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INVESTIGATIVE REPORT

Cerebral Networks Linked to Itch-related Sensations Induced by Histamine and Capsaicin

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This functional magnetic resonance imaging (fMRI) study explored the central nervous processing of itch induced by histamine and capsaicin, delivered via inactivated cowhage spicules, and the influence of low-dose naltrexone. Scratch bouts were delivered at regular intervals after spicule insertion in order to temporarily suppress the itch. At the end of each trial the subjects rated their itch and scratch-related sensations. Stepwise multiple regression analyses were employed for identifying cerebral networks contributing to the intensities of "itching", "burning", "stinging", "pricking" and "itch relief by scratching". In the capsaicin experiments a network for "burning" was identified, which included the posterior insula, caudate and putamen. In the histamine experiments networks for "itching" and "itch relief" were found, which included operculum, hippocampus and amygdala. Naltrexone generally reduced fMRI activation and the correlations between fMRI signal and ratings. Furthermore, scratching was significantly less pleasant under naltrexone. ***Key words: naltrexone; histamine; capsaicin; itch; fMRI.***

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Cerebral responses to itching stimuli have been explored using functional magnetic resonance imaging (fMRI) in several studies (1–3). It is generally accepted that experimental itch stimuli activate a cerebral network similar to that involved in acute pain stimulation (4–6). Many questions remain, however; in particular, regarding the processing of different types of inputs leading to itch and the different sensory qualities intermingling with itch sensations.

In a recent psychophysical study we employed 3 different itching agents introduced into the superficial layers of the skin via cowhage spicules (7, 8). In addition to the native agent mucunain contained in the spicules (9), histamine and capsaicin were applied via inactivated cowhage spicules. Since the stimuli were introduced into the skin with the same applicator, their application could be blinded for the subjects. Three agents were chosen that excite different pathways in the periphery and spinal cord (10–12). The study revealed that the 3 itching stimuli all induce itch, albeit mixed with other sensory qualities, such as burning and stinging (13).

It is well known that a unique characteristic of itching is itch suppression by scratching, regardless of whether the subject scratches the affected skin site themselves or the scratching is performed by another person (14, 15). In a previous fMRI study we have shown that cerebral scratch responses are characteristically stronger on a background of itch sensations in the cortical and subcortical fields belonging to an itch-processing network (16). Scratch responses are easier to visualize in fMRI than the itching itself, due to the more synchronized input from myelinated and unmyelinated afferents. Since itch-related scratching is pleasurable (17), the processing of scratching under itch is inversely, but closely, related to the itch-processing itself. Therefore, for evaluation of the brain mechanisms of itch processing not only the itching sensations themselves should be regarded, but also the scratch responses for gaining insight into the cerebral processing of the itch-scratch cycle.

In previous psychophysical (18) and fMRI studies (16) we employed a paradigm in which itch was induced for several minutes by application of native and modified cowhage spicules and this sensation was interrupted several times by passive scratching. In these experiments the strongest itch sensations were observed in periods immediately before the scratching that led to temporary itch suppression.

The aim of this study was to compare cerebral responses during the itch-scratch cycle for 2 different itch stimuli applied via modified cowhage spicules: histamine and capsaicin. These experiments were conducted in double-blind cross-over experiments under placebo or medication with a low dose of naltrexone, a μ-opioid antagonist, since itch suppression by naltrexone has been experimentally proven in man and animals (19, 20).

We hypothesized that different enhancements of the fMRI signal (blood-oxygen-level dependent [BOLD] responses) during periods of high itch and itch-suppression by scratching, and also different itch and itch-relief-related sensations, should occur after application of histamine and capsaicin. A second question was how these BOLD responses and sensations were affected by a low dose of naltrexone.

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METHODS

Subjects

Sixteen healthy male subjects participated in this study (mean age 23 ± 5 years). All subjects provided written consent prior to participation in accordance with the Declaration of Helsinki. Subjects were free to withdraw from the study at any time. The study was approved by the local ethics committee (approval number 4069). All subjects were free of allergies, atopic eczema and/or other dermatological diseases. In an initial training session all subjects were familiarized with the experimental procedure. Subjects with generally very low itch ratings were excluded. None of the subjects had used opioid drugs during the months before the experiment.

The subjects were informed about the itching compounds and the possible side-effects of naltrexone. They were asked to abstain from alcohol on the day of the experiment. Subjects received financial compensation for their participation.

Experimental procedure

Preparation and application of pruritic agents. Cowhage spicules (courtesy of M. Ringkamp, Johns Hopkins University, Baltimore, USA) were used for application of their native compound mucunain. Inactivated spicules were used for the application of histamine and capsaicin. The procedure has been described in a previous paper (18) and a brief description is given here (Appendix S1[1]).

The spicules were coated with the respective agent by dipping them in the solution, followed by drying for at least 30 min. This procedure was repeated twice. A bunch of approximately 30 spicules was fixed on a cotton applicator with a drop of glue, as described previously. Pressing this applicator to the skin of the forearm left approximately 20 spicules in the upper layers of the skin.

Naltrexone application. Naltrexone (25 mg) or placebo (25 mg) in a neutral capsule prepared by the pharmaceutical service of the University Hospital in Erlangen were swallowed with water by the subjects 1 h before the fMRI session. On the day after the experiment the subjects were asked about possible side-effects of the drug. One subject reported experiencing headaches after the intake of naltrexone. Some subjects reported a slight diffuse indisposition, which was found to be related to placebo intake. In general the subjects could not distinguish between naltrexone and placebo.

Experimental protocol

Application of naltrexone or placebo was double-blinded for subjects and experimenter in 2 different sessions separated by 7–10 days. In each session the 3 kinds of spicules were also applied double-blind in randomized order to the left volar aspect of the forearm. The experimenter, but not the subjects, was able to observe the forearm during the experiment and might have been able to guess the applied stimulus from the absence of a flare response (after cowhage), or occurrence of an extensive flare after histamine and a smaller one after capsaicin.

The first stimulus was applied to a spot 2 cm distal from the elbow crease, the second 2 cm distal of the end of the scratching area for the first stimulus (see below) and 1.5 cm medial of the first stimulus. The third stimulus was placed 2 cm distal of the end and 1.5 cm laterally of the scratching area of the second stimulus. The exact positions of the application and scratching sites were marked with a pen before the experiment. Scratching was applied to a 4×1 cm area oriented along the long axis of the forearm and was also marked with a pen before the experiment. This area ended 1 cm from the application site to avoid interaction of the scratch and chemical stimuli at the level of the nerve terminals. The scratch stimulator was identical to that used in a previous study (18). The experimenter applied this stimulator with a force of approximately 2.5 N, which bent it slightly. Each scratching bout lasted 9 s and consisted of 7 evenly spaced strokes over the whole marked area delivered at constant force and velocity. Four such bouts were delivered in each test run, the first 90 s after spicule application, the others followed at 51 s intervals (for the time-course see also (18)).

Immediately after the fMRI measurements the subjects had to rate the "itch relief by scratching", "itching" (subjects were asked to rate likeness to a mosquito bite), "burning", "stinging" and "pricking". Ratings were performed with the right hand by showing 0–5 fingers. For the sensory qualities 0 fingers indicated "no sensation of this quality", 5 fingers "very strong sensation of this quality". For the scratching 0 fingers indicated "no relief", and 5 fingers "very pronounced relief". Since the 3 kinds of spicules were applied in each fMRI session, these sensory ratings were performed 3 times in each session in the magnetic resonance (MR) scanner.

After the ratings, when the itch sensations had subsided, the spicules were removed by stripping with adhesive tape. This procedure reliably prevented recurrence of the sensation.

Psychophysical experiments

Since the ratings in the MR imaging (MRI) scanner may have been hampered by the experimental conditions, the experiments were repeated 12–15 weeks later in the environment of a psychophysical laboratory. Medications and itch stimuli were applied in identical fashion. Eleven subjects from the fMRI study participated in this psychophysical experiment. The other 5 subjects were no longer available.

Acquisition and evaluation of fMRI data

Magnetic resonance imaging protocol. Three functional imaging sequences, one for each of the itching substances, were recorded. The spicules were applied 30 s after the start of a sequence. The same protocol as in a previous psychophysical study (18) was then used, as described above. Each imaging sequence lasted 5 min. The next sequence with spicule application was started as soon as the subjects reported no more itching sensations, but at least 20 min later. The first of these pauses was used to record the anatomical images.

Magnetic resonance imaging. The subjects' heads were fixed in the scanner with rubber pads and their ears plugged to minimize stress induced by the scanner noise. MRI was carried out using a Siemens Trio 3T system (Siemens, Erlangen, Germany). A brief description of the recording parameters is given in Appendix S1[1].

Subject was included as a random effect. For the purpose of this study, the "high itch" and "itch suppression by scratch" episodes were used as predictors for the analyses, while the other factors were regarded as covariates of no interest (see Appendix S1[1]).

Multiple regression analysis of ratings and "regions of interest" (ROI) activations are shown in Appendix S1[1].

RESULTS

Sensory ratings under naltrexone and placebo

Immediately after the end of the fMRI measurements, the subjects rated their sensory experiences, while

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they were still recumbent in the scanner. These ratings were statistically tested by 2-way analysis of variance (ANOVA) with the factors "agent" (capsaicin, histamine) and "medication" (naltrexone, placebo). No significant differences were found between naltrexone and placebo experiments (Table I). Several significant differences were found, however, between the itching agents: capsaicin was significantly more "burning" ($p < 0.01$), more "stinging" ($p = 0.005$) and "pricking" ($p = 0.006$). The differences between "itching" and "itch relief by scratching" were, however, not significant.

As mentioned in the Methods section, the cowhage experiments had to be excluded from the analysis, since in the placebo experiments 40% of the subjects did not experience itch in these tests, indicating that part of the native spicules may have lost their mucunain effect.

Since the sensory ratings may have been hampered by the conditions in the MR scanner the experiment was repeated with 11 of the subjects in a psychophysical lab (see Methods). In these experiments (which were blinded in the same way as the fMRI experiments) scratching was assessed differently: a 9-point rating scale was used, with the end-points "extremely unpleasant" (–4), "neutral" (0) and "extremely pleasant" (+4). In these experiments the mean rating of "scratching" was 0.36 under naltrexone and 2.18 in the placebo experiments following application of histamine. For capsaicin the respective mean ratings were neutral (0) under naltrexone and 1.27 under placebo. In the respective ANOVA the medication effect ($p = 0.03$) and the "pleasantness of scratching" ($p = 0.035$) were significant, i.e. scratching was more pleasant under placebo.

Functional imaging data

Fig. 1 shows the scratch-induced activations in the histamine experiments under placebo and naltrexone, respectively. The same scale was used to mark activated areas under both conditions (see also Table SI[1] showing the Talairach coordinates of the centres of the activation clusters in the ROIs and the sizes of the clusters). The locations of activation clusters were identical, but the sizes of the activated areas were smaller under naltrexone (Table SII[1]).

Figs 2A and B show the *t*-values of the BOLD changes during the scratching periods obtained under placebo compared with those from the naltrexone experiments. This figure demonstrates the high reliability of the BOLD measures obtained in the 2 experiments separated by one week or more. The coefficients of determination (R^2) were 0.9 for both hemispheres. There was no significant difference between histamine and capsaicin experiments (red and green symbols, respectively), but a significant difference between naltrexone and placebo experiments. The slope of the regression in particular in the right hemisphere (0.85) is less than unity, indicating larger *t*-values under placebo. This is corroborated by direct comparison with a paired *t*-test ($p < 0.001$).

The figure also shows that in both the placebo and the naltrexone experiments part of the *t*-values were negative, indicating deactivation during the itch-scratch cycle (see Table SII[1]). An example is depicted in a specimen record in Fig. 2C. These deactivations were predominantly found in limbic structures, which is in agreement with previous findings by our group (21) and others (22). A BOLD decrease was also observed, however, in the left M1 cortex, ipsilateral to the stimulated side (see Fig. 1). This might reflect inhibition of the urge to scratch in our experiments in which active scratching was not allowed.

Scratch period

In general, the BOLD changes during the "scratch bouts" period were greater than during the "high itch period", probably due to the fact that scratching induced more synchronized input from different primary afferents. For the scratch bouts the mean *t*-value in the group general linear model (GLM) study of 27 clusters tested was $t = 7.19$ (standard error of the mean (SEM) 0.74) for capsaicin/placebo and $t = 7.29$ (SEM 0.79) for histamine/placebo. The highest *t*-values for the "scratch bouts" in the group study were found in the left operculum ($t = 15.4$ and 14.7, respectively, under naltrexone) and in the right posterior insula ($t = 14.4$ and 15.0 under placebo).

High itch period

The mean *t*-values derived from the GLM group study during the "high itch" period were much lower: $t = 2.1$ (SEM 0.3) and 2.2 (SEM 0.2) under placebo. Relatively high *t*-values were found in limbic structures: in the left and right amygdala, the left and right hippocampus, and in the left subgenual anterior cingulate cortex (sACC) the *t*-values were > 3. The highest *t*-value was found, however, in the left hippocampus

Table I. *Sensory ratings (mean ± SEM) obtained during functional magnetic resonance imaging (fMRI) experiments*

		Rating				
Substance	Medication	Relief by scratching	Itching	Burning	Stinging	Pricking
Capsaicin	Placebo	1.94 ± 0.38	1.13 ± 0.30	2.31 ± 0.31	1.75 ± 0.35	2.25 ± 0.25
	Naltrexone	2.00 ± 0.34	1.53 ± 0.43	2.40 ± 0.25	2.27 ± 0.33	2.07 ± 0.33
Histamine	Placebo	2.19 ± 0.41	1.50 ± 0.32	1.75 ± 0.30	1.38 ± 0.22	1.44 ± 0.26
	Naltrexone	1.80 ± 0.34	1.73 ± 0.28	1.67 ± 0.23	1.13 ± 0.26	0.80 ± 0.24

No significant differences between placebo and naltrexone.
Differences between substances: "burning": capsaicin > histamine ($p < 0.01$). "Stinging": capsaicin > histamine ($p = 0.005$). "Pricking": capsaicin > histamine ($p = 0.006$).
"Itching" and "itch relief by scratching": n.s.
SEM: standard error of the mean.

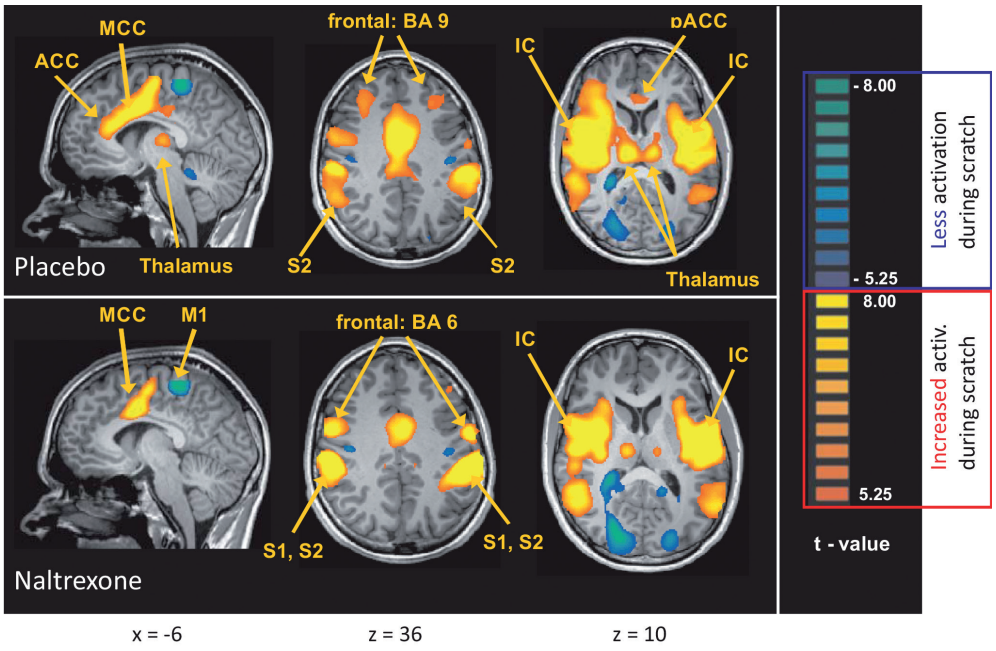

Fig. 1. Scratch-induced activations during itch evoked by histamine in selected brain areas. The locations of the activation centres were nearly identical under placebo (*upper row*) and naltrexone (*lower row*), but the sizes of the activated areas were smaller under naltrexone. In both cases the same scale was used to mark the *t*-values of the blood-oxygen-level dependent changes. The x- and z-values denote the positions of the slices within the Talairach system. ACC: anterior cingulate cortex; BA: Brodmann area; M1: primary motor cortex; MCC: midcingulate cortex; IC: insular cortex; pACC: pregenual anterior cingulate cortex; S1: primary somatosensory cortex; S2: secondary somatosensory cortex.

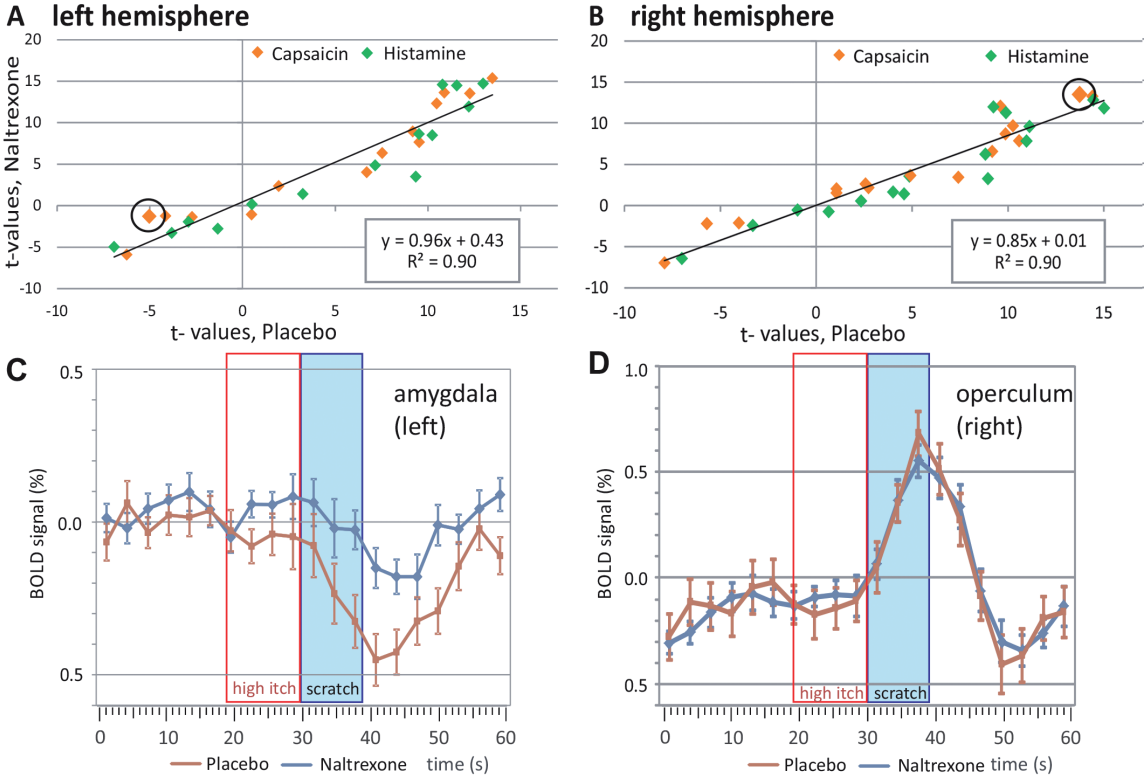

Fig. 2. (A and B) *t*-values conveying the strengths of the blood-oxygen-level dependent (BOLD) responses in the selected regions of interest (ROIs) under placebo (*abscissa*) and naltrexone (*ordinate*) in the left and right hemisphere with regression lines. Each point symbolizes a certain brain region, which is represented by its *t*-values during placebo and naltrexone. Note: negative *t*-values indicate a decrease in the BOLD response. The figure demonstrates that in both hemispheres the activations are quite similar after placebo and naltrexone, resulting in a high correlation of the *t*-values. A slope of the regression <1 indicates that the mean activations were smaller under naltrexone. (A) Left hemisphere: slope 0.96; coefficient of determination R^2=0.90. (B) Right hemisphere: slope 0.85; R^2=0.90. The encircled symbol in (A) represents the time course of the functional magnetic resonance imaging (fMRI) signal during the itch-scratch cycle shown in Fig 2C, the encircled symbol in (B) represents the time course shown in (D). (C) and (D) The lower panel shows the mean BOLD signal changes in (C) the left amygdala and (D) the right operculum, averaged over the 4 scratch periods and all subjects. Averaging started 30 s before start of a scratch bout; the whiskers mark standard errors of the mean (SEM). The marked segments depict the periods that defined the predictors "high itch" and "itch relief by scratch", which were used for analysis of the fMRI experiments. BOLD changes in percent of the grand mean in the respective session. *Red curve*: placebo, *blue curve*: naltrexone data; applied substance capsaicin.

(t=5.1 for capsaicin/placebo and t=4.5 for histamine/placebo). Whereas all 27 clusters in the group study revealed significant t-values during the "scratch bout" period, only 9 clusters also had significant t-values during the "high itch" period. Only the β-values of these 9 clusters were included in the following correlation analysis.

Correlations of blood-oxygen-level dependent changes with sensory ratings

In order to determine the correlation between the BOLD changes and the sensory ratings, the periods "scratch bouts" and "high itch" were extracted from the GLM and the β-values in the activation clusters were assessed. Correlation coefficients (Rs) between the sensory ratings obtained after each trial and the BOLD effects were computed. This analysis was performed separately for the histamine and capsaicin experiments. In the placebo experiments the correlation patterns for histamine and capsaicin were rather different. Only a few significant correlations were found for the naltrexone experiments.

Fig. 3 depicts scatter diagrams for 2 ROIs. In the capsaicin experiment (Fig. 3A) a significant negative correlation was obtained under placebo between the ratings of "burning" and the β-values in the scratch period in the association area BA40 (Rs=–0.6), whereas in the left operculum a high positive correlation was found between the "itch" ratings and the β-values (Rs=0.7) (Fig. 3B). These correlation coefficients indicate 49% and 36% joint variance, respectively, between the rating variables and brain activation. In both cases the correlation was completely abated under naltrexone.

It can also be seen from Fig. 3 that the β-values were mostly positive, as expected from the significant positive t-values in these clusters for the itch/scratch stimulus (see also Table SII[1]).

Multiple regression analysis of scratch bout periods. In the placebo experiments the highest coherence for the capsaicin stimulation was found in the left BA40 cluster (R^2=0.618), where the strongest factor "burning" was negatively correlated with the β-values, and the scale was positively correlated to the β-values. In general, 2 or only one of the rating scales accounted for the significant correlations with the BOLD activations in any of the ROIs. Only in the case of the right posterior insula did 3 scales contribute to the result ("burning", "stinging" and "itching"), which explained 58% of the model. Also, for the left caudate, "burning" was the strongest factor in a model that explained 47% of the variance. For another 5 ROIs a significant model was found; however, only one rating scale contributed to these results and the models explained less than 30% of the variance (see Table SIII[1], which shows a summary of the analyses of the capsaicin stimulation under placebo).

In the naltrexone experiments coherences of the BOLD data and the ratings were generally absent, with 2 exceptions for the capsaicin stimulus: activation of the left caudate was explained as 47% by a model in which "relief by scratching" contributed the major part (t=–3.5, p=0.004), whereas "itching" made a minor contribution (t=2.3, p=0.04). A significant model was also found for the left medial frontal cortex, BA10, explaining 29% of the variance with the scale "itch relief by scratching", which was again negatively correlated with the activation (t=–2.6; p=0.02).

In the placebo/histamine experiments the general picture is completely different: the strongest coherence between sensory ratings and BOLD changes was found in the left operculum, with an R^2 of 0.669 reflecting a common variance of almost 70% (see also the univariate correlation in Fig. 3B). In this case the cerebral response

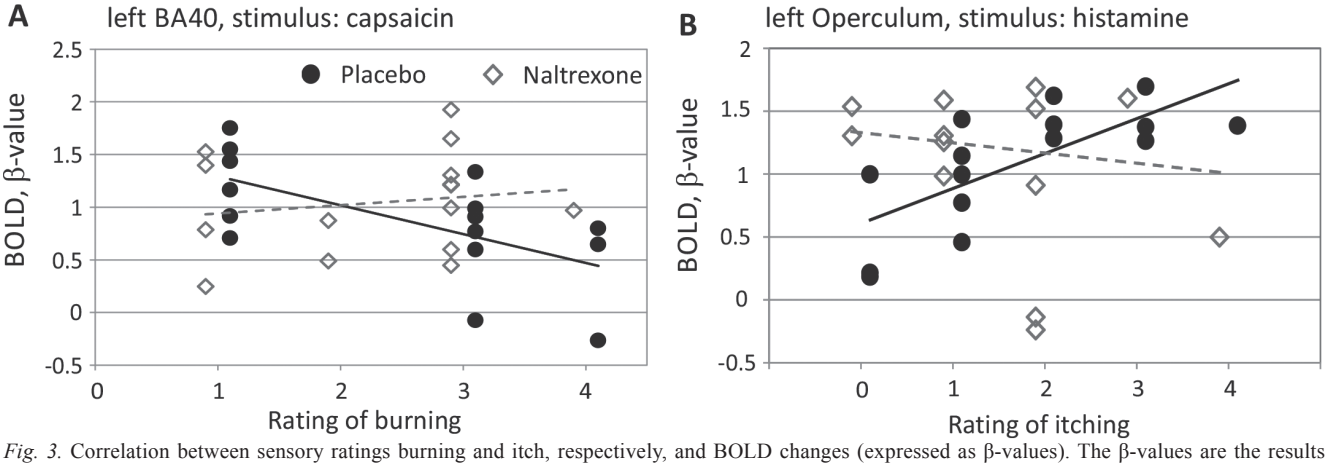

Fig. 3. Correlation between sensory ratings burning and itch, respectively, and BOLD changes (expressed as β-values). The β-values are the results of single studies analyses during placebo (*black bullets*) and naltrexone (*circles*) medication. (A) Rating of "burning" vs BOLD during "itch relief by scratching" in the left BA40 after capsaicin. Placebo: Rs=–0.56; p=0.02. Naltrexone: Rs=0.15; ns. (B) Rating of "itching" vs BOLD during "itch relief by scratching" in the left operculum after histamine. Placebo: Rs=0.74; p<0.01. Naltrexone: Rs=0.05; ns. It has to be noted that a negative correlation between β-values and ratings does not mean that the activation cluster was negatively correlated with the stimulation. It only shows that higher ratings were correlated with lower BOLD effects. If, however, the respective cluster showed a negative BOLD response to the stimulus (as seen in some limbic clusters), a negative correlation between β-values and ratings shows that the BOLD effect was less negative when the ratings were higher.

was mainly due to the "itching" and to a minor degree to "stinging". Nine other activation clusters of the 12 tested revealed significant coherences of β-values and ratings, with $R^2 > 0.21$ and < 0.41 indicating a minor predictive value of ratings for their activation. It is noteworthy that, for 5 activation clusters, "itch relief by scratching" and for another 5 "pricking" were the strongest correlators with the β-values (see Table SIII[1], showing the respective results from the placebo/histamine experiments).

Interestingly, no significant predictions were found in the naltrexone/histamine experiments.

Multiple regression analysis of high itch periods. As explained above, only 9 of the clusters showed significant activations or deactivations during this period. The *t*-values were lower than for the "scratch bouts" (see above). Correspondingly fewer significant models were found in the multiple regression analysis. In the placebo/capsaicin experiments the most significant coherence was found for the right hippocampus ($R^2 = 0.48$). Here "burning" explained most of the variance, followed by "stinging". In the placebo/histamine experiments 2 regions were found in which "relief by scratching" was negatively correlated with the BOLD data: the sACC and the right lateral frontal cortex, BA46 (for summarized data see Table SIV[1]).

DISCUSSION

In this study we combined fMRI data obtained during sequences of itch/scratch cycles with the assessment of ratings depicting different irritant sensations induced by tiny amounts of histamine and capsaicin applied with inactivated cowhage spicules. It has been shown in previous studies that both stimuli induce itch combined with other irritant sensations, such as burning and stinging (8, 13).

As stated in the introduction, our hypothesis was that naltrexone would diminish the "itch" component and decrease the "relief by scratching", while probably enhancing the sensations of burning and stinging. However, the effect of naltrexone on the ratings was not significant in the assessments performed in the MRI scanner, probably due to the low dose of naltrexone chosen (25 mg, single dose by mouth). This dose was below that used for clinical applications and may have been too small to induce significant differences in these psychophysical parameters. The reason for choosing this low dose was 2-fold: in a previous psychophysical study of our group this low dose has been proven effectively to suppress histamine-induced itching and alloknesis (19). The second reason was that we tried to minimize possible side-effects of the drug. In the present fMRI study itching induced by histamine was weaker under naltrexone, but the difference was not significant. Likewise, the differences in itch relief by scratching failed to reach significance. In a successive psychophysical study scratching was significantly more pleasant under placebo than under naltrexone.

In spite of the non-significant effects of naltrexone on the ratings in the MRI scanner, we found a significant difference between the cerebral activations of different regions by the irritant stimuli. Activation clusters were smaller in size and strength of BOLD effect under naltrexone (see Figs 1 and 2), indicating a higher sensitivity of the fMRI measures compared with the sensory ratings. Interestingly, the mitigating effect of naltrexone on the BOLD responses applied not only to the histamine stimuli, but also to capsaicin stimulation, contrary to our initial hypothesis. Naltrexone is supposed to mitigate itch and to enhance pain processing. Our fMRI findings indicate that it affected the processing of pruriceptor input more than the nociceptor component, regardless of whether it was induced by histamine or capsaicin.

As in previous studies we chose a protocol in which standardized scratching bouts were applied by the experimenter at regular intervals close to the application site of the itching stimulus, resulting in regularly alternating phases of itch and itch suppression (18). Three types of itch stimuli were applied in blinded experiments: histamine, capsaicin and cowhage. Unfortunately, the cowhage experiments were inconclusive and could not be regarded further in the data evaluation. The 2 remaining agents, histamine and capsaicin, mediate their itching effect by the excitation of different pathways. In humans, histamine binds to H1 receptors, G-protein coupled membrane receptors of a small sub-population of mechanoinsensitive C-fibres (23). Capsaicin acts on a much larger population of mechanoinsensitive and mechanosensitive small fibres through binding to the TRPV1 membrane receptor (11). In higher concentrations and upon application to a larger area capsaicin induces intense pain. However, it induces a mixture of itching and burning sensations upon punctate application via inactivated cowhage spicules (7, 8). In animal experiments scratching inhibited the transmission of histamine-induced, but not of capsaicin-induced, activations in the spinal cord (12). In the present fMRI experiments the ratings for itch were not significantly different between histamine and capsaicin. However, the sensations induced by capsaicin were significantly more burning, stinging and pricking. This is in agreement with previous psychophysical studies (13).

In general, the areas activated by the itch-related scratch bouts were similar to those found in previous studies on the representation of histaminergic itch (21) and itch-related scratching by our group (16). BOLD activations without a background of itch were found in this study and in a previous study obtained by another group at similar locations (24). This network was rather stable in the 2 experiments under naltrexone and pla-

cebo separated by more than one week. However, the activation peaks were slightly smaller under naltrexone (see Fig. 2). This might reflect the smaller rewarding effect of scratching under this opioid antagonist. There is a parallel with previous findings of suppression by opioid antagonists of rewards associated with eating or alcohol consumption (25).

To determine the relationship between the irritant sensations and the activations in various clusters during the scratch bouts we correlated the sensory ratings with the β-values of the BOLD changes during the "scratch" and during the "high itch" episodes in the single studies. To find out how much variance in the BOLD effects was explained by the sensory ratings, a novel approach was chosen; stepwise multiple regression analysis. This approach identifies the set of ratings that best predicts the BOLD changes.

Due to the stepwise approach, ratings that have only a minimal influence or that would worsen the model are excluded. This can be interpreted such that the respective brain area is not, or is only minimally, involved in the processing of the respective sensation. If a brain area participates in several sensations the ratings that are related to these sensations should be included in the statistical model. In the histamine experiments in all but one ROI the best model is obtained if only one predictor is included (see Table SIII[1]). It is notable that after the histamine stimulus ratings of itch-related sensations (itching, pricking, relief by scratching) are frequently identified as good predictors for brain activation. The strongest covariance was found in these experiments between the left operculum (S2 cortex) and "itching" (see also Fig. 3B). The rating of "relief by scratching" requires an assessment that includes pleasant emotions and memory. From this it is not surprising that brain regions of the limbic system, such as the amygdala and hippocampus, are identified, but also the left BA40 and the left caudate were involved. These results are supplemented by the analyses of the "high itch" period in which the subgenual anterior cingulate (sACC) (nomenclature of the cingulate after (26)) and the right lateral frontal field BA46 showed negative correlations with "relief by scratching" (see Table SIV[1]).

One may assume that some brain areas are activated more intensely during a particular sensation, e.g. itch and itch relief. They may constitute a network generating the itch-related sensation. It can be concluded that the itch relief of scratching is preceded by a significant suppression in the "high itch" period in the sACC and lateral frontal areas, followed by an enhancement of the scratch response in the left BA40 cluster, and a diminution of the negative BOLD response in the hippocampus, left and right amygdala and left caudate.

A network for "burning" seemed to emerge only in the capsaicin experiments. The sensations induced by capsaicin seem to be less clear; therefore up to 3 ratings are required to fit an optimum model that predicts the activation of a brain area. All ratings of "burning" have negative correlations with the activations; i.e. if the sensory quality of "burning" was more pronounced, these regions were less activated during the "scratch bout" (see Table SIII[1]). Brain regions that are activated by the scratch bouts may also participate on the coding of burning. The higher the burning-induced background activity of such a region (i.e. when the rating of "burning" was higher) the less activation increase occurred during the scratch bout (smaller β-values).

Conclusion

Capsaicin and histamine applied in small amounts via inactivated cowhage spicules induce similarly mixed sensations of "itching", "burning" and "stinging". They activate the same cerebral network. However, multiple regression analysis reveals that, within the resolution of our experiment, a network for "burning" can be detected only in the capsaicin experiments, whereas in the histamine experiments activation of the left operculum shows a close correlation with the ratings of "itching". A network for "itch relief by scratching" was also detected in the histamine experiments involving reactions in the "high itch" and in the "scratch bout" periods. Activation of this network seems to consist of preparatory activations in the frontal cortex, followed by a scratch-induced activation in the left BA40 association area and a diminished BOLD decrease in limbic areas and the left caudate. The correlations between sensory ratings and BOLD responses were greatly lowered or even abolished by naltrexone.

Further studies are required for characterization of these different, but overlapping, networks.

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