Itch induced by a novel method leads to limbic deactivations -
A functional MRI study

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Abstract

Functional brain imaging studies on itch usually use histamine as a stimulus and, in consequence, have to cope with the highly variable time course of this particular itch sensation. In this study we describe a novel method of histamine application. To provoke itch, a mixture of histamine and codeine was applied through intradermally positioned microdialysis fiber. The itch was terminated by lidocaine application through the same fiber. During one fMRI session this procedure was repeated four times in four different microdialysis fibers, including one placebo control. Itch ratings of the subjects were correlated with BOLD effects. In a subsequent experiment performed in the same fMRI session, heat pain was provoked in the right forearm with a Peltier thermode. During both experiments, activation clusters were found in brain areas which have been described previously to be frequently activated in response to painful stimuli. This includes prefrontal areas, SMA, premotor cortex, anterior insula, anterior MCC, S1, S2, thalamus, basal ganglia and cerebellum. In general, itch stimulation entailed more activation clusters, in particular on the contralateral brain side. Only upon itch, but not upon heat pain negative BOLD signals were found in the subgenual ACC and the amygdala. The latter results may be associated with the itch induced urge to scratch. Amygdala deactivation may be related to the preparation of scratching by aiming to dissolve the otherwise aversive effects of the noxious scratch stimuli. These negative BOLD effects may also be attributed to the stressful character of itch stimulation.

Key words: fMRI, pruritus, cerebral itch network, limbic forebrain structures, cerebellum
Introduction

Pain and itch have many characteristics in common, but there are some important differences (Drzezga et al. 2001). Both stimuli induce distinct motor reactions. Part of the definition of itch is the urge to scratch and that it can be eased by scratching (Rothman 1941). In contrast, pain is followed by withdrawal reactions or a general increase in muscular tension. Admittedly, some forms of pain may also evoke the urge to shake or touch the painful site. However, this is rarely the case upon acute heat pain. In this study, we investigated similarities and differences in the cerebral processing of itch and heat pain using functional MRI.

Previous functional imaging studies on itch were hampered by the difficulty to control, i.e. to restrict the duration of the experimental itch sensations. Itch can be provoked in healthy subjects using histamine application to the skin either via iontophoresis or via skin prick. This typically leads to waxing and waning itch perceptions over up to 15 minutes and thus precludes stimulus repetition in one experiment. Here we present a novel stimulus method which allowed us to reliably evoke and to discontinue itch perceptions of variable intensities several times within one fMRI scanning period. This allowed testing the co-variability with changes of the BOLD effect in a group study. To provoke itch we perfused intracutaneous microdialysis membranes with a mixture of histamine and codeine. The itch sensation was terminated by perfusing the same membrane with lidocaine. Since the lidocaine perfusion itself did not induce any specific sensations, this resulted in pure modulation of itch. In a subsequent experiment the same subjects received a series of heat pain stimuli to compare the central processing of itch and heat pain.
2. Material and Methods

2.1. Subjects

We performed two experiments on healthy young volunteers. Subjects with a history of allergy, atopic eczema and other current dermatological and non-dermatological conditions were excluded from the study. We used the criteria of Diepgen to identify atopic eczema (Diepgen et al. 1989), except for IgE measurement, which we did not perform. The volunteers had given written informed consent to participate in the study and were free to withdraw from the experiments at any time. They received financial compensation for the time spent in the experiment. The study was approved by the local ethics committee.

In a first psychophysical session, a combination of histamine and codeine was applied through microdialysis fibers in 25 healthy volunteers. Subjects who reported only a weak sensation of itch and subjects who felt a very strong and prolonged itching sensation (i.e. outlasting the 5min between two stimulations; see 2.2), were excluded from the fMRI study.

Ten subjects (8 female, 2 male) fulfilled these criteria and were recruited for the fMRI-experiment on itch. Their mean age was 23 ± 0.6 yrs, they were all right-handed and had normal brain morphologies verified by magnetic resonance imaging. Eight of them participated also in the heat pain stimulation experiment (see below).

2.2. Psychophysical experiment

*Microdialysis stimulation technique:* Intracutaneous microdialysis was performed as described in Schmelz et al. (1997a). Using a 25-gauge canula, four microdialysis fibers (0.4 mm diameter, cut-off 3000 kDa, Asahi Plasmaflow, Japan) were inserted intracutaneously over a length of 1 cm into the skin of the left volar forearm in transversal orientation to the axis of the arm. The distance between the fibers was about 2.5 cm (see fig.1). No local anesthesia was performed, but the skin was cooled over five minutes with an ice bag before needle insertion. After insertion, the skin was rewarmed to above 30°C using bags filled with warm water. The dialysis fibers were continuously perfused with Ringer’s solution (Ringer Injektionslösung, B. Braun, Germany) at a constant flow rate of 4 µL/min applied with a microdialysis pump.
Herde et al: fMRI study on itch induced by a novel method (Pump 22, Harvard Apparatus, U.S.A.) using Tygon tubes (Novodirect, Germany). The dialysate was collected in polyethylene cups, but was not further assayed in these experiments.

After a period of 30 minutes, when all irritant sensations from the insertion and the local flare response had subsided, the perfusion was stopped and the stimulations were performed. In random sequence and at intervals of five minutes, stimulation was applied through the four fibers (see also figure 2a) using each fiber only once. To provoke itch, a combination of histamine $10^{-4}$ M (Sigma, Germany) and codeine 0.1% (Fagron, Germany) was used. Codeine releases endogenous histamine from mast cells. The concentrations of mediators used were based on previous reports (Steinhoff et al. 2003).

The four fibers were used for four stimulation protocols on after the other in a random order. Each of these stimulation protocols lasted 5 minutes during which the following procedures were performed (see also figure 2a):

- 60 s stimulation period during which the histamine-codeine solution was perfused through the fiber. During one of these four stimulation periods (randomly selected) Ringers solution was used and served as placebo control. During this period 200 µl solution were manually passed through the fiber with a syringe, leading to filtration of the substances into the surrounding tissue.

- 30 s change of the syringe and washout period: The second syringe contained 2% lidocaine-HCl solution (B. Braun, Germany). The remaining volume of the histamine-codeine solution was flashed which was in the tube connecting the syringe and the microdialysis fiber.

- 120 s lidocaine application: At the beginning of this period the lidocaine solution reached the microdialysis fiber and 400 µl of the solution were infused with the same flow rate as before the histamine/codeine solution to stop the itch sensation.

- 90 s baseline: Perfusion was discontinued.

The subjects were able to notice the changing of the syringes but the sight of their arms was occluded and they were unaware of the content of the syringes, the effective start and duration of the substance application.

**Rating procedure:** During the itch experiments the volunteers had to track, i.e. quantify, their itching sensations on an electronic Visual Analogue Scale (VAS) which
they manipulated by turning a rotary switch. The maximum rotation of the switch was 270° which resulted in a maximal deflection on the VAS. The VAS represented a range from 0 (no itch sensation) to 100% (unbearable itching). The scale had only one mark at 30% of its total length. The subjects were instructed that ratings beyond this point should correspond to an itch intensity increasing the urge to scratch to a level which would have been eased in daily life. This kind of scale has been used by our group before, but also by other groups (see Leknes et al. 2006, Valet et al. 2007). The control pain experiments (see below) were performed only during the MRT and not in a psycho-physical training session. A similar rating procedure was employed for itch and for heat pain. In the pain experiments 100% scale deflection indicated "unbearable pain". The 30% marking was not used in the pain experiments.

2.3. fMRI experiment

**Experimental procedure**: The microdialysis fibers for the fMRI experiment were inserted 30 minutes prior to the onset of the experiment.

Five minutes before the beginning of the fMRI session, the individual heat pain sensitivity of the respective subject was assessed. Heat pain was produced using a Peltier thermode (Medoc, Israel) fixed to the right volar forearm. The contact area of the thermode was 2.5 x 2.5 cm. Stimuli started from a temperature of 32°C rising at a rate of 1°C/s until the subjects indicated burning pain at an intensity of 60% on the VAS scale. The procedure was repeated four times at an interval of 20s on the same skin area. The mean peak temperature of the four measurements in each individual was used as the stimulation temperature in the fMRI experiment.

The first part of the fMRI study consisted in the itch stimulation experiment. The microdialysis technique was used on the left forearm as described above. Rating movements were performed with the thumb and index of the right hand.

In the second part of the experiment, heat pain was applied to the right forearm. In this experiment, baselines and pain stimuli alternated every 28s. The experiment consisted of four painful stimuli and finished with a baseline period of 30s (see Fig.2c). Pain ratings were performed just as the itch ratings in the experiment before, albeit with the left hand.
Data acquisition: MR imaging was done with a 1.5 Tesla Sonata MRI scanner (Siemens, Erlangen, Germany). The volunteers’ ears were plugged and their heads were fixed with rubber pads in the scanner. A mirror was adjusted above the eyes to allow the subject to see the visual analogue scale positioned outside of the scanner. Functional T2 weighted images were obtained using an EPI (echo planar imaging) technique that consisted of 30 axial slices (TR = 5000 ms for the itch experiment and TR = 4000 ms for the heat pain experiment respectively, TE = 60 ms, flip angle = 90°, slice thickness 4.0 mm, Distance factor = 0). The whole cerebral cortex and two-thirds of the cerebellum were included. The lower limit of the analysis was at the level of the pons. Four dummy volumes were acquired before each sequence to reduce possible effects of T1 saturation. Movements of the subjects were compensated by the online motion correction option which is included in the acquisition software (Thesen et al. 2000).

The session started with the itch experiment followed by the heat pain experiment and a final MPRAGE sequence (magnetization prepared rapid gradient echo) consisting of 160 sagittal slices of 1 mm thickness and in-plane resolution of 265 x 265 pixels (field of view: 220 x 220 mm²).

2.4. Data analysis and statistics

Analysis of the fMRI data were performed using BrainVoyager® QX, Version 1.6. The functional data were pre-processed including motion correction, temporal Gaussian smoothing of 4 sec, and spatial Gaussian smoothing of 4 mm. The individual brains were transformed into a standard stereotactic space (Talairach and Tournoux 1988) and the functional data were co-registered with the 3D MPRAGE data set. The resulting transformations were used to overlay the activation maps into a 3D data set. For group analysis all functional data were transformed into Talairach space. For each participant and experiment, general linear models (GLMs) were created. Model descriptors were defined which described the periods of application of histamine, of placebo, of lidocaine or of the heat pain stimulus, respectively. The individual rating was used as a predictor to test for brain areas showing significant correlations to the itch or pain ratings, respectively. As additional predictor for the GLM we used the time course of the rating movements obtained from the output signal of the potentiometer controlling the VAS. This predictor was introduced to
distract variance from those BOLD signals that are due to motor activity in response to the rating procedure. The resulting β-values were used to estimate the magnitude of correlation between the predictor and the BOLD response. Clusters were regarded as activated when the predictor “rating” was significant. Clusters related to the model predictors or to the predictor “rating movement” were not further considered. To avoid false positive activations due to type 1 errors (Downar et al. 2002), only activation clusters of a minimum size of 50 mm³ were included. An uncorrected threshold of $p<0.000001$ was referred to as significant. Resulting brain areas were identified by comparing their location with a printed atlas (Kretschmann and Weinrich 1992) and Talairach coordinates (http://ric.uthscsa.edu).

To test for brain activations which could be induced by the application of lidocaine an additional contrast analysis was performed which compared the BOLD signals during the periods of the placebo applications with those of the succeeding lidocaine applications.

### 3. Results

#### 3.1. Psychophysics

All subjects showed an initial flare reaction following the insertion of the microdialysis fibers. After 30 minutes, when the flare had vanished and interfering sensations were at a minimum, the experiment was started. In all subjects injection of the histamine-codeine-mixture lead to a flare reaction of 2–5 cm diameter and a wheal in the skin covering the top of the dialysis fiber (see fig.1). The restricted weal size substantiates that the histamine/codeine mixture exerted its effect only in the immediate surroundings of the microdialysis fiber. Certainly it did not reach the other fiber locations were it could have interfered at the nerve terminals with subsequent stimuli. Neither weal nor flare were observed after placebo control and lidocaine.

The onset of sensory responses followed the start of histamine application with a variable delay of 20-60 s. The quality of these sensory responses was always described as itch, sometimes with a minor burning or stinging component as described in other studies on histamine induced itch (Magerl and Handwerker 1988). All subjects regarded this sensation as unpleasant. The maximal rating amounted to
40 - 90% of the rating scale. The itch sensations declined within 10–120 s after the beginning of lidocaine perfusion. The second and third itch stimulus generally provoked a higher rating than the first one. Average ratings during the histamine periods were higher than 50% of the rating scale and well above the threshold for the "urge to scratch" (30%, see methods). Itch ratings dropped below 5% of the rating scale during the perfusion of the fiber with lidocaine, which itself did not induce any specific sensation. In particular, no feeling of numbness was perceived due to the small amount of lidocaine reaching the tissue and the restricted area of its action. Only two subjects rated the placebo control test as itchy, but they rated clearly below the "urge to scratch" threshold. During the placebo control trial the subjects were not able to distinguish the lidocaine from the preceding saline perfusion.

In the following experiment heat pain was applied by a Peltier thermode. Since pain interferes with itch no balanced cross-over design could be applied in this study. The aim of the pain experiment was only to investigate if intense heat pain stimuli activate the same or different brain areas. No comparison of the magnitudes of the BOLD effects, or the extensions of the activation clusters was intended.

In the heat pain experiments mean stimulation temperature was 46.8 ± 0.5°C. All subjects regarded the stimulus as painful. The mean pain rating at the end of each stimulus was 86.4 ± 1.6% of the rating scale, clearly higher than the average ratings on the pruritus scale during the itch experiments. However, the time course of the pain stimuli did not match the long-lasting itch sensations - which would have been difficult to be mimicked by heating without risking skin damage. The rating performance was also different for the itch and the pain experiment (see fig.2). Whilst the average duration of itch was 3 minutes in a stimulus interval, pain stimuli lasted only 28 s (see methods). In contrast to the feeling of itch the onset and the decline of the heat-induced pain was perceived immediately and hence the ratings showed less variability.

3.2. Cortical and subcortical activations

The detailed results are shown in table 1 and 2.
**Itch experiment:** The right hemisphere, contralateral to the itch stimulus, was activated more intensely. The group study revealed 31 activated clusters, in contrast to 21 in the left hemisphere.

Bilateral activations were encountered in prefrontal areas, but they were larger on the contralateral side (BA44 in both, BA46 in the left and BA 9 and 10 in the right hemisphere). Bilateral activations were also found in the premotor cortex and in supplementary motor areas, in the anterior insula and in the anterior midcingulate cortex (aMCC). As for the cingulate cortex, we refer to the classification suggested by Vogt (2005). In addition, the secondary somatosensory cortex and the inferior parietal lobe showed bilateral activations. Several visual regions in the occipital gyrus showed a positive BOLD effect. Among subcortical structures, the posterior part of the thalamus was activated bilaterally. Large areas in the upper 2/3 of the cerebellum were activated, including more posterior than anterior parts and both lateral and medial regions. The lower third of the cerebellum was not included in our study. Activations limited to the right contralateral brain side were found in the primary sensory cortex, but also in temporal areas BA 41 and 42 and in the precuneus. Also some subcortical structures showed a positive BOLD signal only contralaterally: the pulvinar and the ventral lateral nucleus of the thalamus, the claustrum and the caudate nucleus.

Ipsilateral activations were observed in the medial dorsal nucleus of the thalamus. The amygdala and part of the subgenual anterior cingulate cortex showed a bilateral decrease of the BOLD signal during itch stimulation (see fig.3 and 4).

The additional contrast analysis to test for the effects of the lidocaine application showed no significant results.

**Pain experiment:** This experiment resulted in less clusters of activation than itch stimulation. In the contralateral hemisphere, i.e. on the left side, we found 12 activation clusters, compared to 15 clusters in the ipsilateral hemisphere.

In the cortex, bilateral activations were found in prefrontal areas, supplementary motor areas and the midcingulate cortex. Also the anterior insula showed bilateral activations, but more pronounced on the contralateral (left) side. The primary sensory cortex showed a positive BOLD signal bilaterally, but it was stronger accentuated in the right hemisphere, ipsilateral to the stimulus. Bilateral activations were also found in occipital and parietal areas. Among the subcortical regions, the putamen and
medial, anterior parts of the cerebellum showed a bilateral positive BOLD effect. In contrast, the precuneus, the claustrum, the dorsal part of the thalamus and its ventral lateral nucleus showed only contralateral activations. Activation ipsilateral to the stimulus (but contralateral to the rating hand, see methods) was found in the primary motor and premotor cortex. The caudate and the globus pallidus were also activated exclusively in the right hemisphere, ipsilateral to the stimulus.

There were no negative BOLD signals found in the pain experiment.

**Comparison between itch and pain activations:** Figure 3 compares the activations during the itch experiments with selected slices obtained from the pain experiment (group study). The complete data set on all activations is documented in the tables 1 and 2. The main findings are:

- The **midcingulate cortex** showed a positive BOLD signal in both experiments. This activation was located more frontally in the itch experiment.
- In response to itch, the **anterior insula** was intensely activated on both sides, whereas during pain the contralateral insula showed a more pronounced activation than the ipsilateral (right) insula.
- In both experiments, **visual areas** such as the middle occipital gyrus and the fusiform gyrus showed positive BOLD effects, possibly reflecting the visuo-motor task of pain and itch rating.
- Also **thalamic** activation was bilateral and more widespread in the itch experiment, but only contralateral in the pain experiment.
- The **cerebellum** was activated by pain and by itch stimulation, but the cerebellar activation was more widespread during itch. Whereas 8 activation clusters were found in the itch experiment, only 3 were encountered in the pain experiment. Furthermore, pain activations were restricted to the medial parts (vermis) upon heat pain, whereas itch activations extended over medial parts and the hemispheres.
- **Negative BOLD effects:** Only in response to itch negative BOLD effects in parts of the limbic system were observed (see fig.3 and 4).
4. Discussion

4.1 Previous PET and fMRI studies on itch

Studying brain activity related to itch sensations poses major problems as compared to pain: the correlation between stimulus and provoked sensation is much less uniform than the correlation between heating and ensuing pain perception. In previous studies histamine iontophoresis or skin pricks were used to induce itch (see table 3). Both typically lead to itch perceptions that tend to increase and decline at irregular intervals in the range of seconds of up to 15 minutes. In a recent psychophysical study changes of the skin temperature were employed to modulate histamine induced itch at 20 second intervals. This procedure allows a modulation of the itch perception by about 20% of the rating scale (Pfab et al., 2006). Subsequently this method was employed to study cerebral responses in an fMRI experiment (Valet et al., 2007). The aim of this approach was similar to the rationale behind our strategy, to modulate itch sensations in a regular manner. Some of the itch activations found by Valet et al. were similar to our results, but there are also some differences, e.g. Valet et al. found a deactivation in the MCC (see table 3). The differences may be related to the circumstance that decrease of itch coincided with temperature perceptions in the experiment of Valet et al.

In the present study, a stimulus method was employed that achieved strong repeatable pruritus during three stimulus periods and no or little itch sensations during placebo application (see fig.2b). Generally, in most of our subjects the ensuing perception of itch was stronger compared to other studies (Hsieh et al. 1994, Drzezga et al. 2001), inciting ratings of up to 70% of the itch VAS and hence well above the threshold for the urge to scratch.

To account for the variability of stimulus provoked itch sensations, functional brain imaging studies should be performed "percept related" (Davis et al. 2002) with a time resolution in the range of seconds. Early PET studies had a low time resolution. Therefore a less precise relationship between itching and activation of different brain regions could be achieved (Hsieh et al. 1994; Darsow et al. 2000; Drzezga et al. 2001; Mochizuki et al. 2003). Recent fMRI studies with high time resolution only performed itch ratings at 60 or 30s intervals (Walter et al. 2005; Leknes et al. 2006).
But the interpolated time course of the ratings probably does not represent well to the waxing and waning character of the itch sensations.

### 4.2. Cerebral representation of itch

In spite of the technical differences many of the itch provoked activations found in the present study were also reported previously, most frequently in the contralateral prefrontal cortex and in the mid-cingulate area. Table 3 shows the techniques and main results of previous itch studies in comparison to the present study. The activations found in our itch experiment were more multi-focal than in previous studies, probably due to the induction of a strong and widely modulated itch signal. In agreement with previous itch and pain studies prefrontal areas were strongly activated. With the exception of the medial frontal gyrus these activations were contralateral to the itch stimulus (table 1). The dorso-lateral prefrontal cortex (DLPC) is presumed to play a role in cognitive control and to participate in cortico-subcortical control loops (Koechlin et al. 2003; Lorenz et al. 2003). In states of hyperalgesia, these areas were more strongly activated compared to control pain stimuli (Maihofner and Handwerker 2005; Maihofner et al. 2004).

Interestingly, most studies including ours did not find activation in the posterior insula. Only the study of Drzezga et al. (2001) had found activation in this area, correlating with the histamine concentration and with the unpleasantness of the itching sensation. The lack of activation in the other studies - including the newest by Valet et al. - is remarkable, since this region has been regarded as the "interoceptive" cortex (Craig 2003). Furthermore, it has been shown that histamine sensitive "itch fibers" in the cat project to lamina 1 of the spinal cord (Andrew and Craig 2001), the area that provides the major input to the posterior insula via the thalamus (Craig et al. 2000). However, the anterior insula receives its "interoceptive" input mainly via the posterior insula and was bilaterally and strongly activated in our itch experiment. Therefore, the lack of a reliable graded BOLD activation in the posterior insula must have intrinsic or methodological reasons which still have to be explored.

Among the subcortical regions activated by our itch stimulation, bilateral clusters in the medio-dorsal thalamus were previously described (Leknes et al. 2006). In our experiment the thalamic activation also comprised the contralateral ventro-lateral nucleus. The latter activation may be related to the urge to scratch. Bilateral
activations in the caudate nucleus were also found in a previous study (Leknes et al. 2006).

Interestingly, only the first publication about itch using PET (Hsieh et al. 1994) also mentions bilateral itch-induced activations in the cerebellum. In the present experiment we found a strong activation of medial and lateral parts of the cerebellum. The activation of the cerebellar hemispheres may be related to the motor coordination of the fingers that normally execute the scratching. Interestingly, cerebellar activation during heat pain was restricted to the vermis (see below).

4.3. How is activation related to the rating task?

In general, areas related to motor activity were strongly activated in the itch experiment. This could be either due to the rating task which entailed moving a switch, or due to the “urge to scratch”. Like previous investigators who did not perform ratings during the fMRI scans, we found activations in the SMA and the premotor cortex (see table 3). Positive BOLD signals were also found in the right inferior parietal lobe which effectuates motor movements under sensory guidance (Hsieh et al. 1994). In addition, there was activation in the contralateral precuneus, which is known to coordinate motor behavior (Cavanna et al. 2006). The BOLD signal in the claustrum may be related to the role of this structure in synchronizing motor, perceptual and cognitive modalities (Crick and Koch 2005).

In the present experiment the subjects would have used their right, contralateral hand to ease the desire to scratch. Therefore we expected activations accentuated ipsilaterally to the itch stimulus. Similarly, the left brain side was expected to be recruited in response to manipulations of the rating switch with the right hand. Both types of motor activation cannot easily be distinguished. Remarkably, the above-mentioned areas related to the planning or execution of movements were mostly activated bilaterally. Surprisingly, the premotor cortex and part of the parietal lobe were activated contralaterally to the itch stimulus, but ipsilaterally to the moving hand (see table 1). Together with the lack of activation of the S1 cortex contralateral to the moving hand this indicates a minor influence of the rating movements on the BOLD activations in this experiment. Probably in the itch experiment the motor areas are mainly activated in relation to the desire to scratch. In contrast, during the pain experiment we observed premotor activations ipsilateral to the stimulated side, but
contralateral to the hand manipulating the rating switch. Rating-related motor effects are likely to be greater in the pain experiment compared to the itch experiment, since ratings reached high values very fast and this requires broader movements (see fig.2). In contrast itch ratings climbed and decreased slowly. Accordingly, we did not see this kind of activation in the mentioned motor regions.

4.4. Comparison of cerebral activations during heat pain and during itch

In the periphery both types of stimuli, pain and itch, recruit a similar, but distinct spectrum of afferent nerve fibers. Heating stimuli activate Aδ-fibers and different types of C-fibers, while histamine application stimulates a subgroup of mechano-insensitive C-fibers (Schmelz et al. 1997b). The central pathways for both types of input are again similar, but the itch pathway seems to consist of a distinct subgroup of dorsal horn neurons with a specific projection to the thalamus (Andrew and Craig 2001). The central structures activated by pain stimuli are well known and reproduced in numerous fMRI studies (for reviews see Peyron et al. 2000; Brooks et al. 2001, Apkarian et al. 2005). Our study reproduced the expected findings, particularly the bilateral increases in the BOLD signal in the primary and secondary sensory cortex, the insular cortex, the MCC, the contralateral thalamus and also in motor-related areas.

Generally, the brain network involved in the processing of cutaneous pain and itch seems to be similar (see fig. 3), as previously discussed (Stander and Schmelz 2006; Ikoma et al. 2006). However, the itching stimuli induced more widespread activation in particular in the cerebellum and in prefrontal areas.

In both experiments the cingulate cortex was activated in the aMCC region. The pain induced clusters were slightly posterior to the itch induced activation. The cingulate cortex is known to be involved in emotion, cognition and motor processing (Peyron et al. 2000). Numerous fMRI studies have proved the activation of the MCC during pain processing (Peyron et al. 2000). Activations in the MCC or in the pACC cortex were also found in previous itch studies (Mochizuki et al. 2003; Hsieh et al. 1994; Drzezga et al. 2001; Darsow et al. 2000). The ACC plays a role in all reports of noxious or non-noxious visceral stimulation, cooling and warming (Hua et al. 2005; Egan et al. 2005). Our study confirmed these results.
We have found cerebellar activations in the itch and in the pain experiment. The latter were largely restricted to the medial parts. These bilateral activations in the pain experiment could be the expression of an increased general muscular tension and suppressed withdrawal reflexes. In the itch experiment activation was also found in the cerebellar hemispheres and this again could be related to the urge to scratch which is a synchronized arm - finger movement (Hsieh et al. 1994).

4.5. Negative BOLD effects

A novel finding of our study was the bilateral negative BOLD signal in the most fronto-caudal part of the cingulate cortex, the subgenual ACC, and in the amygdala (see fig. 4), which was present exclusively in the itch experiment. So far, the sACC has been considered as being activated in the context of negatively valenced affect and sad emotions (Vogt 2005). Interestingly, one study found a context-dependent decrease of the activation of the amygdala when the subjects expected a longer lasting unpleasant pain (Petrovic et al. 2004). In our experiment most subjects showed this very distinct itch related BOLD decrease in the sACC and amygdala (see table 1). We did not see a similar reaction in the pain experiment, though the applied pain stimuli were in the upper range of tolerability (see fig. 2). For this reason, we assume that these BOLD decreases are itch-specific. In this context we tentatively interpret these strictly localized deactivations in relation to the itch induced urge to scratch. Down regulation of sACC and amygdala activity may be related to the preparation for scratching by dissolving the otherwise aversive effects of the noxious scratch stimuli. An alternative hypothesis may be derived from the known role of the amygdala in fear and anxiety. Increased activity has been observed during the viewing of fear-evoking images (Ogino et al. 2006; Whalen et al. 1998; Morris et al. 1996) and it was suggested to be generally related to negative emotions and stress (Davidson 2002). From other experiments a general limbic deactivation in an aversive situation was suggested (Ingvar 1999; Simpson et al. 2001). In the context of pain, deactivations of this region have been observed during capsaicin elicited pain and during anticipatory anxiety (May et al. 1998; Simpson et al. 2001). The latter has been interpreted as a coping mechanism of fear control, as it was found to be more pronounced in subjects that experienced less anxiety. On this background, an alternative explanation of our experiments may be that itch stimuli were more
stressful for the subjects than the strong pain stimuli due to their short and predictable time course. The greater unpredictability of the time course and magnitude of itch sensations might have contributed to this effect. Future experiments will need to discriminate between these two hypotheses.

4.6. Conclusions

The aim of this study was to characterize the cerebral network activated in response to a newly designed itch paradigm. For comparison we performed a second experiment in the same fMRI session in which strong heat pain stimuli were applied to the contralateral forearm. Our study confirmed the conclusion of previous imaging studies that the cerebral networks for the processing of cutaneous pain and itch are similar. However, there are clear differences: there are more distinct activation clusters in the cerebellum and they are found more in the lateral part of the cerebellar hemispheres during itch processing. This may be related to the preparation of scratching. Likewise, the activation of forebrain structures related to motor control is more pronounced and bilateral for itch, whereas the activation of those structures in the pain experiment can mostly be attributed to the confounding effects of the rating movements. Bilateral activations of the anterior insula, the aMCC and the prefrontal cortex were more wide-spread during itch. A striking difference were the negative BOLD effects in the subgenual anterior cingulum and the amygdala induced by the itch stimulus, which may be related to preparing scratch reactions for making them relieving instead of painful. These deactivations may also be a reflection of the stressfulness of the itching stimulation.

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References


Legends:

**Table 1: Brain areas with significant predictor “itch rating”**.
The table comprises significantly activated clusters (p <0.000001). The column 'BA' indicates the respective Brodmann areas. The column 'N' shows the number of subjects in whom significant activation was found. From the group analysis t-values and Talairach coordinates are given. The latter depict the center of gravity of the respective cluster. Negative t-values indicate a negative correlation between the predictor and the BOLD signal.

**Table 2: Brain areas with significant predictor “pain rating”**.
The organization of the table is as in table 1. All clusters represent significant activations in the group study (p<0.000001).

**Table 3: Comparison of the findings in the present study with previous itch studies.**
This table shows the similarities and differences found in previous itch studies as compared to the present study. Leknes et al. tested two groups of subjects: for the induction of pruritus the first group received histamine, the second group received allergen.

+ activation found
↓ deactivation found
- no activation found or not analysed

For methodological differences between the different studies, see text.
Figure 1. Histamine and codeine application through microdialysis fibers.
The photograph shows a volunteer’s forearm shortly after the itch experiment. Four microdialysis fibers are inserted intracutaneously. The flare reactions due to the histamine application are encircled to highlight the effect. Wheals are visible on top of those fibers that were perfused with histamine. The second fiber (from proximal to distal) served as placebo control. This site shows almost no flare or wheal.

Figure 2. Stimulation protocol and ratings.
a) Itch stimulation protocol. White = baseline, respectively changing of syringes and injection of the residual volume; black = histamine (His) or saline (Plac) application; grey = lidocaine (Lido) application.
b) Time courses of the mean itch rating during histamine/codeine or saline (placebo) stimulation (thick line) and standard error of the mean (thin lines SEM). In the experiment, the position of the placebo stimulus was randomized. The bar above represents the underlying protocol of fig. 2a).
c) Mean rating courses during pain stimulation (thick line) with a thermode and SEM (thin lines). The bar on top of the diagram shows the pain stimulation protocol; black square = heat stimulus application.
Figure 3. Selected axial brain slices comparing the BOLD effects related to itch (upper panel) and to pain (lower panel).

a) Upper panel: activated brain areas during itch stimulation are shown in red and yellow, deactivated areas in blue; b) Lower panel: activated brain areas during pain stimulation are shown in green and yellow.

IPL = inferior parietal lobe, MCC = midcingulate cortex, IPL = inferior parietal lobe, PrG = precentral gyrus, Th = thalamus, In = insula, sACC = subgenual anterior cingulate cortex, Am = amygdala, Ce = cerebellum, Pu = putamen, Pa = pallidum, MOG = middle occipital gyrus, CG = cingulate gyrus.

Figure 4. Signal analysis in the amygdala and the sACC during itch stimulation.

Activation maps of a) the amygdalae and b) the subgenual anterior cingulate cortex (sACC). The maps illustrate the results from the group analysis using the predictor “itch rating”. Note that in both slices the blue-green color coding depicts a negative correlation between itch rating and BOLD signal. There is a bilateral deactivation of the amygdala and the sACC during the perception of itch.

Panels c) and d) show in red the time courses of the BOLD signals of two individual subjects derived from the encircled brain areas shown in a) and b). The black lines depict the concomitant itch ratings. Note that placebo stimulation in c) followed in the second position while in the experiment of d) it was the first stimulus.
### Table 1: Itch experiment

<table>
<thead>
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<th>Region</th>
<th>BA</th>
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<th>Right (contralateral)</th>
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<td></td>
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<td>t-value</td>
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<tr>
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<td>X  Y  Z</td>
<td>X  Y  Z</td>
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<td>I. Positive BOLD effects</td>
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Table 1. Brain areas with significant predictor “itch rating”.

The table comprises significantly activated clusters (p < 0.000001). The column ‘BA’ indicates the respective Brodmann areas. The column ‘N’ shows the number of subjects in whom significant activation was found. From the group analysis t-values and Talairach coordinates are given. The latter depict the center of gravity of the respective cluster. Negative t-values indicate a negative correlation between the predictor and the BOLD signal.
### Table 2: Brain areas with significant predictor “pain rating”.

The organization of the table is as in table 1. All clusters represent significant activations in the group study (p<0.000001).
Table 3: Comparison of the findings in the present study with previous itch studies.

This table shows the similarities and differences found in previous itch studies as compared to the present study. Leknes et al. tested two groups of subjects: for the induction of pruritus the first group received histamine, the second group received allergen.

+ activation found, ↓ deactivation found, - no activation found or not analysed.

For methodological differences between the different studies, see text.
Herde et al.: fMRI study on itch induced by a novel method
Fig 2

Herde et al.: fMRI study on itch induced by a novel method
Herde et al.: fMRI study on itch induced by a novel method

Fig 3