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.