

ADDITIONAL DETAILS

To accompany:

Brain mediators of predictive cue effects on perceived pain

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Supplementary Materials and Methods

Genetic-algorithm based normalization.

The genetic algorithm-based warping is a refinement of the standard SPM5 warping to MNI space, which improves the inter-subject registration of brains to some degree. Images are still in MNI space; the only difference is that the gyral and sulcal patterns show greater overlap across subjects. Structural images were first segmented to gray and white matter and spatially normalized to a standard template brain (the MNI avg152T1.img) using SPM5's iterative segmentation/normalization algorithm (Ashburner and Friston, 2005) using default options (7 x 8 x 7 nonlinear basis functions). As the standard MNI template brain has a relatively low spatial resolution, we used the SPM5 normalization solution as the starting point for additional refinement using a genetic algorithm (GA, based on a similar implementation to (Wager et al., 2008b)). A study-specific, higher-resolution template was created by averaging the normalized SPM5-warped T1 images. We then used a GA to re-normalize each subject's T1 to the group-average template. The objective function was the variance explained by the best-fitting monotonic function (Kruskal, 1964), matching the individual subject T1 image to the group-average template. Each "generation" of the GA involved generating 30 candidate sets of warping parameters, evaluating the objective function on each, and then inter-mixing the

normalization parameter estimates for the best 50% of designs. 10 such “generations” of inter-mixing were performed for each subject. Warping all subjects to the template in this fashion constituted a “cycle”. At the end of each cycle, a new group mean was created by re-averaging the warped images. As the inter-subject registration becomes more precise, the template thus contains more anatomical detail. Ten total cycles were performed.

We assessed both correlation with the SPM5 “avg152T1.nii” template and mutual information between subjects, and found that the warping is a significant advance on the quality of SPM5’s default normalization algorithm. The results showed better gray-white matter separation and better gray matter/cerebrospinal fluid separation in the group anatomical image. This increases the validity of results, as subject anatomy is more closely normalized to the same space. We note that because we started with a group mean registered to MNI space, the resulting warped images were still registered with MNI space.

Single trial analysis.

The single trial, or “single-epoch”, design and analysis approach that we employ is a relatively common design in the study of pain processing using fMRI. This design allows researchers to characterize responses to nociceptive stimulation on a trial-by-trial basis, and to separate stimulation-related activity from rating period activity, while conserving power and minimizing the total amount of noxious stimulation that subjects must endure. There have been several papers demonstrating that single trial analyses are reliable and offer increased sensitivity, especially in modeling responses to pain (Koyama et al., 2003).

In this study, 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 of Rissman et al. (Rissman et al., 2004). However, we used a flexible basis set to model each trial,

thus allowing the shape of the modeled HRF to vary across trials and voxels (Bornhovd et al., 2002; Buchel et al., 2002; Duann et al., 2002). The basis set consisted of three curves shifted in time and was customized for thermal pain responses based on previous studies (Lindquist et al., 2008). A GLM design matrix was constructed that included three trial-specific regressors for each trial as well as several types of nuisance covariates: 12 regressors reflecting estimated head movement (x, y, z, roll, pitch, and yaw) and movement squared, the high-pass filtering matrix (highpass filtering at 120 Hz), indicator vectors for the first two images in each run, and indicator vectors for time points estimated as outliers based on analyses of global signal time series. Global outlier time points were identified by computing both the mean and the standard deviation of values in each image for each slice. Mahalanobis distances for the matrix of mean values (one per slice) x functional volumes were computed, and images with a value above 3 standard deviations were considered outliers. The same procedure was used for standard deviation values. The data for each voxel were regressed on this design matrix. For each trial in each voxel, we re-constructed the fitted response. We used the area under the curve (AUC) of each fitted response (for each trial within each voxel) as a summary estimate of pain-period activity.

This method requires event-related designs in which trials are spaced far enough in time so that the fMRI signal can return to baseline after each trial. The interval between thermal pain stimuli was 36 sec in this study, and the next event (the rating prompt) occurred 14 sec after pain stimulation had terminated. This study design and model were chosen because they allowed us to examine relationships among manipulated variables (temperature and expectation), brain responses, and pain reports across trials.

One important consideration in using trial-wise estimates rather than fixed canonical hemodynamic response functions is that estimates for a given trial will be strongly affected by acquisition artifacts that occur during that trial (e.g. sudden motion, scanner pulse artifacts, etc.). For this reason, trial-by-trial variance inflation factors (VIFs; a measure of design-induced uncertainty due in this case to colinearity with nuisance regressors) were calculated, and any trials with VIFs that exceeded 2 were excluded from first-level GLM analysis ($M = 1.94$). We also ensured that overall signal was relatively constant by downweighting subjects with extreme global signal values.

Voxel-wise trial-by-trial AUC parameters were then passed into first-level GLM analyses, excluding any trials with problematic VIFs or global signal values.

In Analysis 2, as well as to verify that our pain-evoked responses were not affected by anticipatory activity, we examined cue-evoked responses. These were also modeled using single trial analysis, though the underlying period was fit with a canonical hemodynamic response function, rather than the flexible basis sets employed to fit pain-evoked responses. In addition we truncated the anticipatory HRF in order to ensure that fitted anticipatory responses were not affected by pain-evoked activity.

Whole-brain multi-level mediation.

A typical fMRI model, such as the one we use to define our pain-related ROIs (HE-high – LE-low; described above), assesses the relationship between experimental manipulations (reflected in comparisons across trial types) and brain activity. These relationships are typically analyzed using a two-stage “summary statistics” procedure in which within-subjects effects (i.e., differences in fMRI activity between trial types) are estimated for each subject using a first-level analysis, and between-subjects effects are tested using a separate, second-level model, conducted

on the regression slopes or contrast values. This two-step procedure is a simplification of a full univariate mixed-effects model that considers both within-subjects (experimental manipulations) and between-subjects (individual differences) variation in the same model (Wager et al., 2008b). Our analyses incorporate a weighted least squares-based mixed-effects model that considers both sources of variation in the same model, and therefore makes fewer assumptions about homogeneity of variances across subjects.

The multi-level path modeling approach we employed extends the univariate mixed-effects model by including an additional outcome variable, reported pain. The path model thus assesses the standard analysis of experimental effects on fMRI activity (i.e. differences in fMRI activity between HE-Medium and LE-Medium trials), as well as several other effects in the context of a single structural equation model. In the current analysis, we examine relationships between expectancy manipulation (X), brain activity (M), and pain reports (Y); see Figure 2. As our mediation analysis focused on the pathway from manipulated expectancy to reported pain, only HE-Medium and LE-Medium trials were included in the multi-level mediation.

In addition, because it is a mixed-effects model, it incorporates both “first-level” (within-subjects; expectancy effects on pain period brain activity) and “second-level” (between-subjects; individual differences in expectancy effects) effects. Analyses are still performed using data from each brain voxel in a separate analysis, so that the full path model and effects of interest are tested substituting each brain voxel’s data for M . This approach, which we have referred to as Mediation Effect Parametric Mapping (Wager et al., 2008a), retains the flexibility of the statistical parametric mapping approach in locating voxels that show particular effects of interest, but extends it to evaluating effects of interest in a simple structural equation model. Voxel-wise whole-brain multi-level mediation analyses were conducted using a custom Matlab toolbox

(T.D.W.). MEPM was conducted as in (Wager et al., 2008a; Wager et al., 2009), with additional facilities incorporated to address the multi-level case. We describe statistical modeling of mediation effects below.

The $a*b$ mediation test is not equivalent to testing whether the conjunction (intersection) of both Path a and Path b effects are significant. In a single-level path model, the mediation test is significant in a subset of models that show both a and b effects. However, in a single- or multi-level path model, both a and b paths can be significant without an $a*b$ mediation effect under two conditions: First, the results for one path or the other may be too weak, even if the other is highly significant. Secondly, in a multi-level model, mediation implies that the a and b paths are functionally linked, so that if positive average a and b paths exist, but participants who show a strong a effect show a weak b effect (i.e., there is negative covariance between a and b), then the significance of the mediation test will be reduced in the case of positive mediation. Likewise, negative average a and b effects are opposed by negative covariance between a and b . This is expressed concisely by Kenny, Korchmaros, and Bolger (2003)(Kenny et al., 2003) in Eq. 9, which captures the following relationship:

$$\text{mean}(a*b) = \text{mean}(a)*\text{mean}(b) + \text{Cov}(a,b)$$

Hence, the $a*b$ effect can be driven by two different sources, the product of the means of a and b and the covariance between a and b . Therefore the average mediation effect can result from covariance as well as average $a*b$ effects. The covariance term reflects cases in which subjects that are below (or above) the mean on a are also below (or above) the mean on b . If positive average a and b paths exist, then the significance of the mediation test will be enhanced if there is positive covariance between a and b . On the other hand, it will be suppressed if there is negative covariance. Likewise, mediation effects in the presence of negative average a and b

effects are strengthened by positive covariance between a and b , and weakened by a negative covariance.

Thus, unlike single-level mediation, the multi-level mediation test can also identify regions that show evidence for mediation, even if the average a and b effects are not significant alone, due to the additional covariance term. The interpretation in this case is that there is a functional relationship between the magnitude of paths a and b , implying a functional pathway, but with substantial individual differences in the regression slopes. A significant $a*b$ effect in this case cannot be easily attributed simply to individual differences in the scale of the response variables. For example, individual differences in the scale of M will result in negative ab covariance, i.e., large path a effects for those with large M , and small path b coefficients for the same individuals. Individual differences in X or Y will affect only the a or b paths, respectively. Thus, unexplained individual variability in the variables themselves will tend to reduce the significance of either positive or negative mediation. Such results may point to the existence of a second-level moderator (i.e., individual differences variable) that might explain the covariance between the paths.

Covariance estimates are presented in Supplementary Table S4 for all mediator regions. In the current dataset, we verified that our observed mediation effects were not driven by individual differences in magnitude of evoked BOLD response or scanner artifacts, by 1) Verifying the covariance between Path a and Path b coefficients across brain regions followed a Normal distribution (suggesting this is not a function of global signal); and 2) Including average evoked response (trial-wise AUC) as a second-level moderator. None of the paths were significantly explained by this second-level moderation analysis for our regions of interest, so we believe that any covariance is more likely to be related to psychological processing, causing

subjects who show greater differences in activity between high and low pain expectancy to also report higher pain as a function of activity in that region.

Multi-level path modeling of fMRI data.

The advantages of using the structural model over a standard GLM approach are that: 1) It can provide tests of mediation effects; 2) It estimates several GLM equations in the context of a single path model, making it easy to localize regions that show a pattern of interest across multiple effects; and 3) within-subject measurement error is taken into account when conducting group analyses, providing increased efficiency if data quality is better for some subjects.

The system of linear equations underlying the path model are defined as follows. The first level (within-person) equations are of the form $Y_j = Z_j\beta_j + \varepsilon_j$, for $j=1, \dots, N$, where Z is a design matrix for participant j , β_j is a person-specific vector of regression coefficients, and ε_j is a residual vector. Each of the Z_j matrices in the multi-level mediation model contains an intercept column and one or two regressors of interest, as well as regressors for a set of nuisance covariates $Q_1 \dots Q_n$. Adopting the notational convention of (Kenny et al., 2003), the equations can be written:

$$Y_{ij} = d_{0j} + c_j X_{ij} + q_{1j} Q_{1ij} + \dots + q_{nj} Q_{nij} + r_{ij} = Z_j \beta_j + r_{ij} \quad (1)$$

$$M_{ij} = d_{1j} + a_j X_{ij} + q_{1j} Q_{1ij} + \dots + q_{nj} Q_{nij} + e_{ij} \quad (2)$$

$$Y_{ij} = d_{2j} + c_j' X_{ij} + b_i M_{ij} + q_{1j} Q_{1ij} + \dots + q_{nj} Q_{nij} + f_{ij} \quad (3)$$

Here the subscript i indexes observation (trial number) and j indexes participant. The parameters d_{0-2} represent intercept values, and $q_1 \dots q_n$ represent nuisance regression parameter estimates, neither of which are of further interest in our analysis. The parameters a and b estimate linear regression slopes, as described in our main Methods section. In addition, c represents the total relationship between X and Y , and c' represents the direct relationship controlling for M . As we

are interested in mediation effects, and complete mediation of observed behavioral effects by any single brain region is unlikely in the present analyses (i.e., a non-significant c'), c and c' are not discussed in detail in this report. Finally, r , e , and f represent error terms.

The second-level (between-person) equations are of the general form $\beta_j = \gamma + u_j$, indicating that an individual regression slope (β_j) is modeled as the sum of a population regression slope (γ) and a person-level error term (u) for that effect. Thus, the second-level structural equations as follows:

$$a_j = Z_g \gamma_a + u_{aj}; b_j = Z_g \gamma_b + u_{bj}; c_j = Z_g \gamma_c + u_{cj}; c_j' = Z_g \gamma_{c'} + u_{c'j} \quad (4-7)$$

The group second-level design matrix (Z_g) is in this case simply an intercept column. Equations for the intercept terms are not shown, but follow the same form. Thus, person-level intercept and slope estimates are treated as random effects. The within- person error terms (r_{ij} , e_{ij} and f_{ij}) and the between-person error terms (u_{0j} , u_{1j} , u_{2j} , u_{aj} , u_{bj} , u_{cj} and $u_{c'j}$) are each assumed to be normally distributed with a mean of zero and a unique, but constant, variance for each effect.

Model and variance component estimation. Full mixed-effects models typically iterate between estimation of model parameters and the within- person and between- person variance components using algorithms such as expectation-maximization (Dempster et al., 1977) or iterative generalized least squares or Fisher scoring (e.g., (Goldstein, 1986)). Variance component estimators are obtained from the residuals using restricted maximum likelihood (ReML) estimates (Goldstein, 1989; Johnson and Thompson, 1995). However, this procedure can be extremely computationally intensive, and much of the computation time is spent in iterative estimation of how much of the total error variance is attributed to within-subject (r , e , and f) and between-subject error terms (u 's). Here, we approximate the iterative solution using a 3-step re-weighting. This technique allows us to estimate the multi-level path model over

200,000 brain voxels in a reasonable amount of time (~6 hours on a 2008 Intel Mac 8-core workstation).

Specifically, the following procedures are used separately for each regression equation in the path model (e.g., Eqs. 1, 2, and 3), which are described in more detail below: a) Ordinary least squares estimation of regression parameters and variances; b) Estimation of precision values based on the inverse of naïve total (between-person plus within-person) variance estimates; c) Precision-weighted updating of regression parameters (re-weighting Step 1); d) Empirical Bayes updating of the between-person error variance and precision estimates (Step 2); and e) Estimation of final regression parameters using weighted least squares (Step 3). All of these procedures are implemented in the Multilevel Mediation/Moderation Toolbox (M3) v.0.9 (T.D.W. and M.L.), available from the authors.

$$\hat{\beta}_j = (Z_j^T Z_j)^{-1} Z_j^T Y_j \quad (8)$$

with residual variance

$$\hat{\sigma}_j^2 = (Y_j - Z_j \hat{\beta}_j)^T (Y_j - Z_j \hat{\beta}_j) / (t - k) \quad (9)$$

and parameter variance/covariance matrix

$$V_j = \text{cov}(\hat{\beta}_j) = (Z_j^T Z_j)^{-1} \hat{\sigma}_j^2 \quad (10)$$

For the mediation ($a*b$) effect, however, we replace Eq. 10 with the formula from Kenny, Korchmaros, and Bolger (2003)(Kenny et al., 2003):

$$V_j = \text{Var}(ab_j) = b_j^2 \text{var}(a_j) + a_j^2 \text{var}(b_j) + \text{var}(a)^2 \text{var}(b)^2 \quad (11)$$

We note that an autoregressive level-1 error structure was not specified in the model because although allowing for autocorrelation can produce maximally efficient inferential statistics, autocorrelation parameter estimates based on noisy level-1 data are not necessarily more efficient (Friston et al., 2000), and parameter estimates are unbiased in either case.

Let \hat{B} be the $N \times k$ matrix of parameter estimates across all subjects. An initial estimate of the between-subjects covariance component (U) is the $k \times k$ matrix:

$$\hat{U} = \text{cov}(\hat{B}) = (\hat{B}^T \hat{B}) / (N - 1) \quad (12)$$

where N is the number of participants. The precision matrix of each individual participant's estimates is thus the inverse of the sum of between- and within-subjects error estimates:

$$\hat{P}_j = (\hat{U} + \hat{V}_j)^{-1} \quad (13)$$

These initial estimates are used to provide weighted least squares estimates of the population regression slope and intercept parameters (the k -length vector $\hat{\gamma}$) (Raudenbush & Bryk, 2002(Raudenbush and Bryk, 2002), eq. 3.31):

$$\hat{\gamma} = \left(\sum_j \hat{P}_j \right)^{-1} \sum_j \hat{P}_j \beta_j \quad (14)$$

The regression slope parameters for each participant are re-estimated using an Empirical Bayes average of the original estimates and $\hat{\gamma}$ (Raudenbush & Bryk, 2002(Raudenbush and Bryk, 2002), eq. 3.56):

$$\hat{\beta}_j^* = \hat{U} \hat{P}_j \hat{\beta}_j + (I - \hat{U} \hat{P}_j) \hat{\gamma} \quad (15)$$

The original estimate of U will usually be an over-estimate, as no attempt is made to factor out within-subjects measurement error as a source of variance. Rather than perform a series of computationally demanding iterations, we use the Empirical Bayes estimates to provide a revised estimate of U that is shrunk towards zero: $\hat{U}^* = \text{cov}(\hat{\beta}^*)$. Finally, subject weights are calculated based on the total variance of each parameter estimate for each subject and normalized to sum to 1:

$$w_{.j} = \left(\sum_j \hat{P}_j^* \right)^{-1} \hat{P}_j^* \quad (16)$$

This new estimate of the population parameters is used to update the between-subjects covariance estimate.

These weights are used in the final second-level weighted least squares analysis for each effect of interest (a , b , $a*b$). For each effect, if W is a diagonal matrix containing the weight values w_j for each participant, then:

$$\hat{\gamma}_g = (Z_g^T W Z_g)^{-1} Z_g^T W \beta_j \quad (17)$$

In our primary model, the group design matrix Z_g consisted of an intercept only. The intercept term provides a test of significance for whether the average β_j differs from zero, and thus tests the reliability of Paths a , b , and $a*b$ across participants. In exploratory tests, Z_g included the intercept as well as an additional predictor of average trial-wise AUC estimates, which allowed us to verify that individual differences in response magnitude were not driving our observed effects (we discuss in the Methods section of the main manuscript).

Significance testing and inference for each effect was performed as follows. The residual-inducing matrix R aids in the estimation of variances and degrees of freedom:

$$R = W^{1/2} (I - Z^T (Z_g^T W Z_g)^{-1} Z^T W) \quad (18)$$

The error degrees of freedom are estimated based on the weighting using the Satterthwaite correction (Satterthwaite, 1946):

$$dfe = \text{trace}(RW^{-1})^2 / \text{trace}(RW^{-1}RW^{-1}) \quad (19)$$

and the parameter variance/covariance matrix is:

$$\text{cov}(\hat{\gamma}) = \frac{(R\hat{\beta}_j)^T (R\hat{\beta}_j)}{dfe} (Z_g^T Z_g)^{-1} \quad (20)$$

Standard t-values are obtained for purposes of inference using the equation:

$$t_{\gamma_k} = \frac{\hat{\gamma}_k}{\text{cov}(\hat{\gamma})^{kk}} \quad (21)$$

Uncorrected P-values are obtained from this t-test. We calculated p-values required to achieve $p < .05$ corrected for multiple comparison in a priori ROIs defined using our independent pain-processing localizer (see below). Using AFNI's AlphaSim program (Cox, 1996), the cluster extent threshold used in our analyses (3 voxels at $p < .001$) corresponded to an alpha level of $p < .05$ for most of our nine pain-processing ROIs. Only right middle insula and left cerebellum required a higher threshold (6 and 5 voxels, respectively). Because $p < .001$ is the most commonly used threshold in neuroimaging studies (Wager et al., 2007), we used the same threshold for whole-brain analyses as well, which serve the important functions of generating hypotheses for future studies and characterizing distributed patterns of activation.

Bootstrap significance testing. Bootstrap tests (Efron and Tibshirani, 1993) have been shown to be a useful way to assess mediation in small samples (Bollen and Stine, 1990; Stone and Sobel, 1990; Efron and Tibshirani, 1993; Shrout and Bolger, 2002). Bootstrapping provides a more accurate and generally more sensitive test for assessing the magnitude of indirect ($a \times b$) effects than the Sobel test (Sobel, 1982), which assumes a normal distribution of $a \times b$ estimates. Even if a and b path estimates may both be Normally distributed, the $a \times b$ product is not expected to be Normal. In our second-level analysis, we employ Efron's bias corrected, accelerated bootstrap (Efron and Tibshirani, 1993) in order to test the significance of all effects (a , b , and $a \times b$ p-values). We estimated distributions of subject-level path coefficients by randomly sampling with replacement 10,000 observations (rows) from the matrix of [a b ($a \times b$)] path coefficients for each voxel. Two-tailed P -values were calculated from the bootstrap confidence interval.

Intensity-processing localizer

Intensity processing regions were localized by a mega-analytic approach that assessed intensity processing across three experiments that all contrasted high- vs. low-intensity noxious thermal stimulation of the left volar forearm in the absence of pain-predictive cues (total $n = 75$). Individual contrasts between high and low painful stimulation were transformed to z-scores within study and normalized to MNI space. The group model included an intercept and covariates coding for differences between the three studies. We included these normalized images in a one-sample t-test of [high – low intensity] contrast values across the three different studies. Family-wise error correction ($p < .05$) using Gaussian Random Fields as implemented in SPM5 was used in order to identify voxels showing a significant [high – low intensity] effect, which was used to define the “pain-processing network.” Anatomical localization was determined based on the LONI Probabilistic Brain Atlas (Shattuck et al., 2007). This independent localizer approach revealed the following regions as comprising the PPN: bilateral superior, inferior, and middle frontal gyrus; bilateral precentral gyrus; right middle and bilateral lateral orbitofrontal gyrus; bilateral postcentral gyrus; bilateral supramarginal gyrus; bilateral superior and middle temporal gyrus; right hippocampus; right parahippocampal gyrus; left lingual gyrus; bilateral insular cortex; bilateral cingulate gyrus; bilateral caudate; bilateral putamen; brainstem; cerebellum, bilateral thalamus, and medial thalamus.

Supplementary Results

Effect of temporally delayed ratings.

In an earlier experiment, subjects ($n=21$) rated perceived pain either immediately after stimulation, or after a 16-second delay. Delay-to-rate was varied within-subjects, and we compared pain ratings during medium trials as a function of delay, expectancy, and expectancy-by-delay interaction. In our analyses, there was no effect of delay on reported pain ($t=.12$, $p=.91$). There was a marginally significant interaction between expectancy and delay ($t=2.12$, $p=.051$); this interaction is driven by delay causing LM trials to be rated as less painful than trials without delay, with no difference in HM trials. However, the interaction was only a fifth as large as the overall expectancy effect. Controlling for these differences, the main effect of expectancy was still highly significant ($t=4.21$, $p<.001$).

As the overall magnitude of the interaction was minimal, and separating pain period activity from rating period activity would allow us to examine brain responses separately for each of these processes, we decided to include a delay during the imaging study. We shortened the delay to 10 seconds post-offset of thermal stimulation, and expect that the effect of the expectancy-delay interaction is even less given this change.

Dynamics of cue effects on reported pain.

We used a linear mixed model in SAS to examine whether expectancy effects change over time. Analyses revealed a robust effect of expectancy on perceived pain ($t=5.53$, $p<.0001$). However, there was no evidence for any effect of time on perceived pain ($t=.046$, $p=0.65$), nor was there any interaction between time and expectancy ($t=1.11$, $p=.28$). In addition, the random effect of subject was not significant, suggesting that there were no reliable individual differences in dynamics of expectancy effects over time. Supplementary Figure S1 presents ratings for HM

and LM conditions by run (Supplementary Figure S7, Page 21), and illustrates that expectancy effects in our paradigm were quite stable.

Reverse mediation model.

Our paradigm is designed to make causal claims about pain-predictive cue effects on pain-evoked brain responses, and pain-predictive cue effects on reported pain. The third component of our path model (the relationship between pain-evoked responses and effects on reported pain) is correlational, even though pain-evoked responses precede reports in time. Our primary path model, which assumes that brain activity leads to effects on report, was based on animal and human literature showing that direct stimulation of these regions causes effects on perceived pain. However, it is possible that a reverse model (reports lead to pain-evoked responses) could also describe activity. We tested this alternative in regions identified as PPN mediators in our *a priori* path model (left anterior insula, right thalamus, and rdACC). We found that this reverse model did not explain activity in left anterior insula or right thalamus ($p > 0.1$). RdACC was found to be adequately explained by this model ($p < .05$). However, our forward model provided a better fit for activity in this region ($p < 0.005$).

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