Appendix S1.

EXPERIMENTAL PROCEDURES

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 application of histamine and capsaicin. The procedure has been described in a previous paper (18) and a short description is given here: part of the spicules were inactivated by autoclaving for destroying mucunain, the active ingredient of cowhage. Inactivated spicules were coated with histamine or capsaicin. The coating solutions were prepared as follows: (*i*) histamine supplied by Sigma Aldrich (number H7250) was dissolved in distilled water for preparing a 1% solution. (*ii*) Capsaicin (N-vanillyl-nonanamide), supplied by Sigma Aldrich (number V9130), was prepared by dissolving 500 mg in 3.5 g ethanol and titrated with Ringer solution to a 10% capsaicin solution. After coating the spicules the ethanol quickly evaporates and plays no role in the sensory stimulation.

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 before. Pressing this applicator to the skin of the forearm left approximately 20 spicules in the upper skin layers.

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.

Materials and methods

Recording parameters. 110 functional T2* weighted images of the cortex were obtained using an echo planar imaging (EPI) technique consisting of 34 axial slices (TR = 3000 ms, TE = 60 ms, flip angle = 90°, slice thickness = 4 mm, field of view 220×220 mm^2, 64×64 pixel, nominal in-plane resolution 3.44×3.44 mm^2). Possible head movements of the subjects were corrected online using the prospective acquisition correction of the scanner software (27). Anatomical images of the heads were recorded using a magnetization prepared rapid gradient echo (MPRAGE) sequence consisting of 176 sagittal slices of 1-mm thickness and a nominal in-plane resolution of 859×859 μm^2 using a 256×256 pixel matrix with a 220×220 mm^2 field of view.

Data analysis and statistics

All post-processing and statistical analyses of the data were performed using BrainVoyager® QX v2.3 (Brain Innovations, Netherlands, www.brainvoyager.com). Pre-processing of the functional EPI sequences included motion correction, slice scan time correction, spatial Gaussian smoothing of 4 mm, linear de-trending, and temporal Gaussian filtering of 4 s. For a group analysis of the cortical activations all functional data were transformed into Talairach space.

Analysis of the functional imaging data was performed with a general linear model (GLM) random effect analysis, which included the following 4 predictors: (a) application of spicules; (b) "high itch": Four periods starting 21 s before each scratch bout. During this period the itch sensations were strongest, as found in previous experiments (18). (c) "Scratching": Four periods of scratch bouts in which itch suppression occurred (18); (d) "Low itch": Four periods starting immediately after a scratch bout and lasting 21 s.

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.

According to our previous experiences (16, 21) and after a preliminary view on the present data, we selected 15 "regions of interest" (ROIs) on the left, ipsilateral side, and 17 ROIs on the contralateral side for further evaluations (Table SI[1]). Only clusters belonging to 1 of the ROIs were selected for further analysis. This resulted in a corrected *p*-value of 0.001 ($< 0.05/32$) for the detection of activated brain areas. On the basis of a clustering algorithm a cortical brain site was considered to be activated only if a threshold cluster size of 150 mm^3 was matched or exceeded (28).

Evaluation masks were obtained from a "group study", which included the fMRI data from the 2 remaining types of spicules and under placebo conditions (after exclusion of native cowhage spicules). To ascertain the position of an evaluation cluster in a ROI, we compared the evaluation mask with the extensions of the respective ROIs in the "Talairach Demon" (www.talairach.org/applet/). Since the centres of the clusters under the different treatments (medication, agents) were rather similar, we were able to use the same masks for the quantitative evaluation of all types of treatment (medications, agents) (for Talairach coordinates and cluster sizes; see Table SI[1]).

Multiple regression analysis of ratings and regions of interest activations

For each subject and each ROI the β-values of the predictors "high itch" (b) and "scratching period" (c) were calculated using a GLM analysis with random effects (subjects). These β-values were used as a measure of the strength of the individual BOLD changes within the respective ROI during the "high itch" and the "scratch periods", respectively. This was done separately for each of the 4 combinations of medication (naltrexone or placebo) and substance (capsaicin or histamine).

Stepwise linear regression was carried out using SPSS 21.0 (IBM Corp.) to build a statistical model of the relationship between cerebral activation in given ROIs and sensations. For this purpose we identified the set of the sensory ratings (among "itch", "burning", "stinging", "pricking" and "itch relief by scratching") that was most predictive for the BOLD changes in a brain region. The power of the prediction was expressed by the coefficient of determination R^2. Briefly, this analysis is performed as follows: first, the rating with the highest correlation with the β-value of the ROI is identified and the respective adjusted R^2 is calculated. If R^2 is significant, the model computation continues by adding the rating with the next highest correlation to the model. Again, the correspondent adjusted R^2 is calculated. If the new R^2 is larger, the procedure is repeated and the next rating is included. If the new R^2 was less than the R^2 of the model before the preceding one (without the newly added rating) is used as the final multiple regression model. This model provides a *t*-value for each rating, which expresses the significance of the rating within the multiple regression model. The finally chosen R^2 expresses the joint variance of the sensory rating and the BOLD activations expressed as β-values.

Table SI. *Regions of interests (ROI) used for the analysis. The table lists the ROIs together with the Talairach coordinates of their centres and their sizes in mm³. The ROIs are given separately for left and right hemispheres*

		Talairach coordinates			Size
	Abbrevation	x	y	z	(mm³)
Left hemisphere (ipsilateral)					
Thalamus	Thal l	–11.7	–15.22	8	2,212
Caudatus	Caud l	–15.11	4.46	15.8	1,148
Amygdala	Amyg l	–21.82	–8.26	–17.07	1,027
Hippocampus	Hippoc l	–29.36	–19.21	–12.34	1,632
sACC	sACC l	–5.54	25.62	–3.69	338
pACC	pACC l	–2.92	24.73	22.1	1,969
MCC	MCC l	–6.35	4.4	35.95	2,804
Frontal medial BA10	BA10 l	–28	53.43	18.97	1,399
Anterior insular cortex	aIC l	–38.96	6.44	5.96	4,468
Posterior insular cortex	pIC l	–40.16	–16.69	16.27	3,997
Operculum	S2 l	–52.93	–16.92	17.13	2,821
S1	S1 l	–56.55	–26.32	37.28	2,953
BA40	BA40 l	–55.83	–30.72	32.92	4,677
M1 BA4	M1 l	–36.29	–23.62	56.14	885
Right hemisphere (contralateral)					
Thalamus	Thal r	11.22	–14.78	7.95	2,682
Caudatus	Caud r	14	0.31	16.93	1,171
Putamen	Put r	19.42	6.12	8.97	704
Amygdala	Amyg r	21.06	–9.46	–16.98	1,243
Hippocampus	Hippoc r	29.44	–25.4	–11.19	1,672
sACC	sACC r	5.1	26.17	–4.8	705
pACC	pACC r	5.56	24.52	22.64	2,362
MCC	MCC r	5.37	3.84	36.3	2,851
Frontal medial BA9	BA9 r	16.74	50.68	31.36	897
Frontal medial BA10	BA10 r	6.92	54.11	19.61	1,217
Frontal lateral BA46	BA46 r	45.35	44.29	8.21	928
Anterior insular cortex	aIC r	38.99	5.52	6.01	4,492
Posterior insular cortex	pIC r	39.93	–17.67	15.83	4,351
Operculum	S2 r	52.94	–17.58	16.48	3,048
S1	S1 r	53.8	–25.7	36.33	1,626
BA40	BA40 r	56.06	–32.76	32.57	4,587

BA: Brodman area; sACC: subgenual anterior cingulate cortex; pACC: pregenual anterior cingulate cortex; MCC: midcingulate cortex; S1: primary somatosensory cortex; M1: primary motor cortex.

Table SII. *t-values of the scratch-related activations in the regions of interests (ROI) used for the analysis. The higher the value the stronger was the activation during the scratch bouts. Negative values indicate a decrease in the blood-oxygen-level dependent signal during the scratch bouts*

	Capsaicin		Histamine	
ROI	Placebo	Naltrexone	Placebo	Naltrexone
Left hemisphere				
Thalamus	**7.53**	**6.34**	**7.15**	**4.87**
Caudatus	1.95	2.36	3.25	1.41
Amygdala	**–5.03**	–1.03	**–3.82**	–3.28
Hippocampus	**–4.15**	–1.25	–2.92	–1.96
sACC	–2.72	–1.36	–1.33	–2.78
pACC	**6.70**	**4.02**	**9.35**	**3.50**
MCC	**9.53**	**7.65**	**10.23**	**8.48**
Frontal medial BA10	0.49	–1.07	0.51	0.16
Anterior insular cortex	**9.19**	**8.95**	**9.52**	**8.64**
Posterior insular cortex	**12.27**	**13.52**	**12.21**	**11.94**
Operculum	**13.48**	**15.38**	**12.98**	**14.70**
S1	**10.89**	**13.63**	**10.79**	**14.57**
BA40	**10.47**	**12.31**	**11.56**	**14.47**
M1 BA4	**–6.24**	**–5.91**	**–6.92**	**–4.96**
Right hemisphere				
Thalamus	**9.18**	**6.56**	**8.83**	**6.24**
Caudatus	2.74	2.18	**4.01**	**1.63**
Putamen	**4.86**	**3.56**	**4.91**	**3.66**
Amygdala	**–5.70**	–2.21	**–3.30**	–2.44
Hippocampus	**–7.91**	**–6.98**	**–7.00**	**–6.42**
sACC	**–4.03**	–2.12	–0.97	–0.54
pACC	**7.41**	**3.43**	**8.96**	3.27
MCC	**10.58**	**7.86**	**10.97**	**7.84**
Frontal medial BA9	1.06	2.04	2.35	0.56
Frontal medial BA10	1.06	1.54	0.65	–0.75
Frontal lateral BA46	2.57	2.61	**4.58**	1.40
Anterior insular cortex	**9.88**	**8.70**	**11.13**	**9.60**
Posterior insular cortex	**14.41**	**13.28**	**15.01**	**11.84**
Operculum	**13.80**	**13.40**	**14.45**	**12.83**
S1	**9.61**	**12.08**	**9.25**	**12.00**
BA40	**10.26**	**9.70**	**9.90**	**11.27**

Significant *t*-values ($p < 0.001$) are given in bold.
sACC: subgenual anterior cingulate cortex. pACC: pregenual anterior cingulate cortex. MCC: midcingulate cortex. S1: primary somatosensory cortex. M1: primary motor cortex; BA: Brodman area.

Table SIII. *Multiple regression analysis of the correlation between ratings and activation during the "scratch bouts" (β-values derived from the general linear model, single studies) in the placebo experiments, for the independent variables: "itch relief by scratching", "itching", "burning", "stinging", "pricking"*

ROI	Number of ratings	Adjusted R^2	Ratings	T	*p*
Capsaicin					
BA40 left	2	0.618	Burning	–4.945	<0.000
			Relief by scratching	3.628	<0.003
Posterior insular cortex right	3	0.581	Burning	–3.823	<0.002
			Stinging	2.832	<0.015
			Itching	–2.519	<0.027
Caudatus left	2	0.466	Burning	–3.508	<0.004
			Pricking	2.506	<0.026
Hippocampus right	1	0.286	Itching	–2.647	<0.019
Caudatus right	1	0.263	Stinging	–2.521	<0.024
MCC right	1	0.244	Burning	–2.418	<0.030
Thalamus right	1	0.208	Itching	–2.220	<0.043
Putamen right	1	0.203	Burning	–2.197	<0.045
Histamine					
Operculum left	2	0.669	Itching	5.288	<0.000
			Stinging	2.693	<0.018
BA40 left	1	0.407	Relief by scratching	3.361	<0.005
MCC left	1	0.367	Pricking	3.115	<0.008
Hippocampus left	1	0.352	Relief by scratching	3.026	<0.009
Posterior insular cortex right	1	0.344	Pricking	2.980	<0.010
Operculum right	1	0.326	Pricking	2.872	<0.012
Anterior insular cortex left	1	0.279	Pricking	2.611	<0.021
Posterior insular cortex left	1	0.271	Itching	2.566	<0.022
Amygdala right	1	0.23	Relief by scratching	2.343	<0.034
MCC right	1	0.23	Pricking	2.341	<0.035
Lateral front BA46 right	1	0.224	Stinging	2.307	<0.037
Caudatus left	1	0.217	Relief by scratching	2.270	<0.040
Amygdala left	1	0.197	Relief by scratching	2.163	<0.048

The 23 ROIs selected were those that showed significant activations during the itch-scratch cycle under histamine and placebo medication (see Table SI[1]).

ROI: region of interest (dependent variable in the multiple regression analyses); Number of ratings: number of ratings contributing significantly to the multiple regression model; Adjusted R^2: variance explained by the model; Ratings: rating quality contributing significantly to the model; T: *t*-values of the respective rating within the model. A positive *t*-value shows a positive correlation of this rating with the blood-oxygen-level dependent changes (β-value) of the ROI, negative *t*-values conversely. *p*: significance level of the respective *t*-value.

Table SIV. *Multiple regression analysis of the correlation between ratings and activation during the "high itch period" (β-values derived from the general linear model (GLM), single studies) in the placebo experiments. For details, see legend to Table SII*[1]

ROI	Number of ratings	Adjusted R^2	Ratings	*t*-value	*p*-value
Capsaicin					
Hippocampus right	2	0.481	Burning	3.880	<0.002
			Stinging	–2.838	<0.014
S1 left	1	0.194	Relief by scratching	–2.146	<0.050
Histamine					
sACC left	1	0.314	Relief by scratching	–2.804	<0.014
Lateral front BA46 right	1	0.221	Relief by scratching	–2.293	<0.038

ROI: region of interest; sACC: subgenual anterior cingulate cortex.