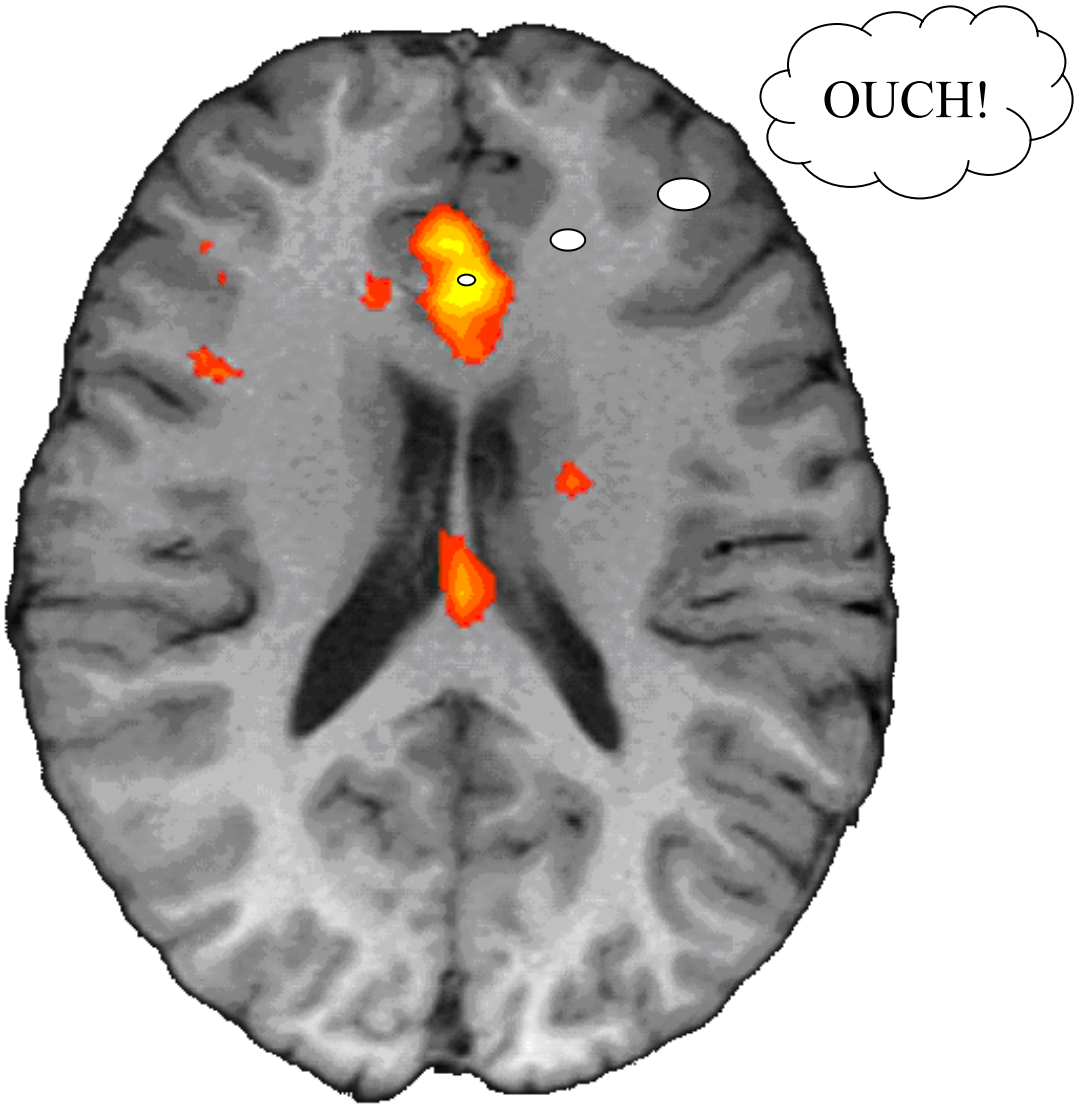
Which areas of the brain say “OUCH!”?

An fMRI study exploring the neural basis of pain



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Abstract

The neural basis of pain was studied using functional magnetic resonance imaging (fMRI). The aim of this study was to a) determine which brain areas are involved with the processing of painfully hot stimulation and b) investigate whether individualized and standardized painfully hot stimuli lead to the same activation patterns. Nineteen participants underwent fMRI scanning while receiving neutral (32°C), warm (37°C) and painfully hot stimuli on the ball of the thumb of the right hand. Half of the painfully hot stimuli were standardized (46°C) and the other half were individualized, using the pain threshold temperature as stimulation temperature (46-48°C). Significant increases of activation during individualized painfully hot stimulation compared to baseline were observed in the insula, anterior cingulate cortex, amygdala, basal ganglia, orbital part of the frontal gyrus, rolandic operculum, superior temporal pole and the superior temporal lobe. These results show great overlap with previous research using fMRI or positron emission tomography (PET). When comparing standardized and individualized painfully hot stimulation, no significant differences in activation were found. The results suggest that the used protocol is overall a good tool to investigate the neural basis of pain. Suggestions for further improvements of the protocol are described in the discussion.

1. Introduction

Pain can cause a lot of discomfort and can be of great influence in someone's life. During an internship at the Department of Neuroscience at the Erasmus Medical Centre (MC) the neural basis of pain in adults will be explored. More specific, the present study will examine the brain areas involved in pain processing. More information about the neural basis of pain will ultimately contribute to the improvement of pain treatment.

1.1 Pain

The definition of pain according to the International Association for the Study of Pain (IASP) is 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'. A distinction should be made between the subjective experience of pain and nociception. The latter is the measurable, physiological event following a noxious stimulus, which does not always result in pain experience (Basbaum & Jessell, 2000).

Pain is a sensation with an important protective function. For example, when you grab something hot, the acute pain evokes a withdrawal reflex. This way the sensation prevents you from getting serious burns. Conscious and unconscious memories of previous pain experiences may lead to the avoidance of stimuli and situations with the potential to cause damage. Contiguously, when someone suffers from an injury, it often hurts too much to move the injured body part. This way pain enhances the healing process (Hudspith, Siddall, & Munglani, 2005). Sometimes a harmful stimulus does not result in withdrawal behaviour, this is called pain indifference. Pain indifference patients suffer from major injuries, which points out the importance of pain experience. However, chronic pain (e.g. migraine) does not serve any purpose, and is therefore often regarded as a disease itself (Hudspith et al., 2005).

Pain can be subdivided in two types; neuropathic and nociceptive pain (see figure 1). When a nerve gets injured directly, this will lead to neuropathic pain. Nociceptive pain, however, is caused by harmful stimuli which activate afferent nociceptors in the skin or in soft tissue (Basbaum & Jessell, 2000). The IASP defines a nociceptor as 'a receptor preferentially sensitive to a noxious stimulus or to a stimulus which would become noxious if prolonged' (IASP).

Three classes of nociceptors can be distinguished. The first class consists of *thermal nociceptors*, which respond to extreme colt or extreme hot temperatures (Basbaum & Jessell, 2000). These nociceptors get activated when you, for example, spill some hot tea on your

hand. High pressure to the skin, for example when you get pinched, causes activation of the *mechanical nociceptors* (Basbaum & Jessell, 2000). The third class of nociceptors responds to noxious mechanical, chemical or thermal stimulation. These are appropriately called the *polymodal nociceptors* (Basbaum & Jessell, 2000).

Both thermal and mechanical nociceptors produce so called 'fast pain', which is experienced as a short, sharp pain. The polymodal nociceptors produce a 'slow pain', which is perceived as a burning feeling. The difference between fast and slow pain is caused by two types of fibers (Basbaum & Jessell, 2000). The thermal and mechanical nociceptors are Aδ fibers, which are myelinated. The polymodal nociceptors are C fibers, which do not have a myelin layer. The myelin layer makes fast signal transportation possible, with a conduction velocity of 6-30 m/s (Hudspith et al., 2005). This conduction velocity is less then 2 m/s in C fibers (Hudspith et al., 2005). Therefore, thermal and mechanical nociceptors transport the pain signal much faster (causing fast pain) than polymodal nociceptors (causing slow pain).

The present study focuses on thermal, nociceptive pain.

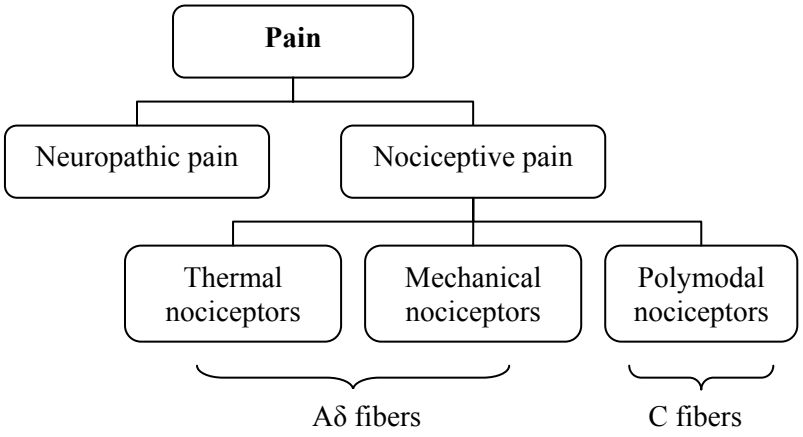

Figure 1. Subdivisions of pain

1.2 Brain areas related to pain

As soon as nociceptors get activated by a noxious stimulus, they carry the signal to the spinal cord. Subsequently, the signal will travel through the spinal cord to the brain. Once arrived in the brain, several areas involved in pain processing will be activated. Numerous studies have been performed in order to find out which brain areas are related to pain processing. These studies used various kinds of pain stimulation (e.g. laser, mechanical, thermal). As the current study uses thermal stimulation, the focus of this report will be on studies performed with the same kind of stimulation.

Previously published studies show a large variation in brain areas allegedly involved in pain processing. Several meta-analyses and reviews on this subject have been published in order to create a clear overview (Apkarian, Bushnell, Treede, & Zubieta, 2005; Brooks & Tracey, 2005; Chen, 2007; Derbyshire, 2000; Peyron, Laurent, & Garcia-Larrea, 2000). Jones, Kulkarni and Derbyshire (2003) created a pain matrix, containing the most significant anatomical areas regarding pain and their possible connections (see figure 2).

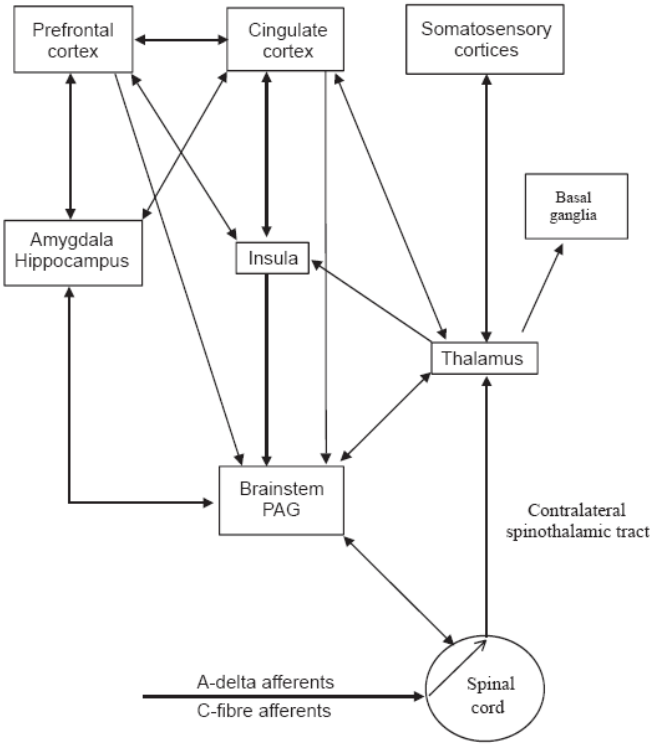

Figure 2. The pain matrix (Jones et al., 2003)

Apkarian et al. (2005) created a list of studies and their results (brain areas activated during different kinds of painful stimulation). From this list, 29 studies used contact heat to administer pain (e.g. Apkarian, Darbar, Krauss, Gelnar, & Szeverenyi, 1999; Derbyshire, Jones, Gyulai, Clark, Townsend, & Firestone, 1997; Peyron, Garcia-Larrea, Gregoire, Costes, Convers, Lavenne et al., 1999). Table 1 shows a list of brain areas and how many out of the 29 studies reported activations in each of these areas. Table 1 also summarizes how these areas are thought to be involved in pain processing, based on current knowledge. Though much is still uncertain or unknown, more and more detailed information about the function of each area is becoming clear.

Table 1
Brain areas activated for pain during previous studies and their functions

Brain area	Function regarding pain	Nr. reported activations
Insular cortex	sensory-discriminative and affective	21
Anterior cingulate cortex (ACC)	affective/motivational and attentional	21
Primary somatosensory cortex (SI)	sensory-discriminative	18
Secondary somatosensory cortex (SII)	sensory-discriminative	18
Thalamus	processing and modulation	14
Prefrontal cortex (PFC)	cognitive and attentional	12
Cerebellum	motor aspects	10
Basal ganglia	initiation of autonomic and emotional states	8
Premotor cortex (PMC)	motor aspects	5
Primary motor cortex (M1)	motor aspects	3
Amygdala	emotional component	1*
Periaqueductal gray (PAG)	part of descending pain inhibitory system	1
Hippocampus	memory functions	1

Note. * Two studies reported a decrease in amygdala activation.
Information retrieved from 29 studies (Apkarian et al., 1999; Apkarian, Gelnar, Krauss, & Szeverenyi, 2000; Becerra, Breiter, Wise, Gonzalez, & Borsook, 2001; Becerra, Breiter, Stojanovic, Fishman, Edwards, Comite et al., 1999; Casey, Minoshima, Berger, Koeppe, Morrow, & Frey, 1994; Casey, Minoshima, Morrow, & Koeppe, 1996; Casey, Morrow, Lorenz, & Minoshima, 2001; Chang, Arendt-Nielsen, & Chen, 2002; Coghill, Gilron, & Iadarola, 2001; Coghill, Sang, Maisog, & Iadarola, 1999; Craig, Reiman, Evans, & Bushnell, 1996; Davis, Kwan, Crawley, & Mikulis, 1998; Derbyshire & Jones, 1998; Derbyshire et al., 1997; Derbyshire, Vogt, & Jones, 1998; Gelnar, Krauss, Sheehe, Szeverenyi, & Apkarian, 1999; Helmchen, Mohr, Erdmann, Petersen, & Nitschke, 2003; Hofbauer, Rainville, Duncan, & Bushnell, 2001; Jones, Brown, Friston, Qi, & Frackowiak, 1991; Kurata, Thulborn, Gyulai, & Firestone, 2002; Kwan, Crawley, Mikulis, & Davis, 2000; Paulson, Minoshima, Morrow, & Casey, 1998; Peyron et al., 1999; Rainville, Duncan, Price, Carrier, & Bushnell, 1997; Strigo, Duncan, Boivin, & Bushnell, 2003; Svensson, Johannsen, Jensen, Arendt-Nielsen, Nielsen, Stodkilde-Jorgensen et al., 1998; Talbot, Marrett, Evans, Meyer, Bushnell, & Duncan, 1991; Tolle, Kaufmann, Siessmeier, Lautenbacher, Berthele, Munz et al., 1999; Tracey, Becerra, Chang, Breiter, Jenkins, Borsook et al., 2000)

Within the pain matrix two pain systems can be distinguished based on anatomy and function; the lateral and medial pain system or pain pathway (e.g. Jones et al., 2003; Brooks & Tracey, 2005; Chen, 2007). The lateral pain system contains projections from the thalamus to the primary and secondary somatosensory cortices (Brooks & Tracey, 2005; Leone, Proietti Cecchini, Mea, Tullo, Curone, & Bussone, 2006) and the parietal operculum (Chen, 2007). This system is thought to be involved with the sensory-discriminative component of pain (Farrell, Laird, & Egan, 2005; Brooks & Tracey, 2005). The thalamus both detects a painful stimulus and provides information about stimulus intensity (Chen, 2007). The primary somatosensory cortex (SI) specifically has been associated with localization of the painful stimulus with respect to the affected body part (Derbyshire, 2000). When SI was damaged, the ability of localization was lost (Peyron et al., 2000). The activity in the secondary somatosensory cortex (SII) increases along with increase of temperature. According to Peyron

et al. (2000), SII is therefore involved in discrimination of stimulus intensity next to general somatosensory integration.

The medial pain pathway contains projections from the thalamus to several areas of the limbic system; the anterior cingulate cortex (ACC) (Brooks & Tracey, 2005), amygdala and hippocampus (Chen, 2007). These areas are associated with the affective and motivational aspects of pain (Farrell et al., 2005). The ACC is specifically thought to be involved in encoding the emotional component (Chen, 2007). It might do so by creating a cortical representation of the negative emotions associated with pain (Derbyshire, 2000). Furthermore, the ACC seems to be involved with attentional aspects of pain, involving attentional shifts and sustained attention to the painful area (Peyron et al., 2000). The exact functions of the amygdala and hippocampus regarding pain are still uncertain. The amygdala could be involved with fear avoidance, while the hippocampus might create memories of the painful stimulus (Derbyshire et al., 1997).

Next to the areas belonging to the lateral and medial pain systems, other brain areas seem to be involved in pain processing as well. First of all, the insula is associated with both sensory-discriminative (stimulus intensity) and affective components of pain (Chen, 2007). Consequently, the insula might be placed between the lateral and medial pain systems, integrating information from both. Lesion studies provide evidence for the emotional function of the insula during painful stimulation. When someone suffers from an insular lesion, this might lead to pain asymbolia. In this case the patient does detect pain, but the emotional reaction to the stimulus is missing (Basbaum & Jessell, 2000).

The prefrontal cortex (PFC) is associated with cognitive and attentional aspects of pain (Chen, 2007). More specifically, Shallice (1988, as cited in Derbyshire, 2000) proposed a supervisory function of the PFC in switching attention. When information about pain reaches the basal ganglia, this area is thought to initiate corresponding autonomic and emotional states (Leone et al., 2006). The lentiform nucleus (LN), which is part of the basal ganglia, might have a preparatory function regarding motor action or could be involved in response selection (Derbyshire et al., 1997). The periaqueductal gray (PAG), located in the brain stem, is involved with analgesia. The PAG is part of the descending pain inhibitory system, which is able to inhibit the nociceptors in the spinal cord (Behbehani, 1995). Finally, several motor related areas might be involved in pain processing; caudate nuclei, cerebellum, the primary motor cortex (M1) (Peyron et al., 2000) and the premotor cortex (PMC) (Paulson et al., 1998). These areas might be related to a withdrawal reaction, as a response to a painful stimulus.

1.3 Individualized versus standardized stimulation

Based on stimulation temperature, a distinction can be made between the previously performed studies using thermal stimulation. Some of the studies used the same painfully hot stimulation temperature for all participants (Becerra et al., 1999; Casey et al., 1994; Coghill, Talbot, Evans, Meyer, Gjedde, Bushnell et al.1994; Craig et al., 1996; Talbot et al., 1991). For instance, each participant was stimulated at 47°C in order to induce pain. In this case stimulation is standardized. Other studies first determined a participant's pain threshold (Derbyshire, Jones, Devani, Friston, Feinmann, Harris et al., 1994; Jones et al., 1991; Tolle et al., 1999; Vogt, Derbyshire, & Jones, 1996). Subsequently, this individual pain threshold temperature was used during painfully hot stimulation. This kind of stimulation is individualized.

It is not clear whether standardized stimuli lead to the same results as individualized stimuli. When using standardized stimulation (e.g. 47°C), a participant could be stimulated below his or her individual pain threshold (e.g. 48°C). This might cause an activation pattern different from the pattern resulting from individualized stimulation, which is known to be painful.

Table 2 summarizes results from four studies using individualized (Derbyshire et al., 1994; Jones et al., 1991; Tolle et al., 1999; Vogt et al., 1996) and five studies using standardized painfully hot stimuli (Becerra et al., 1999; Casey et al., 1994; Coghill et al., 1994; Craig et al., 1996; Talbot et al., 1991). The table specifically shows how often a certain brain area was reported to be involved with pain processing. The majority of the brain areas do not show a large difference in the number of reported activations between the two types of studies. The somatosensory cortices (SI and SII) on the other hand, do show a remarkable difference. Both areas are found to be activated during standardized painfully hot stimulation in all five studies, however none of the studies using individualized painfully hot stimuli reported activations in either SI or SII.

Table 2
The number of studies reporting activation in each brain area

Brain area	Nr. of reported activations during individualized stimuli	Nr. of reported activations during standardized stimuli
Anterior cingulate cortex	4	5
Thalamus	4	3
Basal ganglia	2	1
Primary somatosensory cortex	0	5
Secondary somatosensory cortex	0	5
Insular cortex	3	4
Prefrontal cortex	3	1

Note. Four studies used individualized stimuli (Derbyshire et al., 1994; Jones et al., 1991; Tolle et al., 1999; Vogt et al., 1996) Five studies used standardized stimuli (Becerra et al., 1999; Casey et al., 1994; Coghill et al., 1994; Craig et al., 1996; Talbot et al., 1991)

1.4 Neuroimaging techniques used in pain research

During the last few decades, neuroimaging techniques have evolved into useful, non-invasive tools to explore the brain. Within the research area investigating the neural basis of pain, previous studies mainly used positron emission tomography (PET) and functional magnetic resonance imaging (fMRI).

The PET technique assumes that an increase of regional cerebral blood flow (rCBF) indicates an increase of activity in the corresponding brain area (Peyron et al., 2000). In order to detect the rCBF, a radioactive material is administered to the patient. The PET scan is able to receive the amount of radioactivity in the brain, with high levels indicating increased rCBF (Devlin).

The present study uses fMRI, during which the blood oxygen level dependent (BOLD) signal is a measure of neural activity. fMRI using BOLD response works according to the following principle. When a neuron becomes more active, it simultaneously needs more oxygen. Hemoglobine (Hb) in oxygenated blood (oxyhemoglobine) is diamagnetic, while Hb without oxygen (deoxyhemoglobine) is paramagnetic (Noll, 2001). When neural activity increases, so does the blood flow in order to deliver more oxygen. The increase of oxygen in the blood is much more then what is actually needed by the active brain areas. As a result the blood contains relatively more diamagnetic oxyhemoglobine (Clare, 1997). This shift in magnetism can be detected using an MRI scanner. In short, an increase of diamagnetic oxyhemoglobine leads to an increased fMRI signal, which indicates more neural activation in the corresponding brain area.

According to Peyron et al. (2000), results from pain studies using either PET or fMRI show great resemblance. Even though the results are quite similar, the fMRI technique shows

some advantages over PET. First of all, no radioactive materials are injected during fMRI (Jones et al., 2003; Peyron et al., 2000), which is obviously healthier to the participant or patient and enables repeated measure experiments. The latter is problematic during PET, because the restraint regarding the radioactivity dose allows only a limited number of scans (Chen, 2007). Secondly, both PET and fMRI offer great spatial resolution but a relatively low temporal resolution in comparison to electrophysiological techniques (e.g. EEG and MEG). Nevertheless, fMRI offers a better temporal resolution than PET (Peyron et al., 2000).

A disadvantage of both techniques concerns the results, which only show areas of activation. No information about connections between areas or the direction of the signal flow is acquired by either PET or fMRI (Chen, 2007; Jones et al., 2003).

1.5 The current study

The current study has an explorative and preparative function. It is part of a project which focuses on pain sensitivity in children who suffered from neonatal pain and tissue damage. Research in this area has shown that pain sensitivity in these children is different from the pain sensitivity in healthy, control children (Peters, Schouw, Anand, van Dijk, Duivenvoorden, & Tibboel, 2005). This alteration in pain perception might be explained by abnormalities during early brain development due to neonatal pain.

To investigate whether neonatal pain actually affects brain development, a pain experiment using fMRI will be performed in children who suffered from neonatal pain. However, some preparation is necessary before starting this experiment. The first step will be made by the present study, during which a pain experiment protocol using fMRI will be developed and tested in healthy adults. In a following study, the same experiment will be performed on children without a history of neonatal pain. At that point the neural basis of pain processing in adults and children can be compared. Following these preliminary studies, the pain experiment will finally be performed on children who experienced neonatal pain.

The general goal of the current study is to test and improve the experimental protocol, so it can be used to study children in the future. In order to do so, we investigate which brain areas in healthy adults are involved with pain processing. We expect to find pain related activations in several areas, which are summarized in table 1. Based on previous research, we do not expect all these areas to show activations, but specifically the areas reported most frequently (insula, ACC, SI and SII). Furthermore, this study examines whether standardized and individualized painfully hot stimuli lead to different activation patterns. Based on previous research, we expect to find similar results regarding the majority of the brain areas,

with exception of the somatosensory cortices. We hypothesize that pain related activations in SI and SII will occur during standardized, but not during individualized painfully hot stimulation.

2. Methods

2.1 Subjects

Initially, 20 healthy subjects (10 males, 10 females) participated voluntary. One male participant was excluded from further analysis because of morphological brain anomalies. The majority of the remaining 19 volunteers were students at the Erasmus University Rotterdam. Ages ranged from 19 to 29 years (M = 22.74, SD = 2.49 years). Informed consent was obtained in accordance with ethical approval by the Erasmus Medical Ethical Committee (METC). Each participant was screened for MRI contraindications according to the Erasmus MC Department of Radiology. Absolute contraindications are for instance pregnancy and ferromagnetic materials in the body. For a complete list of MRI exclusion criteria, see the MRI checklist in appendix A. Additional exclusion criteria for the present study were the use of drugs related to pain suppression, a pain threshold above or below stimulation limits (46-48°C) and a history of neonatal pain.

2.2 Materials

2.2.1 Apparatus

Image acquisition. Each participant was scanned at the department of Radiology in the Erasmus MC, Rotterdam. Anatomical and functional MRI scans were performed using a 1.5 T MRI scanner (Signa CV/I; General Electric Milwaukee, USA) with a dedicated 8-channel head coil. Pillows were used to support the participant's head in order to minimize head movements. To reduce the noise of the scanner and to enable communication with the experiment leader, each participant wore an MRI-compatible headphone.

An anatomical whole brain image was acquired with a 3D high resolution inversion recovery FSPGR T1 weighted sequence (TR/TE/TI 9.9/2.0/400 ms, flip angle 20°, 320 x 224 matrix with a field-of-view of 24 cm, 1.6 mm slice thickness with no gap; ASSET factor 2; acquisition time 3 m 10 sec).

Functional imaging was performed with single-shot gradient-echo echo-planar imaging (EPI) sequences in transverse orientation, which is sensitive to blood oxygenation level dependent (BOLD) contrast. The following parameters were used: TR/TE 3000/40 ms, flip angle 60°, 96 x 96 matrix with a field-of-view of 26 cm, 5 mm slice thickness with 1 mm gap, 22 slices and voxel sizes of 2.7 x 2.7 x 5 mm^3. Acquisition time was 7 m 3 sec per session, including 15 seconds of dummy scans.

Thermal stimulation. In order to apply thermal stimuli we used the MRI-compatible Thermal Sensory Analyzer II (TSA-II, Medoc Advanced Medical systems). The TSA-II is a computer controlled device, which is able to repeatedly generate thermal stimuli over a range of 0°C-50°C. The thermode, a device with a 9 cm^2 contact surface, was placed at the ball of the thumb on the right hand. The TSA-II was used to measure individual pain thresholds and to produce tonic thermal stimulation during the fMRI experiment.

Numerical Rating Scale. The Numerical Rating Scale (NRS), which can be delivered verbally, was used to collect information about pain intensity and pain unpleasantness. To measure pain intensity the following question was asked: 'How much pain did you experience?' The NRS scale ranged from 0 (no pain) to 10 (worst imaginable pain). In order to measure pain unpleasantness the question 'How unpleasant was the pain stimulus?' was asked. The NRS pain unpleasantness scale ranged from 0 (not unpleasant at all) to 10 (extremely unpleasant).

State-Trait Anxiety Inventory (STAI). The State-Trait Anxiety Inventory (STAI) is an instrument used to measure anxiety in adults. A Dutch version of the STAI, developed by Van der Ploeg, Defares and Spielberger (1980), was used in the present study. This scale is a 40-item self-report questionnaire containing a 4-point Likert response scale. The test contains two parts, the STAI version DY1 (first 20 items) and STAI version DY2 (last 20 items). These two parts differentiate between the present 'state anxiety' (DY1) and the general 'trait anxiety' (DY2). During the current study the STAI-DY1, concerning state anxiety, was used. The DY1 Likert scale ranges from 1 (not at all) to 4 (very much so). See appendix B for the complete Dutch version of the STAI-DY1.

2.2.2 Stimuli and Design

During the present study an experimental design was used. The dependent variable was the blood oxygenation level dependent (BOLD) response, as reflected by the MRI signal. Two independent variables have been manipulated within-subjects, 'temperature' and 'stimulus type'. The two experimental temperature conditions were 'warm' (37°C) and 'painfully hot' (temperature depends on stimulus type). In order to calculate the BOLD response, each experimental condition was compared to the baseline condition. The baseline temperature of the thermode was 32°C.

The stimulus type was either standardized or individualized. The standardized painfully hot stimulus (46°C) was determined beforehand and was equal for all participants.

The individualized painfully hot stimulation was determined separately for each participant, based on their pain thresholds. The individual pain threshold temperature (rounded up to half or whole degrees) was used as stimulation temperature for the concerning participant. The method used to measure individual pain thresholds will be described in the procedure section.

For tonic pain stimulation, which was used during the experimental blocks, the maximum stimulation temperature was set at 48°C. Using a higher stimulation temperature for a long period of time would cause tissue damage. The mean individualized hot stimulation temperature over 19 participants was 47.58 (SD = .63). Table 3 shows the three conditions formed by combinations of the variables 'temperature' and 'stimulus type'.

Tabel 3
Conditions during fMRI

Condition	Stimulus type	Temperature
1	Standardized	Baseline (32°C)
2	Standardized	Warm (37°C)
3	Standardized	Hot (46°C)
4	Individualized	Hot (46°C - 48°C)

2.3 Procedure

In order to investigate whether the environment of the MRI scanner might influence pain sensitivity, the pain thresholds were measured both outside and inside the scanner. Repeated tonic pain stimulation can lead to sensitization or habituation, which would cause distorted results. To avoid this, the pain thresholds outside and inside the scanner were measured during two separate sessions, with at least three days in between.

2.3.1 First session: measuring pain thresholds outside the MRI scanner

The first session started with each participant verbally answering the STAI-DY1, concerning state anxiety. It was necessary to perform this test verbally, because it was performed again during the second session, while the participant was lying in the scanner. Following the STAI-DY1, the thermode was placed at the participant's ball of the thumb on the right hand, in order to measure the pain threshold in a neutral environment, outside the MRI scanner.

The pain threshold was measured using the 'Method of Levels' (MLE), see figure 3 for an example. Each participant had to keep his or her eyes closed throughout the pain threshold determination. The baseline temperature of the thermode was 32°C. From this point the temperature initially increased with 3°C steps at a speed of 2°C/sec. As soon as the thermode reached the target temperature (which was 35°C the first time), the temperature

immediately returned back to baseline. Afterwards, the participant was asked whether or not this stimulation was painful (yes or no). As long as the participant would answer 'no' to this question, the target temperature increased with 3°C. As soon as the answer was 'yes' the subsequent step sizes would decrease at one half of the previous step. This method was repeated until the step size was decreased to .5°C. The definition of the pain threshold determined with the MLE is 'the lowest temperature which is considered to be painful'.

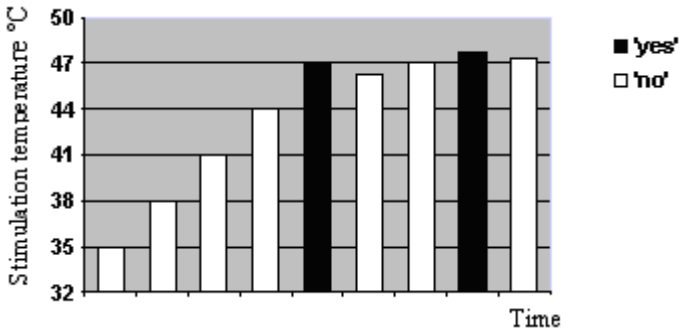

Figure 3. Example of pain threshold determination, with pain threshold determined at 47.5°C. The color of the bar indicates whether the participant considered the target temperature to be painful.

During pain threshold determination, the maximum stimulation temperature was 50°C. Using a higher stimulation temperature would increase the risk of tissue damage. This stimulation temperature limit is higher than the tonic hot stimulation limit (48°C), which was used during the experimental blocks. Since the stimulations at target temperature during pain threshold determination last very briefly, the skin is able to tolerate a higher temperature than during tonic stimulation.

Following pain threshold determination, one individualized block of the experiment was performed, containing four painfully hot and four warm, tonic stimuli separated by baseline temperature (blocks will be described in detail later). During this block, the determined pain threshold temperature (rounded up to half or whole degrees) was used as painfully hot stimulation. Again the eyes had to be closed throughout the entire block. After each painfully hot stimulus the participant's NRS score for pain intensity and pain unpleasantness was determined. Afterwards, the participant's pain threshold was measured again, in order to check for habituation or sensitization to pain after repeated tonic painfully hot stimulation.

2.3.2 Second session: measuring pain thresholds inside the scanner and fMRI

During the second session, the anatomical whole brain scan was performed first. Following the anatomical scan, each participant had to verbally answer the STAI-DY1, measuring state anxiety. Thirdly, the participant's pain threshold was determined as described before, but now while lying in the MRI scanner. The achieved pain threshold (rounded up to half or whole degrees) was used as painfully hot stimulation during fMRI.

In some cases the pain threshold turned out to be either too high (the participant experienced unbearable pain) or too low (no pain experience) during tonic stimulation. In these situations another method was used to find the correct individualized painfully hot stimulation temperature. The participant received tonic stimulation (21 sec) several times, each time a different temperature was used. In case the initial pain threshold was too high, the tonic stimulation temperature started .5°C below pain threshold and decreased with .5°C steps (vice versa when the initial pain threshold was too low). Following each tonic stimulation, the NRS pain intensity was scored by the participant. The correct individualized painfully hot stimulation temperature was found when the participant's NRS score was 6 or higher and the temperature was bearable for 21 sec. The NRS pain intensity threshold of 6 was used according to convention.

Some participants' pain thresholds were above the maximum stimulation temperature (48°C). In these cases the participants received a tonic stimulation during 21 sec at 48°C. Afterwards they had to rate the NRS pain intensity. All concerning participants experienced enough pain (NRS $\geq$ 6) to proceed the experiment with 48°C individualized painfully hot stimulation.

The fMRI experiment consisted of four blocks with tonic stimulation, during which the participant had to keep the eyes closed. After each block the participant was asked to rate the pain intensity and pain unpleasantness on the numerical rating scale (NRS).

During each block the temperature of the thermode alternated between baseline and stimulation temperature (warm or painfully hot). The duration of a warm or painfully hot stimulus was 21 seconds, during which seven brain volumes were scanned (7 TR). In order to prevent anticipation to the stimuli as much as possible, the baseline duration was either short (8 TR = 24 sec) or long (10 TR = 30 sec). Four combinations of baseline (B) and stimulation (S) were possible (short B warm S, short B hot S, long B warm S and long B hot S). Each combination was used twice during one block and every block was completed with an additional short baseline. Figure 4 shows an example of stimulation in one block.

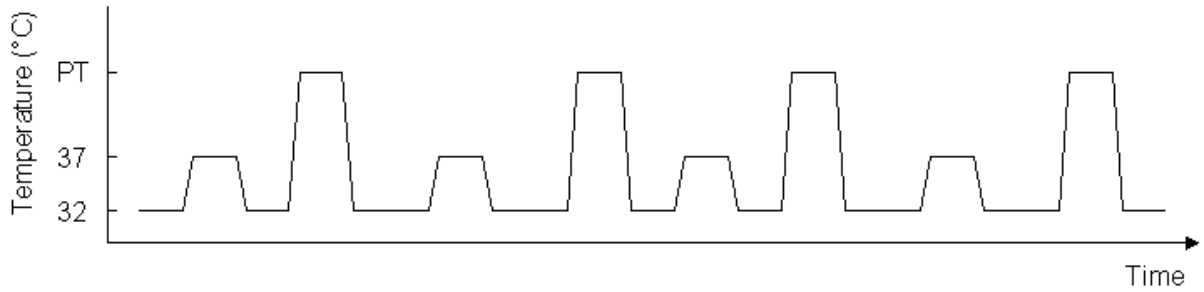

Figure 4. Example of an experimental block with neutral (32°C), warm (37°C) and individualized hot stimuli (PT = pain threshold)

The order of the four combinations was balanced according to a latin square, resulting in four experiments (see table 4). Painfully hot stimuli in these four experiments were either standardized or individualized, indicated by an 'S' or 'I' in front of the experiment number. For each participant two experiment numbers were picked; one was used during two blocks with standardized hot stimulation and the other experiment was used during two blocks with individualized hot stimulation. The individualized and standardized blocks were scanned alternately. For ten of the participants the even blocks were standardized and the odd blocks individualized. The order was switched for the remaining nine participants. Appendix C shows the 19 used block combinations, one for each participant.

After the four fMRI blocks, each participant's pain threshold was determined once again (with the eyes closed) in order to check for habituation or sensitization due to tonic painful stimulation.

Table 4
Balanced latin square with the four combinations of baseline and stimulation

Experiment number	**Conditions: Alternating baseline (short, long) & Stimulation (warm, hot)**																
	B	**St**	**B**	**St**	**B**	**St**	**B**	**St**	**B**	**St**	**B**	**St**	**B**	**St**	**B**	**St**	**B**
S1 or I1	short	W	short	H	long	W	long	H	short	W	short	H	long	W	long	H	short
S2 or I2	short	H	long	H	short	W	long	W	short	H	long	H	short	W	long	W	short
S3 or I3	long	H	long	W	short	H	short	W	long	H	long	W	short	H	short	W	short
S4 or I4	long	W	short	W	long	H	short	H	long	W	short	W	long	H	short	H	short

Note. B = baseline, St = stimulation, W = warm, H = hot, S = Standardized, I = Individualized

2.4 Analysis

2.4.1 Functional imaging data

The functional imaging data were analyzed using Statistical Parametric Mapping software (SPM2, distributed by the Wellcome Department of Cognitive Neurology, University College London, UK) implemented in MATLAB (Version 6.5, Mathworks, Sherborn, MA, USA).

Preprocessing. Preprocessing of the fMRI data consisted of four steps. The first step, realignment, was used to correct for movements of the participant's head during or between the four scanning blocks. Following, co-registration was performed to match the functional images to the anatomical image. Thirdly, both anatomical and functional images were normalized to the standard space defined by the Montreal Neurological Institute (MNI) template. The normalized anatomical data had a resolution of 1 x 1 x 1 mm^3 and the resolution of the normalized functional data was 3 x 3 x 3 mm^3. During the final step of preprocessing the functional data were smoothed using a Gaussian kernel, with a full-width-half-maximum (FWHM) of 6 mm.

Individual analysis. During individual analysis, a design matrix was composed for each participant separately. To correct for residual motion artifacts after realignment, the movement parameters (resulting from the realignment step) were included as regressors of no interest. The preprocessed data were subsequently compared to the created design matrix. This estimation was performed using a high-pass filter with a cut-off period of 128 seconds. For each participant a t-contrast map was calculated between the following conditions: individualized hot versus baseline, standardized hot versus baseline and warm versus baseline.

Group analysis. To investigate group effects, a second level random effects group analysis was performed. The individual t-contrast maps were used in three tests.

First, a one-sample t-test was performed to investigate the warm versus baseline comparison. Secondly, a one-sample t-test was performed to investigate the individualized hot versus baseline comparison. In this case, the NRS pain intensity scores determined during fMRI were used as a weight factor. The mean NRS pain intensity score over the individualized fMRI blocks was calculated for each participant and was used to give a weight to the functional data. The data with high NRS scores received more weight than the data with lower NRS scores. Thirdly, a paired t-test was performed in order to compare activations between individualized and standardized hot stimulation, corrected for baseline activations.

In all three tests a .05 p-value with a family wise error (FWE) correction for multiple comparisons was used. Only clusters with a significant p-value and a size of 15 voxels or greater are reported.

The final step, anatomical labeling of the significant areas of activation, was performed using the macroscopic anatomical parcellation procedure of the Montreal Neurological Institute (MNI) MRI single-subject brain (Tzourio-Mazoyer, Landeau, Papathanassiou, Crivello, Etard, Delcroix et al., 2002).

2.4.2 Pain threshold data

Pain thresholds were determined four times during this study; before and after repeated tonic hot stimulation, during both the first and the second session. The pain threshold data were abnormally distributed, due to the stimulation limit of 50°C. Therefore, the data were analyzed using non-parametric tests.

In order to investigate possible habituation or sensitization to the painful stimuli, two Wilcoxon tests for two related samples have been performed. The first Wilcoxon test was used to compare pain thresholds before and after repeated tonic pain stimulation during the first session. The second test was used to compare pain thresholds before and after repeated tonic pain stimulation during the second session. The dependent variable in both tests was the pain threshold (°C) and the independent variable was time of determination (before versus after repeated tonic pain stimulation).

To investigate whether the environment (out- versus inside the MRI scanner) has influenced the pain thresholds, an additional Wilcoxon test for two related samples has been performed. This test compared the two pain thresholds which were measured before tonic pain stimulation (one during the first and one during the second session). The dependent variable was the pain threshold (°C) and the independent variable was place of determination (outside versus inside the MRI scanner). The pain thresholds determined after tonic pain stimulation could not be compared, since the number of tonic pain stimuli was much higher during the second session than during the first.

A Mann-Whitney test for two independent samples has been performed to investigate whether pain thresholds differed significantly between men and women. The dependent variable was mean pain threshold over four determinations (°C) and the independent variable was gender (male versus female).

2.4.3 State anxiety scores

A paired t-test was used to examine whether the state anxiety (STAI-DY1) scores differed between the first (outside the MRI scanner) and the second session (inside the MRI scanner). The dependent variable was the total state anxiety score over 20 items, the independent variable was testing environment (in- or outside the MRI scanner).

2.4.4 Numerical rating scale scores

Two mean NRS pain intensity scores were calculated for each participant; one concerning the individualized and one concerning the standardized painfully hot stimuli during fMRI. A paired t-test was performed to investigate whether these scores differed significantly from one another. The dependent variable was the NRS pain intensity score (0-10) and type of stimulation was the independent variable (individualized versus standardized). The same procedure was used to investigate the NRS pain unpleasantness scores.

A third paired t-test was performed to compare the NRS pain intensity scores from the first with the second individualized block during fMRI. This test provides information about whether the participants were experiencing the same amount of pain during these two individualized blocks, which is particularly important when habituation or sensitization has occurred after repeated tonic stimulation.

3. Results

3.1 Functional imaging results

3.1.1 Warmth related activations

Group analysis using the individual t-contrast maps 'warm versus baseline' revealed no significant positive or negative activations.

3.1.2 Pain related activations

The random effects group analysis concerning individualized hot stimulation, with NRS pain intensity correction, showed significant positive activations in two clusters. Table 5 shows the full list of anatomical and their corresponding functional areas, which belong to these clusters. Figure 5 shows the major group activations observed during individualized painfully hot stimulation, superimposed on axial anatomical slices. See appendix D for images of all activations.

Table 5
Areas of activation during painfully hot stimulation

Cluster size (voxels)	T-value	MNI Coordinates (mm)			Anatomical area	Laterality	nº voxels	Functional area
		x	y	z				
399	**8.18**	**-51**	**3**	**-12**	Insula	L	70	
					Sup. temporal lobe	L	65	Auditory cortex
					Sup. temporal pole	L	63	
					Amygdala	L	33	Limbic system
					Putamen	L	26	Basal ganglia
					Inf. frontal gyrus, orbital part	L	26	Association cortex
971	**7.95**	**42**	**9**	**-15**	Insula	R	165	
					ACC	R	123	Limbic system
					Sup. temporal pole	R	116	
					ACC	L	79	Limbic system
					Amygdala	R	31	Limbic system
					Putamen	R	30	Basal ganglia
					Inf. frontal gyrus, orbital part	R	28	Association cortex
					Caudate nucleus	R	23	Basal ganglia
					Rolandic operculum	R	17	Sensorimotor cortex

Note. L = Left, R = Right

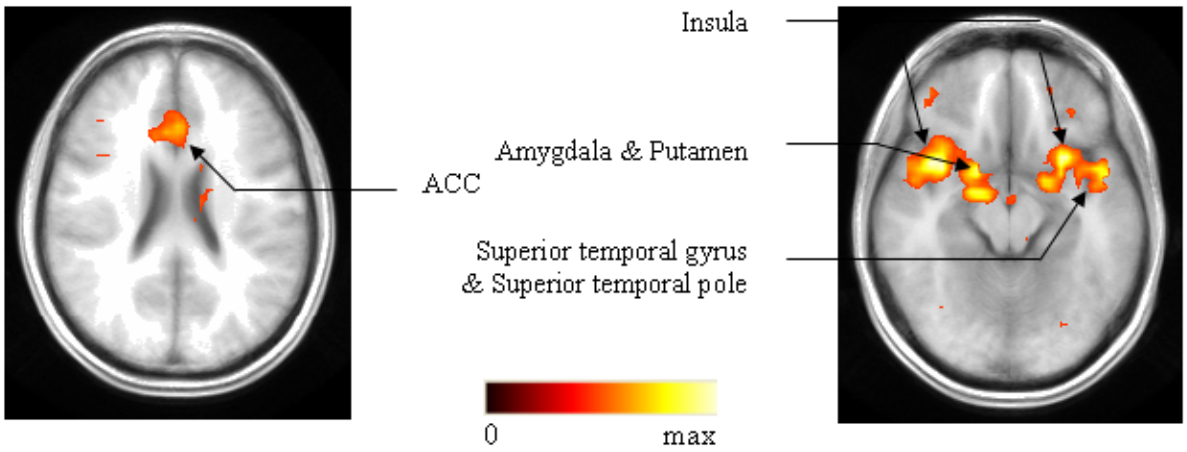

Figure 5. Mean group activations observed during individualized painfully hot stimulation superimposed on axial anatomical slices.

3.1.3 Individualized versus standardized hot stimulation

The paired t-test showed no significant differences in activation between individualized and standardized painfully hot stimulation.

3.2 Pain threshold data

During the first session the mean pain thresholds show an increase between before (M = 47.48) and after (M = 48.60) repeated tonic stimulation. The Wilcoxon test proved that this increase was significant, z = 3.18, N – Ties = 13, $p < .01$. During the second session, the difference between the mean pain threshold before (M = 47.51) and after (M = 49.01) tonic pain stimulation was significant as well according to the Wilcoxon test, z = 3.73, N – Ties = 18, $p < .001$.

The third Wilcoxon test for two related samples showed no significant difference between the pain threshold measured inside and the pain threshold measured outside the MRI scanner, z = .26, N – Ties = 16, $p = .80$.

The Mann-Whitney test revealed a significant difference in mean pain threshold ranks (MR) between men (MR = 13.22) and women (MR = 7.10), U = 16.00, $N_{male} = 9$, $N_{female} = 10$, $p < .05$.

3.3 State anxiety scores

The mean state anxiety (STAI DY-1) score during the first session was 33.00 and was 33.26 during the second session. This difference was not significant according to the paired t-test, t(18) = -.15, $p = .88$.

3.4 Numerical rating scale scores

Both of the paired t-tests comparing NRS scores from individualized with NRS scores from standardized stimulation showed significant results. The NRS pain intensity score concerning individualized stimulation (M = 6.87) differed significantly from the one concerning standardized stimulation (M = 4.09), $t(18) = 7.93$, $p < .001$. Similarly, the NRS pain unpleasantness score concerning individualized stimulation (M = 6.22) differed significantly from the one concerning standardized stimulation (M = 2.88), $t(18) = 6.00$, $p < .001$.

The NRS pain intensity scores from the first individualized block during fMRI (M = 6.78) did not differ significantly with the NRS scores from the second individualized block (M = 6.96), $t(18) = -.90$, $p = .38$.

4. Discussion

4.1 Brain activations during pain

4.1.1 Comparison with previous research

Pain related activations were expected to be discovered in areas which have been reported in previous studies. The areas found to be significantly activated during individualized painfully hot stimulation, correspond mainly with previous research. Table 6 summarizes the results from previous research and shows the reported results from the current study.

Table 6
Pain related activations during previous studies and the present study

Brain area	Function regarding pain	Nr. reported activations	Current results
Insular cortex	sensory-discriminative and affective	21	√
Anterior cingulate cortex (ACC)	affective/motivational, attentional	21	√
Primary somatosensory cortex (SI)	sensory-discriminative	18	
Secondary somatosensory cortex (SII)	sensory-discriminative	18	√
Thalamus	processing and modulation	14	
Prefrontal cortex (PFC)	cognitive and attentional	12	√
Cerebellum	motor aspects	10	
Basal ganglia	initiation of autonomic and emotional states	8	√
Premotor cortex (PMC)	motor aspects	5	
Primary motor cortex (M1)	motor aspects	3	√
Amygdala	emotional component	1*	√
Periaqueductal gray (PAG)	part of descending pain inhibitory system	1	
Hippocampus	memory functions	1	

Note. √ = Significant increase of activation, * Two studies reported a decrease in amygdala activation.
Information retrieved from 29 studies (Apkarian et al., 1999; Apkarian et al., 2000; Becerra et al., 2001; Becerra et al., 1999; Casey et al., 1994; Casey et al., 1996; Casey et al., 2001; Chang et al., 2002; Coghill et al., 2001; Coghill et al., 1999; Craig et al., 1996; Davis et al., 1998; Derbyshire & Jones, 1998; Derbyshire et al., 1997; Derbyshire et al., 1998; Gelnar et al., 1999; Helmchen et al., 2003; Hofbauer et al., 2001; Jones et al., 1991; Kurata et al., 2002; Kwan et al., 2000; Paulson et al., 1998; Peyron et al., 1999; Rainville et al., 1997; Strigo et al., 2003; Svensson et al., 1998; Talbot et al., 1991; Tolle et al., 1999; Tracey et al., 2000)

The insula and anterior cingulate cortex (ACC) are the two areas with the largest amount of activated voxels in the current study. In accordance, over 70% of the studies summarized in table 6 reported these areas to be activated during painful stimulation (e.g. Coghill et al., 2001; Tracey et al., 2000). The orbital part of the inferior frontal gyrus is part of the prefrontal cortex, which has been reported in about 40% of the studies. Putamen and the caudate nucleus both belong to the basal ganglia, which are reported in approximately 25% of the previous research in this field.

The rolandic operculum (RO), known as the sensorimotor cortex (Yetkin, Papke, Mark, Daniels, Mueller, & Haughton, 1995), is situated at the base of the pre- and postcentral gyri (Cerf-Ducastel, Van de Moortele, MacLeod, Le Bihan, & Faurion, 2001). It is the part of the operculum which surrounds the rolandic fissure, or central sulcus. Activation in the rolandic operculum indicates secondary somatosensory cortex involvement. First of all, Yetkin et al. (1995) found activation in RO during tactile stimulation of the palm of the hand. Furthermore, SII is known to be located in the fronto-parietal operculum (Treede, Apkarian, Bromm, Greenspan, & Lenz, 2000), which shows at least partial overlap with the RO. Rolandic operculum activation indicates involvement of the primary motor cortex (M1) as well, which is located at the precentral gyrus (Williams, White, & Mace, 2004).

The current study also found the amygdala to be involved in the processing of pain. Previous pain research showed contradictory findings regarding this area. Becerra et al. (2001) found an increase in amygdala activation from baseline to painful stimulation, however in other studies a decrease has been reported in this area (Becerra et al., 1999; Derbyshire et al., 1997). Although the actual involvement of the amygdala is still uncertain, results from the current study indicate participation of the amygdala in pain processing.

A significant increase of activation has also been found in the contralateral superior temporal lobe and in both of the superior temporal poles. Activation in these areas has not been reported before in pain research involving thermal stimulation. The superior part of the temporal lobe is known to be involved in the processing of auditory information. It contains areas such as Wernicke's area, involved in language comprehension, and the primary auditory cortex (Williams et al., 2004). The MRI scanner produces a lot of noise during fMRI, so activity in auditory areas could be expected. However, this does not explain an increase of activation in this area while one is experiencing pain. It is unknown what variable might have caused the temporal activation.

Based on previous research, the primary and secondary somatosensory cortices were expected to show increased activation during painfully hot stimulation. These areas were found to be involved in pain processing in 18 out of 29 studies. Results from the present study indicate SII involvement in pain processing, since RO activation was found. However, SI activation was not found during painfully hot or warm stimulation. This lack of SI activation could be due to the stimulation of a relatively small area of the skin (9 cm^2). Peyron et al. (2000) show that most studies using large stimulation areas report SI activation, while the majority of the studies using small stimulation areas fail to do so. This could be explained by

the fact that a larger stimulation area simultaneously activates a larger part of the sensory homunculus in SI. When a small focal part of SI is activated the signal will tone down during averaging over subjects and no significant activation will remain (Derbyshire, 2000).

4.1.2 Laterality

All significant activations during pain stimulation were bilateral, except for those in the superior temporal lobe, caudate nucleus and rolandic operculum. The first structure showed contralateral activation and the second and third showed activity ipsilateral to stimulation.

4.1.3 Anxiety during fMRI

The state anxiety scores showed no differences between the first and the second session. This shows that the participants were not experiencing more anxiety while lying in the scanner as to being in a neutral environment. These results might be surprising, because the majority of the participants seemed to be a bit nervous before entering the MRI scanner. The STAI was deliberately performed áfter the anatomical scan, so each participant got used to the environment before answering the questions. The anxious feelings were apparently gone after the anatomical scan, so during fMRI the participants were just as calm as outside the scanner. These results demonstrate that the functional imaging data have not been influenced by anxiety.

4.1.4 Pain thresholds: habituation, environment and gender

During both sessions, the pain thresholds increased after repeated tonic stimulation. This indicates that habituation to painfully hot stimuli occurred after repeated tonic stimulation. In the first session, participants were exposed to only four painfully hot stimuli, after which habituation occurred already. These results show how fast the habituation effect develops.

Strong habituation could result in less or no pain experience after repeated stimulation. In order to prevent distortion of the fMRI results because of this effect, it is necessary to ask for NRS pain intensity scores several times throughout the experiment. This way it is possible to verify whether the participant is still experiencing the same amount of pain after several tonic stimulations. The NRS pain intensity scores from the two individualized blocks during fMRI, did not differ significantly for one another. This means that the participants experienced the same amount of pain throughout the experiment. Therefore, the observed pain-related activations were not influenced by habituation.

Pain thresholds measured inside the MRI scanner did not differ significantly from the pain thresholds measured outside the scanner. It can be concluded that the environment of the MRI scanner does not influence pain sensitivity.

Finally, pain thresholds appear to be related to gender. During the present study pain thresholds were higher in men than in women, indicating that women are more sensitive to hot stimulation. This result is in accordance with previous studies (e.g. Berkley, 1997; Sarlani, Grace, Reynolds, & Greenspan, 2004). Furthermore, this effect is not specific for thermal stimulation, since the sex differences also appeared during other types of painful stimulation. For instance, Sarlani et al. (2004) showed that women are more sensitive to mechanical pain than men.

4.2 No warmth related activations

No significant activations were found during warm stimulation compared to baseline. This means that a small temperature increase (32°C to 37°C) does not result in any changes of neural activation.

Previous research has shown various results regarding this topic. Similar to our observations, Brooks, Nurmikko, Bimson, Singh and Roberts (2002) and Jones et al. (1991) did not find any significant activation during warm stimulation. Contradictory to these findings, Becerra et al. (1999) and Moulton, Keaser, Gullapalli and Greenspan (2005) found several areas of significant activation (e.g. SI, ACC and insula) during non-noxious stimulation.

A possible explanation could be that the warm stimulation temperature of 37°C, which was used in the recent study, was too low to find a significant increase of activation. Becerra et al. (1999) and Moulton et al. (2005) for example used 41°C as non-noxious stimulation temperature.

4.3 Individualized versus standardized hot stimulation

Based on prior studies, a difference in the amount of SI and SII activation was expected when comparing stimulus types. These areas were thought to show pain related activations during standardized but not during individualized painfully hot stimulation. However, the comparison between the two stimulus types showed no significant difference in activations. Both conditions triggered activations in the rolandic operculum, which indicates SII activation. SI activation was missing during both standardized and individualized painfully hot stimulation.

As explained before, the lack of SI activation during the present study might be due to the relatively small stimulation area. The chance of finding activations in the primary somatosensory cortex would increase when stimulating a larger area of the skin. In this case, a difference in the amount of SI activation based on stimulus type can not be ruled out. However, we can conclude that, when using a relatively small stimulation area, standardized and individualized stimuli will lead to the same results.

The NRS pain intensity and pain unpleasantness scores did show a difference between these two conditions. Both mean NRS scores were below the threshold of 6 during standardized stimulation. The majority of the participants told the experiment leader they did not experience any pain during these blocks. These results suggest that the areas activated during individualized painfully hot stimulation are not pain specific, but are also involved in the processing of non-noxious hot stimuli.

4.4 Suggestions for future research

The present study was mainly performed to investigate whether the current protocol is a valid tool to study pain related activations in the brain. Overall, the results from the present protocol are in accordance with previous studies. The only important limitation to the results is the lack of activation in the primary somatosensory cortex. As explained before, stimulation of a relatively small skin area might be the cause of this. Increasing the stimulation area to, for instance, the entire palm of the hand might be a solution.

However, enlarging the currently used thermode will cause a problem. This thermode has a flat surface which is not able to bend. The palm of the hand is uneven, so an enlarged thermode will not be touching the entire surface. A flexible thermode, for instance shaped like a glove, would be more suitable to stimulate an uneven surface with. This way a large area of the hand could be stimulated at once, which would increase the chance of finding SI activation during painful stimulation.

Secondly, the results indicate that individual pain threshold determination is not necessary when stimulating with the currently used thermode. The standardized and individualized conditions did not show significant differences in activation patterns, so both methods will lead to roughly the same results. In case an enlarged thermode will be used, a difference in SI activation between the two stimulus types can not be ruled out yet. Future studies might provide more information about this subject.

Overall, when this protocol will be used with children, it would be better to take the pain threshold determination out of the experiment. Not only would it save time, but it will

also be less unpleasant to the children. In order to actually measure pain, it would be necessary to use the NRS pain intensity scores. Each participant with a score below 6 will have to be excluded from further participation.

Pain threshold determination might not be necessary in studies investigating brain activations during pain, but it can still be used in other research areas. The method of levels, which is commonly used to determine pain thresholds, has its limitations though. During the recent study it appeared to be a bad indicator of a participant's pain threshold for tonic stimulation. The pain threshold was initially used in order to keep the pain as low as possible for each participant. But it turned out that the pain threshold was too high in several cases (and too low in one participant). When a participant was stimulated with his or her individual pain threshold for several seconds, the pain was sometimes unbearable (or no pain was experienced at all in case of one participant). In these cases the experiment was interrupted and a lower (or higher) stimulation temperature was used subsequently.

The reason for the overrated pain threshold is probably because of the short exposure to the target temperature during pain threshold determination. Immediately after the target temperature has been reached, the temperature of the thermode drops back to the 32°C baseline. This way the pain threshold for short, hot stimulation was determined. In order to determine someone's pain threshold for tonic hot stimulation, the method of levels should be changed. For instance the thermode should stay at the target temperature for 3 seconds before returning to baseline.

In summary, the current study showed pain related activations which generally correspond with previous research. With a few adjustments, like using standardized in stead of individualized stimulation and increasing the stimulation area, this fMRI protocol can be used in the future to perform imaging of pain in children.

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Appendix A - MRI checklist

VRAGENLIJST VOOR MRI ONDERZOEK BIJ PROEFPERSONEN

Naam:	Geboortedatum:
Geslacht:	Gewicht in kg:
Naam & Woonplaats van uw huisarts:	

MRI is een onderzoeksmethode waarbij gebruik gemaakt wordt van een zeer sterk magneetveld. In bepaalde gevallen zou dit magneetveld echter een gevaar kunnen vormen. Om ieder risico in dit opzicht uit te sluiten, verzoeken wij u onderstaande vragenlijst in te vullen.

Heeft u of draagt u:

een pacemaker of (oude) pacemakerdraden	ja / nee
een medicijnpomp (bv insulinepomp)	ja / nee
een neuro-stimulator	ja / nee
een uitwendige prothese (bv kunstarm)	ja / nee
één of meerdere piercings op uw lichaam	ja / nee
tatoeages of permanente make-up	ja / nee
tandtechnische constructies (beugels, draadjes e.d.)	ja / nee
medicijnpleisters (nicotine-, hormoonpleisters e.d.)	ja / nee

Heeft u ooit een operatie ondergaan aan:

het hoofd (bv plaatsen vaatclip of pompje)	ja / nee
het hart (bv kunstklep)	ja / nee
de ogen (bv geïmplanteerde lenzen)	ja / nee
de oren (gehoorbeentjesprothese; niet te verwijderen hoorapparaat)	ja / nee
de botten (waarbij platen en schroeven zijn gebruikt)	ja / nee
anderszins?	ja / nee
Zo ja, aan	

Bent u (oud) metaalbewerker?	ja / nee
Bestaat er kans op metaalsplinters in de oogkas?	ja / nee

Heeft u last of ooit last gehad van:

engtevrees/claustrofobie (bv bent u bang in een lift?)	ja / nee
kortademigheid (bij plat liggen)	ja / nee

Zou u zwanger kunnen zijn?	ja / nee

Heeft u kennis genomen van de schriftelijke informatie van MRI-onderzoek?	ja / nee
Heeft u deze informatie begrepen?	ja / nee
Indien er door het MRI-onderzoek toevalsbevindingen zijn gevonden, willen wij uw huisarts hiervan berichten. Gaat u hiermee akkoord?	ja / nee

Rotterdam, datum .....-.....- 20.....

.....	.....	.....
(naam)	(handtekening)	(paraaf uitvoerder MRI)

Appendix B – State-Trait Anxiety Inventory DY1

ZELF-BEOORDELINGS VRAGENLIJST
Ontwikkeld door H.M. van der Ploeg, P.B. Defares en C.D. Spielberger.

STAI · versie DY·1

Naam: ..... Sekse: ..... Datum: .....
Afgenomen bij: Bepaling pijndrempel / fMRI

Toelichting: Hieronder vindt U een aantal uitspraken, die mensen hebben gebruikt om zichzelf te beschrijven. Lees iedere uitspraak door en zet dan een kringetje om het cijfer rechts van die uitspraak om daarmee aan te geven hoe U zich *nu voelt*, dus *nu op dit moment*. Er zijn geen goede of slechte antwoorden. Denk niet te lang na en geef Uw eerste indruk, die is meestal de beste. Het gaat er dus om dat U weergeeft wat U *op dit moment* voelt.

	geheel niet	een beetje	tamelijk veel	zeer veel
1. Ik voel me kalm	1	2	3	4
2. Ik voel me veilig	1	2	3	4
3. Ik ben gespannen	1	2	3	4
4. Ik voel me onrustig	1	2	3	4
5. Ik voel me op mijn gemak	1	2	3	4
6. Ik ben ik de war	1	2	3	4
7. Ik pieker over nare dingen die kunnen gebeuren	1	2	3	4
8. Ik voel me voldaan	1	2	3	4
9. Ik ben bang	1	2	3	4
10. Ik voel me aangenaam	1	2	3	4
11. Ik voel me zeker	1	2	3	4
12. Ik voel me nerveus	1	2	3	4
13. Ik ben zenuwachtig	1	2	3	4
14. Ik ben besluiteloos	1	2	3	4
15. Ik ben ontspannen	1	2	3	4
16. Ik voel me tevreden	1	2	3	4
17. Ik maak me zorgen	1	2	3	4
18. Ik voel me gejaagd	1	2	3	4
19. Ik voel me evenwichtig	1	2	3	4
20. Ik voel me prettig	1	2	3	4
	geheel niet	een beetje	tamelijk veel	zeer veel

Appendix C – Block combinations

Table 7
Experiment number and block order for each participant

Participant number	Block 1&3	Block 2&4
1	I1	S2
2	I1	S3
3	I1	S4
4	I2	S1
5	I2	S3
6	I2	S4
7	I3	S1
8	I3	S2
9	I3	S4
10	I4	S1
11	S3	I1
12	S4	I1
13	S1	I2
14	S3	I2
15	S4	I2
16	S1	I3
17	S2	I3
18	S4	I3
19	S1	I4

Note. I = Individualized stimulation, S = Standardized stimulation

Appendix D – fMRI activations during pain

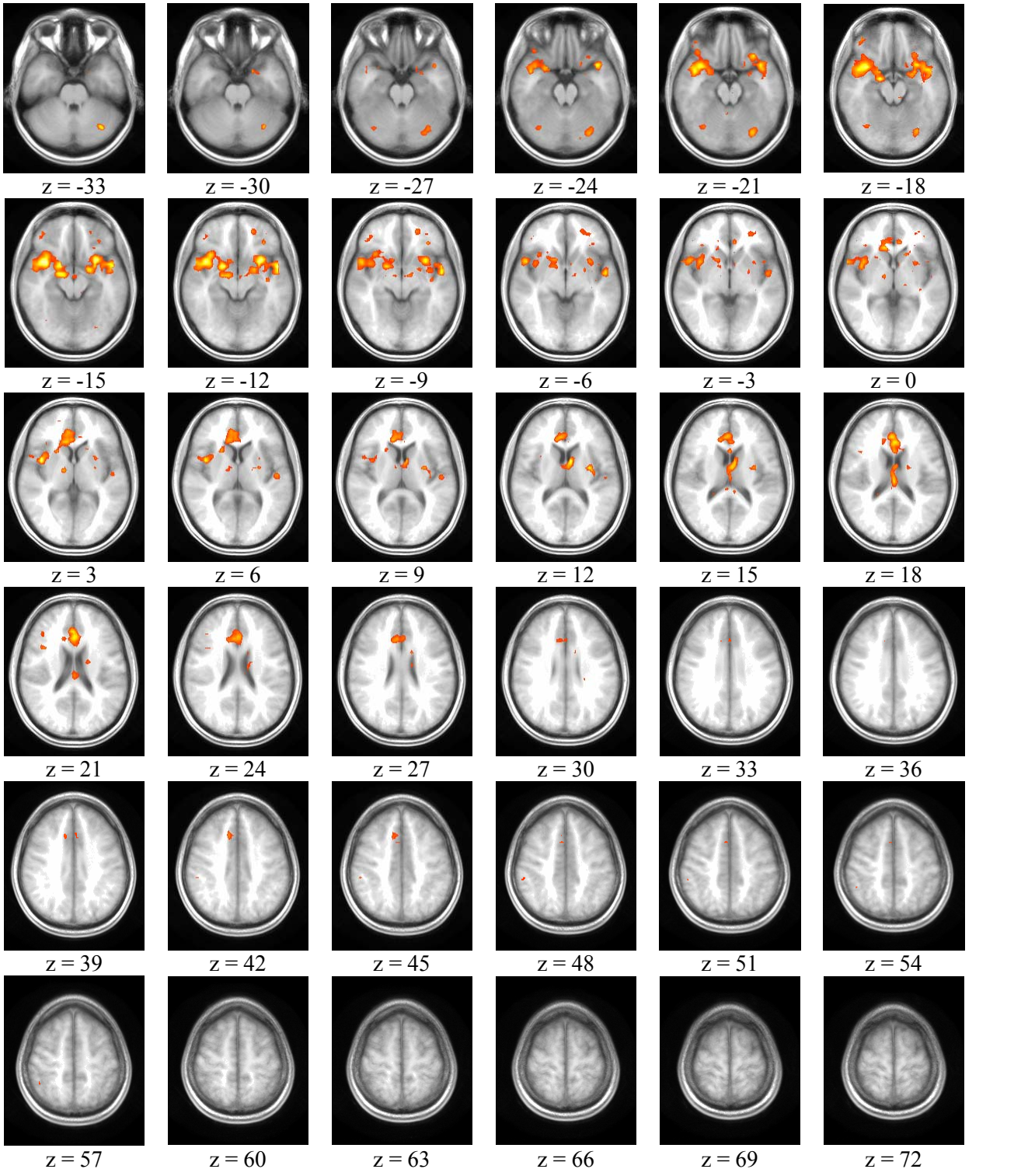

Figure 6. Mean group activations observed during individualized painfully hot stimulation, superimposed on axial anatomical slices.