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Mesocorticolimbic pathways encode cue-based expectancy effects on pain

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Competing interests

All authors declare no conflict of interest.

30 **Abstract**

31 Expectation interacting with nociceptive input can shape the perception of pain. It has been
32 suggested that reward-related expectations are associated with the activation of the ventral
33 tegmental area (VTA), which projects to the striatum (e.g., nucleus accumbens [NAc]) and
34 prefrontal cortex (e.g., rostral anterior cingulate cortex [rACC]). However, the role of these
35 projection pathways in encoding expectancy effects on pain remains unclear. In this study, we
36 leveraged a visual cue conditioning paradigm with a long pain anticipation period and collected
37 magnetic resonance imaging (MRI) data from 30 healthy human subjects (14 females). At the
38 within-subject level, whole brain functional connectivity (FC) analyses showed that the
39 mesocortical pathway (VTA-rACC FC) and the mesolimbic pathway (VTA-NAc FC) were
40 enhanced with positive expectation but inhibited with negative expectation during pain
41 anticipation period. Mediation analyses revealed that cue-based expectancy effects on pain were
42 mainly mediated by the VTA-NAc FC, and structural equation modeling showed that VTA-
43 based FC influenced pain perception by modulating pain-evoked brain responses. At the
44 between-subject level, multivariate pattern analyses demonstrated that gray matter volumes in
45 the VTA, NAc, and rACC were able to predict the magnitudes of conditioned pain responses
46 associated with positive and/or negative expectations across subjects. Our results therefore
47 advance the current understanding of how the reward system is linked to the interaction between
48 expectation and pain. Furthermore, they provide precise functional and structural information on
49 mesocorticolimbic pathways that encode within-subject and between-subject variability of
50 expectancy effects on pain.

51 **Keywords:** Expectancy effects, Conditioned pain responses, Ventral tegmental area (VTA),
52 Mesocortical pathway, Mesolimbic pathway
53

54 **Significance statement**

55 Studies have suggested that reward-related expectation is associated with the activation of the
56 ventral tegmental area (VTA), which projects to the striatum and prefrontal cortex. However, the
57 role of these projection pathways in encoding expectancy effects on pain remains unclear. Using
58 multi-modality MRI and a visual cue conditioning paradigm, we found that the functional
59 connectivity and gray matter volumes in key regions (the VTA, nucleus accumbens, and rostral
60 anterior cingulate cortex) within the mesocorticolimbic pathways encoded expectancy effects on
61 pain. Our results advance the current understanding of how the reward system is linked to the
62 interaction between expectation and pain, and provide precise functional and structural
63 information on mesocorticolimbic pathways that encode expectancy effects on pain.

64

65 Introduction

66 Pain is a highly subjective experience that can be influenced by a variety of psychological factors
67 (Wiech et al., 2008). One clear example is that expectation of nociceptive input can modulate the
68 perception of pain (Atlas and Wager, 2012; Fields, 2018). To investigate the relationship
69 between expectation and pain, studies invoke conditioning to establish a link between a cue and
70 increased or reduced pain that follows, thus creating predictive knowledge (i.e., expectation) that
71 modulates future pain responses to the same cue (Wager et al., 2004; Jensen et al., 2012).
72 Although the neural mechanisms underlying the interaction between expectation and pain are
73 still under investigation, neuroimaging studies using functional magnetic resonance imaging
74 (fMRI) and positron emission tomography (PET) have offered insights into how predictive cues
75 are processed by the coordination of complex brain circuits (e.g., brainstem, higher-order cortical
76 and subcortical brain regions), and then modulate pain and pain-related brain responses (Wager
77 et al., 2004, 2007; Koyama et al., 2005; Seymour et al., 2005; Atlas et al., 2010; Kong et al.,
78 2013; Shih et al., 2019).

79 Among several neurobiological models proposed for explaining the relationship between
80 expectation and pain-related behaviors (Enck et al., 2008; Büchel et al., 2014), brain reward
81 circuitry has been widely studied in both basic and clinical settings. It has been suggested that
82 expectation is closely related to the activation of tegmental or prefrontal dopaminergic neurons,
83 which project to the dorsal and ventral striatum (de la Fuente-Fernandez et al., 2001; de la
84 Fuente-Fernández et al., 2002; Lidstone et al., 2010), and may affect the circuitry of the basal
85 ganglia (Benedetti et al., 2004). In particular, the mesocorticolimbic pathways, which originate
86 from the ventral tegmental area (VTA) and project primarily to the frontal lobe (e.g., rostral
87 anterior cingulate cortex [rACC]) and ventral striatum (e.g., NAc) (Gardner and Ashby, 2000),

88 form part of the key circuits that are typically implicated in reward and motivation behaviors
89 (Enck et al., 2008; Scott et al., 2008; Schweinhardt et al., 2009).

90 While previous studies have focused on investigating reward-related brain responses in the
91 frontal lobe and striatum, it is unlikely that isolated brain regions would be sufficient to achieve
92 relevant functions (e.g., reward, aversion). The role of mesocorticolimbic pathways (e.g., from
93 VTA to rACC and from VTA to NAc) in mediating cue-based expectancy effects on pain
94 remains elusive, which is the primary aim of the present study. Moreover, evidence from fMRI
95 and structural magnetic resonance imaging (sMRI) has suggested that a more efficient reward
96 system, e.g., larger NAc responses to reward cues (Scott et al., 2007) and higher gray matter
97 densities of NAc and prefrontal cortex (Schweinhardt et al., 2009), may be associated with
98 stronger reward-related behaviors. Therefore, we also aimed to investigate whether the
99 functional and structural properties of mesocorticolimbic pathways can explain individual
100 differences of conditioned pain responses.

101 To answer these questions, we leveraged a visual cue conditioning paradigm with a long pain
102 anticipation period (15 seconds) for each trial and collected sMRI and fMRI data from 30
103 healthy participants. At the within-subject level, we performed a VTA-based whole brain
104 functional connectivity (FC) analysis to identify pathways involved in cue-based pain
105 modulation. In addition, we conducted a mediation analysis to assess the potential mediatory
106 effect of anticipatory VTA-based FC on the relationship between expectation and pain
107 perception. We applied structural equation modeling to explore whether VTA-based FC
108 influences pain perception by modulating pain-evoked brain responses. At the between-subject
109 level, we applied a multivariate pattern analysis (MVPA) to assess the possibility of functional

110 and structural features (VTA-based FC and gray matter volume, respectively) associated with
111 mesocorticolimbic pathways to predict conditioned pain responses.

Material and Methods

Subjects

Thirty healthy participants without any psychiatric or neurologic disorders were enrolled in this study. One subject was excluded from data analysis due to an incomplete MRI scan. The final sample consisted of 29 participants (14 females; aged 22.0 ± 1.9 years). To detect the effectiveness of sample size for exploring the cue-based expectancy effects on pain, we performed a power analysis for F tests using G*power (a free online software for power analysis, available at <http://www.gpower.hhu.de/en.html>) and set statistical power at 0.8 with a medium effect size ($f = 0.25$) and significance level at 0.05 (Cunningham and McCrum-Gardner, 2007). The result showed a minimum sample size of 28 for detecting main effects. As for the power to determine the effects of conditioned expectation on brain responses, the sample size of our study was determined based on the sample sizes of previous studies that similarly aimed to investigate expectancy-based pain modulation using fMRI techniques (Wager et al., 2004, 2011; Atlas et al., 2010; Jensen et al., 2015). All protocols were approved by the Institutional Review Board of Southwest University, and all participants provided written and signed informed consent.

Experimental procedure

The experiment consisted of three phases: a calibration phase, conditioning phase, and test phase. Calibration and conditioning phases were performed 24 hours before the test phase.

Calibration phase

133 Nociceptive somatosensory stimuli (intraepidermal electrical stimuli, IES) were delivered using
134 an electrical stimulator (model DS7A; Digitimer, UK) with three stainless steel concentric
135 bipolar needle electrodes separated by an equal distance of 6 mm. Each electrode consisted of a
136 needle cathode (length: 0.1 mm, Ø: 0.2 mm) surrounded by a cylindrical anode (Ø: 1.4 mm).
137 Each stimulus consisted of 6 rapidly succeeding constant-current, square-wave pulses at 60 Hz
138 (0.5-ms duration for each pulse; 100 ms for the whole stimulus). The electrodes were placed over
139 the left volar forearm. For each subject, the ascending method of limits (pulses with an ascending
140 current starting from 0.2 mA and increasing in steps of 0.1 mA) were applied in the calibration
141 phase to determine the stimulus intensity that would represent “low pain” (2 on a 0-10 numerical
142 rating scale [NRS]; 0: no pain; 10: worst pain that subject can tolerate), “moderate pain” (4 on
143 the NRS), and “high pain” (6 on the NRS). Subjects were instructed to move a slider to rate the
144 intensity of pain perception. This procedure was repeated three times for each subject, and the
145 averaged stimulus intensities were calculated for the following experiment. In addition, the
146 averaged stimulus intensities were presented several times in random orders to ensure rating
147 consistency. Please note that IES was shown to preferentially activate the A δ nociceptive fibers,
148 and the simultaneous activation of the A β fibers cannot be completely ruled out considering that
149 a wide range of stimulus intensities were used (Mouraux et al., 2010).

150

151 Conditioning phase

152 After the calibration phase, participants were given the following instruction: “You are about to
153 see some pictures on the screen. Each picture is paired with a pain stimulus on your arm. Your

154 task is to focus on the screen at all time, and after each pain stimulus, you are required to rate
155 how much pain you felt on your arm using the same 0-10 NRS as in the calibration phase.”

156 A 60 Hz, 19-inch Dell monitor was used for visual presentations, and the screen resolution was
157 1024 × 768 pixels. The experiment was programmed in E-Prime 3.0 software (Psychology
158 Software Tools, Pittsburgh, PA). In each trial, a visual cue presented on the screen was followed
159 by a pain stimulus 15 seconds later (from the offset of visual cue to the onset of pain stimulus).
160 Subjects were required to rate the intensity of pain perception on the same 0-10 NRS. In the
161 conditioning phase, two types of cues (a white plus mark and a white minus mark) were
162 presented 20 times each in a random order. The white plus mark cue was followed by a high pain
163 stimulus, and the white minus mark cue by a low pain stimulus (Figure 1A). The timings of a
164 typical trial in the conditioning phase are displayed in Figure 1C. The inter-trial interval (ITI)
165 ranged from 10 to 12 seconds. To ensure that subjects remained attentive to external sensory
166 stimuli, the conditioning phase was divided into two sessions (about 15 minutes each). Subjects
167 had the opportunity to rest for approximately 1 minute between sessions.

168 After the conditioning phase, all participants verbally confirmed that the stimuli following the
169 white plus mark was more painful than the stimuli following the white minus mark using the
170 same 0-10 NRS.

171

172 Test phase

173 The test phase was performed during the MRI scan, and subjects had a response-device in their
174 right hand to rate the intensity of pain perception in the scanner. Before fMRI data collection, the

175 stimulus intensity was calibrated again in the MRI scanner, and five low-pain trials and five
176 high-pain trials were delivered to the subjects to familiarize them with the sensation.

177 Subjects were then instructed, “You are about to see the same pictures on the screen, and each
178 picture will be paired with a pain stimulus on your arm, just like before. The only difference is
179 that this time there will be a picture of a circle, which you have not been exposed to before. Your
180 task is to focus on the screen at all time, and after each pain stimulus you are required to rate
181 how much pain you felt on your arm using the same 0-10 NRS.”

182 In the test phase, all cues were followed by pain stimuli of the same intensity (i.e., moderate pain,
183 4 on the NRS) in order to test the conditioning effect (Figure 1B). The timings of a typical trial in
184 the test phase are detailed in Figure 1D. The ITI was between 10 and 12 seconds. The test phase
185 was divided into three sessions of 15 trials (45 trials in total), each of which contained 5 trials for
186 each of the 3 different cues (a white plus mark, white minus mark, and white circle mark).
187 Subjects could rest for 1 minute between consecutive sessions.

188 After the test phase, all participants confirmed that they expect to receive more painful stimuli
189 following the white plus mark compared to the white minus mark.

190

191 **Behavioral data analysis**

192 The positive expectancy effect was defined as the difference between perceived intensity to
193 moderate pain stimuli following the low cue (white minus mark) and following the neutral cue
194 (white circle mark) in the test phase. The negative expectancy effect was defined as the
195 difference between perceived intensity to moderate pain stimuli following the high cue (white
196 plus mark) and following the neutral cue (white circle mark) in the test phase. Adding a neutral

cue (i.e., white circle mark) enabled us to differentiate the positive expectancy effects (low cue vs. neutral cue) from the negative expectancy effects (high cue vs. neutral cue). The comparison of perceived pain intensities following different cues were assessed using a one-way repeated measures analysis of variance (ANOVA; Figure 2A). When the main effect was significant, post-hoc paired-sample t-tests were performed and p values were Bonferroni corrected.

MRI data acquisition

MRI data were acquired using a Siemens 3.0 Tesla Trio scanner with a standard 32-channel head coil at the Key Laboratory of Cognition and Personality (Ministry of Education) of Southwest University (China). Functional images were obtained using a gradient echo-planar-imaging sequence with the following parameters (repetition time = 1500 ms, echo time = 29 ms, 25 slices, slice thickness = 5.0 mm, interslice gap = 0.5 mm, in-plane resolution = 3 mm × 3 mm, field of view = 192 × 192 mm², data matrix = 64 × 64, flip angle = 90°). A high-resolution, three-dimensional T1-weighted structural image was acquired using a Magnetization Prepared Rapid Acquisition Gradient-Echo (MPRAGE) sequence (1 mm³ isotropic voxel, field of view = 256 × 256 mm²) after functional imaging.

fMRI data analysis

fMRI data preprocessing

fMRI data were preprocessed using SPM12 (Wellcome Trust Center for Neuroimaging, London, UK). The first five volumes were discarded to allow for signal equilibration. Images were slice

218 timing corrected using the middle slice and realigned to the first scan. The resulting images were
 219 normalized to the Montreal Neurological Institute (MNI) space (resampling voxel size = $3 \times 3 \times 3$
 220 mm^3) (Ashburner and Friston, 2005). To minimize the effect of head motion in the following
 221 fMRI analyses, 6 motion estimates and 2 physiological time series (white matter and
 222 cerebrospinal fluid) were regressed out of the normalized images.

223 Due to the small size of the VTA and its proximity to other brainstem structures, the brainstem,
 224 isolated using the SUIT toolbox (<http://www.diedrichsenlab.org/imaging/suit.htm>), was not
 225 spatially smoothed, and the time course of the isolated VTA seed was extracted. The rest of the
 226 brain, excluding the brainstem, was smoothed with a 5 mm full width at half maximum (FWHM)
 227 Gaussian smoothing kernel.

228

229 General linear model (GLM) analysis

230 Single-subject fMRI data were analyzed using a GLM approach, including regressors for three
 231 types of visual cues (low, neutral, and high cues) and pain stimuli. The BOLD signals were
 232 modeled as a series of events (visual cues and pain stimuli) using a stick function, and convolved
 233 with a canonical hemodynamic response function (HRF). To identify brain responses to pain
 234 stimuli, group-level statistical analyses were carried out using a random effects analysis with a
 235 one-sample t-test, as implemented in SPM12. The significance threshold was set as $p < 0.001$ at
 236 the voxel level and $p_{\text{FDR}} < 0.05$ at the cluster level (false discovery rate [FDR] correction for
 237 multiple comparisons) in the whole-brain exploratory analyses.

238 To assess conditioned expectancy effects, contrast images were computed for the low cue versus
 239 neutral cue and for the high cue versus neutral cue for anticipation and experience of pain

240 separately, using a random effects analysis with a paired-sample t-test in SPM12. Anticipation of
 241 pain was defined as the period between the presentation of visual cues and the delivery of pain
 242 stimuli, and experience of pain was defined as the period between the delivery of pain stimuli
 243 and the rating of pain perception. The significance threshold was set as $p < 0.001$ at the voxel
 244 level and $p_{\text{FDR}} < 0.05$ at the cluster level in the whole-brain exploratory analyses.

246 VTA-based functional connectivity analyses

247 To investigate the role of mesocorticolimbic pathways in mediating conditioned expectancy
 248 effects, we selected the VTA as the seed for the following seed-based FC analyses (Figure 4A).
 249 The probabilistic atlas of VTA was defined in an independent sample of 50 participants from a
 250 previous study (mean volume $\pm$ standard deviation: $440.98 \pm 100.6 \text{ mm}^3$) (Murty et al., 2014)
 251 and thresholded to 75% (as suggested in [Murty et al., 2017]) in the present study. The VTA-
 252 based FC during the pain anticipation period was estimated using seed-based correlation analysis
 253 (Cisler et al., 2014; Cole et al., 2014). For each single trial, the averaged time series across all
 254 voxels of the VTA seed in the anticipation period (15 seconds in total; 10 scans) was extracted as
 255 the reference time series. Pearson's correlation analysis was performed to produce individual-
 256 level correlation maps of all voxels within the whole brain that represented their relationship
 257 with the reference time series of the VTA. Please note that seed-based correlation analysis rather
 258 than a psychophysiological interaction (PPI) approach was adopted in the present study, since
 259 previous studies have suggested that correlation analysis may be more suitable than PPI when
 260 assessing the differences in FC between experimental conditions (Di et al., 2018), especially for
 261 event-related fMRI data (Cisler et al., 2014).

262 The VTA-based whole brain FC (r value) maps were Fisher's z transformed to increase
 263 normality of the data for the following statistical analyses: 1) A one-sample t-test against zero
 264 was performed to reveal brain regions that were significantly correlated with VTA during pain
 265 anticipation and averaged over all cues. The resulting statistical map was set at a threshold of $p <$
 266 0.001 at the voxel level and $p_{\text{FDR}} < 0.05$ at the cluster level in the whole-brain exploratory
 267 analyses. 2) Paired-sample t-tests were performed to show significant differences in positive
 268 expectation (low cue vs. neutral cue) and negative expectation (high cue vs. neutral cue)
 269 contrasts. The resulting statistical maps were set at a threshold of $p < 0.001$ at the voxel level and
 270 $p_{\text{FDR}} < 0.05$ at the cluster level (i.e., within the brain regions showing significant VTA-based FC
 271 [Figure 4A]).

272 After identifying brain regions with significant differences in VTA-based FC in positive
 273 expectation and negative expectation contrasts, we calculated their overlaps (by finding the
 274 conjunct regions of Figure 4B and C) for the following analysis (i.e., $r_{\text{ACC}_{\text{overlap}}}$ and $\text{NAC}_{\text{overlap}}$;
 275 see Results section for details). Specifically, single-trial FC between VTA and $r_{\text{ACC}_{\text{overlap}}}$, as
 276 well as between VTA and $\text{NAC}_{\text{overlap}}$, was estimated and correlated with the corresponding
 277 single-trial perceived pain intensities using Pearson's correlation analysis to investigate their
 278 relationship in the pain anticipation period. In order to minimize the influence of individual
 279 differences (Hu and Iannetti, 2019; Tu et al., 2019b), single-trial perceived pain intensities were
 280 normalized within each subject by subtracting their mean and dividing by their standard
 281 deviation before performing the correlation analysis. The obtained correlation coefficients were
 282 Fisher's z transformed, and the resulting z values were compared against zero using a one-
 283 sample t-test.

284

285 Control seed-based analyses

286 Due to the small size of the VTA and its adjacency to substantia nigra (SN), we performed
 287 control analyses with SN as the seed to demonstrate the robustness of results obtained from
 288 VTA-based analyses. The SN is the origin of the nigrostriatal pathway, which transmits
 289 dopamine from SN to the dorsal striatum (e.g., the caudate, putamen). Different from
 290 mesocortical and mesolimbic pathways, the nigrostriatal pathway and the dorsal striatum are not
 291 usually associated with conditioned expectation on pain. The probabilistic atlas of SN was also
 292 defined in an independent sample of 50 participants from the previous study (Murty et al., 2014)
 293 (mean volume $\pm$ standard deviation: $972.4 \pm 267.5 \text{ mm}^3$) and thresholded to 75%. The SN-based
 294 FC analyses were identical to the VTA-based FC analyses, which have been detailed in the
 295 previous section. A high-resolution illustration of the VTA and SN seeds is provided in Figure 4-
 296 1.

297
 298 Mediation analyses

299 We performed bootstrapped mediation analyses to assess the mediatory role of anticipatory
 300 VTA-based FC (i.e., VTA-rACC and VTA-NAc) on the relationship between cue and pain
 301 perception. The PROCESS macro (version 2.16.3) in SPSS (IBM, version 22.0) was used with
 302 1000 bootstrap samples, which identified 95% confidence intervals for model components. With
 303 categorical values as the independent variable (coded as 1, 0, and -1 for low, moderate, and high
 304 cues, respectively) (Hayes and Preacher, 2014) and perceived pain intensities as the outcome, we
 305 tested three different models: 1) VTA-NAc FC as a mediator, 2) VTA-rACC FC as a mediator,
 306 and 3) VTA-NAc and VTA-rACC FCs as two mediators. A significant mediation occurs when

307 bootstrapped upper and lower 95% confidence intervals (CIs) do not include zero (Hayes and
308 Preacher, 2014).

309

310 Structural equation modeling

311 To explore whether anticipatory VTA-based FC influences pain perception by modulating pain-
312 evoked brain responses, structural equation modeling (SEM) with maximum likelihood
313 estimation was performed using Amos (IBM, version 22.0). Specifically, anticipatory VTA-
314 based FC was the predictor and loaded by VTA-NAc FC and VTA-rACC FC. The outcome was
315 perceived pain intensity. Pain-evoked brain responses were the mediator and loaded by BOLD
316 responses in the bilateral thalamus and bilateral insula. These two brain regions were selected
317 because 1) they were significantly activated by pain stimuli (Figure 2B), 2) pain-evoked brain
318 responses were significantly stronger following neutral cues than low cues (Figure 2C), and 3)
319 pain-evoked brain responses were significantly stronger following high cues than neutral cues
320 (Figure 2D). The ROIs of the thalamus and insula were defined as the overlapping portions of
321 the thresholded statistical maps in Figure 2B-D, and beta estimates at the individual level,
322 extracted from the two ROIs following different cues, were used as the BOLD responses.

323 The model fit was assessed using the following criteria: ratio of chi-square to degrees of freedom
324 (χ^2/df) < 2 (Kline, 2015), root mean square error of approximation (RMSEA) $\leq$ 0.06 (Hu and
325 Bentler, 1999), both goodness-of-fit index (GFI) and adjusted goodness-of-fit index (AGFI) $\geq$
326 0.90, both comparative fit index (CFI) and normed fit index (NFI) $\geq$ 0.95 (Hooper et al., 2008).

327 To assess the significance of the indirect and direct effects, bias-corrected 95% CIs were

328 calculated using the bootstrapping procedure (Hayes and Preacher, 2014). The estimate was
329 considered statistically significant if the 95% CIs (based on 1000 bootstrap samples) excluded
330 zero.

331

332 Multivariate pattern analysis

333 A MVPA was used to investigate the association between VTA-based FC and positive
334 expectancy / negative expectancy effects at the between-subject level (Anzellotti et al., 2017;
335 Anzellotti and Coutanche, 2018). First, multivariate FC between the VTA and each voxel in
336 rACC (resulting in a vector pattern of VTA-rACC FC; the number of features equals to the
337 number of voxels in rACC) and between the VTA and each voxel in left NAc (resulting in a
338 vector pattern of VTA-NAc FC; the number of features equals to the number of voxels in NAc)
339 were extracted for each single trial in each subject and averaged across trials for each type of cue.
340 The differences in VTA-based multivariate FC (neutral cue vs. low cue or high cue vs. neutral
341 cue) were calculated and used to predict the magnitude of conditioned pain responses. Second,
342 the relationship between VTA-based multivariate FC (independent variables, i.e., VTA-rACC
343 multivariate FC and VTA-NAc multivariate FC) and the magnitude of conditioned pain
344 responses (dependent variable) was described using a multivariate linear regression (MVLRL)
345 model (Tu et al., 2016, 2018a). In order to minimize the influence of different levels of pain
346 perception following neutral cues across subjects, the percentage difference of perceived pain
347 intensities between neutral cue and low/high cues was calculated to represent the magnitudes of
348 conditioned pain responses for positive and negative expectations respectively. The MVLRL
349 model was decoded using a support vector regression (SVR), as implemented in the LIBSVM

350 toolbox (Chang and Lin, 2011), and this calculation resulted in a pattern of prediction weights
351 for all independent variables (Tu et al., 2019a). The prediction of conditioned pain responses was
352 achieved based on the 5-fold cross-validation. Specifically, we partitioned all subjects into five
353 groups, four of which were used for training and the remaining one was used for testing. This
354 procedure was repeated five times to ensure that each subject was used in the test sample once.
355 The predicted magnitude of conditioned pain responses was calculated by taking the dot product
356 of the pattern of prediction weights obtained from the training samples and the VTA-rACC and
357 VTA-NAc FC from subjects in the test sample. To evaluate the prediction performance, we
358 calculated the prediction-outcome correlation, which was defined as the correlation coefficient
359 between the actual and predicted magnitudes of conditioned pain responses (Wager et al., 2011,
360 2013; Doehrmann et al., 2013; Lindquist et al., 2016).

361 The significance of the prediction performance was estimated using a permutation test, in which
362 we randomly permuted the labels of the data (conditioned pain responses) prior to training.
363 Cross-validation was performed on the permuted data, and the whole procedure was repeated
364 1000 times. If the model trained on real data labels had a prediction-outcome correlation (z-
365 scored) that exceeded the 95% CI generated from the results of the models trained on randomly
366 relabeled data, the prediction model was considered to be well performing.

367 We also examined whether BOLD signals in isolated brain regions (i.e., rACC and NAc; beta
368 estimates at the individual level) during the pain anticipation period were predictive to
369 conditioned pain responses. In brief, predictors consist of BOLD signals at brain voxels within
370 rACC and NAc, and the relationships between the extracted BOLD signals and conditioned pain
371 responses were modeled using the MVLR and decoded using the SVR.

372

373 **Structural MRI data analysis**

374 Structural MRI images were analyzed using the Computational Anatomy Toolbox (CAT12;
375 <http://www.neuro.uni-jena.de/cat/>) in SPM12. Data preprocessing included bias-field and noise
376 removal, skull stripping, segmentation into gray and white matter, and normalization to MNI
377 space. The quality of images was assessed with the built-in image-quality rating and manually
378 checked by authors. Gray matter was spatially smoothed using the same 5 mm FWHM Gaussian
379 smoothing kernel as used in the analysis of fMRI data.

380 Structural properties of ventral striatum (NAc is a core part of ventral striatum) and prefrontal
381 cortex have been found to be related to dopamine-related personality traits (Schweinhardt et al.,
382 2009). To determine whether structural properties of mesocorticolimbic pathways can predict
383 conditioned pain responses at the between-subject level, we performed voxel-based
384 morphometry (VBM) analysis (Ashburner and Friston, 2005) using CAT12 toolbox and voxel-
385 wise estimation of gray matter volume (GMV) for the rACC (consisting of a vector of
386 multivariate GMV values of rACC for each subject) and left NAc (consisting of a vector of
387 multivariate GMV values of left NAc for each subject) was extracted. Additionally, the GMV of
388 the VTA was also extracted from each subject. The ability of the structural properties (as
389 represented by multivariate GMV values) of these regions to predict the between-subject
390 variability of conditioned pain responses was assessed using the same MVPA approach detailed
391 in the previous section.

392

Results

Behavioral results

The perceived intensities to pain stimuli following low cue ('-'), neutral cue ('o'), and high cue ('+') were 2.69 ± 0.18 , 3.32 ± 0.23 , and 4.10 ± 0.28 (mean $\pm$ SEM), respectively (Figure 2A). A one-way repeated measures ANOVA showed significant differences among the three conditions ($F_{(2,26)} = 57.6$, $p < 0.001$). Post-hoc comparisons showed that perceived pain intensities were significantly higher for neutral cue than for low cue ($p < 0.001$) and significantly higher for high cue than for neutral cue ($p < 0.001$). Cue-based expectancy effects on pain did not significantly change over time, as the one-way repeated measures ANOVA did not reveal significant differences across three sessions in the test phase for both positive ($F_{(2,26)} = 1.71$, $p = 0.19$) and negative ($F_{(2,26)} = 2.48$, $p = 0.09$) expectancy effects, although both effects exhibited a trend of decrease across time.

Cue-based expectancy effects during pain experience and anticipation periods

Pain stimuli elicited brain activations within a wide range of brain regions, including the bilateral insula, supplementary motor area (SMA), dorsal striatum (i.e., putamen and caudate), thalamus, primary and secondary somatosensory cortices (SI and SII), and dorsal ACC (dACC) ($p_{FDR} < 0.05$, the same hereinafter; Figure 2B; Table 1). Pain-evoked deactivations were observed in the bilateral parahippocampus (Table 1).

During the pain experience period, positive expectation induced decreased brain activations in the bilateral thalamus, bilateral insula, middle/posterior cingulate cortex (MCC/PCC), and

414 midbrain (Figure 2C; Table 2). In contrast, negative expectation induced increased brain
 415 activations in the bilateral insula, dACC, SMA, bilateral thalamus, bilateral putamen, MCC/PCC,
 416 bilateral dorsolateral prefrontal cortex (DLPFC), and midbrain (Figure 2D; Table 2), and induced
 417 decreased brain activations in the bilateral angular (Table 2). Please note that these brain regions
 418 associated with cue-based expectancy effects on pain were largely overlapping with the
 419 neurological pain signature (Wager et al., 2013). To illustrate pain-evoked brain responses
 420 following different cues in the brain regions associated with cue-based effects on pain (Atlas et
 421 al., 2010; Jensen et al., 2015; Shih et al., 2019), dACC, SMA, bilateral insula, bilateral putamen,
 422 and bilateral thalamus were selected as regions of interest (ROIs). Pain-evoked brain responses
 423 in these ROIs were extracted and are shown in Figure 2E.

424 During the pain anticipation period, different cues elicited brain activations within a wide range
 425 of overlapping brain regions (Figure 3A). Compared to neutral cues, low cues induced increased
 426 orbitofrontal cortex (OFC) activations and decreased SMA activations (Figure 3B; Figure 3-1).
 427 In contrast, high cues induced increased brain activations in the subgenual ACC (sgACC), rACC,
 428 and PCC (Figure 3C; Figure 3-1).

429

430 **VTA-based FC during pain anticipation period**

431 During the pain anticipation period, VTA exhibited significant FC with a broad range of cortical
 432 and subcortical regions, including the thalamus, caudate, ACC, mPFC, insula, hippocampus,
 433 amygdala, NAc, inferior frontal gyrus (IFG), and putamen (Figure 4A; Table 3). Compared to
 434 neutral cues, the VTA showed increased FC with the rACC and NAc following low cues (Figure
 435 4B; Table 4). In contrast, the VTA had reduced FC with the rACC and NAc following high cues

436 compared to neutral cues (Figure 4C; Table 4). In the control seed-based analyses, no significant
437 difference of SN-based FC was observed between low cues and neutral cues, as well as between
438 high cues and neutral cues (Figure 4D). Further, single-trial correlation analysis revealed
439 significant negative correlations between VTA-rACC/NAc FC and perceived pain intensities
440 (VTA-rACC: $r = -0.38 \pm 0.11$, $p = 0.001$; VTA-NAc: $r = -0.35 \pm 0.15$, $p = 0.02$; Figure 4E),
441 indicating that a stronger FC within mesocorticolimbic pathways during the pain anticipation
442 period was associated with a lower perceived pain intensity during the pain experience period.
443 Additionally, no significant difference in BOLD responses evoked by different cues was
444 observed in VTA, rACC, and NAc, suggesting that the observed differences in VTA-based FC
445 during the pain anticipation period were not likely to be contributed by differences in the
446 magnitude of cue-evoked brain responses (Figure 5).

447

448 **VTA-based FC mediates cue-based expectancy effects on perceived pain intensity at**
449 **within-subject level**

450 Three models in the mediation analyses describing the relationships between cues, VTA-based
451 FC, and perceived pain intensities are summarized in Figure 6. In model 1 (Figure 6A), we found
452 that VTA-NAc FC significantly mediated cue-based expectancy effects on perceived pain (direct
453 effect = 0.49, $p = 0.01$; indirect effect = 0.28, 95% CI: [0.09~0.52]). In model 2 (Figure 6B), we
454 found that VTA-rACC FC significantly mediated cue-based expectancy effects on perceived
455 pain (direct effect = 0.63, $p = 0.002$; indirect effect = 0.13, 95% CI: [0.004~0.298]). In model 3
456 (Figure 6C), two mediators (VTA-NAc FC and VTA-rACC FC) were adopted, and we found
457 that VTA-NAc FC significantly mediated cue-based expectancy effects on perceived pain (direct

effect = 0.48, $p = 0.02$; indirect effect = 0.26, 95% CI: [0.07~0.53]), while VTA-rACC FC did not (indirect effect = 0.02, 95% CI: [-0.16~0.14]).

460

Pain-evoked brain responses mediate effects of VTA-based FC on perceived pain intensity at within-subject level

The model used in the SEM analysis described the mediatory role of pain-evoked brain responses in the relationship between anticipatory VTA-based FC and perceived pain intensities (Figure 6D). The fitted model produced adequate fit indices: $\chi^2/df = 1.41$, RMSEA = 0.04, GFI = 0.99, AGFI = 0.99, CFI = 0.99, NFI = 0.99. All observed variables had high loadings (bs range = [0.81, 0.89]) in their respective unobserved variables. Anticipatory VTA-based FC showed a significant indirect effect ($b = -0.42$; 95% CI: [-0.83~0.17], $p = 0.007$) on perceived pain intensities through pain-evoked brain responses in the thalamus and insula, but not a direct effect ($b = 0.025$, 95% CI: [-0.36~0.44], $p = 0.94$).

471

Functional and structural patterns in mesocorticolimbic pathways encode conditioned pain responses at between-subject level

Functionally, we found that both VTA-rACC and VTA-NAc multivariate FCs were not able to predict positive expectation-conditioned pain responses (Figure 7A; $r = 0.13$, $p = 0.58$; $r = 0.19$, $p = 0.33$; respectively) or negative expectation-conditioned pain responses (Figure 7B; $r = 0.40$, $p = 0.03$; $r = 0.03$, $p = 0.89$; respectively; the significance threshold was adjusted to $p_{FDR} < 0.05$) at the between-subject level. Similarly, BOLD signals at brain voxels within the rACC and NAc during the pain anticipation period were unable to predict positive or negative expectation-

480 conditioned pain responses (positive expectation: $r = 0.32$, $p = 0.08$ for rACC, and $r = 0.32$, $p =$
481 0.08 for NAc; negative expectation: $r = 0.20$, $p = 0.13$ for rACC, and $r = 0.41$, $p = 0.03$ for NAc;
482 Figure 8).

483 Structurally, gray matter volumes in the rACC and NAc were able to predict the magnitude of
484 the positive expectation-conditioned pain responses across subjects (Figure 7A; $r = 0.51$, $p =$
485 0.005 ; $r = 0.46$, $p = 0.01$; respectively; the significance threshold was adjusted to $p_{\text{FDR}} < 0.05$). In
486 addition, the gray matter volume in the rACC, but not in the NAc, was able to predict the
487 magnitude of negative expectation-conditioned pain responses across subjects (Figure 7B; $r =$
488 0.49 , $p = 0.007$; $r = 0.14$, $p = 0.46$; respectively). Additionally, the gray matter volume in the
489 VTA was able to predict the magnitude of the positive ($r = 0.44$, $p = 0.02$; Figure 8A) but not the
490 negative ($r = 0.18$, $p = 0.35$; Figure 8B) expectation-conditioned pain responses across subjects.

491

Discussion

In the present study, we demonstrated the relevance of mesocorticolimbic pathways in the encoding of cue-based expectancy effects on pain. At the within-subject level, the function of the mesocortical pathway (represented by the VTA-rACC FC) and the mesolimbic pathway (represented by the VTA-NAc FC) was enhanced during pain anticipation with positive expectation and inhibited during pain anticipation with negative expectation (Figure 4B-4C). Single-trial correlation analysis revealed negative relationships between FC within mesocorticolimbic pathways during the pain anticipation period and perceived pain intensities during the pain experience period (Figure 4E). In addition, mediation analyses revealed that the cue-based expectancy effects on perceived pain intensities was mainly mediated by the VTA-NAc FC, and SEM showed that VTA-based FC influenced pain perception by modulating pain-evoked brain responses (Figure 6). At the between-subject level, we found that FC within mesocorticolimbic pathways during the pain anticipation period was unable to predict the magnitude of conditioned pain responses (Figure 7). In contrast, gray matter volumes in the VTA, rACC, and NAc were able to predict the magnitude of conditioned pain responses across subjects (Figures 7&8). In summary, our results emphasize the important role of the mesocorticolimbic pathways underlying the cue-based expectancy effects on pain.

Cue-based expectancy effects on pain

Conditioning is known to play an important role in establishing a link between a context (cue) and the following pain stimulus, thus creating expectation for future pain responses in the same situation (Atlas and Wager, 2012). Using a conditioning model and cue-based manipulations of

514 stimulus expectation, previous behavioral studies have revealed that short-term expectations that
515 vary as a function of cue have strong effects on pain perception (Colloca et al., 2010). Further,
516 predictive cues can be recognized subliminally, and conditioned pain responses can be elicited
517 without conscious awareness (Jensen et al., 2012). Recent studies have extended these theories to
518 observational conditioning, wherein subjects observe others and learn from their experience.
519 These studies have demonstrated that social observation can also elicit expectancy effects on
520 pain (Egorova et al., 2015; Tu et al., 2018b). In parallel, studies using fMRI have demonstrated
521 the cue-based expectancy effects on pain-evoked brain responses (Koyama et al., 2005; Atlas et
522 al., 2010; Jensen et al., 2015; Shih et al., 2019). Consistent with these studies, our results showed
523 that cue-based expectation modulated both perceived intensity and neurobiological responses of
524 pain (Figure 2A-2E).

525 Two neurotransmitters, endogenous opioid and cholecystokinin (CCK), are generally considered
526 important in mediating the general placebo analgesia and nocebo hyperalgesia responses,
527 respectively (Levine et al., 1978; Benedetti et al., 2006). However, they are less likely to underlie
528 cue-based expectancy effects since expectation, unlike the effects of endogenous opioid and
529 CCK, can vary from trial to trial. As suggested by Atlas and Wager, neuromodulatory signals
530 that mediate cue-based expectancy effects on pain are expected to be transient and reversible
531 (Atlas and Wager, 2012). Phasic dopaminergic (DA) signaling, which has been linked to the
532 computation of prediction error and expected value, would be an appropriate candidate for
533 mediating cue-based expectancy effects (Haruno and Kawato, 2006; Büchel et al., 2014). Indeed,
534 a number of studies have investigated the role of DA responses in generating placebo and nocebo
535 effects (de la Fuente-Fernandez et al., 2001; Scott et al., 2007, 2008). For example, Scott et al.
536 (2007, 2008) found that DA activity in the NAc was associated with the formation of placebo

537 and placebo pain responses. Results from our present study align with findings from general
538 placebo and placebo studies. Our study also extends previous theories by demonstrating that
539 expectation towards pain can modulate the synchronization of key components (i.e., VTA, NAc,
540 and rACC) in the reward circuitry. These brain regions may be associated with DA responses
541 (Enck et al., 2008) and DA projections from tegmental to striatum and prefrontal cortex (de la
542 Fuente-Fernandez et al., 2001; Lidstone et al., 2010).

543

544 **Neural coding of cue-based expectancy effects on within-subject variability of pain**

545 Previous studies have shown that expectancy effects on pain can be mediated by brain activities
546 in localized regions during both pain anticipation and pain experience (Atlas et al., 2010; Woo et
547 al., 2017). To investigate the role of mesocorticolimbic pathways in mediating cue-based
548 expectancy effects, we designed a cue-based paradigm that had a long pain anticipation period
549 (15 seconds, 5 times longer than previous studies [Jensen et al., 2012, 2015]). We observed that
550 while the VTA exhibited significant FC with several cortical and subcortical brain regions during
551 the pain anticipation period (Figure 4A), only the FC between the VTA and NAc and between
552 the VTA and rACC were modulated by cue-based expectations (Figure 4B-3C). It should be
553 noted that while VTA-rACC FC can serve as a functional indicator of the mesocortical pathway
554 that is responsible for executive brain function, VTA-NAc FC is the main projection in the
555 mesolimbic pathway related to reward and aversive processing (Gardner and Ashby, 2000).
556 Therefore, our results indicate that both the mesolimbic and mesocortical pathways are involved
557 in the formation of cue-based expectancy effects on pain (Ashar et al., 2017). The mediation
558 analysis demonstrated that the effect of cue-based expectation on perceived pain was mainly

559 mediated by VTA-NAc FC (Figure 6A-6C), suggesting that the mesolimbic pathway related to
560 reward and aversive processing plays a major role in mediating cue-based expectation to relieve
561 or enhance subsequent pain perception.

562 Additionally, SEM analysis showed the mediatory role of pain-evoked brain responses (in the
563 bilateral thalamus and insula) in the relationships between anticipatory VTA-based FC and
564 perceived pain intensities (Figure 6D). Specifically, anticipatory VTA-based FC showed a
565 significant indirect effect on perceived pain intensities through pain-evoked brain responses in
566 the thalamus and insula, suggesting that anticipatory VTA-based FC modulated pain-evoked
567 brain responses and consequently influenced pain experience. This finding is similar to that in a
568 previous study (Atlas et al., 2010), which found that anticipatory activities in the medial OFC
569 and ventral striatum mediated cue effects on pain-evoked brain responses in the thalamus,
570 rostradorsal anterior cingulate cortex, and anterior insula, and consequently modulated pain.

571 Taken together, the mesocorticolimbic pathways may be one of the brain systems mediating
572 expectancy effects (as indicated by the significant direct and indirect effects in Figure 6A-C) on
573 pain perception through pain-evoked brain responses (as indicated by the significant indirect
574 effect but not direct effect in Figure 6D). It is worth noting that both mediation and SEM
575 analyses have the limitation of interpreting a causal inference between variables (e.g., there may
576 be reciprocal causation between the mediator and the dependent variable) (MacKinnon et al.,
577 2007), and caution should be taken when interpreting the obtained results.

578

579 **Neural coding of between-subject variability of conditioned pain responses**

580 Since previous studies have suggested that a more efficient reward system may lead to stronger
581 reward-related behaviors (Scott et al., 2007; Schweinhardt et al., 2009), it would be interesting to
582 assess whether the mesocorticolimbic pathways can also encode between-subject variability of
583 conditioned pain responses. To achieve this, we performed an MVPA to decode between-subject
584 variability of conditioned pain responses associated with positive and negative expectations
585 based on functional and structural features of the mesocorticolimbic pathways. We observed that
586 functional features (i.e., VTA-rACC and VTA-NAc FC) could not significantly predict
587 magnitudes of conditioned pain responses (Figure 7), suggesting that functional features that
588 varied from trial to trial may play a major role in capturing the phasic information in modulating
589 expectancy effects on pain (e.g., expectation towards the subsequent pain stimulus). In contrast,
590 we found that the structural features (i.e., gray matter volumes in the NAc, rACC, and VTA)
591 were able to predict magnitudes of conditioned pain responses across subjects. This observation
592 is consistent with a previous study that demonstrated the role of gray matter density in the
593 mesolimbic reward system in encoding individual differences of the placebo effect
594 (Schweinhardt et al., 2009).

595

596 **Limitations and future directions**

597 There are several limitations of the present study that merit consideration in the future. First, the
598 sample size was limited, especially when assessing between-subject variability of conditioned
599 pain responses. Second, whereas the anticipatory VTA-based FC may be associated with DA
600 responses, we were not able to measure DA responses using fMRI and did not collect DA-related
601 psychological traits (Schweinhardt et al., 2009). Future studies with dopamine antagonists will

602 be helpful to investigate the relationship between our findings and the DA system, and to
603 contrast recent studies with dopamine antagonists reporting no modulation of placebo effects in
604 the context of pain (Skyt et al., 2018; Zunhammer et al., 2018). Third, a fixed time interval
605 during the pain anticipation period was adopted in the present study, which enabled us to make
606 full use of data samples for a reliable estimation of the seed-based FC analyses. However, a
607 variable time interval should be considered in the future, as it would enable a better separation of
608 BOLD responses to successive stimuli. Fourth, the 0-10 NRS (11 points) was used to evaluate
609 the subjective intensity of pain perception throughout the present study, which may limit the
610 appreciation of variability in scoring pain as compared to the 0-100 NRS (101 points).

611

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- 756

Figure Legends

Figure 1. Experimental design. A. In the conditioning phase, two types of cues (white plus mark and white minus mark) were presented 20 times each in a random order. The white plus mark cue was followed by a high pain stimulus, and the white minus mark cue by a low pain stimulus. B. In the test phase, three types of cues (white plus mark, white circle mark, and white minus mark) were presented 15 times each in a random order. All cues were followed by stimuli of the same intensity (i.e., moderate pain). C. There were two sessions in the conditioning phase, and each session included 20 trials with two types of cues (white plus mark and white minus mark) displayed in random order. Subjects could rest for 1 minute between each session. D. There were three sessions in the test phase, and each session included 15 trials with three types of cues (white plus mark, white minus mark, and white circle mark) displayed in random order. Subjects could rest for 1 minute between consecutive sessions.

Figure 2. Pain and conditioned pain-related brain responses. A. Significant differences of perceived pain intensities following low, neutral, and high cues were observed across all subjects. B. Pain stimuli elicited brain activations in the dorsal anterior cingulate cortex (dACC), supplementary motor area (SMA), putamen (PUT), caudate (CAU), thalamus (TH), and insula (INS). C. During the pain experience period, positive expectation induced decreased brain activations in the midbrain, middle cingulate cortex (MCC), INS, and TH. D. Negative expectation induced increased brain activations in the midbrain, dorsal lateral prefrontal cortex (DLPFC), MCC, SMA, dACC, TH, and INS during the pain experience period. All results in B-D were thresholded at $p < 0.001$ at voxel level and $p_{\text{FDR}} < 0.05$ at cluster level. E. Pain-evoked brain responses at representative regions of interests. dACC, SMA, bilateral INS, bilateral PUT, and bilateral TH were selected as ROIs. We built these ROIs based on the conjunction of pain-responsive regions in B and the standard regions in Automated Anatomical Labeling atlas. Pain-evoked brain responses in these ROIs were extracted and compared between different cues (low, neutral, and high) using a one-way repeated-measures ANOVA and post-hoc paired-sample *t* tests when the main effect was significant. Asterisks indicate $p < 0.05$ FDR correction.

Figure 3. Brain responses associated with cue-based expectancy effects. A. Brain responses to low, neutral, and high cues. Visual cues elicited brain activations in a wide range of brain regions. B. During the pain anticipation period, positive expectation induced increased orbitofrontal cortex (OFC) activations. C. Negative expectation induced increased brain activations in the rostral and subgenual ACC (rACC, sgACC) during the pain anticipation period. All results were thresholded at $p < 0.001$ at voxel level and $p_{\text{FDR}} < 0.05$ at cluster level. Clusters that exhibited significant differences between cues during pain anticipation are summarized in Figure 3-1.

Figure 4. Ventral tegmental area (VTA)-based functional connectivity (FC) during pain anticipation period. A. The VTA exhibited significant FC with several brain regions, including the thalamus (TH), caudate (CAU), anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), insula (INS), hippocampus (HIP), amygdala (AMY), nucleus accumbens (NAc), putamen (PUT), and inferior frontal gyrus (IFG). B. Compared to neutral cues, the VTA showed increased FC with the rACC and left NAc following low cues. C. Compared to neutral cues, the VTA showed decreased FC with the rACC and left NAc following high cues. D. Substantia nigra (SN) based functional connectivity during the pain anticipation period. No significant difference of SN-based FC was observed between low cues and neutral cues, as well as between high cues and neutral cues. The SN seed is shown in Figure 4-1. All results in A-D were thresholded at $p < 0.001$ at voxel level and $p_{FDR} < 0.05$ at cluster level. E. The FC between the VTA and rACC/NAc was significantly and negatively correlated with perceived pain intensities. Each colored line represents an individual subject.

Figure 5. Cue-evoked brain responses in VTA, NAc, and rACC. A and B. Brain regions that were significantly activated by cues are marked in hot; VTA seed is marked in blue; NAc is marked in yellow; rACC is marked in green. NAc and rACC in A were identified using the contrast 'low vs. neutral' in Figure 4B. NAc and rACC in B were identified using the contrast 'high vs. neutral' in Figure 4C. C, D, and E. Time courses of BOLD signals following three different cues from the VTA, NAc_{overlap}, and rACC_{overlap} in Figure 4E.

Figure 6. Path analyses of cue-based expectancy effects on perceived pain intensity. A. Mediation model 1 (One mediator variable: VTA-NAc FC). Cue-based expectancy effects on perceived pain intensities were mediated by VTA-NAc FC. B. Mediation model 2 (One mediator variable: VTA-rACC FC). Cue-based expectancy effects on perceived pain intensities were mediated by VTA-rACC FC. C. Mediation model 3 (Two mediator variables: VTA-NAc FC and VTA-rACC FC). VTA-NAc FC mediated cue-based expectancy effects on perceived pain intensities, while VTA-rACC FC did not mediate cue-based expectancy effects on perceived pain intensities. D. Structural equation modeling (SEM). VTA-based FC during pain anticipation was the predictor and loaded by VTA-NAc FC and VTA-rACC FC. Perceived pain intensity was the outcome. Pain-evoked brain responses were the mediator and loaded by the BOLD responses in the bilateral thalamus and bilateral insula. VTA-based FC during pain anticipation showed a significant indirect effect on subjective pain perception through pain-evoked brain responses in the thalamus and insula, but not a direct effect. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Figure 7. Brain patterns in mesocorticolimbic pathways encode individual variability of conditioned pain responses. A. Gray matter volumes in the rACC and NAc were able to predict the magnitude of positive expectation-conditioned pain responses across subjects. In contrast, the functional connectivity (FC) between the VTA and rACC/NAc during the pain anticipation period could not predict the magnitude of positive expectation-conditioned pain responses at the

835 between-subject level. B. The gray matter volume of the rACC, but not of the NAc, was able to
836 predict the magnitude of negative expectation-conditioned pain responses across subjects. The
837 FC between VTA and rACC/NAc could not predict the magnitude of negative expectation-
838 conditioned pain responses at the between-subject level. FDR correction was applied ($p_{\text{FDR}} <$
839 0.05), and the significance threshold was adjusted to $p < 0.03$.

840

841 **Figure 8. Prediction of individual variability of conditioned pain responses using**
842 **anticipatory BOLD responses and VTA gray matter volume.** A. BOLD responses in the
843 rACC and NAc during the pain anticipation period could not predict the magnitude of positive
844 expectation-conditioned pain responses at the between-subject level. In contrast, the gray matter
845 volume in the VTA was able to predict the magnitude of positive expectation-conditioned pain
846 responses across subjects. B. BOLD responses in the rACC and NAc during the pain anticipation
847 period, as well as VTA gray matter volume, were not able to predict the magnitude of negative
848 expectation-conditioned pain responses across subjects. FDR correction was applied ($p_{\text{FDR}} <$
849 0.05), and the significance threshold was adjusted to $p < 0.03$.

850

851 **Table 1. Clusters that exhibited significant brain activations and deactivations to pain**
852 **stimuli.**

853

854 **Table 2. Clusters that exhibited significant differences between cues during pain experience.**

855

856 **Table 3. Clusters that exhibited significant functional connectivity with the VTA during**
857 **pain anticipation.**

858

859 **Table 4. Clusters that exhibited significant differences of functional connectivity with the**
860 **VTA between cues during pain anticipation.**

861 **Extended Data Legends**

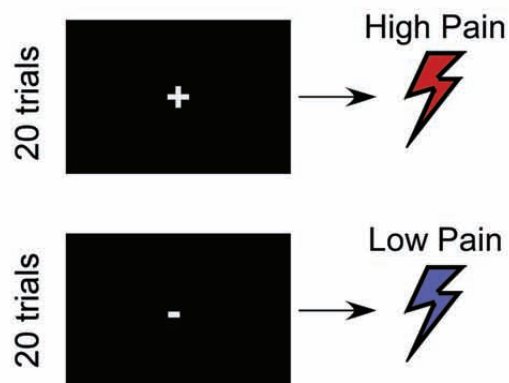
862 **Figure 3-1. Clusters that exhibited significant differences between cues during pain**
863 **anticipation.**

864

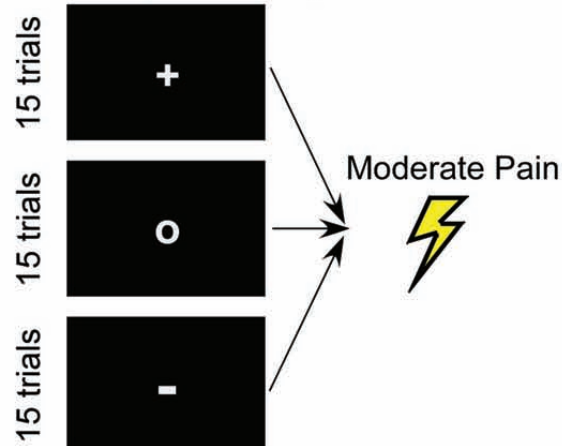
865 **Figure 4-1. Axial view of the VTA and SN seeds.** The probabilistic atlas from Murty et al.,
866 2014 was threshold at 75%, resampled at $1\times 1\times 1$ mm, and overlaid on the group-averaged
867 anatomical image.

868

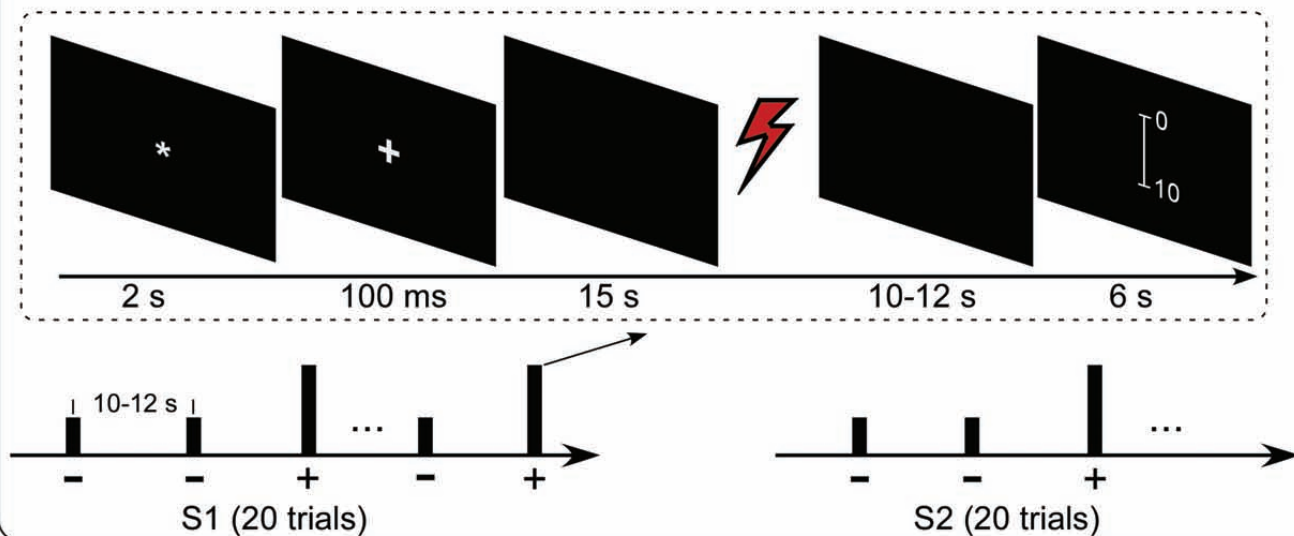
A. Conditioning (40 trials)



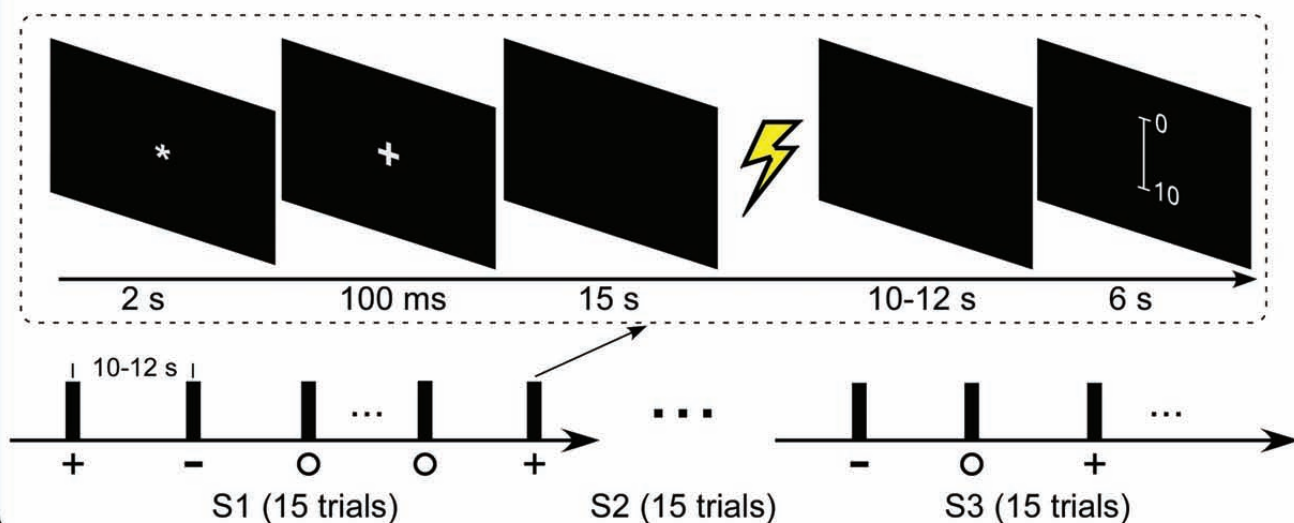
B. Test (45 trials)



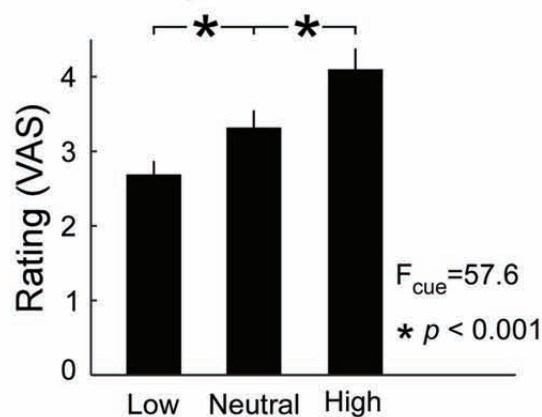
C. Conditioning sequence



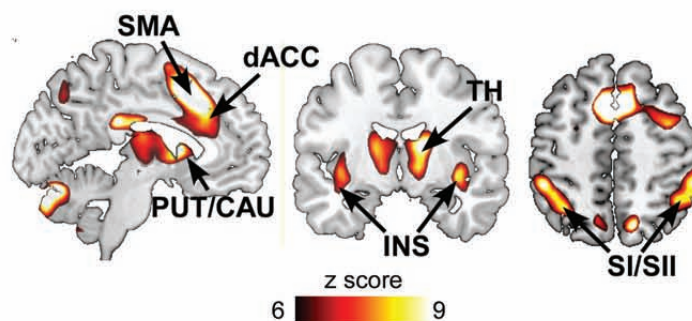
D. Test sequence



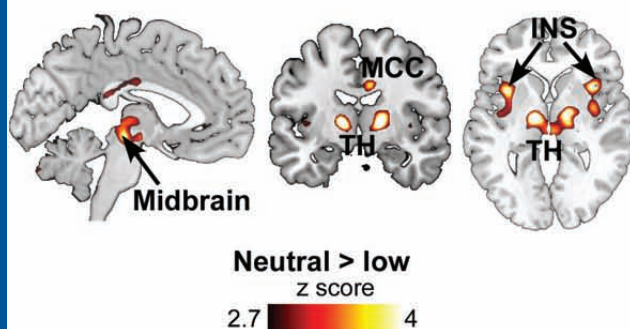
A. Pain ratings



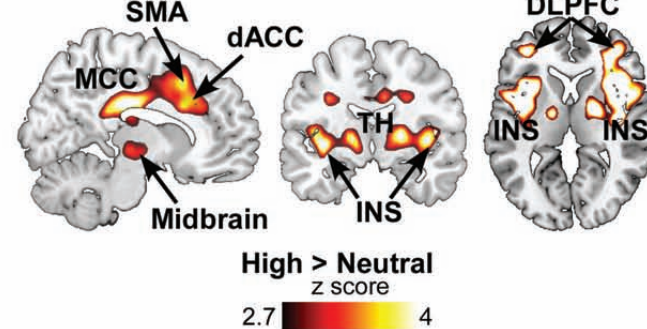
B. Pain-responsive regions



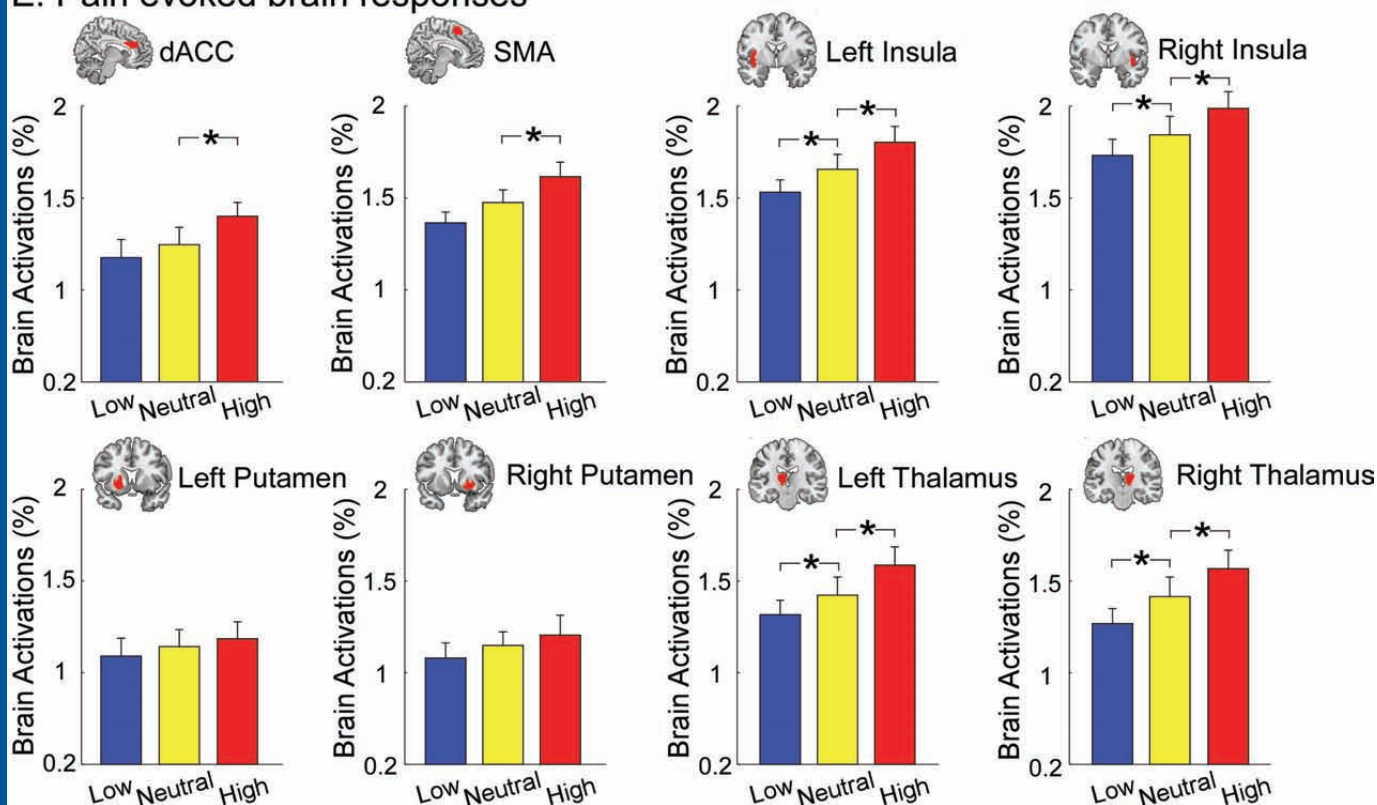
C. Neutral vs. Low during stimulation



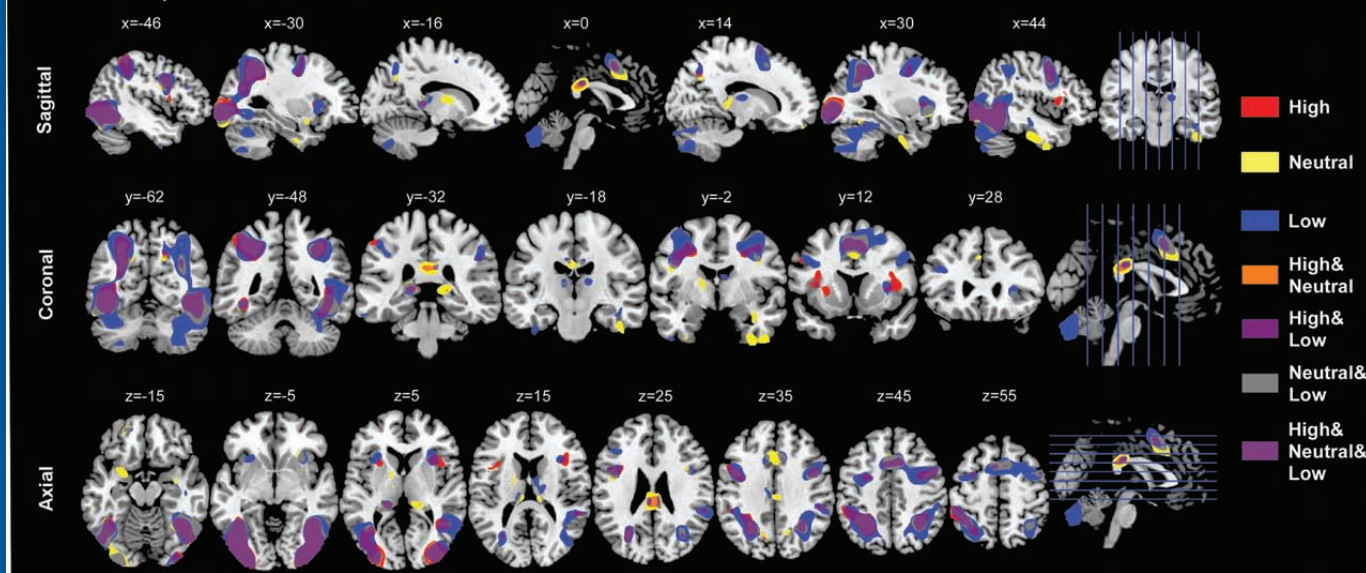
D. High vs. Neutral during stimulation



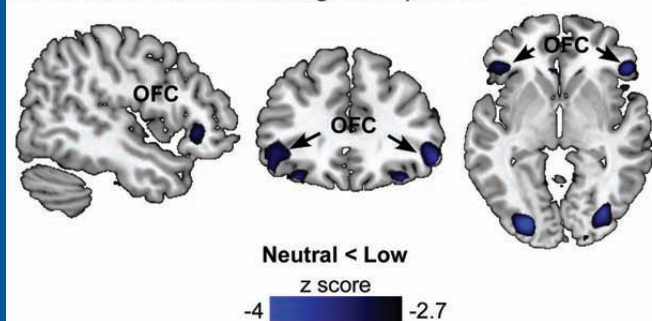
E. Pain evoked brain responses



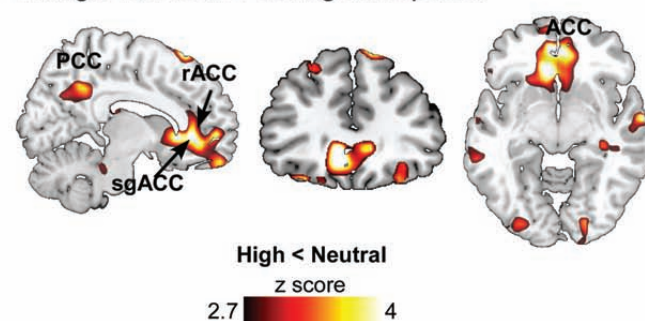
A. Brain responses to cues



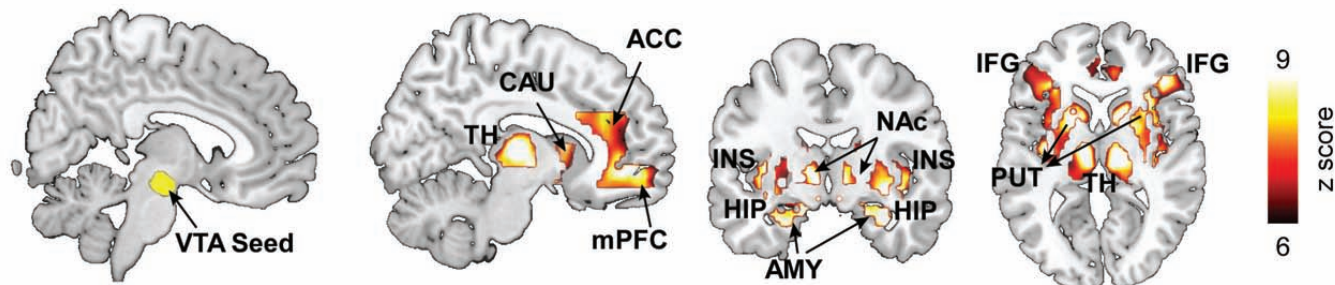
B. Neutral vs. Low during anticipation



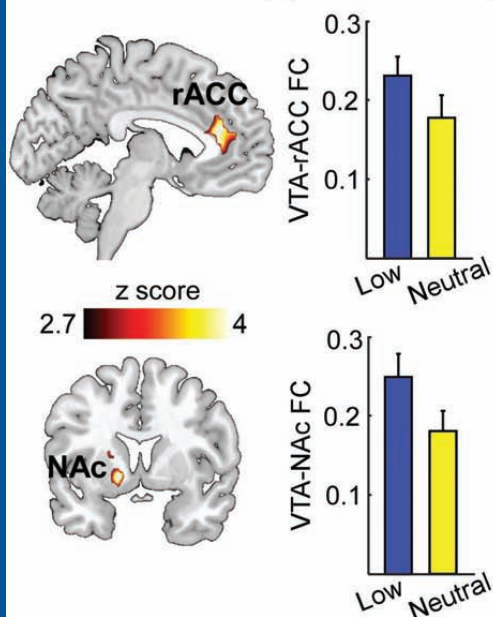
C. High vs. Neutral during anticipation



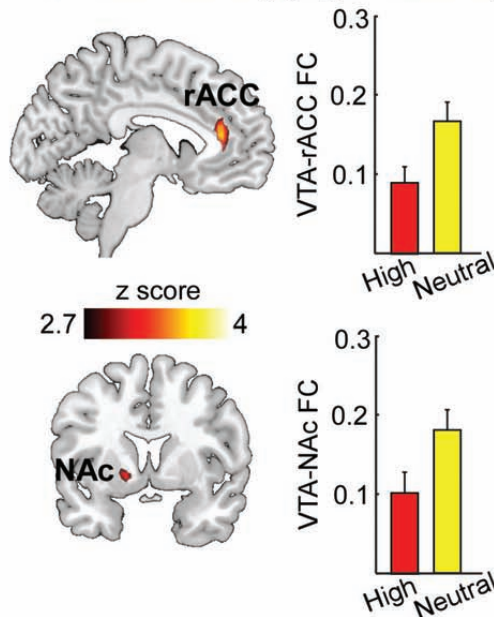
A. VTA connectivity during anticipation



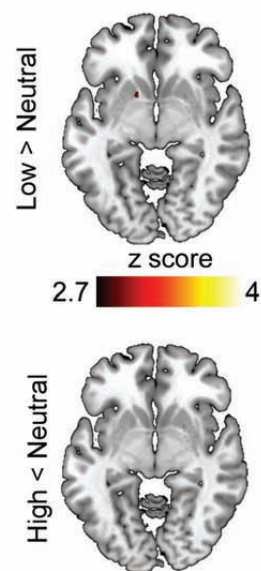
B. VTA connectivity (Low > Neutral)



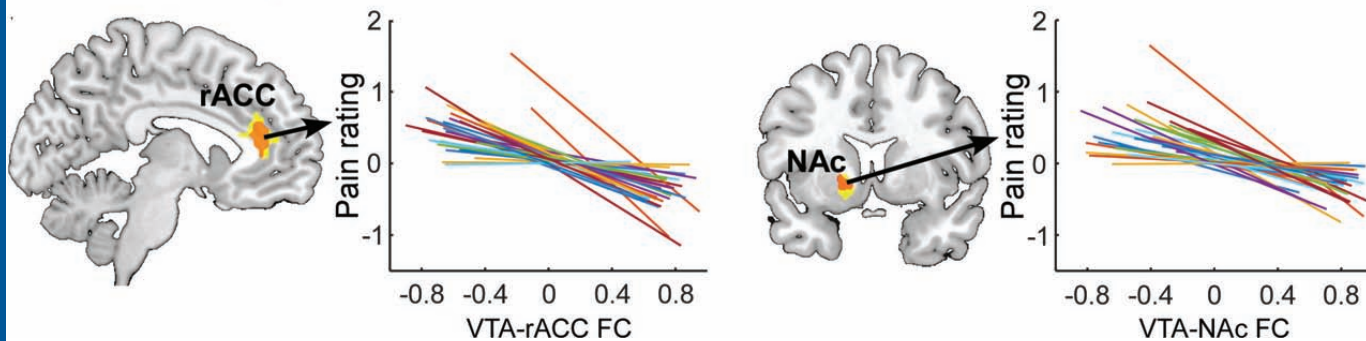
C. VTA connectivity (High < Neutral)

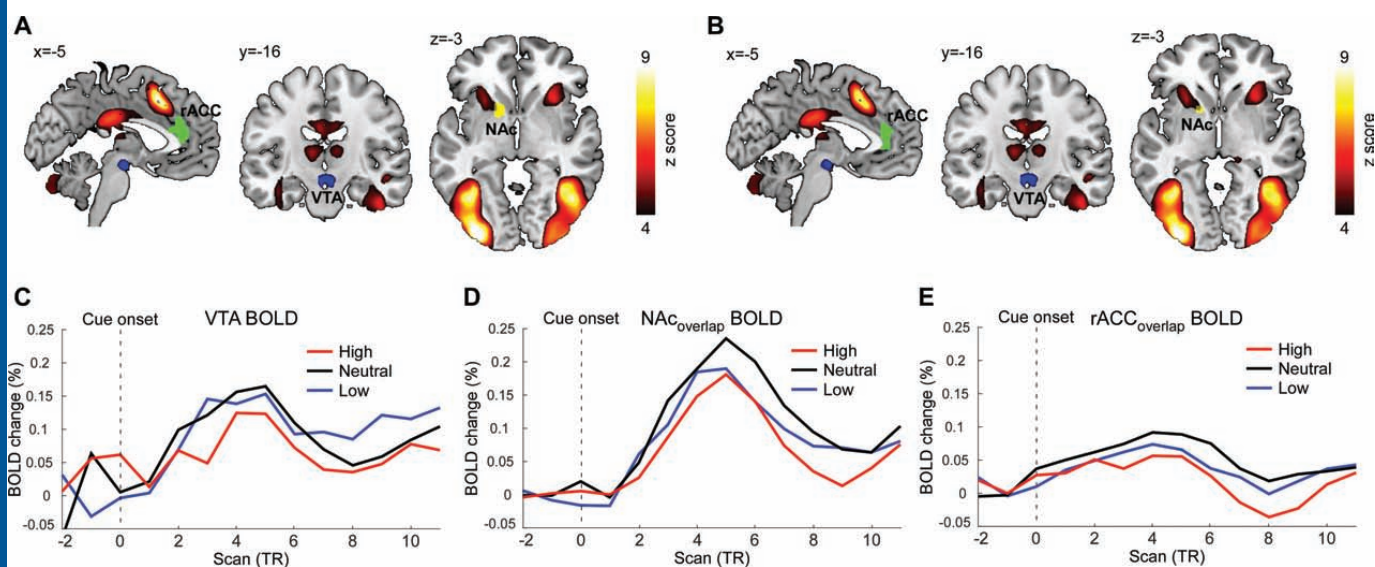


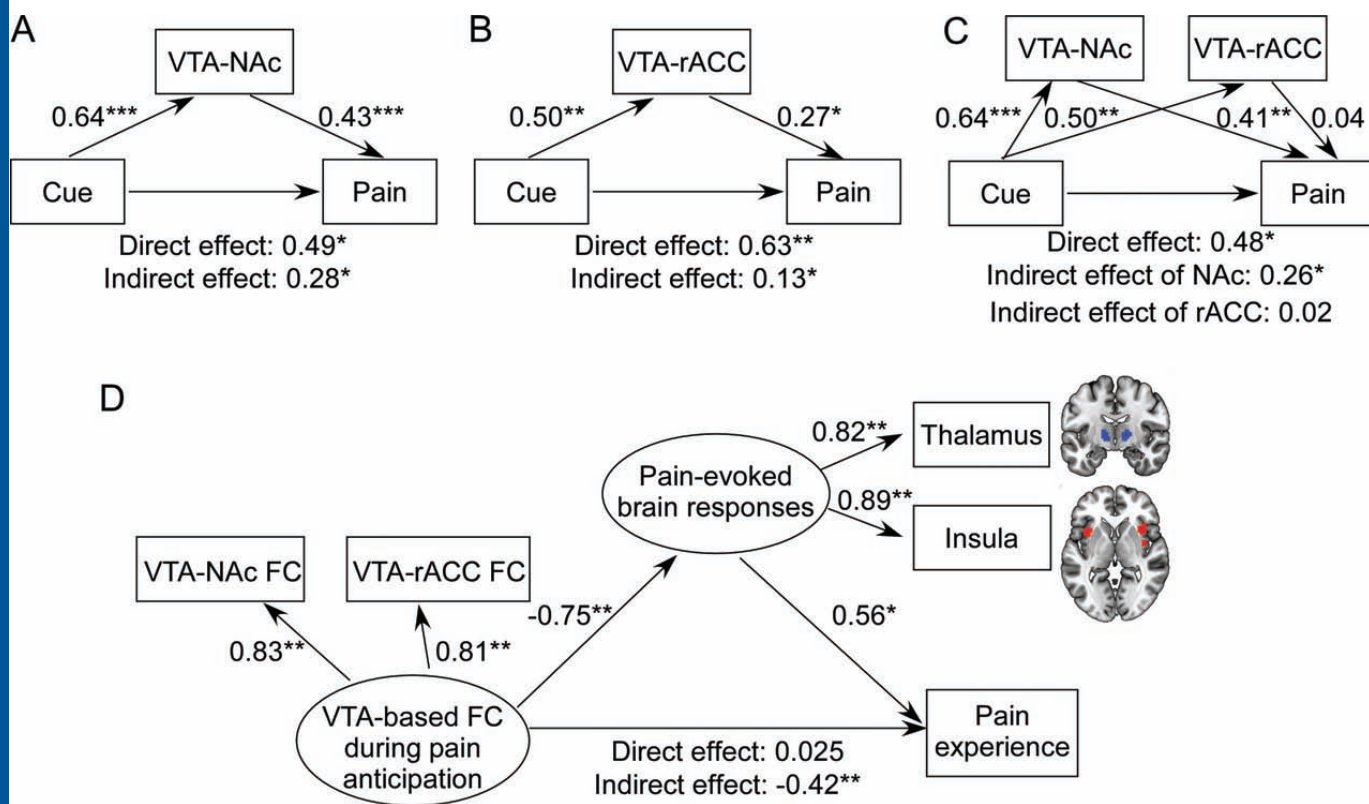
D. SN connectivity



E. VTA connectivity and Pain



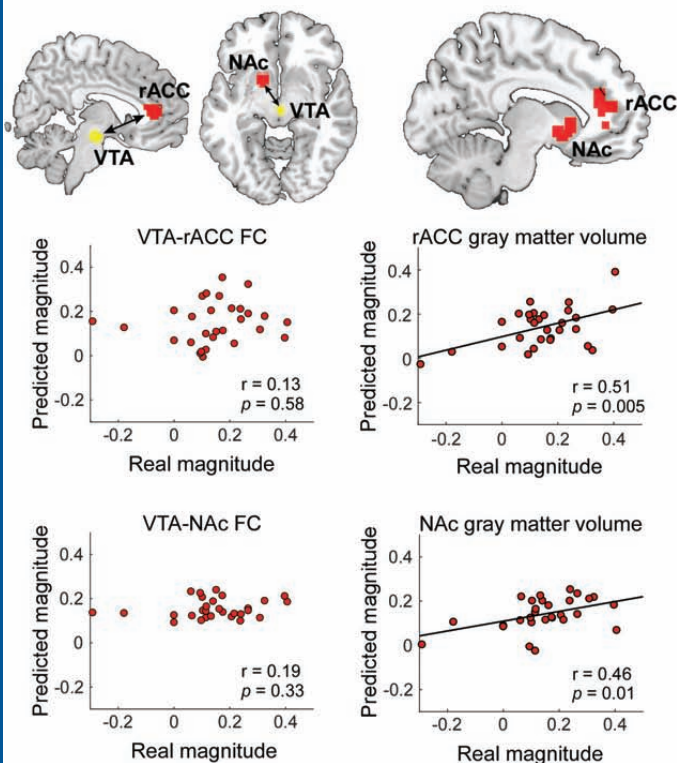




A Positive expectancy effects

Functional Connectivity

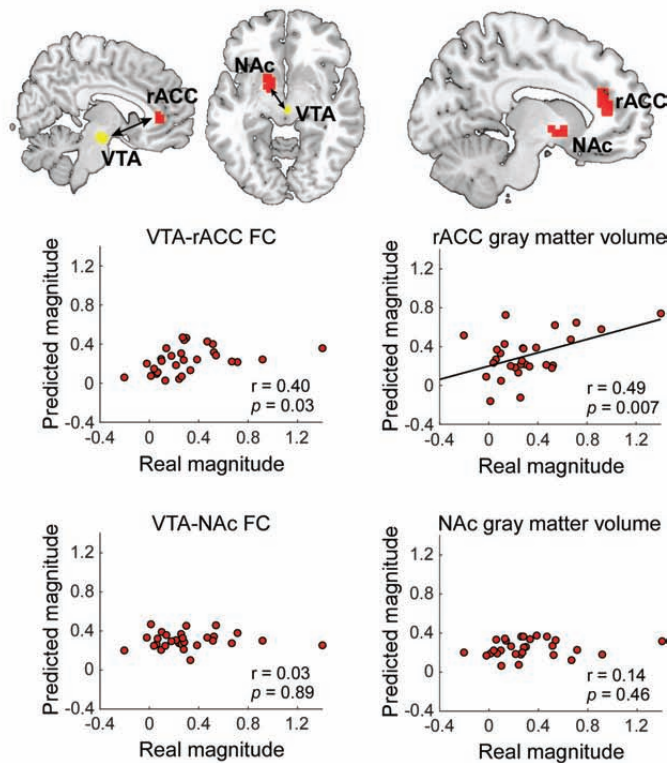
Gray Matter Volume



B Negative expectancy effects

Functional Connectivity

Gray Matter Volume



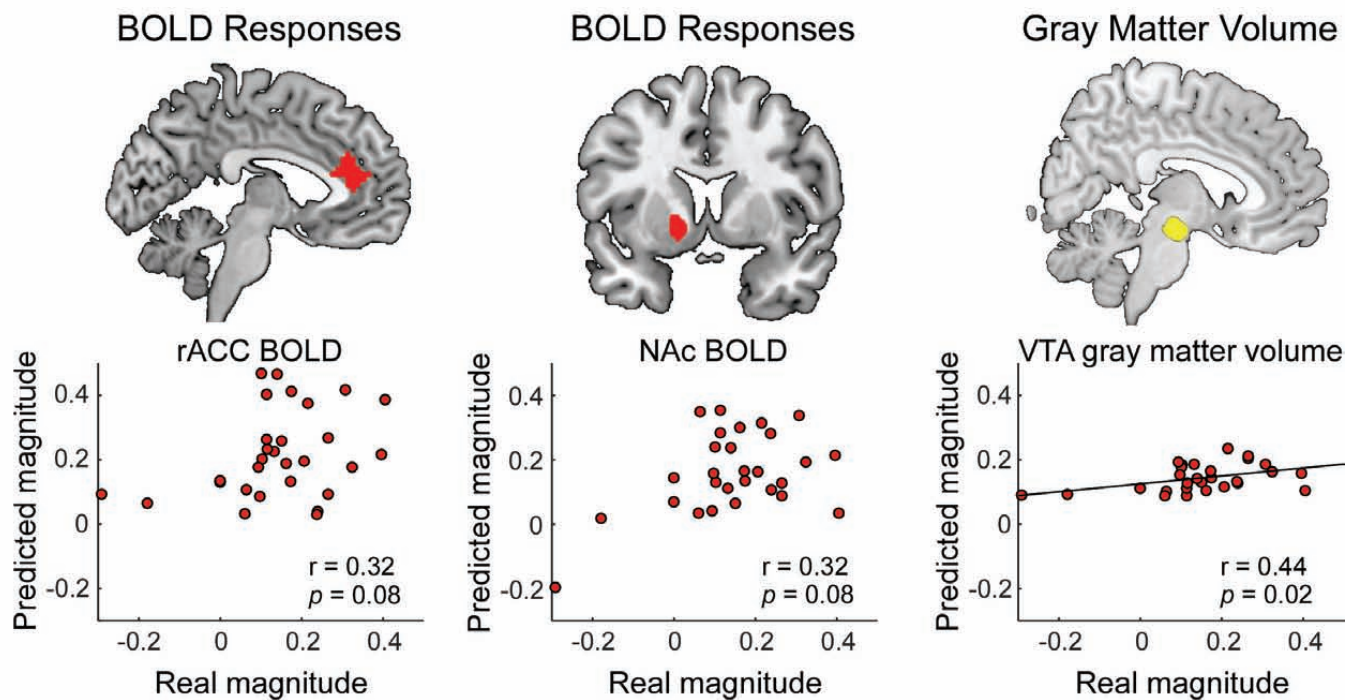
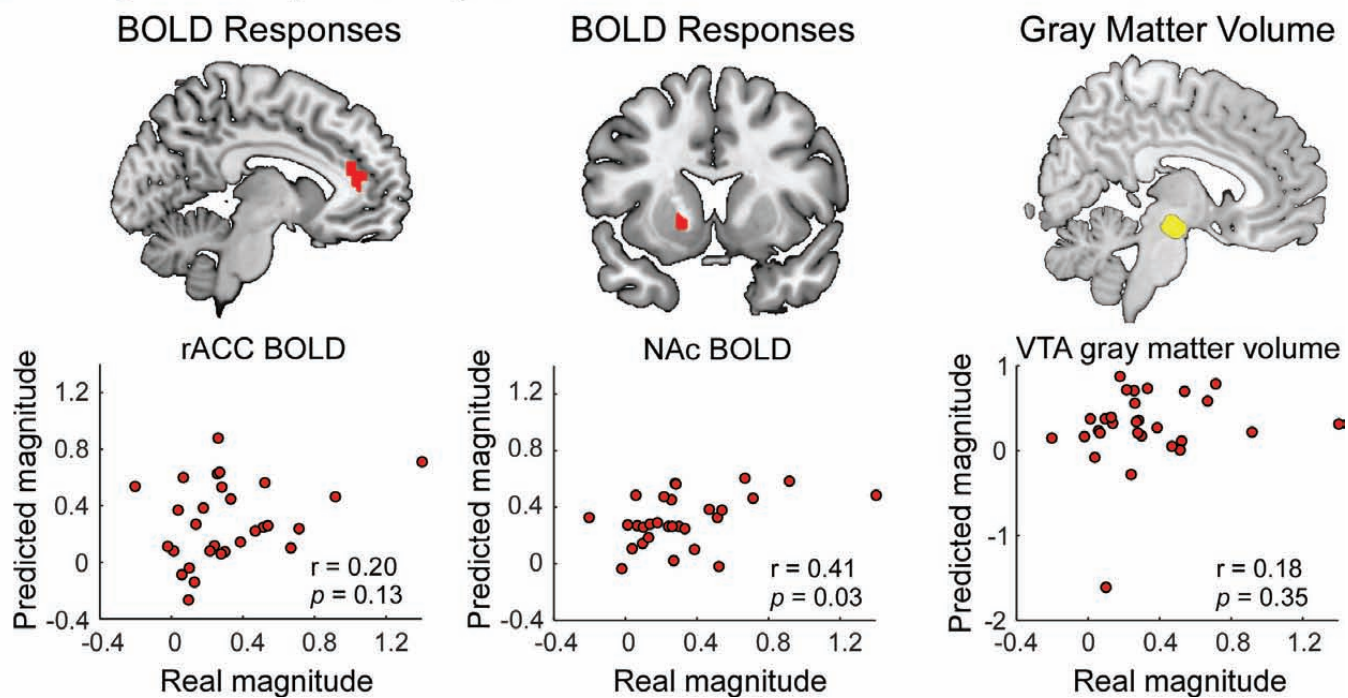
A Positive expectancy effects**B Negative expectancy effects**

Table 1. Clusters that exhibited significant brain activations and deactivations to pain stimuli.

Activation clusters					Deactivation clusters				
Area	Side	Peak x/y/z	T	Size	Area	Side	Peak x/y/z	T	Size
SMA	L	-6 14 49	8.5	283	ParaHipp	L	-30 -34 -17	-7.8	30
SMA	R	8 20 39	8.2	208	ParaHipp	R	27 -34 -17	-7.6	31
Insula	L	-33 20 1	7.5	361					
Insula	R	36 17 1	7.7	323					
Thalamus	L	-9 -13 4	7.4	164					
Thalamus	R	13 -13 4	8.0	165					
Caudate	L	-11 11 4	6.6	179					
Caudate	R	14 10 4	6.7	168					
Putamen	L	-17 14 5	6.4	119					
Putamen	R	17 7 -6	6.6	128					
ACC	L	-6 34 26	6.2	102					
ACC	R	10 30 27	6.4	126					
SI/SII	L	-33 -58 43	9.8	283					
SI/SII	R	60 -46 40	10.0	426					
Precuneus	L	-12 -67 52	8.7	53					
Precuneus	R	15 -67 40	11.7	110					
Cerebellum	L	-6 -76 -20	12.1	989					
Cerebellum	R	36 -58 -29	10.5	397					

SMA: supplementary motor area, ACC: anterior cingulate cortex, SI: primary somatosensory cortex, SII: secondary somatosensory cortex, ParaHipp: parahippocampus.

Table 2. Clusters that exhibited significant differences between cues during pain experience.

High > Neutral					Neutral > Low				
Area	Side	Peak x/y/z	T	Size	Area	Side	Peak x/y/z	T	Size
Insula	L	-37 5 -5	6.8	300	Insula	L	-36 11 1	3.7	33
Insula	R	39 11 -5	6.9	320	Insula	R	36 14 -2	4.0	36
ACC	L,R	-11 24 31	3.7	290					
SMA	L,R	6 17 46	3.6	75					
Putamen	L	-31 6 -5	6.1	54					
Putamen	R	30 10 -4	6.2	116					
Thalamus	L	-14 -6 0	3.8	42	Thalamus	L	-15 -13 7	5.2	58
Thalamus	R	16 -10 0	3.9	92	Thalamus	R	15 -10 7	5.1	80
MCC/PCC	L,R	9 -25 31	5.6	257	MCC/PCC	L,R	9 -28 31	3.6	43
DLPFC	L	-32 51 6	4.4	33					
DLPFC	R	41 50 4	4.6	69					
Midbrain	L,R	9 -21 -5	3.1	35	Midbrain	L,R	-5 -22 -9	3.8	55
High < Neutral					Low > Neutral				
Area	Side	Peak x/y/z	T	Size	Area	Side	Peak x/y/z	T	Size
Angular	L	-45 -76 31	-4.0	30					
Angular	R	53 -68 28	-4.1	42					

SMA: supplementary motor area, ACC: anterior cingulate cortex, MCC: middle cingulate cortex, PCC: posterior cingulate cortex, DLPFC: dorsal lateral prefrontal cortex.

Table 3. Clusters that exhibited significant functional connectivity with VTA during pain anticipation.

Area	Side	Peak x/y/z	T	Size
ACC	L,R	9 32 25	8.5	360
NAC	L	-16 11 2	13.9	63
NAC	R	15 18 -5	12.2	55
mPFC	L,R	3 50 -2	10.6	294
IFG	L	-40 32 14	8.1	298
IFG	R	45 36 8	9.2	223
Insula	L	-27 17 -11	12.5	146
Insula	R	27 23 -11	11.1	177
Thalamus	L	-8 -15 7	11.2	249
Thalamus	R	13 -15 4	11.2	250
Caudate	L	-16 14 6	11.8	108
Caudate	R	14 10 4	11.4	151
Putamen	L	-22 12 -2	12.1	243
Putamen	R	18 8 -8	10.7	240
Hippocampus	L	-17 -10 -18	10.2	176
Hippocampus	R	21 -10 -20	6.4	184
Amygdala	L	-19 -3 -20	9.8	46
Amygdala	R	22 -3 -20	10.0	50

ACC: anterior cingulate cortex, NAC: nucleus accumbens, mPFC: medial prefrontal cortex, IFG: inferior frontal gyrus.

Table 4. Clusters that exhibited significant differences of functional connectivity with VTA between cues during pain anticipation.

High < Neutral					Neutral < Low				
Area	Side	Peak x/y/z	T	Size	Area	Side	Peak x/y/z	T	Size
NAc	L	-15 11 -2	3.3	26	NAc	L	-14 10 -5	5.9	37
rACC	L	-12 38 13	4.5	67	rACC	L	-3 35 19	4.7	63

NAc: nucleus accumbens, rACC: rostral anterior cingulate cortex.