Cerebral Processing of Histamine-Induced Itch Using Short-Term Alternating Temperature Modulation – An fMRI Study

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Human neuroimaging studies on the physiology of itch have been hampered by the lack of reproducible "on–off" stimuli. Using a previously established biphasic temperature stimulus model, we investigated the cerebral activation pattern of itch processing in 12 healthy volunteers with functional magnetic resonance imaging. Itch was provoked on the right forearm with skin prick application of 1% histamine-dihydrochloride. Local temperature modulation allowed reproducible itch provocation above scratch threshold (defined as 33/100 on a visual analogue scale) during 25°C, whereas itch declined below scratch threshold during the 32°C stimulation period. No itch sensation was reported using 0.9% saline with temperature modulation. Itch sensation above scratch threshold was associated with increased activation of the thalamus, presupplementary motor area, anterior insular, inferior parietal, and dorsolateral prefrontal cortex, and decreased activation of the orbitofrontal, medial frontal, mid-cingulate, and primary motor cortex in comparison to saline. The biphasic temperature model allows rapid modulation of histamine-induced itch. The evoked itch sensation above scratch threshold is processed by a network of brain regions contributing to the encoding of sensory, emotional, attention-dependent, cognitive-evaluative and motivational aspects of itch.

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INTRODUCTION

The sensation of itch is defined as an ''unpleasant sensation that provokes the desire to scratch'' (Hafenreffer, 1660), and itch is the most prevalent symptom of allergic and inflammatory skin diseases (Behrendt *et al.*, 2001; Charlesworth and Beltrani, 2002; Ring *et al.*, 2005; Sicherer and Leung, 2006). Itch-specific C-receptors in the human skin have been identified and itch is thought to be mediated by histamine sensitive and histamine-independent C-fibers (Schmelz *et al.*, 1997, 2003). There is evidence, that despite psychophysiological similarities to pain, itch and pain pathways seem to be distinct (Schmelz *et al.*, 1997, 2003; Andrew and Craig, 2001; Drzezga *et al.*, 2001; Mochizuki *et al.*, 2003; Ikoma *et al.*, 2006).

There are only few neuroimaging studies investigating the patho-/physiological cerebral mechanisms of itch processing (Hsieh *et al.*, 1994; Darsow *et al.*, 2000; Drzezga *et al.*, 2001; Mochizuki *et al.*, 2003; Walter *et al.*, 2005; Leknes *et al.*, 2007). In these studies, the anterior cingulate cortex, pre-/supplementary motor area, prefrontal, and inferior parietal cortex have been found to play an important role in the processing of itch.

From a general point of view, it is highly desirable for functional magnetic resonance imaging (fMRI) to work with on – off stimuli, that is, in terms of itch to be able to produce and reduce itch rapidly. However, experimental itch can usually not be easily turned on and off within a short time and the experimental investigation of itch is therefore a methodological challenge. Previous fMRI studies have approached this problem by using correlation analyses of subjective itch ratings with the fMRI Blood Oxygenation Level Dependent signal (Walter *et al.*, 2005; Leknes *et al.*, 2007). However, continuously obtaining itch ratings during fMRI scanning might induce motor and cognitive interactions confounding itch related imaging results.

In a previous study, we were able to evidence that short-term low-intensity temperature stimulation allows the rapid modulation of histamine-induced itch. This paradigm seemed to be promising for the application in an fMRI setting (Pfab *et al.*, 2006). Here, we present the first fMRI study

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Abbreviations: dACC, dorsal part of the anterior cingulate cortex fMRI, functional magnetic resonance imaging; M1, primary motor cortex; MRI, magnetic resonance imaging; pre-SMA, presupplementary motor area; VAS, visual analogue scale

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using this previously validated biphasic stimulus model (Figure 1).

RESULTS

Visual analogue scale (VAS) ratings for itch intensity

Median baseline itch ratings on a VAS (range 0–100) obtained 120 seconds after application of histamine and directly before fMRI scanning were 40.0 and 0.0 for saline. During fMRI scanning, the median itch intensity ratings were 20.0 during the 32°C stimulation periods and 42.5 during the 25°C stimulation periods ($P<0.001$, paired Wilcoxon test; Figure 2). Using saline, the subjects reported itch ratings of 0.0 during the 32°C, as well as during the 25°C stimulation periods. Sensation of pain was not reported by any of the subjects.

Cerebral itch processing

When analyzing the effects of increased itch during 25°C stimulation (first 8 seconds) of histamine-treated skin *versus* 32°C baseline, significant brain activation was observed in the presupplementary motor area (pre-SMA), the anterior insular cortex, and the inferior parietal cortex ($P<0.001$; Table 1, no figure). The caudal part of the SMA, medial frontal cortex, orbitofrontal cortex, primary motor cortex (M1), and primary somatosensory cortex (s1) were less activated during the 25°C stimulation as compared to the 32°C baseline ($P<0.001$, Table 1, no figure). The 25°C stimulation of saline-treated skin *versus* 32°C baseline was not associated with any significant increase or decrease in brain activation ($P<0.001$).

The calculation of itch-specific activation maps for the first 4, 8, 12, 16, and 20 seconds of the 25°C stimulation period confirmed that the behavioral changes during the first 8 seconds are reflected by the higher brain activations during this time period than during the other time periods. Focusing on the first 8 seconds of 25°C stimulation, the thalamus, pre-SMA, lateral prefrontal cortex, anterior insular cortex, and inferior parietal cortex were more active than during the saline condition ($P<0.001$; Table 1; Figure 3). The medial frontal cortex, the orbitofrontal cortex, the dorsal part of the anterior cingulate cortex (dACC) and the M1 were less active during histamine-induced itch, than during saline ($P<0.001$; Table 1; Figure 3) treatment. Figure 4 shows all itch-specific activation maps demonstrating a dynamic process with increase and decrease of regional brain activation.

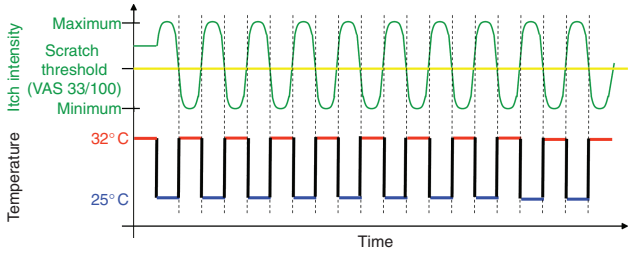

Figure 1. Schematic illustration of the itch stimulation protocol during the fMRI measurement. Lower part: temperature was changed block wise from 32°C (red) to 25°C (blue). Upper part: in green, the schematic time course of the mean itch ratings, which depends on the actual temperature, with maximum ratings during 25°C and minimum ratings during 32°C.

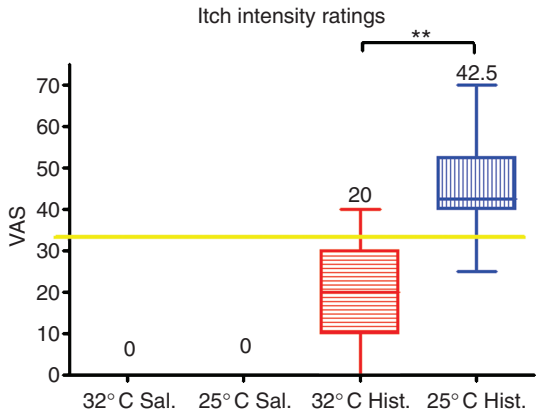

Figure 2. Itch intensity ratings. Box-and-whiskers graph of the itch intensity ratings of 12 healthy subjects during short-term temperature modulation with 25°C (blue columns) and 32°C (red columns) after applying saline or histamine dihydrochloride. The box extends from the 25th percentile to the 75th percentile, with a line at the median (50th percentile). The whiskers above and below the box show the highest and lowest-rating values. The yellow line represents the scratch threshold (33/100 VAS itch intensity). Asterisks indicate significant differences between columns. $^{**}P<0.001$ (two-tailed Wilcoxon matched pairs test). No itching was reported after applying saline.

DISCUSSION

The use of a biphasic low-intensity thermal stimulus model with application of neutral (32°C) and slightly cold (25°C) stimulation periods in alternating order allowed the rapid modulation of histamine-induced itch. Subjects consistently reported high itch ratings (above scratch threshold) during the 25°C stimulation period, in contrast to low itch ratings (below scratch threshold) during the 32°C stimulation period. These results confirmed the findings of our previous methodological study (Pfab *et al.*, 2006). The current study investigated the neuronal correlates of cerebral itch processing with fMRI. It provides the advantage of statistical power being significantly increased by multiple blocks of evoked itch. This reduces methodological problems resulting from longer scanning times (e.g. head movements, signal drifts) and cognitive or motivational interactions.

It was not the aim of our study to perform correlation analyses of itch ratings and cerebral Blood Oxygenation Level Dependent signals as similar data have been previously published by other groups (Walter *et al.*, 2005; Leknes *et al.*, 2007). Therefore we restricted the VAS ratings to one rating after each run to reduce possible rating associated artifacts.

Possible mechanisms of itch provocation by cooling from 32 to 25°C

In contrast to common knowledge, that intensive cooling and heating inhibits itch (Fruhstorfer *et al.*, 1986; Bromm *et al.*, 1995; Yosipovitch *et al.*, 2005), short-term low-intensity cooling has been shown to be associated with an increased sensation of itch above scratch threshold (Pfab *et al.*, 2006). However, a recently published study systematically

Table 1. Significant cerebral activations during the first 8 seconds of cooling (25°C)

	Histamine condition Regions more active			Histamine condition Regions less active			Histamine *versus* Saline Regions more active			Histamine *versus* Saline Regions less active		
Region		***x/y/z***	***Z*-score**		***x/y/z***	***Z*-score**		***x/y/z***	***Z*-score**		***x/y/z***	***Z*-score**
Thalamus							L	−9/−12/6	2.76 ns			
							R	9/−6/0	4.47			
Pre-SMA (BA6)	R	12/33/42	4.45				R	9/33/39	4.08			
SMA (BA6)				R	3/−18/51	3.50						
DLPFC (BA45)							L	−48/39/9	3.34			
							R	36/60/12	3.42			
Anterior insula	L	−36/18/–6	4.03				L	−45/18/−9	3.31			
	R	33/18/−9	3.67									
IPC (BA40)	L	−54/–45/54	3.40				L	−57/−48/48	4.01			
	R	45/−60/48	4.03				R	48/−69/45	4.00			
MFC (BA10)				R	3/60/12	4.04				R	3/60/12	3.45
OFC				R	3/24/−9	4.24				L	−6/45/−12	3.52
dACC										R	12/−27/45	3.45
M1 (BA4a)				L	−39/−18/57	3.46				L	−33/−21/54	3.78
S1 (BA2,3b)				L	−45/−30/54	3.56						
				R	39/−27/48	3.54						

dACC, dorsal part of the anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; IPC, inferior parietal cortex; L, left side; M1, primary motor cortex; MFC, medial frontal cortex; ns, not significant; OFC, orbitofrontal cortex; pre-/SMA, pre-/supplementary motor area; R, right side; S1, primary somatosensory cortex.
No significant activation was found for the saline condition.
Significant itch-related activation maxima for each brain region, with *x*, *y*, *z* coordinates according to the standardized brain template of the Montreal Neurological Institute (x=−78 mm left to 78 mm right; y=−112 mm caudal to 76 mm rostral; z=−50 mm bottom to 85 mm top), thresholded at $P<0.001$ uncorrected for multiple comparisons. Minimum activation size is 5 voxel (1 voxel=3 × 3 × 3 mm).

investigated the influence of different repetitively applied thermal stimuli on the modulation of experimentally induced itch and failed to modulate itch with innocuous biphasic thermal stimulation (Yosipovitch *et al.*, 2007). Methodical differences concerning the application of histamine (prick *vs* iontophoresis), thermode size, and location of application, as well as differences in the applied temperatures, are possible explanations why the biphasic modulation of itch was not successful with histamine iontophoresis (Yosipovitch *et al.*, 2007), but worked with pricking (Pfab *et al.*, 2006).

A conceivable explanation for the increase in the sensation of itch by our model is that the stimulation of A-delta fibers by cooling on a fast but low-intensity level (temperature decrease of 5°C per second from 32 to 25°C) might lead to a temporary spinal disinhibition of pruritoceptive spinal neurons, thereby enhancing pruritoceptive responses. Similar mechanisms of disinhibition have been proposed for the ''thermal grill illusion'', where a sensation of strong, often painful heat is elicited by touching interlaced innocuous heated and cooled bars (Craig and Bushnell, 1994). Here, the insular cortex, an important area for thermosensory perception, is thought to play a major role (Craig *et al.*, 2000). In our study, the simultaneous input of thermal and pruritoceptive signals might be associated with a paradoxical interpretation of the brain signaling an increase of itch sensation. However, this view is speculative, as our study design was not designed to determine whether the insular cortex is specifically involved in encoding of this phenomenon.

Cerebral processing of itch

We observed itch-induced activation in a distributed network of neural structures that subserves sensory, emotional, cognitive-executive, and motor aspects of itch processing (Figure 3; Table 1). Similar activation patterns have been reported in previous neuroimaging studies on itch processing (Hsieh *et al.*, 1994; Darsow *et al.*, 2000; Drzezga *et al.*, 2001; Mochizuki *et al.*, 2003; Walter *et al.*, 2005; Leknes *et al.*, 2007), thereby sharing similarities to the pain processing network (Peyron *et al.*, 2000; Apkarian *et al.*, 2005).

Thalamus and primary somatosensory cortex

The activation of the thalamus and primary somatosensory cortex can be attributed to sensory aspects of itch processing. The ability to locate itch plays an important role in the initiation of withdrawal behavior. These brain structures are performing important functions regarding detection, localization, discrimination, and intensity encoding of sensory stimuli (Apkarian *et al.*, 2005).

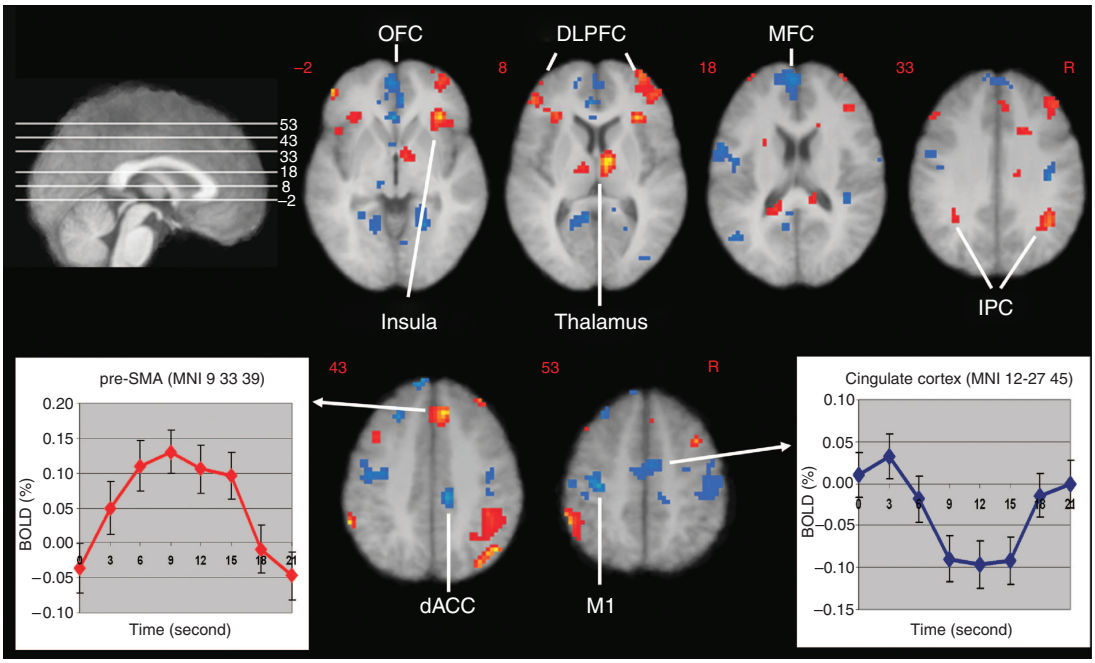

Figure 3. Cerebral activation map of histamine-induced itch. The increase of histamine-induced itch during the first 8 seconds of the 25°C stimulation periods (as compared to saline) is associated with an increase (red) and decrease (blue) of activation in various brain structures subserving sensory, emotional, cognitive, and motivational aspects of itch processing. As an example, the averaged relative fMRI Blood Oxygenation Level Dependent signal of all subjects during 20 seconds of the 25°C period is shown – for the pre-SMA region with increased and the cingulate cortex with decreased activation in comparison to saline. Abbreviations: OFC – orbitofrontal cortex, DLPFC – dorsolateral prefrontal cortex, MFC – medial frontal cortex, IPC – inferior parietal cortex, dACC – dorsal anterior cingulate cortex, M1 – primary motor cortex, R – right side of the brain.

Insular cortex

Activation of the anterior insular cortex was observed during the histamine condition *versus* baseline as well as in comparison to the saline condition. The anterior insula is assumed to subserve subjective feelings (Singer *et al.*, 2004) and to integrate sensory and emotional experiences (Gracely *et al.*, 2004). It has been suggested that the insular cortex is part of an interoceptive system providing the basis for a cortical image of homeostatic activity that reflects all aspects of the physiological condition (Craig, 2003). In this context, the activation of the insular cortex might indicate an interference on the homeostatic balance by the sensation of itch, leading to the desire to scratch (Leknes *et al.*, 2007).

Inferior parietal cortex and dorsolateral prefrontal cortex

The inferior parietal cortex is known to be involved in the spatial representation of the intra- and extrapersonal space (body scheme), and is regarded as polymodal association area integrating multisensory information from the thalamus, insula, anterior cingulate cortex, and prefrontal cortex (Freund, 2001). It is known that lesions of this region in the nondominant hemisphere are highly associated with neglect and inattention syndromes. Activation of this region may therefore reflect a spatially directed attention to the itching stimulus.

The dorsolateral prefrontal cortex is associated with cognitive evaluative, attention-dependent, working memory and executive functions (Fuster, 1997). Besides the input from the thalamus and cingulate cortex, it receives and processes multisensory information mainly from the inferior parietal cortex (Fuster, 1997). The sensory convergence and integration is required in the preparation of motor action.

Presupplementary motor area and M1, motor part of the cingulate cortex

In accordance with other neuroimaging studies about itch (Hsieh *et al.*, 1994; Drzezga *et al.*, 2001; Mochizuki *et al.*, 2003; Leknes *et al.*, 2007), itch-specific activation of the presupplementary motor area (pre-SMA) was observed. Furthermore, the contralateral M1 and the dACC are less activated in comparison to saline.

The pre-SMA is thought to encode motor actions before self-initiated voluntary movements and during imagination of motor action (Cunnington *et al.*, 2005). M1 is typically involved in motor planning and execution highlighting the definition of itch which includes the intention to scratch (Hafenreffer, 1660). As the subjects were not allowed to scratch the deactivation might indicate a suppression of motor activity. The dACC is also thought to be engaged in premotor planning (Kwan *et al.*, 2000; Vogt, 2005) as well as in stimulus intensity encoding (Tölle *et al.*, 1999; Buchel *et al.*, 2002). Translating this information from pain to itch processing, we hypothesize that the dual function of the dACC and the anatomical neighboring to M1 is advantageous for the generation of an adequate motor response to the itching stimulus in relation to the processed sensory information.

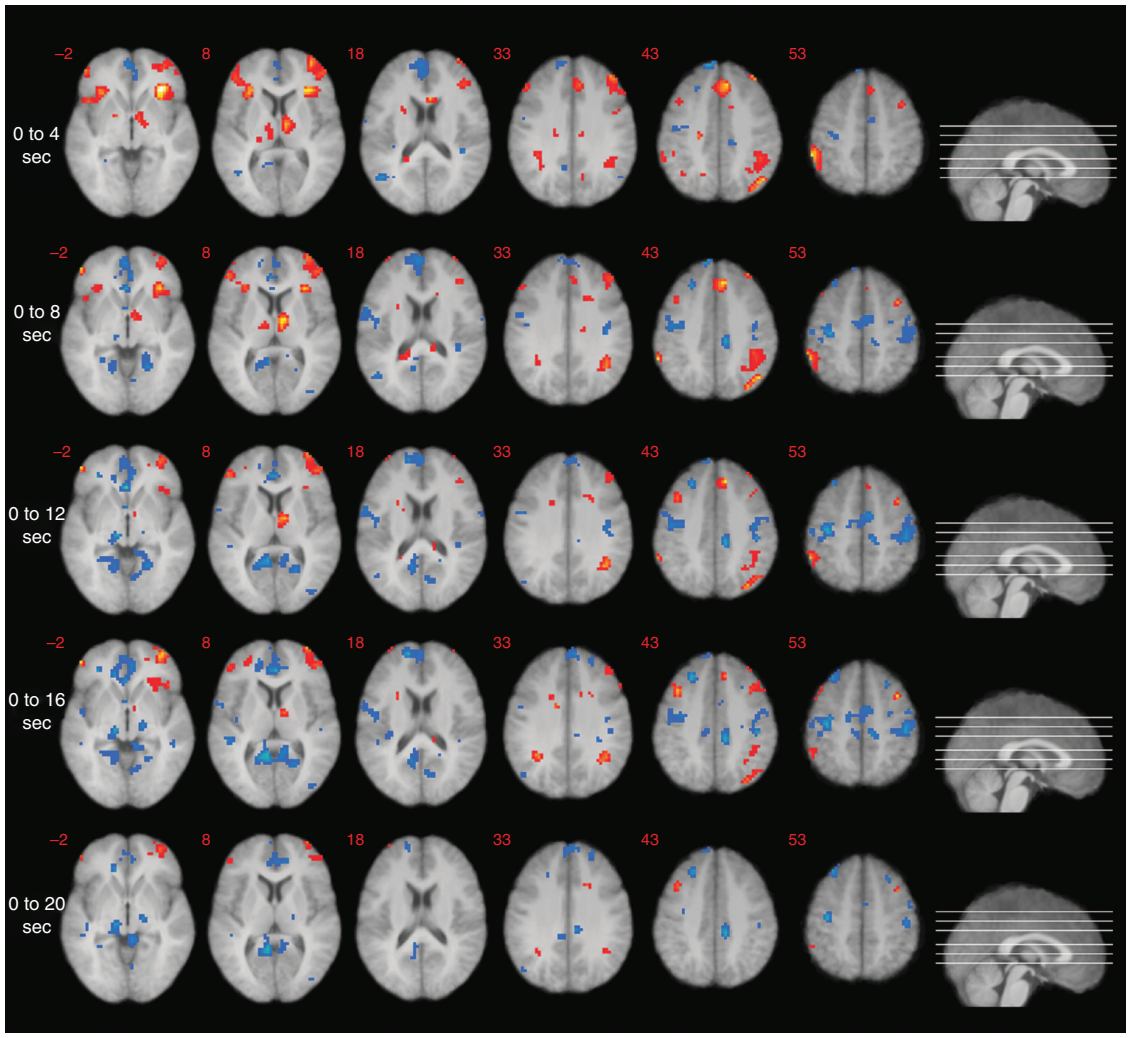

Figure 4. Cerebral activation maps of histamine induced itch for different time periods. Activation maps of histamine-induced itch compared to saline were calculated for the first 4, 8, 12, 16, and 20 seconds of the 25°C stimulation period. A dynamic process with increase and decrease of regional brain activation can be observed in the time course of 25°C stimulation. These results highlight that highest degree of brain activation occurs during the first 4 and first 8 seconds of the 25°C stimulation period.

Orbitofrontal cortex/medial frontal cortex

The orbitofrontal cortex and the medial frontal cortex were relatively less activated during the histamine condition *versus* baseline as well as in comparison to the saline condition. Involvement of both structures has been observed in the processing of aversive aspects of sensory stimuli (Rolls *et al.*, 2003), fear and anxiety reactions (Baker *et al.*, 1997). In view of these functions in the coding of aversive stimuli, one might have expected increased activation instead of decreased activation in the histamine condition. The reason for the observed deactivation state remains elusive. However, another supposed function of the prefrontal cortex is the regulation of inhibition and facilitation of sensory processing in distributed neural networks (Knight *et al.*, 1999), and one might speculate whether the decreased activation of the orbitofrontal cortex/medial frontal cortex might be related to disinhibition of the itch sensation.

Conclusion

We used an experimental stimulation protocol with ''on–off'' characteristics for the investigation of itch using fMRI. The psychophysical ratings above scratch threshold and the corresponding cerebral activation pattern underline the suitability of our model for functional neuroimaging purposes. The itch activation pattern shows similarities with pain activation patterns of previous studies and highlights the multi-dimensional aspects in the processing of itch. In the future, application of this experimental model for the investigation of itch in various allergic, dermatologic, or internal diseases might offer a promising approach for a better understanding of the different itch sensations in pruritic conditions.

MATERIALS AND METHODS

Subjects

Twelve right-handed healthy male volunteers with no history of atopic disease or allergy, inflammatory skin disease, and no

neurological or psychiatric diseases were selected for this study. The subjects neither received any systemic medication nor had topical treatment on the forearm. Age ranged from 25 to 32 years (mean age, 27.9 years). All participants received detailed information about the experimental procedure, were free to withdraw from the study at any time, and provided written informed consent. All procedures were approved by the local ethics committee of the Technische Universität München and were conducted according to the Declaration of Helsinki Principles.

Study paradigm

Two weeks before fMRI scanning, the volunteers received a training session of histamine-induced itch, using short-term alternating temperature modulation to be familiar with the experimental protocol.

There were two different fMRI scanning on two days (one with saline and one with histamine application in pseudorandom order), which were separated by at least 1 week from each other. On each day of scanning, all volunteers underwent two fMRI runs to empower our observations for statistical reasons. During the fMRI scanning, and after histamine or saline application, the sensory perception was modulated by short-term alternating temperature stimulation, as previously described (Pfab *et al.*, 2006).

The histamine stimulus was applied using a skin prick model (Pepys *et al.*, 1975) at the volar side of the right forearm, 3 cm proximal to the distal wrist crease. A 1% histamine dihydrochloride solution was applied as a single drop in aqueous solution on the skin, followed by a superficial puncture of the skin with a lancet. This technique results in a deposit of the histamine solution at the dermal–epidermal junction, where the terminals of itch-related C-fibers are located (Shelley and Arthur, 1957). The skin prick technique has been previously compared to histamine iontophoresis (Darsow *et al.*, 1996). Thereby, the skin prick procedure was more effective in producing itch with stronger sensations than induced by iontophoresis. After a median latency of 35 seconds, an itch sensation develops with peak itch intensity 120 seconds after application. No sensation of punctuate pain was reported by the subjects. Depending on the dose, the duration of a supra-threshold itch sensation ranges between 4 and 12 minutes. In our study, 120 seconds after application of the histamine stimulus, the drop of histamine solution was carefully removed with a cellulose pad. Then, a 30×30 mm-sized MRI suitable thermode (Peltier device, TSA II Medoc, Ramat Yishai, Israel) was placed on the histamine treated skin area. fMRI scanning was started and the skin temperature was modulated by the thermal stimulator. Starting from a neutral skin temperature of 32°C, 10 equal cycles directly following each other were applied. Each cycle started with a neutral block producing a constant skin temperature of 32°C for 20 seconds followed by a transit time of 1.5 seconds (ramp 5°C per second) to change to a relative cold block of 25°C also lasting for 20 seconds (Figure 1); the transit time from 25°C to the following cycle of 32°C was 1.5 seconds again (ramp 5°C per second).

When the subjects reported no further itching after a resting period of 15–30 minutes, a second fMRI scan with the application of another histamine stimulus – 1–2 cm apart from the first stimulus – followed by the above described temperature modulation was performed.

The procedure for the control condition with saline was identical to the above described procedure with the histamine stimulus, except that 0.9% saline solution was used instead of histamine dihydrochloride.

After each fMRI run, the subjects were asked to rate mean itch intensity on a VAS (0–100) for the whole 25°C as well as for the whole 32°C blocks. Thereby, the scratch threshold was defined to be at one-third of the VAS (33/100) (Darsow *et al.*, 1996; Blunk *et al.*, 2004). Above this threshold, each individual felt the clear-cut desire to scratch which, however, was not permitted nor done.

fMRI parameters and statistical analysis

fMRI was performed on a Siemens Symphony 1.5 Tesla MRI scanner with echo planar imaging sequence (155 images, first five images discarded because of T1 equilibration effects, matrix: 64×64; time to echo (TE), 50 milliseconds; time to repeat (TR), 3,000 milliseconds; alpha, 90°; field of view (FOV), 192 mm, 28 axial slices; resulting voxel size, $3 \times 3 \times 5$ mm). This sequence allows indirect measurement of neuronal activity by determining the Blood Oxygenation Level Dependent contrast (Ogawa *et al.*, 1990). Preprocessing and statistical analyses were conducted with SPM2, available from the Wellcome Department of Imaging Neuroscience, London, UK (Friston *et al.*, 1995). The fMRI data were realigned to correct for motion artifacts, normalized to standard reference space according to the echo planar imaging template of SPM (mean brain of 305 healthy subjects determined at the Montreal Neurological Institute) (Collins *et al.*, 1994), and resampled with $3 \times 3 \times 3$ mm voxel size. Finally, data were smoothed with an isotropic Gaussian kernel of 8 mm full-width at half maximum to account for anatomical individual variances and to improve the signal-to-noise ratio.

All statistical analyses were first carried out on a single subject level using a fixed-effects general linear model to calculate activation maps for the histamine and saline conditions. Activation maps were then applied for group analysis to draw a statistical conclusion for the population (random effects analysis). These maps were thresholded at $P<0.001$ with a minimum cluster size of 5 voxel (135 mm^3) uncorrected for multiple comparisons. According to previous results of $H_2^{15}O$-PET and fMRI studies, we expected activation in the thalamus, primary somatosensory, secondary somatosensory, cingulate cortex, insular, parietal, and prefrontal cortex (Hsieh *et al.*, 1994; Darsow *et al.*, 2000; Drzezga *et al.*, 2001; Mochizuki *et al.*, 2003; Walter *et al.*, 2005; Leknes *et al.*, 2007).

In the first analysis, we analyzed possible itch-induced cerebral activations within the first 8 seconds of 25°C stimulation separately for the conditions histamine and saline. In our preceding psychophysiological study, the increase in subjective itch sensation was highest during the first 8 seconds of each 25°C block – which was the reason to focus on this fraction of time (Pfab *et al.*, 2006). The last 12 seconds of each 32°C temperature block served as neuronal baseline as all VAS itch ratings remained stable and below scratch threshold, indicating a stable neuronal baseline during this time interval. The resulting activation maps reveal brain regions, which are more, respectively less active in comparison to the 32°C baseline.

In the second analysis, we aimed to reduce the influence of cooling on cerebral itch processing. We calculated five itch-specific activation maps. Hereby, the first 4, first 8, first 12, first 16, and first 20 seconds of the 25°C stimulation periods of the histamine scans were compared to the according time segments of the 25°C

stimulation periods of the saline scans. The resulting itch-specific activation maps reveal brain regions, which are more, respectively less active in comparison to saline. The segmentation of the 20 seconds stimulation period was performed to guarantee analogy to our previous work, where we obtained itch ratings every 4 seconds (Pfab *et al.*, 2006). We also focused to report brain activations for the first 8 seconds.

The fMRI images obtained during transit time (from 32 to 25°C) were included in each analysis, as we believe that the rapid temperature decrease from 32 to 25°C is an important factor for the provocation of itch.

Psychophysiological parameters were assessed by statistical comparison of these parameters between conditions (histamine *vs* saline) with a two-tailed Wilcoxon matched pairs test using Prism 4 for Windows (GraphPad Software Inc., San Diego).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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