**Manuscript outline:**

**Title: Network effect sizes and reliability of the multivariate neurological signatures of pain**

**Summary:**

There is an increasing movement towards establishing neurophysiological markers of mental processes. A priori markers can provide quantitative predictions that can be tested across laboratories, serve as targets for interventions, and increase reproducibility by decreasing analytic flexibility. As markers become more widely shared across labs and translated into practical applications, robust validation of their psychometric properties and performance benchmarks across contexts will become increasingly important. Three such signatures in pain research domain are the Neurologic Pain Signature (NPS); (SIIPS) and (PDM), multivariate ‘signatures’ that track pain induced by nociceptive input.

Here, we analyze the pain effect sizes and reliability of three signatures across more than 20,000 fMRI images related to single-stimulus epochs collected in 408 participants from 12 studies. We compared the effect sizes and reliabilities across three multivariate pain signatures. We also compared three different methods to extract the features of these signatures. Finally, we assessed effect size and reliability in partial and local regions compared with whole pain signatures.

**Research Question:**

There are significant questions about neuroimaging -- standard measures in psychometrics and testing. how reliable is it? how reproducible? can it be useful as as neuromarker? This must be asked in each domain….pain? (IASP task force, Robinson) NPS is interesting example….how well does it perform, and where do we need to go from here? The primary aim of this study is to characterize the NPS to help understand its properties and neuroscientific basis. And: can it be useful in identifying specific pathways and sub-networks? Therefore:

1. we analyzed the reproducibility, reliability, and characteristics of the NPS (including relationship with stimulus intensity and pain report) across 25,000 fMRI images related to single-stimulus epochs collected in 300\*\*\* participants from \*\*\* studies.
2. we explored the connectivity and clustering of the distinct regions of the NPS to characterize the relationships among the primary local regions that contribute to the overall signature, and
3. We assessed the prediction performance of patterns in local regions compared with whole NPS.
4. we performed a large-scale decoding analysis to characterize the relationship between the distributed NPS pattern and a broad set of \*\*\*\* studies in the Neurosynth database.

**Methods:**

**Participants:**

The data used for this study are based on a single trial database on healthy subjects during pain tasks including comprehensive behavioral and fMRI data. Our data set included overall ∼22.000\*\* single-trial images of fMRI activity associated with multiple levels of noxious heat and pain ratings, across over health 410\*\*\* participants from 13\*\*\* studies. Descriptive data on age, sex and other features of each study sample are given in Table 1. In all studies, participants received a series of contact-heat stimuli and rated their individually experienced pain following each stimulus. The number of trials, stimulation sites, rating scales and stimulus intensities and durations varied across studies, but were comparable; these variables are summarized in Table 2. Each study also comprises a specific psychological manipulation, such as cue-induced expectation and placebo treatment. In the studies included, we focused only on residual pain ratings (ratings after removing noxious stimulus intensity) and the NPS irrespective of the study-specific psychological manipulations.

Most data from the studies have been used in previous publications (see Table 1 and ref. Lindquist et al. 2015 and Woo et al. 2017) except for study 9 (bmrk5) and study 12 (MPA1). However, the analyses and findings reported here are novel and have not been published elsewhere. All participants were recruited from New York City and Boulder/Denver Metro Areas between 2009 and 2017. The institutional review board of Columbia University and the University of Colorado Boulder approved all the studies, and all participants provided written informed consent. Preliminary eligibility of participants was determined through an online questionnaire, a pain safety screening form and an MRI safety screening form. Participants with psychiatric, physiological or pain disorders, neurological conditions, and MRI contraindications were excluded prior to enrollment. No participants were excluded from the study after screening other than individuals who, upon screening, provided different responses that made them now ineligible (eg, development of a physiological disorder). In addition, participants had to have sufficient data for data processing and statistical analyses (e.g. at least 23 trials with low variance inflation factors (<2.5), and non-missing painful heat rating and stimulation intensity data). Based on these criteria, an additional \*\*\* participants were excluded, resulting in a total of \*\*\* participants for the final analyses.

>>> *Table 1* “Demographics” <<<

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 1.** Study demographics and prior publications | | | | | |  |  |
| **No** | **Study name** | ***N*** |  | **Gender** | **Ages** | **Prior publications** | **Other experimental manipulations** |
| Study 1 | bmrk3 | 33 |  | 22 F | 27.9 (9.0) | Woo et al., 2015 Plos Biology, Lindquist et al. 2017 (GRIP), \*Woo et al. (SIIPS) 2017 | Cognitive self-regulation intervention to increase or decrease pain |
| Study 2 | bmrk4 | 28 |  | 10 F | 25.2 (7.4) | Chang et al., 2015, Krishnan et al., under review, Lindquist et al. 2017 (GRIP), \*Woo et al. (SIIPS) 2017 | Combination of painful stimuli with heat-predictive visual cues |
| Study 3 | scebl | 26 |  | 11 F | 28 (9.3) | Koban et al., in preparation; Lindquist et al. 2017 (GRIP), \*Woo et al. (SIIPS) 2017 | Combination of painful stimuli with heat-predictive visual cues and unreinforced social information |
| Study 4 | nsf | 26 |  | 9 F | 27.8 (7.5) | Wager et al., 2013; Atlas et al., 2014, \*Lindquist et al. 2017 (GRIP), \*Woo et al. (SIIPS) 2017 | Combination of painful stimuli with masked emotional faces evenly crossed with temperature |
| Study 5 | ie (ie for tor) | 50 |  | 27 F | 25.1 (6.9) | Roy et al., 2014 Nature Neuroscience, Lindquist et al. 2017 (GRIP), \*Woo et al. (SIIPS) 2017 | Combination of painful stimuli with heat-predictive visual cues and with a placebo manipulation |
| Study 6 | exp | 17 |  | 9 F | 25.5 (?) | Atlas et al., 2010 Journal of Neuroscience; Lindquist et al. 2017 (GRIP), \*Woo et al. (SIIPS) 2017 | Combination of painful stimuli with heat-predictive auditory cues |
| Study 7 | ilcp (ilcp\_wani) | 29 |  | 16 F | 20.4 (3.3) | Schmidt et al., in preparation; Lindquist et al. 2017 (GRIP), \*Woo et al. (SIIPS) 2017 | Combination of painful stimuli with intervention for perceived control (making vs. observing cue choice) and expectancy (80% vs. 50% probabilities of low pain) |
| Study 8 | dpsp | 59 |  | 31 F | 20.8 (3.0) | Kross et al., 2011; Woo et al., 2014 | Combination of painful stimuli with placebo intervention |
| Study 9 | bmrk5\_painsound | 88a |  | 44 F | 28.82 (5.67) | Losin et al. In review | None |
| Study 10 | IE2 | NA |  | NA | NA | NA | NA |
| Study 11 | REMI | 21 |  | 11 F | 24.7 (4.18) | Atlas et al. 2012, Wager et al. 2013 | Combination of painful stimuli with remifentanyl application (under two conditions: open and hidden administration). |
| Study 12 | MPA1\_A | 32b |  | 11 F | 25.1 (7.8) | in prep | Noone |
| Study 14 | placebo\_value\_stephan | 40 |  | 0 F | NA | Geuter et al.  2013 NeuroImage, Büchel et al. 2014 Neuron, Zunhamer et al. in prep. | Combination of painful stimuli with placebo intervention |
| **\***  **a)** 9 subjects were excluded of the initial data set for matching reasons; b) 8 subjects were excluded of the initial data set because of incomplete/bad data quality (pain intolerable, movement, no ratings, co-morbidities) | | | | | | | |

**Materials and Procedures**

**Thermal stimulation**

In each study, we delivered thermal stimulation to multiple skin sites using a TSA-II Neurosensory Analyzer (Medoc Ltd., Chapel Hill, NC) with a 16 mm Peltier thermode endplate (Study SCEBL: 32 mm). On every trial, after the offset of stimulation, participants rated the magnitude of the warmth or pain they had felt during the trial on a visual analog scale or labelled magnitude scale. Other thermal stimulation parameters varied across studies, with stimulation temperatures ranging from 41 °C to 50 °C\*\*\* and stimulation durations from 1,85 to 16 seconds\*\*\*. Most studies applied thermal stimulation to the glabrous skin of the left forearm; Study BMRK 4 (additionally to the dorsum of the left foot) and SCEBL (leg only) applied the stimulation to the lower extremity. See Table 2\*\*\*\* for stimulation duration, intensity levels and location of stimulus site, details of the rating scales, duration of inter-stimulus interval, and number of trials per subject. .

>>> *Table 2a* “Pain stimuli information” <<<

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| **Table 2.** Pain stimuli information | | | | | | | | | |  |  |
| **Study number** | **Stimulated locations** | **Number of sites** | **Mean pain ratings by intensity level (within subjects SEM)** | **Number of intensity levels** | **Fixed vs. calibrated** | **Stimulus Duration (seconds)** | **Rating Scale** | **Other experimental manipulations** |
| Study 1 (bmrk3) | Arm | 2 | 49.1, 56.6, 74.3, 99.4, 133.0, 159.3 (3.12) | 6 (44.3-49.3ºC) | Fix | 12,5 | 0-200 VASa | Cognitive self-regulation intervention to increase or decrease pain |
| Study 2 (bmrk4) | Arm, Foot | Arm: 4, Foot:4 | UL: 31.7, 40.5, 53.6 (0.9787) LL: 31.5, 40.2, 53.3 (0.96) | 3 (46-48ºC) | Fix | 11 | 0-100 LMSb | Heat-predictive viCombination of painful stimuli with heat-predictive visual cues sual cues for low, medium, and high pain |
| Study 3 (scebl) | Leg | 6 | 26.0, 33.3, 40.4 (1.12) | 3 (48, 49, 50) | Fix | 1,85 | 0–100 VAS | Combination of painful stimuli with heat-predictive visual cues and unreinforced social information |
| Study 4 (nsf) | Arm | 3 | 2.0, 2.8, 4.2, 6.6 (0.14) | 4 (PT/L/M/H) | Cal | 10 | 0-10 VASc | Combination of painful stimuli with masked emotional faces evenly crossed with temperature |
| Study 5 (ie) | Arm | 6 | 29.4, 38.9, 51.9 (0.64) | 3 (46-48ºC) | Fix | 11 | 0-100 VASd | Combination of painful stimuli with heat-predictive visual cues and with a placebo manipulation |
| Study 6 (exp) | Arm | 4 | 2.5, 4.3, 7.4 (0.13) | 4 (L/M/H) | Cal | 10 | 0-10 VASc | Combination of painful stimuli with heat-predictive auditory cues |
| Study 7 (ilcp) | Arm | 2 | 24.3, 46.7 (1.14) | 2 (L44.7°/H46.7) | Cal | 10 | 0-100 VASd | Combination of painful stimuli with intervention for perceived control (making vs. observing cue choice) and expectancy (80% vs. 50% probabilities of low pain) |
| Study 8 (dpsp) | Arm | NA | NA | 2 | NA | 15 | NA | Combination of painful stimuli with placebo intervention |
| Study 9 (bmrk5) | Inner forearm | 4 | L: M=32.10, SD= 20.22; M: M= 41.94, SD= 20.36;  H: M=50.34, SD= 21.20\* | 3 (47°C, 48°C, 49°C) | Fix | (8 and) 11 | 0-100 LMSb | none |
| Study 10 (ie2) | NA | NA | NA | NA | NA | NA | NA | NA |
| Study 11 (remi) | Arm | 3 | NA | 2 (L41.16°C±2.64/ H47.05°±1.69C) | Cal | 10 | 0-10 VASc | Combination of painful stimuli with remifentanyl application (under two conditions: open and hidden administration). |
| Study 12 (mpa1) | Thumb | 1 |  | 4 | Fixed | 10 | gLMS avoidance | none in this session (MPA1\_A) |
| Study 14 (stephan) | Arm | 4 | 57.2 (SD: 21.03 ) | 1 | Cal | 16 plateau (20 total) | 0-100 VAS | Combination of painful stimuli with placebo intervention |

Note: PT, pain threshold; L, Low painful heat; M, Medium painful heat; H, High painful heat; F, Fixed; C, Calibrated; VAS, visual analog scale. LMS, labelled magnitude scale. aPain vs. no-pain decision followed by 0-100 VAS for either warmth or pain rating (=2x100). b:0, no sensation; 1.4, barely detectable; 6.1, weak; 17.2, moderate; 35.4, strong; 53.3, very strong; 100, strongest imaginable sensation. c:0, no sensation; 1, non-painful warmth; 2, lopain; 5, moderate pain; 8, maximum tolerable pain. d:0, no pain; 100, worst imaginable pain. \*The data of study 9(bmrk5) in the rating column it is not AUC of the continuous rating transformed into a 0-100 scale but the “rating” measure is actually the peak of the continuous rating.

**fMRI Analysis**

**Preprocessing**. >>> *Table S* “Acquisition parameters” <<<

Structural T1-weighted images were co-registered to the mean functional image for each subject using the iterative mutual information based algorithm implemented in SPM (Ashburner and Friston, 2005), and were then normalized to MNI space using SPM. Following SPM normalization, Studies 4 (nsf) and study 6 (exp) included an additional step of normalization to the group mean using a genetic algorithm-based normalization (Atlas et al., 2010, 2014; Wager and Nichols, 2003). In each functional dataset, we removed initial volumes to allow for image intensity stabilization (see Table \*\*\* for details on acquisition and preprocessing parameters). We also identified image-intensity outliers (that is, ‘spikes’) by computing the mean and standard deviations (SD, across voxels) of intensity values for each image for all slices to remove intermittent gradient and severe motion-related artefacts present to some degree in all fMRI data. To identify outliers, we first computed both the mean and the S.D. of intensity values across each slice, for each image. Mahalanobis distances for the matrix of (concatenated) slice-wise mean and standard deviation values by functional volumes (over time) were computed. Any values with a significant χ2 value (corrected for multiple comparisons based on the more stringent of either false discovery rate or Bonferroni methods) were considered outliers. In practice, less than 1% of images were deemed outliers. The outputs of this procedure were later included as nuisance covariates in the first level models. Next, functional images were corrected for differences in the acquisition timing of each slice and were motion-corrected (realigned) using SPM. The functional images were warped to SPM's normative atlas (warping parameters estimated from co-registered, high-resolution structural images), interpolated to 2 × 2 × 2 mm3 voxels, and smoothed with an 8 mm FWHM Gaussian kernel. This smoothing level has been shown to improve inter-subject functional alignment, while retaining sensitivity to mesoscopic activity patterns that are consistent across individuals (Shmuel et al. 2010).

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| **Table 3.** Acquisition parameters | | |  | |  | |  |  |  |  |  |  |  |
| **Study number** | **No. of trials/ subjects** | **Mean No. of excluded trials  (high VIFs)** | **Study location** | **Scanner details** | **EPI parameters** | **Voxel size (mm3)** | **Acquisition parameters** | **Discarded volumes** | **Stimulus software** | **Analysis software** |
| **Study 1** (bmrk3) | 97 | 6,8 | Columbia | 3T Phillips Achieva TX | TR = 2000ms; TE = 20ms; FOV = 224mm; Matrix = 64 x 64; Flip angle = 72° | 3.0 x 3.0 x 3.0 | 42 Slices Interleaved SENSE = 1.5 | 4 | E-prime | SPM8 |
| **Study 2** (bmrk4) | 81 | 6,1 | CU Boulder | 3T Siemens Tim Trio | TR = 1300ms; TE = 25ms; FOV = 220mm Matrix = 64 x 64; Flip angle = 50° | 3.4 x 3.4 x 3.4 | 26 Slices Interleaved iPAT = 2 | 6 | Matlab | SPM8 |
| **Study 3** (scebl) | 96 | 4 | CU Boulder | 3T Siemens Tim Trio | TR = 1300 ms; TE = 25ms; FOV = 220mm Matrix = 64 x 64; Flip angle = 50° | 3.4 x 3.4 x 3.4 | 26 Slices Interleaved iPAT = 2 | 3 | E-prime | SPM8 |
| **Study 4** (nsf) | 48 | 2,7 | Columbia | 1.5T GE Signa TwinSpeed Excite HD | TR = 2000ms; TE = 3 ms; FOV = 224mm Matrix = 64 x 64 | 3.5 x 3.5 x 4.0 | 29 Slices | 5 | E-prime | SPM5, 8 |
| **Study 5** (ie) | 48 | 5,7 | CU Boulder | 3T Siemens Tim Trio | TR = 1300ms; TE = 25ms; FOV = 220mm Matrix = 64 x 64; Flip angle = 75° | 3.4 x 3.4 x 3.0 | 26 Slices Interleaved iPAT = 2 | 6 | E-prime | SPM8 |
| **Study 6** (exp) | 64 | 2,1 | Columbia | 1.5T GE Signa TwinSpeed Excite HD | TR = 2000ms; TE = 40ms; FOV = 224mm Matrix = 64 x 64; Flip angle = 84° | 3.5 x 3.5 x 4.55 | 24 Slices T2\*-weighted spiral in/out pulse | 5 | E-prime | SPM5 |
| **Study 7** (ilcp) | 64 | 1 | CU Boulder | 3T Siemens Tim Trio | TR = 1980ms; TE = 25ms; FOV = 220mm Matrix = 64 x 64 Flip angle = 75° | 3.4 x 3.4 x 3.0 | 35 Slices Interleaved iPAT = 0 | 5 | E-prime | SPM8 |
| **Study 8** (dpsp) | 16 | 0 | NA | NA | NA | NA | NA | NA | NA | NA |
| **Study 9** (bmrk5) | 36 heat trials | 3.5 | CU Boulder | 3T Siemens Tim Trio | \*TR = 460ms; TE = 29ms; FOV = 248mm; Flip angle = 44° | \*3.0 x 3.0 x 3.0 | 56 slices, multiband factor: 8 | 20\*\* | MATLAB | SPM8 |
| **Study 10** (ie2) |  | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| **Study 11** (remi) | 64 |  | Columbia | 1.5T GE Signa TwinSpeed Excite HD | TR = 2000ms; TE = 34ms; FOV = 224mm Matrix = 64 x 64 | 3.5 x 3.5 x 4.0 | 28 Slices | 5 | E-prime | SPM5 |
| **Study 12** (mpa1) | 8 per run, 4 runs = 32 total | 0 | CU Boulder | 3T Siemens TrioTIM | TR = 473ms, TE = 29ms, FOV 220mm, Matrix 64 x 64, Flip angle = 44°, multiband factor 8 | 2.7 x 2.7 x 2.7 | 56 slices, interleaved, P>>A, PAT off | NA | Matlab | SPM8 |
| **Study 13** (stephan) | 60 | NA | UKE Hamburg | 3T Siemens Tim Trio | TR = 2580 ms; TE = 26ms; FOV = 220mm  Flip angle = 80° | 2 x 2 x 3 | 42 Slices Interleaved = 1 mm | 4 | Cogent (Matlab Toolbox) | SPM8 |

Note: R, Time to repeat; TE, Time to echo; FOV, Field of view. \*First 25 participants were assessed with TR = 460ms, TE = 29ms, FOV = 248mm, and a Flip angle = 44°, than because of inference problems between multiband sequence and thermal stimulator after software uptdate, the remaining 63 participants were assessed with TR = 1300ms, TE = 25ms, FOV = 220mm, a Flip angle = 50°, and a voxel size of 3.4 x 3.4 x 3.4 mm; standard EPI; \*\* Discarded volumes for the remaining 63 subjects: 7.

**Single trial analyses**

Single trial analysis (Except Study 2/bmrk4 and Study 6/exp). For each study a single trial, or “single-epoch”, design and analysis approach was employed to model the data. Quantification of single trial response magnitudes was done by constructing a GLM design matrix with separate regressors for each trial, as in the “beta series” approach (Mumford et al., 2012; Rissman et al., 2004). First, boxcar regressors, convolved with the canonical hemodynamic response function (HRF), were constructed to model cue, pain, and rating periods in each study. Then, we included a regressor for each trial, as well as several types of nuisance covariates. Because each trial consisted of relatively few volumes, trial estimates could be strongly affected by acquisition artifacts that occur during that trial (e.g. sudden motion, scanner pulse artifacts, etc.). Therefore, trial-by-trial variance inflation factors (VIFs; a measure of design-induced uncertainty due, in this case, to collinearity with nuisance regressors) were calculated, and any trials with VIFs that exceeded 2.5 were excluded from the analyses. For Study 4 (nsf), we also excluded global outliers (trials that exceeded three SDs above the mean), and employed a principal components based denoising-step during preprocessing to minimize artifacts. This approach generated single trial estimates that reflect the amplitude of the fitted HRF on each trial and refer to the magnitude of anticipatory and pain-period activity for each trial in each voxel. Single trial analysis for Study 2 (bmrk4) and Study 6 (exp) \*\*\* were based on fitting a set of three basis functions, rather than the standard HRF used in the other studies. This flexible strategy allowed the shape of the modeled hemodynamic response function (HRF) to vary across trials and voxels. This procedure differed from that used in other studies because (a) it maintains consistency with the procedures used in the original publication on Study 6 (exp) (Atlas et al., 2010), and (b) it provides an opportunity to examine predictive performance using a flexible basis set. For both studies, the pain period basis set consisted of three curves shifted in time and was customized for thermal pain responses based on previous studies (Atlas et al., 2010; Lindquist et al., 2009). To estimate cue-evoked responses for Study 6, the pain anticipation period was modeled using a boxcar epoch convolved with a canonical HRF. This epoch was truncated at 8 s to ensure that fitted anticipatory responses were not affected by noxious stimulus-evoked activity. As with the other studies, we included nuisance covariates and excluded trials with VIFs > 2.5. In Study 6 we also excluded trials that were global outliers (those that exceeded 3 SDs above the mean).We reconstructed the fitted basis functions from the flexible single trial approach to compute the area under the curve (AUC) for each trial and in each voxel. We used these trial-by-trial AUC values as estimates of trial-level anticipatory or pain-period activity.

**Computing Neurologic Pain Signature (NPS) responses.**

We computed for each subject a single scalar value representing their expression of the NPS pattern in response to the thermal pain stimulus (using the contrast [Pain Stimulation minus Baseline] images as detailed below). For this analysis, we separated NPS regions likely to be related to nociceptive pain (associated with pain-evoked activation in the NPS) from those that play other modulatory roles (associated with pain-evoked deactivation in the NPS). In most of the regions in the NPS, pain is associated with increased overall activity. Such regions include the major targets of ascending nociceptive afferents, including the thalamus, secondary somatosensory regions (SI/SII), posterior, mid and anterior insula and adjacent opercula, midbrain, dorsal anterior cingulate cortex (dACC), inferior frontal gyrus and amygdala (REF). We refer to pattern responses in this set of regions as the “Nociception-positive NPS” (NPSp). In a subset of other medial regions, including the perigenual ACC (pgACC) and the PCC (posterior cingulate)/precuneus/paracentral lobule, pain was associated with deactivation in the original NPS pattern. These regions are not strongly linked to nociception and are not direct targets of nociceptive afferents, rather they have been associated with a variety of affective, autonomic, social, self-referential, and decision-making functions [Roy et al]. We refer to responses in this set of regions as the “Nociception-negative NPS” (NPSn), and analyze this pattern separately from the NPSp due to its differential functional characteristics and considering the particular role of these regions, mostly the pgACC, in chronic pain. Of note, the local pattern of voxel weights is exactly the same as in the original NPS within the two NPS components (NPSp and NPSn).

**Connectivity analyses:**

Defining a brain pattern sensitive and specific to pain is a critical first step towards developing meaningful models of brain networks of nociceptive processing. The NPS offers the opportunity to characterize the basis of this pattern representation within and across brain networks. We therefore explored the distinct subnetworks of the NPS. For this analysis, we calculated pattern responses within each of the largest regions in the NPS (*p* < .001, k = 10 voxels; for every individual trial within each participant and used a robust clustering algorithm to group the NPS regions into separate networks based on similar patterns of trial-by-trial covariation. The best solution contained three separate clusters for positive weights, and three for negative weights, which provides a descriptive characterization of the subnetworks that comprise the NPS.

We used hierarchical agglomerative clustering to find predictive regions that showed similar response profiles across trials. For this analysis, we extracted contiguous regions from the NPS that survived the *p* < .001 uncorrected threshold and contained a minimum of ten voxels. These regions provided the strongest contributions to the NPS. Region-specific pattern response values to each trial (*n*= xxx\*\*\*) were rank ordered and normalized within-subject to (a) provide statistically robust connectivity estimates, as in nonmetric multidimensional scaling algorithms (Shepard 1980), and (b) reflect within-subject “beta-series” connectivity, which is both less susceptible to imaging artifacts than raw connectivity (Rissman et al 2004) and insensitive to individual differences in hemodynamic variables. Inter-region connectivity matrices were calculated aggregating across trials and subjects and subjected to hierarchical agglomerative clustering with Euclidean distance using the Ward minimum variance algorithm. Clusters were determined using an arbitrary threshold of 31%\*\*\* of the maximum distance, which resulted in nine distinct clusters.

**Metaanalytic large-scale decoding analysis**

De La Vega CoSinus Linearity (google neurosynth) Pauli et al. 2016, PNAS:

“We relied on the NeuroSynth database to gain a comprehensive and unbiased window into coactivation of the NPS other networks. The NeuroSynth database contains activation coordinates for 5,809 functional MRI (fMRI) studies that were not selected for specific criteria, or with regard to the psychological processes under investigation, but only for the presence of reported brain activations; hence, it is highly representative of the broader neuroimaging field (Haber et al 2010). In this database, 10-mm boxcar smoothing was applied to activation coordinates to increase the robustness against differences in smoothing kernels across studies. We used k-means clustering to identify functional activity, based on whether they showed similar coactivation patterns across studies. Details are provided in Pauli et al 2016).

To identify the psychological functions associated with the Neurological Pain Signature (NPS) with an unbiased, data-driven approach, we calculated for each psychological term included in the NeuroSynth database its likelihood ratio of appearing in a study, given that activation was reported anywhere in the NPS (i.e., the ratio between the number of studies reporting activation in the NPS when this term was vs. was not used in the article). It takes into account both the mean word frequency of each term across studies and the base rate of activation. The terms, however, were not associated with any specific fMRI activity, but only with the article as a whole.” In addition, the association of the NPS pattern with different functional networks was explored. To identify functional networks associated with the Neurological Pain Signature (NPS), we calculated for each functional network included in the NeuroSynth database its likelihood ratio of appearing in a study, given that activation was reported anywhere in the NPS.

**Results**

**Evoked pain response**

**fMRI Results**

**Subheader 1.** **Characteristics of the Neurological Pain Signature pattern**

Brief description of the NPS based on whole sample (all studies) to derive the regions of interest used for the connectivity analyses

*>>> Figure \*\*\*: NPS and Stimulus intensity-induced brain activity*

*A screenshot of a map

Description automatically generated.*   
A close up of a map

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Figure \*\*\* A) The multivariate pattern of fMRI activity predictive of pain ratings represents the NPS. B) Brain activity of the NPS in regard to stimulus intensity. The sharp bend in the brain response curve at 45° is in line with the activation threshold of thermal nociceptors. C) Z-scored quartile pain ratings versus crossvalidated (leave-one-participant-out) prediction (also z-scored and quartile binned) with the NPS stratified for the different single-trial-studies.

*Figure 1: NPS and Stimulus intensity-induced brain activity.*

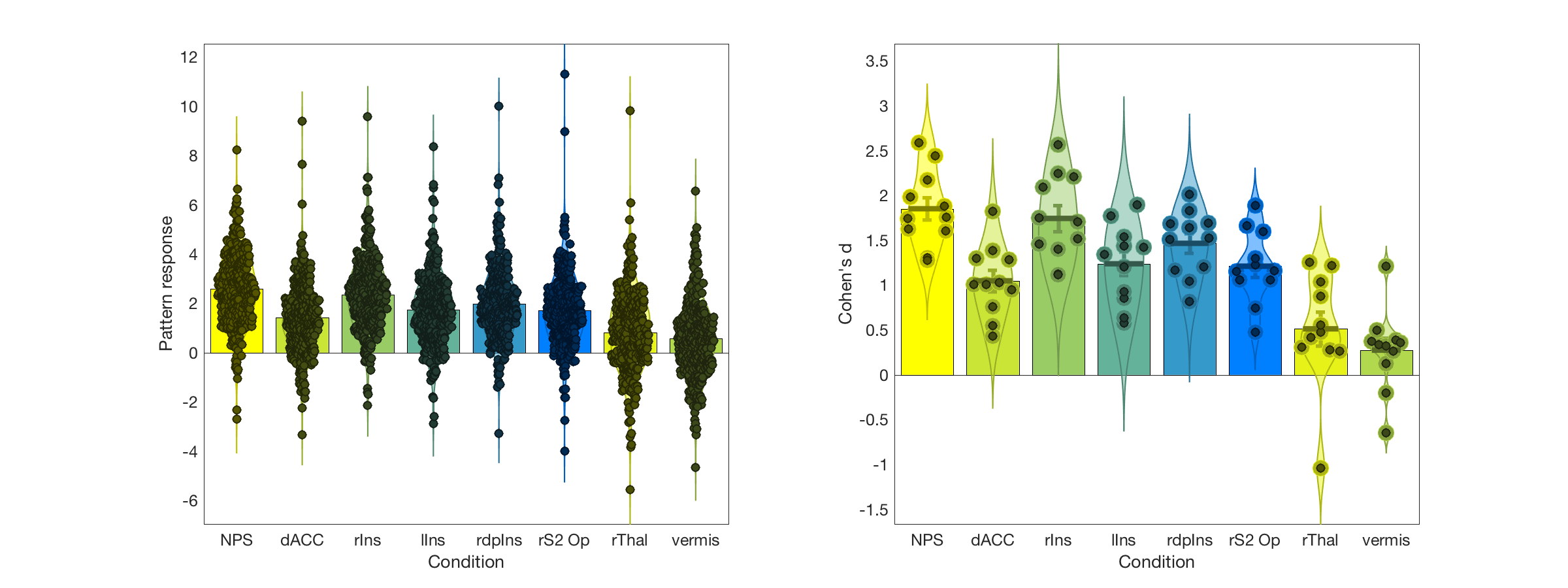
A screenshot of a cell phone

Description automatically generatedFigure \*\*\* A) The multivariate pattern of brain activity of the NPS correlates with the subjective experience of pain. Shown are the correlation coefficients of the NPS with subjective pain report stratified for the different single-trial-studies.

>>>Table**.** Top 7 regions of the NPS that show positive and negative correlations with stimulus intensity and residual pain ratings response

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table \*\*\*.** Top 7 regions of the NPS that show positive correlations with stimulus intensity and residual pain ratings response | | | | | | | | | | | |
| **Correlations with:** | **Stimulus intensity** | | |  | **Residual pain ratings** | | |  | **NPS response** | | |
|  | regions | r | p |  | regions | r | p |  | regions | r | p |
| Rank 1 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 2 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 3 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 4 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 5 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 6 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 7 |  |  |  |  |  |  |  |  |  |  |  |

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| **Table \*\*\*\*.** Top 7 regions of the NPS that show negative correlations with stimulus intensity and residual pain ratings response | | | | | | | | | | | |
| **Correlations with:** | **Stimulus intensity** | | |  | **Residual pain ratings** | | |  | **NPS response** | | |
|  | regions | r | p |  | regions | r | p |  | regions | r | p |
| Rank 1 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 2 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 3 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 4 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 5 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 6 |  |  |  |  |  |  |  |  |  |  |  |
| Rank 7 |  |  |  |  |  |  |  |  |  |  |  |

>>>*Figure*. Benchmarking of the NPS and involved brain regions

*Figure legend: Thal: thalamus; S2: secondary sensory cortex; Op: operculum; vermis: cerebellar vermis; ACC: anterior cingulate cortex, Ins: insula, r: right, l: left; d: dorsal.*

**Subheader 2. Connectivity network of the Neurological Pain Signature**

*Figure 2.* Connectivity network in the NPS: Figure of the Connectivity Network of the NPS based on connectivity clusters (above), distinguishing between pro and anti-pain Pain Network Regions (below).  
A close up of a map

Description automatically generatedA close up of a map

Description automatically generated

*Figure 3.* reliability and temporal stability

>>>HereTable Weights (“energy”) ???<<<

**Subheader 3. Large-scale decoding analysis of the Neurological Pain Signature**

*Figure xxxx.* Large-scale decoding analysis of the neurological pain signature

*A screenshot of a cell phone

Description automatically generated*

*Figure legend: 1.) Association of the NPS pattern with different psychological concepts. To identify the psychological functions associated with the Neurological Pain Signature (NPS), we calculated for each psychological term included in the NeuroSynth database its likelihood ratio of appearing in a study, given that activation was reported anywhere in the NPS. Distribution of likelihood ratios across functional zones indicates a clear functional dissociation.*

*2.) Association of the NPS pattern with different functional networks. To identify functional networks associated with the Neurological Pain Signature (NPS), we calculated for each functional network included in the NeuroSynth database its likelihood ratio of appearing in a study, given that activation was reported anywhere in the NPS. Distribution of likelihood ratios across functional networks indicates an strong positive association with the ventral attentional network and to a lesser extent the somatomotor network and negative association with the default network.*