not be only viewed as a higher-order processing area but is also involved in the early processing of somatosensory inputs. S2 may also acts as the center of integretion, modulation and interpretation of sensory input coming from different types of receptors. In the setting of brain reorganisation following tooth loss and its replacement by an osseointegrated implant, we might hypothetize that S2 represent one of the main areas able to sustain the restored sensory function by integrating input coming from new nerve ending located in the adjacent bone and softissue. Activation of both S1 and S2 may represent the underlying physiologic mechanism of osseoperception explaining the superior tactile and stereognosic abilities, and therefore the better mandibular function. Moreover the shape of the BOLD signal-to-time curve correlated the best with the stimuli in somatosensory areas for both patients and controls. This indicates that these areas are the most directly connected to the periphal receptors for both implants and natural teeth.

6.4.2.2. Cortical network outside the somatosensory areas

The large cortical network elicited by the stimumated implant outside the somatosensory areas may be regarded as a compensatory mechanism for the lower level of sensory input as compared to natural teeth. This network was considerably larger in the contralateral side, arguing again for a more extensive reoganisation on this side.

The areas where the signal-to-time curve was linked to the stimulation (but at a low level) should be considered as the most direct compensatory areas for integrating the tactile stimuli. This kind of signal was almost exclusively found in patients. For I21-p, this concerned the supramarginal gyrus and the precentral gyrus bilaterally, and the contralateral insula. For T23-p, this implicated the superior temporal gyrus (strongly linked to the stimulus), the contralateral inferior frontal gyrus and the contralateral insula. The decreased level of sensory input for I21-p may favor the activation of brain areas that can be intrinsically activated by tactile stimulation and/or are physiologically connected to somatosensory areas.

The activation found in the superior temporal gyrus might correspond to a spillover of the activation of the parietal operculum or correspond to a distinct

activation in this area known as a multi-sensory region that responds to tactile, auditory and visual stimulation ((Macaluso and Driver, 2005).

It is well known that the insula is involved in the processing of painful and non painful sensations from various body regions, including trigeminal nerve sensation (Penfield and Faulk, 1955; Ostrowsky et al, 2002). These authors have also demonstrated a somatotopic organization in the human insula with the extremities being represented more posteriorly than orofacial structures. A PET study demonstrated bilateral involvement of the insula in oral tactile sensation during the injection of pure water into the mouth (Zald and Pardo, 2000). Moreover, activation of the anterior insula has been reported during painless vibrotactile dental stimulation (Ettlin et al., 2004, Trulsson et al. 2010) as well as in unpleasant or painful dental stimulation (Jantsch et al, 2005; Ettlin et al., 2009).

The supramarginal gyrus (BA 40) contains neurons responsive to tactile and visual stimulation (Robinson and Burton, 1980). It is believed to correspond to a region homologous to the monkey area 7b or feline tertiary somatosensory cortex (S3) and is regularly activated by light touch (Hagen and Pardo, 2002).

On the other hand, the precentral gyrus is part of the sensorimotor network with usual coactivation of somatosensory and motor cortices during biting, mastication and any oral movements.

Areas with a signal-to-time curve maintained or delayed should be regarded as involved in more secondary processes. They may be part of the attention network engaged to compensate for the lower level of sensory input or participate in the integration of somatosensory information for planning movements, or for feeding complex cognitive or emotional networks. This concerned the inferior and middle frontal gyri in both patients and controls, extended more in the contralateral side in patients. Such curves were also observed in the ipsilateral medial frontal gyrus and bilateral middle temporal gyri in controls, and in the ipsilateral inferior parietal lobule in patients. The cortical network involved in vibrotactile attention is largely distributed in fronto-parietal areas. The following areas were identified by Burton et al. (2008) as being part of this network: contralateral parietal opercular OP1, BA 4 (precentral gyrus), middle frontal gyrus including frontal eye field and dorsal premotor, anterior and posterior BA 7, and bilateral superior temporal gyrus (BA 22), inferior frontal gyrus including the ventral premotor and frontal operculum, insula and medial frontal gyrus

including the supplementary motor area. We may note that this network is bilateral with a contralateral dominance. If it is more activated when stimulating the implant, this might explain why the overall activation induced by I21-p was more bilateral as compared to the other teeth. The enhanced activity in the tactile attention network may in turn exerts a top-down modulation on the sensorimotor system with an increased activation in the network involved in movements planning, including the frontal premotor and supplementary motor areas and posterior parietal areas (Porro et al., 2004). Indeed, such interplay between sensory and motor systems is frequently observed even without actual movement (Fink et al, 1997, Maruno et al., 2000). Moreover, activation of some of these areas has been described after vibrotactile dental stimulation (Ettlin et al., 2005, Trulsson et al., 2010) and in studies involving trigeminal stimulation (Dresel at al., 2008, Huang and Sereno, 2007)

6.4.3. Cortical plasticity beyond I21 in patients

Our results indicate that the cortical plasticity induced by the osseointegrated implant extends beyond the projections of the lost tooth, and also involves natural teeth in the vicinity. Indeed, the network activated by T23-p was somewhat intermediate between this elicited by I21-p and this found for T21 and T23 in controls. In our study design, we paid attention to stimulate non contiguous teeth. Indeed, it is known that about half of single nerve afferents originating from human periodontal mechanoreceptors have receptive fields responding to 3 teeth: the main one and the two adjacent ones (Trulsson, 1993). This is due to a simple mechanical coupling of the contacting crowns (Johnsen and Trulsson, 2003) that could also occur between the implant and adjacent natural tooth. When stimulating the implant, this coupling may transmit some information to the periodontal mechanoreceptors of the adjacent teeth and therefore might contribute to the activation of the somatosensory network via the afferents from T22 and T11. However, this simple mechanical coupling is unlikely to play a major role in the cortical reorganization related to T23 in patients. Brain plasticity observed for T23-p must rather be understood as global changes in the sensory input from oral structures. This can influence mandibular, tongue and lips movements, and is only partially restored by the osseointegrated implant. Indeed, it has been shown in the macaque that in area S1, 37% of neurons responding to mechanical tooth stimulation had also receptive fields in the gingiva, lip, and tongue mucosa (Toda

and Taoka, 2001). This allows the integration of sensory information from the periodontal ligament and from other oral structures representing a combination of the regions stimulated simultaneously during food intake. It is therefore understandable that T23-p is also affected by the cortical reorganization occurring after T21 loss and its replacement by an implant, and must not be considered as a "control" tooth.

6.5. Limitations of our study and further perspectives

Our study describes for the first time the basis of pure tactile osseoperception at a cortical level after tooth replacement by bone-anchored implant. However, it must be considered as a preliminary work. First of all, we used a quite liberal statistical threshold ($p<0.05$, not corrected) and we cannot exclude the occurrence of false positive activated clusters. This was imposed by limited number of subjects (as in many studies including patients) and by our choice to work without spatial smoothing to improve the accuracy of spatial localisation. However, we think that false positive clusters were reduced by the use of an extend threshold of 45 mm^3 and by the fact that we considered only the clusters where the time course of the signal could be correlated with the stimulus. Nevertheless, before being able to generalize our results, more subjects should be included and various implanted sites should be studied. To decrease the heterogeneity of the cortical response, the strength of the stimulation (calibrated by the VFF number) could be adjusted to the individual sensory threshold. To better understand the relationship between the activated cortical network and the restoration of the sensory feedback, psychophysical measurement of the tactile function of the implant and peri-implant tissues should be performed and correlated to the brain activations. For instance, we do not know wether activation in S1 could be a marker of improved sensory recovery. Correlations between the activation pattern and implant design and surgical timing and technique should also be established. Our pilot study open the doors to further research aimed at understanding the relationship between the cortical activation pattern and functional recovery in light of individual oral dental characteristics and implantation techniques. This should allow us to optimize the osseoperception phenomenon and to improve the neurophysiological integration of implants. This may also serve as a guide in the design of novel bone-anchored prosthetic appliances and bionic limbs for helping patients to regain a better quality of life.

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Discussion and General Conclusions

Chapter 7

6. DISCUSSION AND GENERAL CONCLUSIONS

The present thesis addresses the phenomenon of osseoperception compared to the exteroceptive function of periodontal mechanoreceptors when teeth are still present.

Part I mainly focused on investigating the impact of the presence of oral implants on the tactile functionality of the neighboring mucosa. (Chapters 2 & 3)

Part II focused on identifying the brain structures involved in the projections of intra-oral inputs by the use of functional magnetic resonance imaging (fMRI). (Chapters 4, 5 & 6)

Jaw function has some unique properties when compared to limbs. Jaw muscles are attached to a rigid structure, the mandible, which crosses the body midline and thus a bilateral synergism of agonist muscles is needed during jaw movements. Furthermore a sudden impact occurs during jaw closing when the occlusal plane, formed by the masticatory surfaces of the teeth, is reached. Thus a very fast servo-system, especially when the natural dentition is present, is needed to avoid damage to the teeth. The trigeminal nerve which conveys afferent and efferent signals to the oro-facial structures is also unique as it has its primary synapses intra-cranially and since it also provides somatosensory innervation to specialized structures such as the tympanic membrane, cornea and part of the conjunctiva.

Peripheral inputs during jaw function are manifold because of its complexity. They interact with central pattern generators during rhythmic jaw chewing function (Pereira et al., 2006). Central pattern generators are characterized by two or more processes which sequentially decrease or increase their activity. The resulting interaction returns the generator to its starting condition. For voluntary jaw movements central pattern generators are turned off and the interplay of many inputs and motor programs insure a smooth jaw function.

In dentate subjects the main inhibitory peripheral input, after occlusal contact is reached, originates from mechanoreceptors located in the periodontal ligaments. The latter are densely populated by free and organized nerve endings. The periodontal ligaments disappear after tooth loss and the periodontal innervation involutes sensibly, even though it does not completely disappear (Linden and Scott, 1989). What happens subsequently with periodontal sensory inputs is a pertinent question. Vibrations caused by impact forces on the dental occlusal surfaces may trigger distant receptors due to

transmission of vibrations. Whether this feedback will be efficient enough to avoid overload is questionable.

Partially edentulous patients who wear removable dentures have them carried by, or anchored, to neighboring teeth. Thus some resilience of the prosthesis is provided by the periodontal ligaments of the supporting teeth. This resilience is even more present when fully edentulous patients have their dentures supported by the underlying mucoperiosteum. The damping of the impact forces, occurring when dental occlusal contact is reached during chewing, allows less stringent feedback mechanisms. How this is dealt with when prostheses are rather supported by osseointegrated oral implants - the latter offering no resilience whatsoever - constitutes another pertinent question. The latter question is also clinically relevant. Indeed the rigid anchorage of the implants to the surrounding jaw bone, without the cushion effect of a periodontal ligament, leads to an immediate transfer of impact forces to the jaw bone. Overload may jeopardize the long-term survival of such bone-anchored prostheses which would constitute a major clinical issue.

A proper jaw function also implies the capacity to detect and/or discriminate objects kept in between antagonistic teeth. Many neural structures may contribute to this function in edentulous people, reaching from jaw muscle spindles to temporo-mandibular innervation. Experiments revealed that the interocclusal detection capacity of even small-sized objects exists but is nevertheless reduced when compared to that of dentate subjects (Jacobs et al., 1997).

Fully edentate subjects wearing bone-anchored prostheses also reveal their ability to identify modalities of touch as for example the consistency of the food bolus. This ability has also been reported by subjects with an amputated limb replaced by a bone-anchored prosthesis. They could identify the consistency of the floor they are walking on rather than only detect when contact is established. However, to become conscious, sensory inputs require transmission of nerve impulses from the periphery to the somatosensory area of the brain cortex. This route involves the dorsal horns of the spinal cord, and relay stations in the brain stem and thalamus.

This tactile sensation is evidently important for the subject’s comfort whether it is after amputation of a limb or of teeth. Mechanoreceptors involved in either oral or limb osseoperception in patients with implant-supported prostheses need to be identified and their function unraveled.

Already during the late seventies the favorable clinical experience accumulated on fully edentulous patients rehabilitated with implant-supported dental prostheses lead to the conclusion that some (compensatory) neuromuscular feedback mechanisms remain active or even take over from the involuted periodontal mechanoreceoptors (Haraldson and Ingervall, 1979). Evidence was also provided that mechanical stimuli applied to oral osseointegrated implants could be perceived although at a higher tactile threshold level then for teeth (Yoshida, 1988). Research involving the registration of evoked potentials in the electroencephalogram (EEG) gave a neurophysiological base to these psychophysical observations (Van Loven et al., 2000). However, evoked potentials do not allow identifying the cortical or subcortical location of the sensory projections.

Furthermore, the vicinity of the intra-oral stimulating device and the brain leads to inevitable noise during the EEG registrations. Thus the quest for an alternative approach to properly indentify the brain structures involved in the projections of intra-oral inputs led to the use of functional magnetic resonance imaging (fMRI).

6.1. The use of fMRI

fMRI is an established neuroimaging technique providing good spatial resolution of cortical and subcortical structures critical in the processing of tactile inputs with acceptable temporal resolution. Since it does not involve ionizing radiation it use for experimental purposes is acceptable. Lundborg demonstrated that tactile stimuli applied to an osseointegrated prosthetic thumb induced bilateral activations of the primary somatosensory cortex in an area corresponding to that of the hand. This bilateral activation may be regarded as a compensatory mechanism in the brain to substitute for the inferior sensory function in the prosthetic thumb, by recruiting additional areas in sensory functions (Lundborg et al., 2006).

6.1.1. The stimulation device

Since the fMRI has been hardly used for studying intra-oral somatosensory inputs the present thesis involved the development of a proper customized machinery and methodology (see chapter 4). In this study, a new manually controlled device using von Frey monofilaments was tested on a phantom and on eight volunteers.

Indeed the device had to deliver reproducible mechanical stimuli without inducing any magnetic field. Both the rate of force build-up and the intensity, needed to be perceived

by the subject, had to be determined. The methodology used had to be comfortable for the experimental subjects to reflect physiological conditions.

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. Beside the somatosensory system, other cortical areas were also activated, mainly in the temporal lobe and the precentral gyrus, although less consistently.

6.1.2. fMRI and teeth

A description of the entire cortical network involved in the tactile teeth stimulation require more subjects and a more uniform protocol to perform group analysis. Twenty healthy volunteers with a full dentition were recruited. A block design paradigm was used to stimulate tooth (T) 11 and T13 in 10 subjects, and T21 and T23 in 10 other subjects. Random effect group analyses were performed for each stimulated site, and differences between teeth were examined using ANOVA. Our results prove that mechanical stimulation of teeth by means of von Frey hairs leads to clear-cut bilateral cortical activation in the primary and secondary somatosensory areas (see chapter 5).

Significant activations in the somatosensory cortex were found for all stimulated teeth indicating that the response in these cortical areas was dominant and robust. In previous studies, conflicting results emerged when painful and non-painful dental stimulations were compared (Hari and Kaukoranta, 1985)or when non physiologic stimuli were applied like vibrotactile stimuli (Ettlin et al., 2004). The latter identified activations primarily in the insular cortex bilaterally and in the supplementary motor cortex but not in the somatosensory cortex. Using a manually applied torque force, Miyamoto et al. (Miyamoto et al., 2006) were able to map the S1 representation of the stimulated tooth. Very recently, Trulsson et al. (2010) demonstrated that low frequency vibrotactile stumuli (20 Hz) were able to activate somatosensory areas while higher frequency did not. Our study confirms this finding by showing that very low frequency stimulation (1 Hz) triggers periodontal mechanoreceptors and activates mainly 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 and Pardo, 2002; Iannetti et al., 2003). The S1area, located on the inferior lateral aspect of the post-central gyrus, had a light preponderance of the contralateral projection. The bilateral representation of sensory input in the S2 area is probably due to transcallosal projections. Therefore, we believe that these somatosensory areas play a major role in the sensory feedback control of the forces used to hold and manipulate the food between teeth, in a comparable way as for the fingers during the precision grip. (Rausell and Jones, 1991a, b). In humans, bilateral projections to the thalamus from both upper and lower teeth have been demonstrated for nociceptive stimuli (Weigelt et al. 2010). However, we were unable to find any study about the thalamic projections of human periodontal mechanoreptors. Therefore, we may only speculate that a bilateral representation of intraoral structures is present as early as in the thalamus with only minor and non consistant activations in the inferior parietal lobule, superior temporal gyrus, precentral gyrus, middle or inferior frontal gyri, posterior insula and the cerebellum.

6.1.3. fMRI and implants

Loading of endosseous implants is known to activate neural receptors in the vicinity. They can be either intra-osseous or periosteal. Histological studies revealed the presence of neural endings close to the bone-to-implantinterface. The density of this innervation increases over time.

In our fMRI study, we applied 1 Hz punctuate tactile stimuli on teeth and implant to trigger periodontal mechanoreceptors and receptors in peri-implant tissues respectively. A block design paradigm was used to stimulate tooth T21 and tooth T23 in 10 controls, and implant I21 and T23 in 9 patients. Random effect group analyses were performed. This study demonstrates for the first time that punctuated mechanical stimulation on an osseointegrated tooth implant activates cortical somatosensory areas. This activation may represent the underlying mechanism of osseoperception. We also show that tooth loss and its replacement by an osseointegrated implant induce brain plasticity as indicated by the difference between the cortical network activated when stimulating the implant and natural teeth. As a group, patients activated S2 bilaterally for both I21 and T23, while controls activated S1 and S2 bilaterally for T21 and T23. However, at an individual level, S1 was activated by 4/9 implants, mainly on the

ipsilateral side. The implant activated a larger bilateral cortical network outside the somatosensory areas, with activations found in parietal, frontal and insular lobes, the main clusters being located in the inferior frontal gyri. Stimulation of T23 in patients resulted in an activation pattern intermediate between this of the implant and natural teeth.

The large cortical network elicited by the stimulated implant outside the somatosensory areas may be regarded as a compensatory mechanism for the reduced sensory input as compared to that originating from the natural dentition (see chapter 6). These compensatory mechanisms of cortical projection after rehabilitation by means of osseointegrated implants may explain why a bone-anchored prosthetic substitute can become integral part of a subject's bodily image (Blomberg and Lindquist, 1983).

Of course the increased self-confidence resulting from the excellent fixation of the dentures which alleviates any fear of lossing them during function, may also contribute to the psychological integration of the susbtitute.

6.2. Assessment of the sensory tactile function in peri-implant soft tissues

The present thesis did not only address the phenomenon of osseoperception compared to the exteroceptive function of periodontal mechanoreceptors when teeth are still present. It also investigated the impact of the presence of oral implants on the tactile functionality of the neighboring mucosa. (see Chapters 2 & 3) Indeed adjacent tissues may reprogram afferent projections in an attempt to restore function. By documenting changes in the threshold for light-touch sensation and two point discrimination (see chapter 2) and investigating graphaestesia and kinaesthesia (see chapter 3) of the soft tissues surrounding oral implants, compensatory mechanisms could be elucidated.

Mechanoreceptors in the oral mucosa are known to include Meissner's corpuscules, glomerular endings, Merkel cells, Ruffini-like endings, and free nerve endings. Thus the origin of the latter tactile inputs is multifold. Nine patients with one or two missing teeth replaced by osseointegrated implants demonstrated that light touch sensation of the gingival and alveolar mucosa is unaffected by the loss of teeth and the replacement by an endosseous implant. On the other hand, the surgical

interventions and/or implant placement led to increased two-point discrimination acuity, probably due to nerve regeneration.

The prospective evaluation of directional mucosal kinaesthesia and graphaesthesia after implant installation to replace one or two missing teeth shows a reduction of tactile ability at abutment surgery. Later on a recurrence is observed when the prosthetic superstructure is installed. Nevertheless the sensitivity of the control (dentate) side was not reached.

These observations correlate with previous morphological observations which report an increased innervation in the peri-implant epithelium after implant placement. At the morphological level Merkel cells are found in the peri-implant mucosa in humans. The present observations may be due to cortical plasticity and/or local reinnervation patterns after surgical trauma and placement of a foreign biocompatible body.

6.3. General Conclusion

Both the endosseous and mucosal neural inputs may contribute to the mandibular function. The present thesis purposely limited itself to the elucidation of sensory function of oral implants. The contribution of sensory inputs to trigeminal reflex mechanisms will need further investigations. To understand a satisfactory oral rehabilitation by means of implants carrying dental prostheses implies unraveling how humans adapt (or not) to an altered oral environment and how clinical approaches aimed at restoring oro-facial function may produce their rehabilitative effect.

6.4. Future research and clinical implications

The balance between the dynamic and static sensitivities of the mechanoreceptor systems available to edentulous patients rehabilitated by means of implants differs from the time when they were dentate and had periodontal mechanoreceptors. Findings on the neuroplasticity induced in cortical sensory and motor areas by alterations in sensory inputs or learning in both animals and humans underline the need for more studies to clarify the cortical mechanisms associated with changes in the oral sensory environment (Sessle et al., 2005). Such knowledge is very limited for oro-facial sensorimotor functions. It is however fundamental to understand how a person learns a new oro-facial skill or adapts or not to an altered oral

environment. It should also steer clinical approaches aimed at restoring orofacial functions (e.g. opting for a fixed bridge, or removable dentures, anchored to implants).

The underlying mechanism of the 'osseoperception' phenomenon remains a matter of debate. So far the neurophysiological correlation of osseoperception at the level of the brain cortex has only been identified in response to the tactile stimulation of an implant supported prosthetic thumb (Lundborg et al., 2006).

The present thesis describes for the first time at a cortical level the basis of tactile osseoperception after tooth replacement by a bone-anchored implant. More subjects should be included and various implanted sites should be studied before being able to generalize our results. To decrease the heterogeneity of the cortical response, the strength of the mechanical stimulation could be adjusted to the individual sensory threshold. To better understand the relationship between the activated cortical network and the restoration of the sensory feedback, psychophysical measurement of the tactile function of the implant and peri-implant tissues should be performed and correlated to the brain activations. Correlations between the activation pattern and implant design and surgical timing and technique should also be established. Longitudinal fMRI on human subjects undergoing extraction and subsequent oral implant placement will allow to unravel the effect of such interventions at both peripheral and central levels.

Future research could include also high intensity mechanical stimulation to explore the properties of pain perception in patients with osseointegrated prostheses. Our study encourages further research aimed at understanding the relationship between the cortical activation pattern and the functional recovery after different oral rehabilitation methods of edentulism. Focus should be put on the physiological and psychological integration of oral endoprostheses helping patients to regain a better quality of life. More research is required to make practical use of the osseoperception phenomenon in the design of novel bone-anchored prosthetic appliances and bionic limbs.

SUMMARY – SAMENVATTING

SUMMARY

The periodontal ligament, that connects the root of the tooth to the jaw bone, contains many mechanoreceptors that encode tooth load when subjects contact and gently manipulate food with the teeth. Tooth loss will remove these receptors and reduce the intra-oral neural input to the brain.

The rehabilitation of (partial) edentulism by means of endosseous implants leads to an improvement in the sensory and motor functions but fail to reach the same level of sensitivity as dentate subjects. Patients with a lower limb prosthesis anchored by a percutaneous osseointegrated implant reported that this allowed them to feel the kind of soil they walked on. This sensory improvement coined ‘osseoperception’ was defined as a perception of external stimuli transmitted via the implant through the bone by activation of receptors located in the peri-implant environment, the periosteum, the skin, the muscles and/or the joints. Hence, it remains uncertain whether this improvement can be ascribed to neural endings in the implant-bone interface itself or to intraosseous neural endings ('osseoreceptors') located further either in the bone marrow or above in the periosteum.

Histological, neurophysiological and psychophysical evidence of osseoperception is available. Yet, from the current evidence it remains unclear whether an altered innervation (from periodontal to peri-implant) may have changed the tactile function of implant-rehabilitated sites.

The main objective of this thesis was to assess alterations and adaptation in oral tactile function by oral endosseous implant placement using psychophysical and neurophysiological methods.

The sensory tactile function in peri-implant soft tissue has not been previously investigated.. In the first part of this thesis, the impact of the presence of oral implants on the tactile functionality of the neighboring mucosa was investigated. (chapters 2 & 3) Indeed adjacent tissues may reprogram afferent projections in an attempt to restore function. By documenting changes in the threshold for light-touch sensation and two point discrimination (chapter 2) and investigating graphaestesia and kinaesthesia (chapter 3) of the soft tissues surrounding oral implants, compensatory mechanisms could be elucidated.

Nine patients with one or two missing teeth replaced by osseointegrated implants demonstrated that light touch sensation of the gingival and alveolar mucosa is unaffected by the loss of teeth and the subsequent replacement by an endosseous implant. On the other hand the surgical interventions and/or implant placement led to increased two-point discrimination acuity, probably due to nerve regeneration. The prospective evaluation of directional mucosal kinaesthesia and graphaesthesia after implant installation to replace one or two missing teeth shows a reduction of tactile ability at abutment surgery. Later on a recurrence is observed when the prosthetic superstructure is installed. Nevertheless the sensitivity of the control (dentate) side was not reached.

In the second part of this thesis, the identification of the possible sensorimotor cortical adaptive processes that may be associated with the loss of teeth and their replacement by endosseous implants was explored by functional magnetic resonance imaging (fMRI) (chapter 6).

Since the fMRI has been hardly used for studying intra-oral somatosensory inputs the present thesis involved the development of a proper customized machinery and methodology (chapter 4). In this study, a new manually controlled device using von Frey monofilaments was tested on a phantom and on eight volunteers.

A description of the entire cortical network involved in the tactile stimulation of periodontal mechanoreceptors would require more subjects and a more uniform protocol to perform group analysis. Twenty healthy volunteers with a full dentition were recruited. The S1 area seems located on the inferior lateral aspect of the post-central gyrus and had a light preponderance of the contralateral projection (chapter 5). A bilateral representation of sensory input was found in the S2 area.

In conclusion we may say that both the endosseous and mucosal neural inputs contribute to the mandibular function. The present thesis describes for the first time at a cortical level the basis of tactile osseoperception after tooth replacement by a bone-anchored implant. More subjects should be included and various implanted sites should be studied before being able to generalize our results.

SAMENVATTING

Het parodontaal ligament die de tandwortel verbindt met het kaakbeen bevat talrijke mechanoreceptoren. Deze vertalen tandbelasting ten gevolge van manipulatie van/ of contact met de voedselbolus via actiepotentialen in afferente zenuwsignalen. Na tandverlies en rehabilitatie via enossale implantaten, wordt een zekere motorische en sensorische functie behouden, doch deze bereikt nooit het niveau van de natuurlijke dentitie. Patiënten met een botverankerde percutane lidmaatprothese melden dat ze hierdoor de vloersamenstelling kunnen waarnemen, waardoor ze hun gang kunnen aanpassen aan het type ondergrond.

Deze gevoelsperceptie kreeg de naam osseoperceptie. Met deze term duidt men aan dat er een gevoelsgewaarwording wordt opgewerkt via transfer van externe stimuli door implantaten via zenuwuiteinden in de nabijheid van het implantaat (in het beenmerg of ter hoogte van het periosteum, ook wel osseoreceptoren genaamd). Er bestaan histologische, neurofysiologische en psychofysische argumenten die wijzen op osseoperceptie. Maar vooralsnog blijft het onduidelijk of een verandering in de bezenuwing door tandextractie en implantaatplaatsing de gevoelsfunctie beinvloedt.

Het voornaamste doel van deze thesis is een evaluatievan de wijzigingen en adaptatie van de orale gevoelsfunctie in kaakbotregio's waar implantaten werden geplaatst. Daartoe worden neurofysiologische en psychofysische methoden gebruikt. In dit proefschrift lig de nadruk op het zoeken van verklaringen voor het fenomeen van osseoperceptie rond implantaten, tov de exteroceptieve functie van de parodontale mechanoreceptoren rond natuurlijke tanden.

In het eerste deel van de thesis wordt de impact van de aanwezigheid van orale implantaten op de tactiele functie van de omgevende mucosa geobserveerd (hfdst 2 en 3). De gevoelsfunctie van peri-implantaire weke weefsels rondom orale implantaten werd nooit eerder onderzocht. De huidige studie toont aan dat de bezeuwing van deze weefsels, een deel van de parodontale gevoelsfunctie kan overnemen. Deze compenserende mechanismen worden aangetoond door drempelbepalingsstudies voor tastzin, twee-punt discriminatie (hfdst 2), grafestesie en kinesthesie van de mucosa (hfdst 3). Negen patiënten met een of twee ontbrekende tanden die vervangen werden door implantaten blijken vertoonden een onveranderde tastzin van de gingiva en alveolaire mucosa na tandvervanging door enossale implantaten.

Daartegenover blijkt het chirurgisch ingrijpen tijdens het plaatsen van de implantaten een verbetering van de twee-puntdiscriminatie capaciteit te veroorzaken. Dit is wellicht een rechtstreeks gevolg van het helingsproces met zenuwregeneratie. Een prospectieve evaluatie van de directionele mucosale kinesthesie en grafestesie na het plaatsen van implantaten toont een zekere respons van de parodontale receptoren en herstel van de natuurlijke functies zonder echter het niveau van natuurlijke tanden te benaderen.

In het tweede deel van deze thesis worden de potentiële sensorimotorische adaptieve processen op corticaal niveau bestudeerd na tandextractie en plaatsing van implantaten, aan de hand van functionele magnetische resonantie (fMRI) (hfdst 6).

Daar fMRI nooit eerder gebruikt werd voor het bestuderen van intra-orale sensorimotorische was de ontwikkeling van een specifieke methodologie en apparatuur onontbeerlijk (hfdst 4). Daarom werd een houten basis met ingebouwde von frey stimulator ontwikkeld en gevalideerd bij een controlegroep. Dit was ook nodig om de corticale projecties te kunnen beschrijven die bij een normale tandstimulatie in gezonde proefpersonen opgewekt worden.

Twintig gezonde vrijwilligers met elk een volledige dentitie kregen een mechanische stimulus op een een bovenfronttand. De S1 regio bleek gelokaliseerd ter hoogte van het onderste lateraal aspect van de post-centrale gyrus, met een lichte voorkeur voor contralateraal projecties (hfdst 5). Bilaterale activatie was wel zichtbaar in de S2 regio.

Algemeen kan men besluiten dat mucosale en enossale neurale inputs een invloed uitoefenen op de kaakfunctie.

Deze thesis biedt het eerste rapport over het osseopercetiefenomeen en haar corticale activatie na tandvervanging door een enossaal oraal implantaat. In de toekomst is er evenwel nood aan onderzoek op grotere groepen vrijwilligers om onze huidige resultaten te kunnen bevestigen en veralgemenen.

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CURRICULUM VITAE

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Pascale Habre was born on June 25th 1969 in Mansourieh, Lebanon. She is married and has two boys (twins) of 10 years old.

In 1993, she graduated as a dentist at Saint-Joseph University in Beirut. Afterwards, she received a scholarship from the French Government to continue her postgraduate training in Paris. She spent five years in Paris where she had many diplomas. In 1995 she obtained a Postgraduate Certificate in Prosthodontics « CESB » at « Université Paris 7 », France and a Certificate in Biological and Medical Sciences (MSBM) at « Université Paris 5», France. In 1997, she obtained a Master in Neurosciences from «Université Paris 6», France and a University Diploma in Surgical and Prosthetic Implantology (DUICP) from «Université Paris 7», France. Shortly after, she was recruited by Saint-Joseph University in Beirut where she has been teaching since September 1998 in the department of Prosthodontics. She gives many lectures to the under-graduate and post-graduate students at the Dental school at Saint-Joseph University. In 2003, she enrolled in the Doctor of Medical Science at the Katholieke Universiteit Leuven. From 2007 to 2009 she had the opportunity to explore osseoperception by the use of the functional Magnetic Resonance Imaging at Saint-Luc University Hospital in Brussels (Université Catholique de Louvain).

Pascale Habre-Hallage is a reviewer for several international journals (COI and IAJD) and is a member of the editorial board of the International Arab Journal of Dentistry (IAJD). She published a number of articles in international journals and presented papers on different international meetings (Clinical Oral investigations, Clinical Implant Dentistry and related research, Journal of Clinical Periodontology, Europeen Association for osseointegration 2009 and 2010…) In October 2010, she won the first Prize in the Basic Research competition of the European Association of Osseointegration in Glasgow, Scotland.

Currently, she is working part-time as an associate Professor at the department of prosthetic dentistry at Saint Joseph University and has a private practice limited to prosthodontics and implant rehabilitation.

Her language knowledge includes Arabic, French and English.