DECONSTRUCTING PAIN: SENSORY AND COGNITIVE MANIPULATIONS OF PAIN ARE MEDIATED BY DISTINCT SYSTEMS

by

CHOONG-WAN WOO

B.A., Seoul National University, 2005

M.A., Seoul National University, 2007

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Master of Arts

Department of Psychology and Neuroscience

2013

This thesis entitled:

Deconstructing pain:
Sensory and cognitive manipulations of pain are mediated by distinct systems
written by Choong-Wan Woo
has been approved for the Department of Psychology and Neuroscience

Tor D. Wager_____

Matt Jones_____

Randall C. O'Reilly _____

Date_____

The final copy of this thesis has been examined by the signatories, and we
Find that both the content and the form meet acceptable presentation standards
Of scholarly work in the above mentioned discipline

HRC protocol # AAAE3743

Woo, Choong-Wan (M.A., Department of Psychology and Neuroscience)

Deconstructing pain: Sensory and cognitive manipulations of pain are mediated by distinct systems

Thesis directed by Associate Professor Tor D. Wager

Cognitive strategies can strongly modulate emotion and pain. However, it is unclear whether cognition primarily influences core affective processes or later decision and valuation processes. We combined fMRI imaging with an experimental pain paradigm, and concurrently manipulated both the intensity of noxious input and a cognitive reappraisal of pain. Both manipulations strongly influenced reported pain, but they did so via two distinct brain pathways. The effects of stimulus intensity were mediated by a distributed brain network recently shown to predict physical pain with over 90% sensitivity and specificity across four studies. Cognitive reappraisal had no effect on activity in this network. Instead, cognitive effects on pain were mediated through a pathway connecting the nucleus accumbens and ventromedial prefrontal cortex. This pathway was unresponsive to noxious input, and has been broadly implicated in valuation and emotional appraisal. These findings suggest that sensory and cognitive manipulations influence pain through distinct brain pathways.

CONTENTS

TABLES

Table

FIGURES

Figure

CHAPTER I

INTRODUCTION

The ability to regulate affective experience, including negative emotion and pain, is critical for physical and mental health (Gross and Munoz, 1995). Among emotion regulation strategies, reappraisal—the use of self-generated cognition to reinterpret emotion-eliciting events—has been found to be particularly effective in promoting psychological well-being (Gross and John, 2003). There is currently intense interest in the brain mechanisms that underlie such regulatory strategies (Ochsner et al., 2012; Tracey, 2010) and the types of affective brain processes they influence, with current theory focusing on prefrontal-subcortical emotion and valuation circuits (Ochsner et al., 2012; Wager et al., 2008). However, because brain markers that are sensitive and specific to particular types of affective experience have not been identified, it is still unclear whether cognitive regulation influences core affective processes that translate sensory input (e.g., aversive images, painful heat) into affective experience, or valuation and decision-making processes involved in evaluating, reflecting on, and acting on such experiences.

It might first appear that adequate brain markers for affective processes already exist. In neuroimaging studies of pain, for example, reductions in activity within the anterior insula and cingulate cortices (ACC) are often taken as evidence for the modulation of core pain-construction processes (Tracey, 2010; Wager et al., 2004). However, these regions are the most frequently activated brain areas in the functional Magnetic Resonance Imaging (fMRI) studies across all task types (Yarkoni et al., 2011), indicating that the overall activity of these regions lack specificity to pain experience. In neuroimaging studies on emotion, amygdala activity has often been used as a brain marker for negative emotion (Ochsner et al., 2012; Wager et al., 2008). However, amygdala neurons respond to both positive and negative events (Paton et al.,

2006). In addition, amygdala activity can be elicited without the conscious experience of emotion (Whalen et al., 2004), and some challenges that elicit strong subjective anxiety and autonomic increases do not involve the amygdala (Davis et al., 2003; Wager et al., 2009). Therefore, in order to test the effects of psychological, pharmacological, or other manipulations on brain representations of affective experience (i.e., pain or a particular emotion), brain markers sensitive and specific to that type of experience must be developed (Poldrack, 2011).

Recently, we identified a distributed fine-grained pattern of fMRI activity that is sensitive and specific to physical pain across four separate experiments (Wager et al., 2013) (Figures 1 and 3B), providing a new leverage point for testing the effects of pain-modulatory interventions. The fMRI pattern, which we term the 'neurologic pain signature' (NPS), is not defined by overall activation of 'pain-related' regions such as ACC and insula, but on more fine-grained patterns within the ACC, insula, thalamus, and other regions. Across four fMRI studies, the strength of the NPS response (a weighted average of activity across the pattern) discriminated physical pain from non-painful warmth, pain anticipation, distressing images related to social rejection, and pain recall in 90-100% of individual participants tested (depending on the comparison and study). This accuracy level was achieved even when applying the NPS pattern to individuals tested on different scanners. The NPS response also tracked subjective pain more closely than the noxious stimulus itself, and was strongly reduced by the opiate analgesic remifentanil, demonstrating sensitivity to active treatment. Together, these findings establish the NPS as a provisional brain marker for experimental, nociceptive pain.

This foundation provides a platform for re-examining the effects of cognitive regulation strategies, by testing whether its effects on pain are mediated through activity in the NPS or in other pathways. This test can yield information about the nature of the systems influenced by

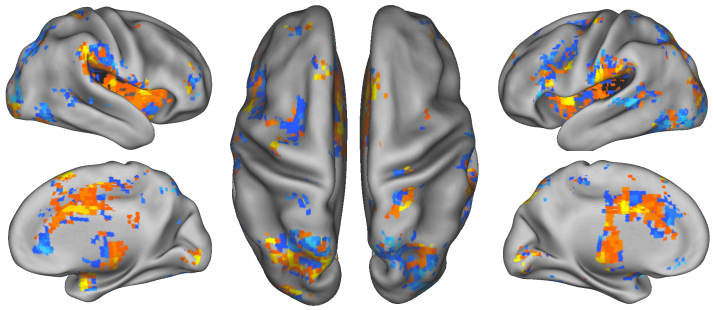


Figure 1. The unthresholded surface image of the neurologic pain signature (NPS; Wager et al., 2013).

cognitive regulation strategies and the stages of processing impacted. Treatments that influence early nociceptive and pain construction processes would be likely to influence the NPS response, as does the opiate analgesic remifentanil (e.g., Wager et al., 2013). Conversely, treatments that mainly affect reflections on and decisions about pain should not impact the NPS, but could have effects on pain reports that are mediated by other brain systems.

Different types of cognitive strategies are likely to modulate pain via different systems (Lawrence et al., 2011). A dominant theory of pain modulation has been that modulatory effects must occur by descending modulation of nociception. However, some recent provocative findings suggest that non-nociceptive brain systems can also mediate pain under different circumstances (Baliki et al., 2010; Baliki et al., 2012; Hashmi et al., 2013). Some cognitive pain-modulation strategies could rely on non-nociceptive systems, but this possibility has not been examined. Particularly, understanding cognitive reappraisal of pain has major implications for many forms of therapy based on self-regulation techniques.

CHAPTER II

METHODS

To assess the effects of cognitive reappraisal, we designed an intervention that would have a high probability of affecting pain based on prior studies (Fernandez and Turk, 1989; Rainville et al., 1999). We instructed participants to use affectively loaded mental imagery (Metcalfe and Mischel, 1999) to both increase and decrease pain (on different trials). For example, on 'Regulate-up' trials, participants imagined that the heat was burning, sizzling, and melting their skin. On 'Regulate-down' trials, they imagined that the heat was pleasant and diffuse, like "a warm blanket on a cold day" (see Experimental Procedures in Appendix). These procedures differ somewhat from reappraisal procedures employed in the regulation of visual images, as a) the narrative component inherent in reappraisal of complex images is less applicable in pain, which is a uniform, primary sensory experience, and b) pain can be effectively modulated by affective imagery (Fernandez and Turk, 1989).

We examined whether the effects of reappraisal on pain were mediated by the NPS (*hypothesis A*; a red path in Figure 3A) or other brain systems involved in pain valuation and affective behavior (*hypothesis B;* a blue path in Figure 3A). Participants ($N = 33$) experienced thermal stimulation at six distinct temperatures during fMRI scanning (44.3-49.3°C in 1-°C increments on the left forearm, with 4~20 repetitions of each stimulus; Figure 2). After each trial, participants judged whether the stimulus was painful or not, followed by a judgment of pain or warmth intensity on a 100-point visual analog scale. On seven of the nine experimental runs, participants passively experienced and rated the stimuli ("Passive experience" condition), but on two "Regulation" runs (the 3rd and 7th), we asked them to cognitively increase or decrease pain intensity. In the Regulate-up run (the 3rd for half the participants, and the 7th for the other half),

participants used reappraisal to increase pain, and in the Regulate-down run, they used reappraisal to reduce it.

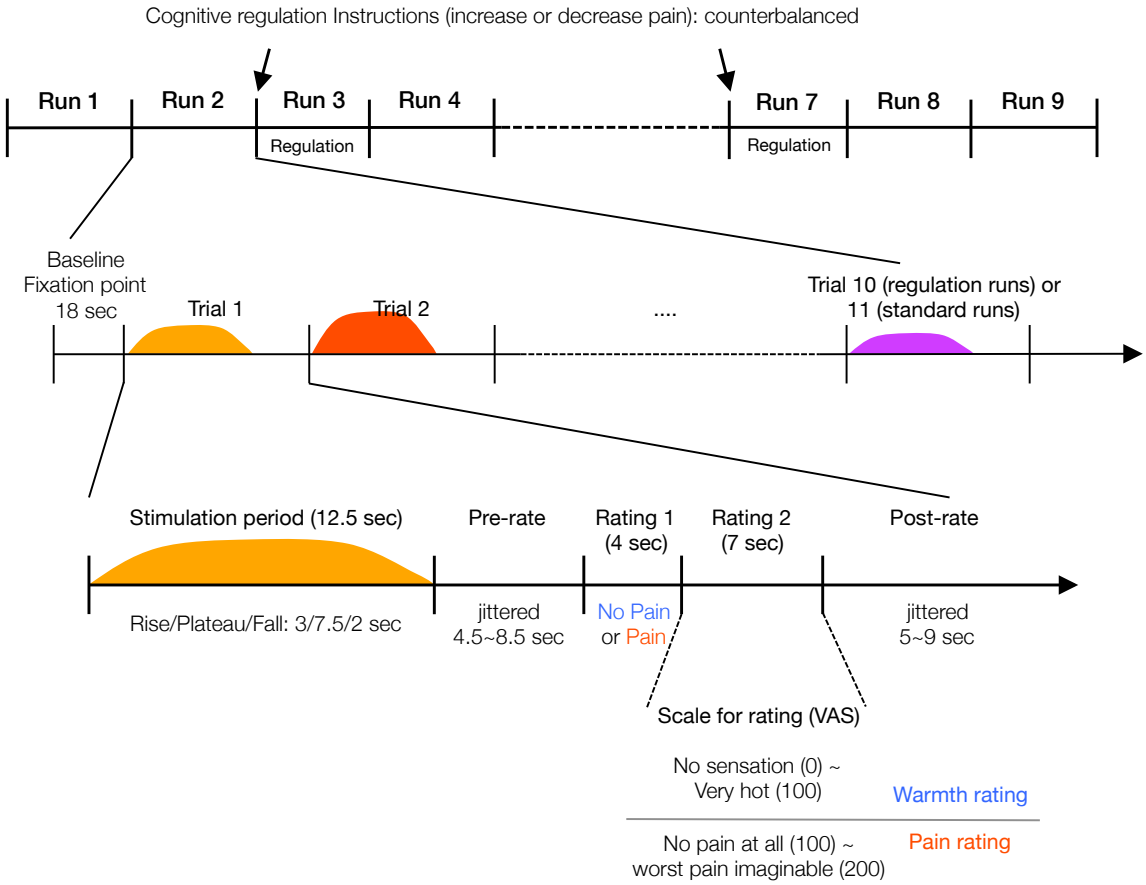


Figure 2. Experimental design. The experiment consisted of 9 runs. Among the runs, third and seventh runs were "Regulation" runs, which consisted of "Regulate-up (increase pain)" and "Regulate-down (decrease pain)". The ordering of the two conditions was counterbalanced across subjects. Passive experience (i.e., no regulation) runs comprised 11 trials, and regulation runs comprised 10 trials. During each run, thermal stimulations that consisted of 6 levels of intensity for the passive experience runs and 5 levels of intensity for the regulation runs were delivered. The Regulate-up or -down instructions were presented before regulation runs (3^{rd} or 7^{th} runs). Every run started with a baseline period during which a fixation cross was presented for 18 seconds. Each trial started with a 12.5-second long thermal stimulation, followed by a 4.5 to 8.5 seconds long pre-rating period. After the pre-rating period, participants were asked to decide if the stimulation was painful or not. Then, participants rated the intensity of the warmth or painful sensation. A 5- to 9-second inter-trial interval followed the rating period.

CHAPTER III

RESULTS

Both heat intensity and reappraisal substantially impacted pain reports. As expected, pain reports were higher with increasing heat intensity ($\hat{\beta}$ = 22.10, t_{32} = 17.60, P < .0001). In addition, pain increased in the Regulate-up (t_{32} = 3.71, P < .001) and decreased in the Regulate-down (t_{32} = -3.82, P < .001) compared to Passive experience conditions, and pain was higher in the Regulate-up than Regulate-down condition ($\hat{\beta}$ = 14.02, t_{32} = 4.75, P < .0001; Figure 3E). There was no interaction between heat intensity and reappraisal instructions ($\hat{\beta}$ = -0.66, t_{32} = -1.03, P = .31). Further, the significant effects of cognitive reappraisal on pain were retained when considering only data in the clearly noxious range, above 47°C ($\hat{\beta}$ = 11.25, t_{32} = 3.88, P < .001), which is judged to be painful on nearly 80% of trials for all participants (Figure 4).

To test whether cognitive reappraisal affects pain reports by changing core nociceptive brain processes (*hypothesis* A), we estimated the linear increase in NPS response as a function of both stimulus intensity and Regulate-up vs. Regulate-down instructions (Figure 3E). NPS response increased substantially with stimulus intensity ($\hat{\beta}$ = 2.41, t_{32} = 10.53, P < .0001), but showed no effect of cognitive reappraisal ($\hat{\beta}$ = 0.05, t_{32} = 0.13, P = .90). In addition, stimulus intensity did not interact with cognitive reappraisal ($\hat{\beta}$ = -0.03, t_{32} = -0.20, P = .84). This pattern of findings were same in the clearly noxious range, above 47°C. There was a strong effect of stimulus intensity on NPS responses ($\hat{\beta}$ = 5.13, t_{32} = 7.95, P < .0001), but no effect of reappraisal on NPS responses ($\hat{\beta}$ = 0.18, t_{32} = 0.34, P = .74).

In addition, we used multilevel mediation (Atlas et al., 2010) to test whether the NPS response mediated the effects of both cognitive reappraisal and stimulus intensity on pain report.

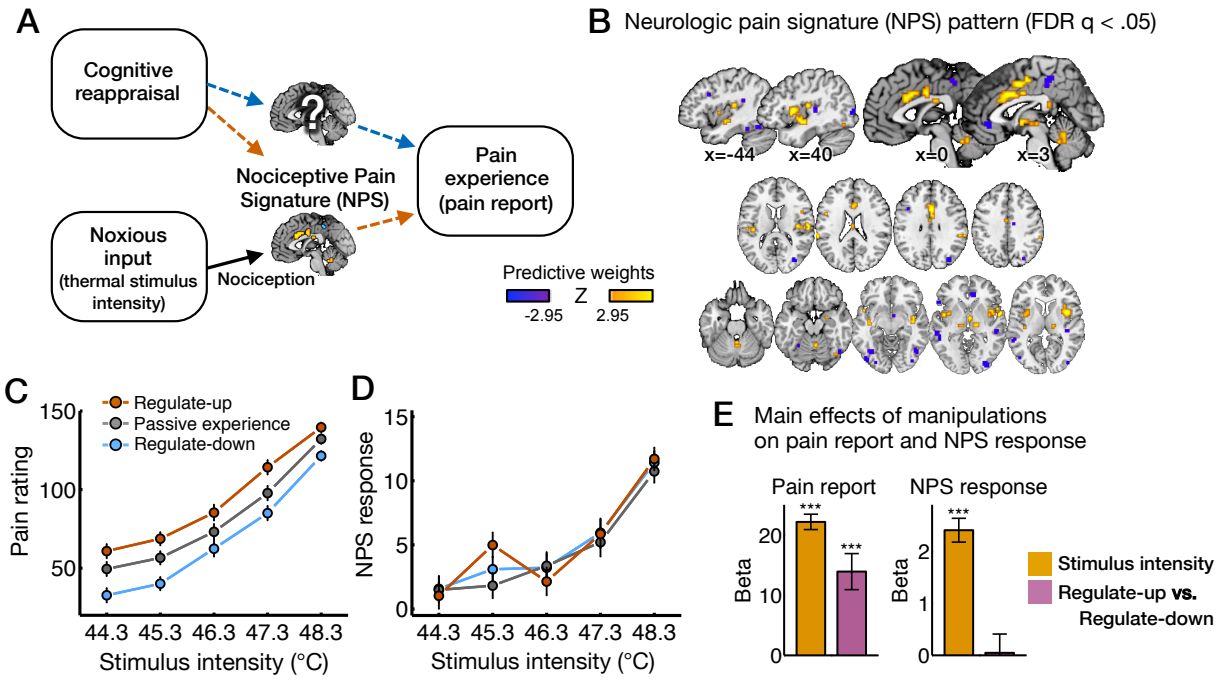


Figure 3. The effects of sensory and cognitive reappraisal on reported pain and brain activity. (A) Hypotheses about the effects of cognitive reappraisal on pain-related brain processes. The effects of cognitive reappraisal on pain could be mediated by the neurologic pain signature (NPS) (*Hypothesis A*, red dotted lines) or other brain systems (*Hypothesis B*, blue dotted lines). **(B)** The NPS pattern, an *a priori* distributed pattern of functional Magnetic Resonance Imaging signal that is sensitive and specific to physical pain (Wager et al., 2013). The pattern map is thresholded at $q < .05$, False Discovery Rate (FDR) for display only. All voxels within NPS were used in analyses. **(C), (D)** Pain rating and NPS response as a function of stimulus intensity and regulation conditions. NPS response values, which indicate the strength of expression of the signature pattern, are calculated by taking the dot-product of the NPS pattern weights and activation maps for each single trial. Error bars represent within-subject standard errors of the mean (S.E.M.). **(E)** The main effects of manipulations (Stimulus intensity and Regulate-up vs. -down) on pain rating and NPS response. Beta (y-axis) represents regression coefficients from a multi-level generalized linear model. Error bars represent S.E.M. ***$P < .001$, two-tailed.

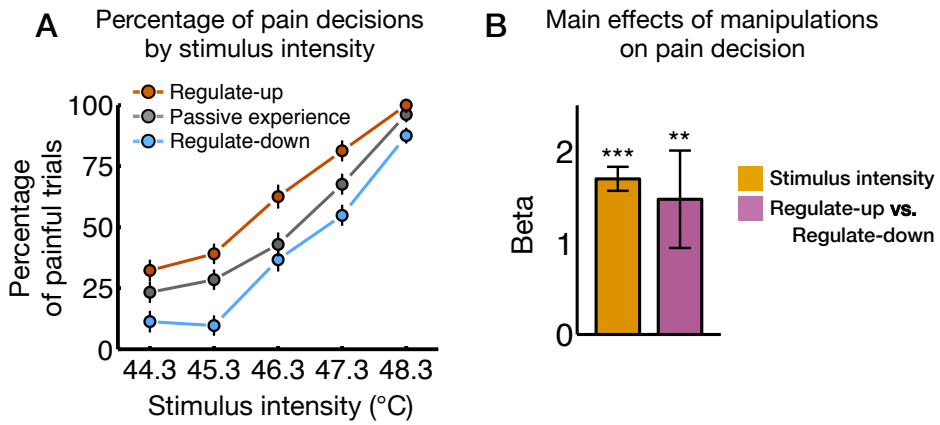


Figure 4. The effects of stimulus intensity and cognitive reappraisal on pain/no-pain decisions. (A) The percentage of trials on which people reported the stimulus was painful, as a function of stimulus intensity and regulation condition. **(B)** The main effects of manipulations (Stimulus intensity and Regulate-up vs. -down) on the percentage of pain decision. Beta (y-axis) represents regression coefficients from logistic regression for each participant, and error bars represent standard errors of the mean (S.E.M.) across participants. $^{**}p < .01$, $^{***}p < .001$, two-tailed.

Mediation analysis tested the joint effects of reappraisal on NPS response (Path *a*) and NPS response on pain (Path *b*), as well as the direct (non-mediated) effect of reappraisal on pain (Path *c*). Regulate-up vs. -down instructions and NPS response magnitude were both associated with increased pain reports (Paths *b* and *c* in Figure 5B), but reappraisal instructions did not impact the NPS response (Path *a*), and therefore the mediation effect (Path *a*×*b*) was not significant. In contrast, the NPS response mediated the effect of heat intensity on pain reports (Figure 5A). Therefore, *hypothesis A* was not supported by the results, indicating that the pain-modulatory effects of cognitive reappraisal are not primarily mediated by changes in early nociceptive processes.

This conclusion was supported by voxel-wise mapping of the effects of stimulus intensity and cognitive reappraisal on responses during pain. Increasing stimulus intensity was associated with increased activity in a number of regions associated with nociceptive processing and pain construction (Coghill et al., 1999), including right (contralateral) dorsal posterior insula (dpINS), bilateral secondary somatosensory cortex (S2), bilateral anterior insula (aINS), ventrolateral and medial thalamus (vlThal/mThal), and dorsal anterior cingulate cortex (dACC; Figure 6A; all voxel-wise results reported are significant at $P < .05$, family-wise error rate [FWER] corrected). Specific patterns within of these regions are part of the NPS.

In contrast, Regulate-up vs. -down instructions yielded a very different pattern of results. Across all voxels, the correlation between the map of stimulus intensity effects and cognitive reappraisal effects was $r = 0.022$. Activity in left nucleus accumbens (NAc) was enhanced for Regulate-down and suppressed for Regulate-up (Figures 7A, 7D, 7E, and Table 1). NAc activation was bilateral at a slightly lower threshold (voxel-wise $P < .001$; Figures 6B and 7A). This region was unresponsive to stimulus intensity ($\hat{\beta} = -.002$, $t_{32} = -0.33$, $P = .76$, Figure 7E),

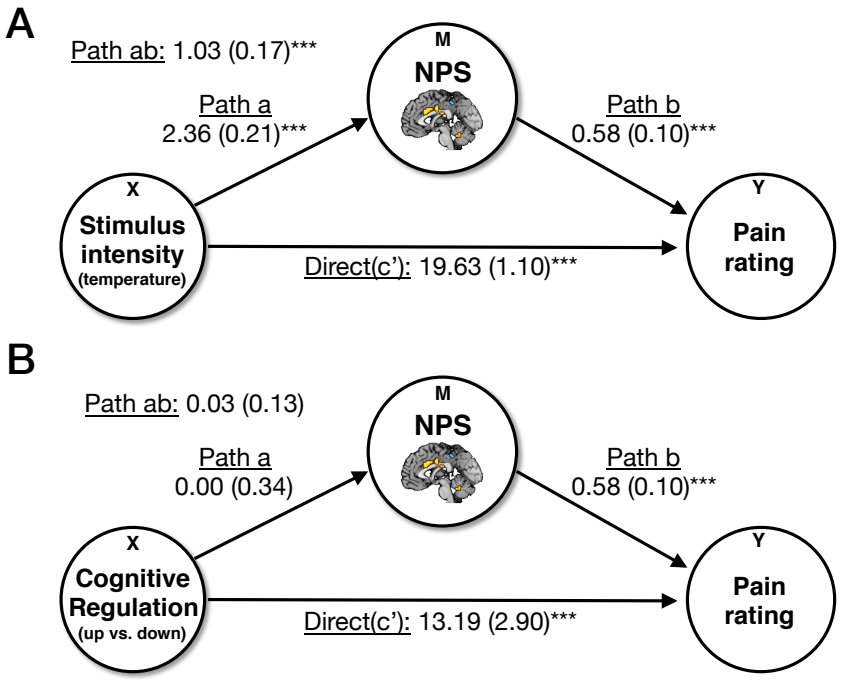


Figure 5. The NPS response mediated the relationship between stimulus intensity and pain ratings, but not between cognitive reappraisal and pain ratings. This figure presents the results of the multilevel mediation analyses, where the neurologic pain signature (NPS) response was the mediator. In **(A)**, stimulus intensity (i.e., temperature) was entered as a predictor, and in **(B),** cognitive reappraisal (Regulate-up vs. –down instructions) was entered as a predictor. In both models, pain ratings were the outcome. The results showed that the NPS response mediated the effects of temperature on pain report, but did not mediate the effects of cognitive reappraisal on pain report. The lines are labeled with path coefficients, and standard errors are shown in parenthesis. ***$p < 0.001$, two-tailed.

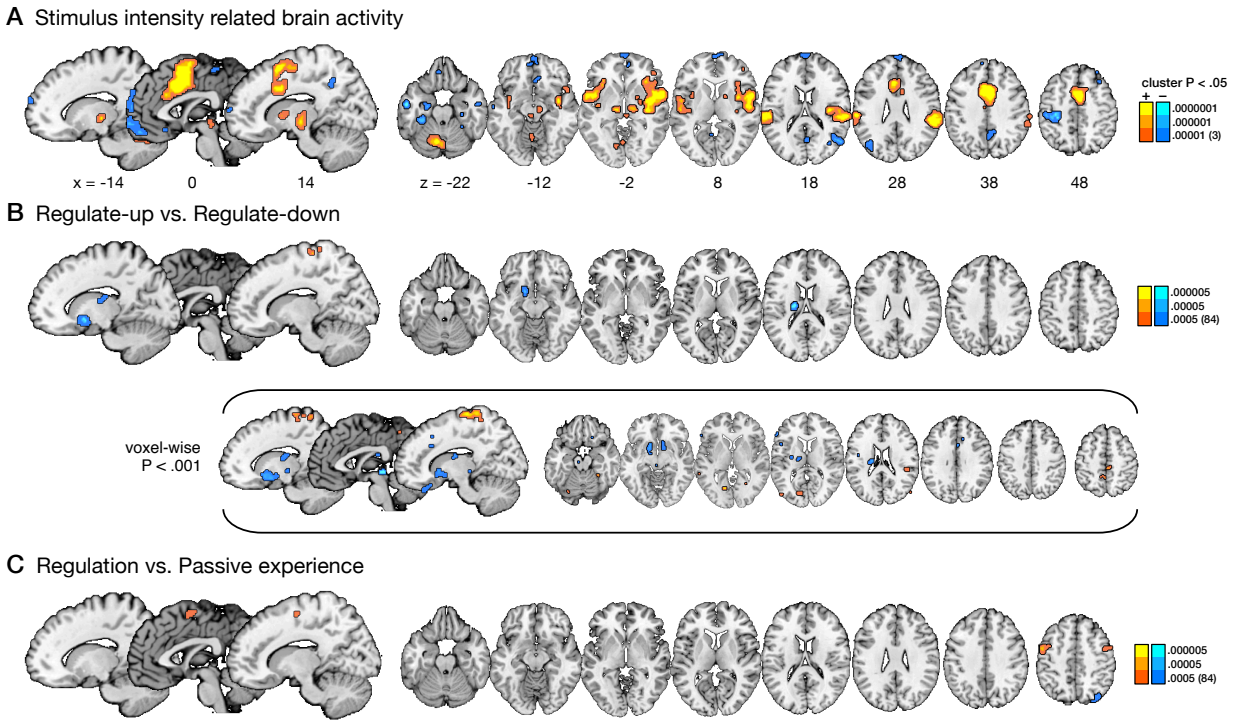


Figure 6. Stimulus intensity- and cognitive reappraisal-induced brain activity. (A) Brain regions that are associated with stimulus intensity. **(B)** Brain regions that are associated with Regulate-up vs. Regulate-down instructions. **(C)** Brain regions that are associated with Regulation vs. Passive experience instructions. In order to obtain these brain maps, we used parametric modulation modeling with regressors for stimulus intensity, cognitive regulation, and their interaction. All colored regions were significant at $p < .05$, family-wise error rate (FWER) corrected based on cluster extent estimated by Monte-Carlo simulation. The legend indicates primary voxel-wise threshold levels and cluster extent threshold (parentheses). For the purpose of display, we pruned the results using two additional higher levels of voxel-wise threshold.

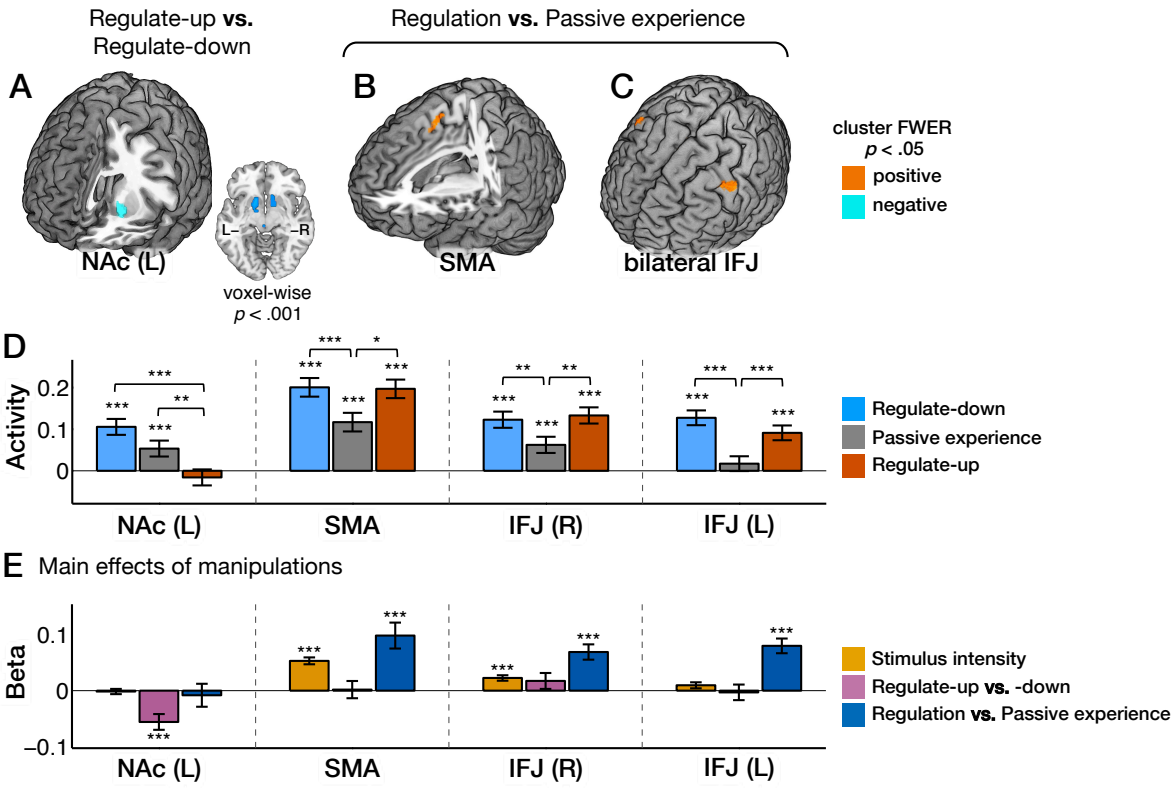


Figure 7. Cognitive reappraisal-induced brain activity. (A) Activity in left nucleus accumbens (NAc) was associated with Regulate-up vs. regulate-down instructions (at $p < .05$, family-wise error rate corrected based on cluster extent, with a primary threshold of $p < .0005$). Bilateral activations were found at a lower threshold (voxel-wise $p < .001$). **(B), (C)** Activity in the supplementary motor area (SMA) and bilateral inferior frontal junction (IFJ) were associated with Regulation vs. Passive experience instructions (for the whole-brain maps, see Figure 6B and 6C). **(D)** Bar plots of the averaged activity (y-axis) across voxels within the corresponding brain region for regulation conditions (x-axis). Significance levels present the results of one-sample or paired-sample t-test. **(E)** Main effects of Stimulus intensity, Regulate-up vs. -down, and Regulation vs. Passive experience on the corresponding brain regions. Beta (y-axis) represents regression coefficients from a multi-level generalized linear model with manipulations (i.e., Stimulus intensity, Regulate-up vs. -down, Regulation vs. Passive experience, and their interactions) as predictors and ROI activity as a dependent variable. Error bars represent standard errors of the mean (S.E.M.). ***$p < .001$, **$p < .01$, *$p < .05$, two-tailed.

Table 1. The effects of manipulations on brain regions associated with cognitive reappraisal

	Regulate-up vs. -down									Regulation vs. Passive experience											
	NAc			Caudate			SMC			SMA			IFJ (R)			IFJ (L)			SPL		
Effects	*β*	*t*	p	*β*	*t*	p	*β*	*t*	p	*β*	*t*	p	*β*	*t*	p	*β*	*t*	p	*β*	*t*	p
Contrasts (paired comparison)																					
Regulate-down vs. baseline	.105	3.71	.0008	.007	0.33	.7460	-.088	-2.93	.0063	.201	7.52	.0000	.123	4.85	.0000	.127	4.97	.0000	-.196	-4.38	.0001
Passive experience vs. baseline	.053	4.14	.0002	-.039	-2.92	.0064	-.049	-2.93	.0063	.117	5.60	.0000	.062	4.33	.0001	.017	1.38	.1765	-.105	-4.02	.0003
Regulate-up vs. baseline	-.016	-0.69	.4947	-.087	-4.39	.0001	.010	0.46	.6515	.197	5.23	.0000	.133	4.33	.0001	.091	3.73	.0007	-.224	-6.78	.0000
Regulate-down vs. passive experience	.052	1.85	.0737	.045	2.23	.0327	-.039	-1.47	.1513	.084	4.00	.0003	.061	3.01	.0050	.110	4.49	.0000	-.090	-2.70	.0111
Regulate-up vs. passive experience	-.069	-2.95	.0059	-.048	-2.39	.0229	.059	3.92	.0004	.080	2.19	.0356	.071	3.00	.0052	.074	3.90	.0005	-.119	-4.53	.0000
Regulate-up vs. Regulate-down	-.121	-4.16	.0002	-.093	-3.96	.0004	.098	3.11	.0039	-.004	-0.10	.9195	.010	0.28	.7808	-.036	-1.19	.2446	-.028	-0.63	.5307
Manipulation effects (multiple regression)																					
a) Stimulus intensity	-.002	-0.33	.7527	.002	0.42	.6544	-.001	-0.22	.8407	.053	7.66	.0001	.010	1.74	.0806	.023	4.61	.0001	-.020	-2.86	.0014
b) Regulate-up vs. Regulate-down	-.056	-3.85	.0001	-.048	-4.02	.0001	.046	3.37	.0001	.002	0.11	.8854	-.003	-0.21	.8302	.018	1.12	.1855	-.012	-0.57	.5001
c) Regulation vs. passive experience	-.008	-0.39	.7153	-.001	-0.03	.9382	.008	0.52	.5848	.099	4.15	.0003	.080	5.30	.0001	.069	5.09	.0003	-.097	-4.91	.0001
d) Interaction between a) and b)	-.002	-0.35	.7291	-.006	-1.33	.1566	.008	1.36	.1182	.002	0.37	.6703	.003	0.56	.5479	.006	1.22	.1848	.001	0.18	.8419
e) Interaction between a) and c)	-.000	-0.08	.9039	.002	0.36	.7025	-.011	-1.68	.0366	.003	0.51	.5767	.008	1.20	.1629	.003	0.45	.5900	.013	1.93	.0439

Note. The brain regions were significantly associated with Regulate-up vs. -down or Regulate vs. Passive experience instructions (for more details about the brain regions, see Appendix C). The statistics (*beta*, *t*, and *p*) were results of multilevel generalized linear model where independent variables were each contrast (for paired comparison, one contrast was entered in a single model) or manipulations (all the independent variables are entered in a single model), and dependent variables were ROI activity. IFJ, inferior frontal junction; NAc, nucleus accumbens; SMA, supplementary motor area; SPL, superior parietal lobe.

suggesting it is not part of the primary nociceptive circuit. A portion of the caudate nucleus also showed the same pattern of effects (Figures 8A). Conversely, sensorimotor cortex showed greater activity in Regulate-up vs. -down (Figure 8B). The supplementary motor area (SMA) showed a different pattern, with increased activation in both Regulate-up and Regulate-down conditions relative to Passive experience (Figures 7B, 7D, and 7E). This finding is consistent with literature on the cognitive reappraisal of aversive images, which shows reappraisal-related increases regardless of the direction of regulation (Urry et al., 2009). The bilateral inferior frontal junction (IFJ) also showed a similar pattern with SMA (Figures 7C and 7D). Both SMA and IFJ have been consistently implicated in cognitive reappraisal according to a recent meta-analysis (Buhle et al., 2013). In contrast, activity in superior parietal lobe decreased in both Regulate conditions relative to Passive experience (Figures 8C and 8D). Thus, overall, stimulus intensity and cognitive reappraisal produced very different effects on regional brain activity.

If cognitive reappraisal does not impact primary nociceptive pain-generation circuits (i.e., the NPS), it may impact pain in other ways (*hypothesis B*). For example, although NAc does not appear to code for nociceptive intensity, it could participate in the pain-generation process by signaling the motivational and hedonic value of the stimuli in context (Fields, 2004; Leknes and Tracey, 2008). To identify brain mediators of cognitive effects on pain, we first conducted a whole-brain search with multilevel mediation analysis to identify voxels that mediated Regulate-up vs. Regulate-down effects on pain reports, controlling for stimulus intensity. No clusters survived multiple-comparisons correction (Figure 9D), and examination of the maps revealed that cognitive reappraisal-induced brain activity (Path *a*, which included NAc) showed little overlap with brain activity predicting pain report (Path *b*; Figure 10C).

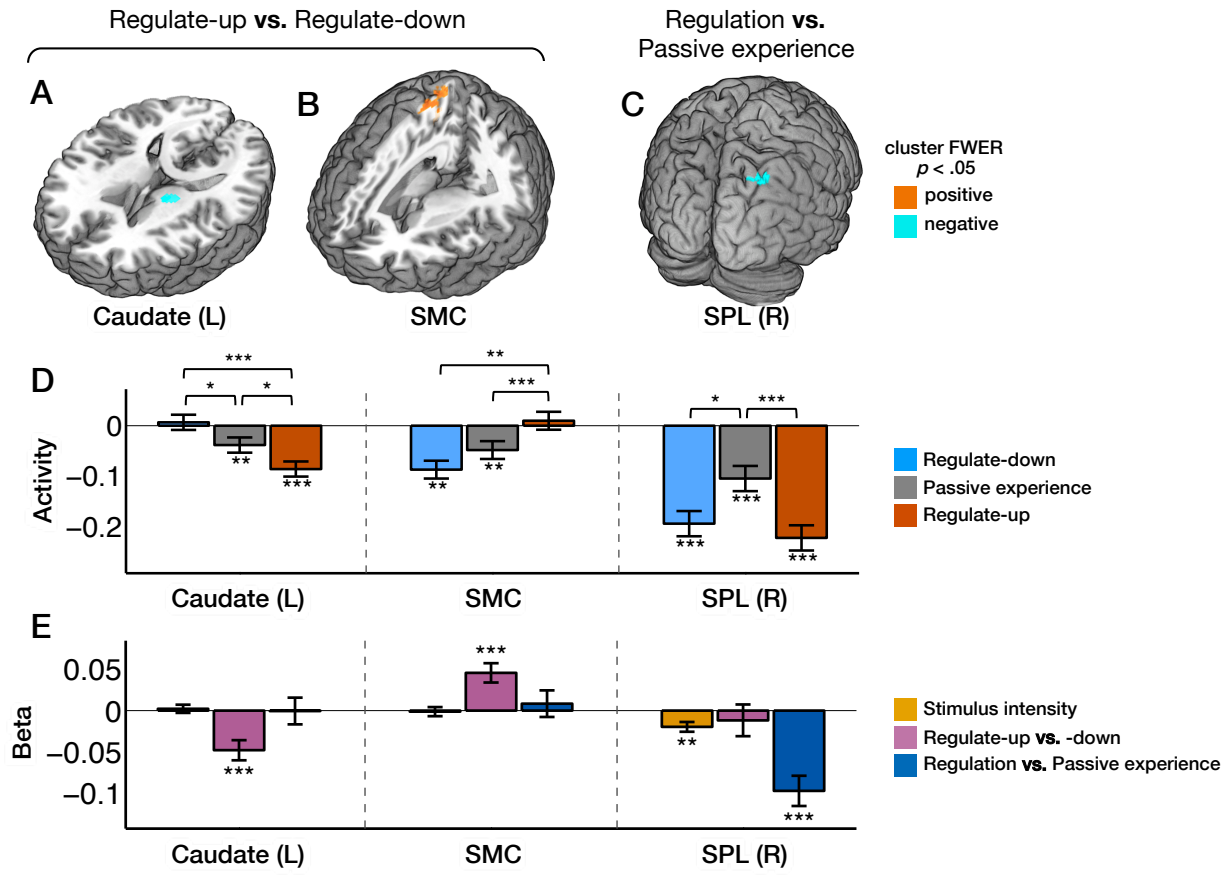


Figure 8. The effects of manipulations on brain regions associated with cognitive reappraisal. (A), (B) Activity in caudate (left) and sensory motor cortex (SMC) were associated with Regulate-up vs. -down instructions (at $p < .05$, family-wise error rate corrected based on cluster extent, with a primary threshold of $p < .0005$). **(C)** Superior parietal lobe (right) were associated with Regulation vs. Passive experience instructions. **(D)** Bar plots of the averaged activity (y-axis) across voxels within the corresponding brain region for regulation conditions (x-axis). Significance levels present the results of one-sample or paired-sample t-test. **(E)** Main effects of Stimulus intensity, Regulate-up vs. -down, and Regulation vs. Passive experience on the corresponding brain regions. Beta (y-axis) represents regression coefficients from a multi-level generalized linear model with manipulations (i.e., Stimulus intensity, Regulate-up vs. -down, Regulation vs. Passive experience, and their interactions) as predictors and ROI activity as a dependent variable. Error bars represent standard errors of the mean (S.E.M.). ***$p < .001$, **$p < .01$, *$p < .05$, two-tailed.

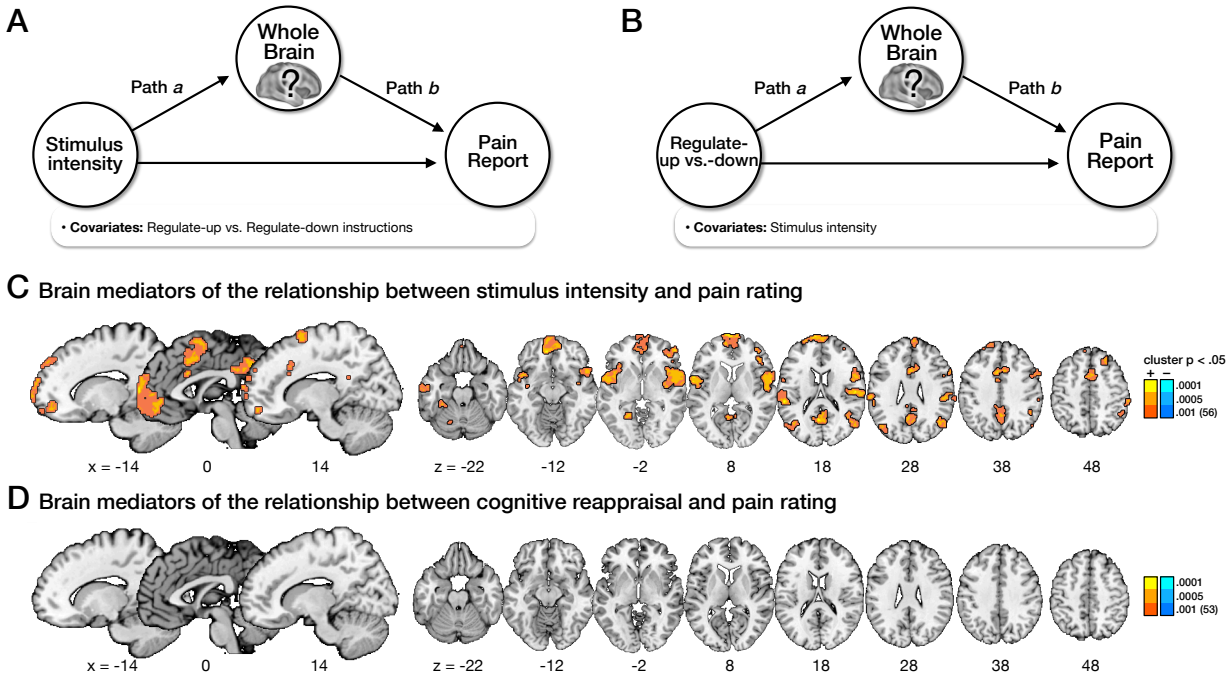


Figure 9. Whole-brain search for mediators of the relationship between stimulus intensity/cognitive reappraisal and pain rating. (A),(B) The mediation models. **(C)** and **(D)** present the results of the whole-brain search using multilevel mediation analysis. Only positive brain mediators were shown because of interpretability. There was no significant brain mediator between cognitive reappraisal and pain rating. The map was thresholded at cluster-extent based threshold $p < .05$, family-wise error rate (FWER) corrected based on Monte-Carlo simulation. The legend indicates primary voxel-wise threshold levels and cluster extent threshold (parentheses).

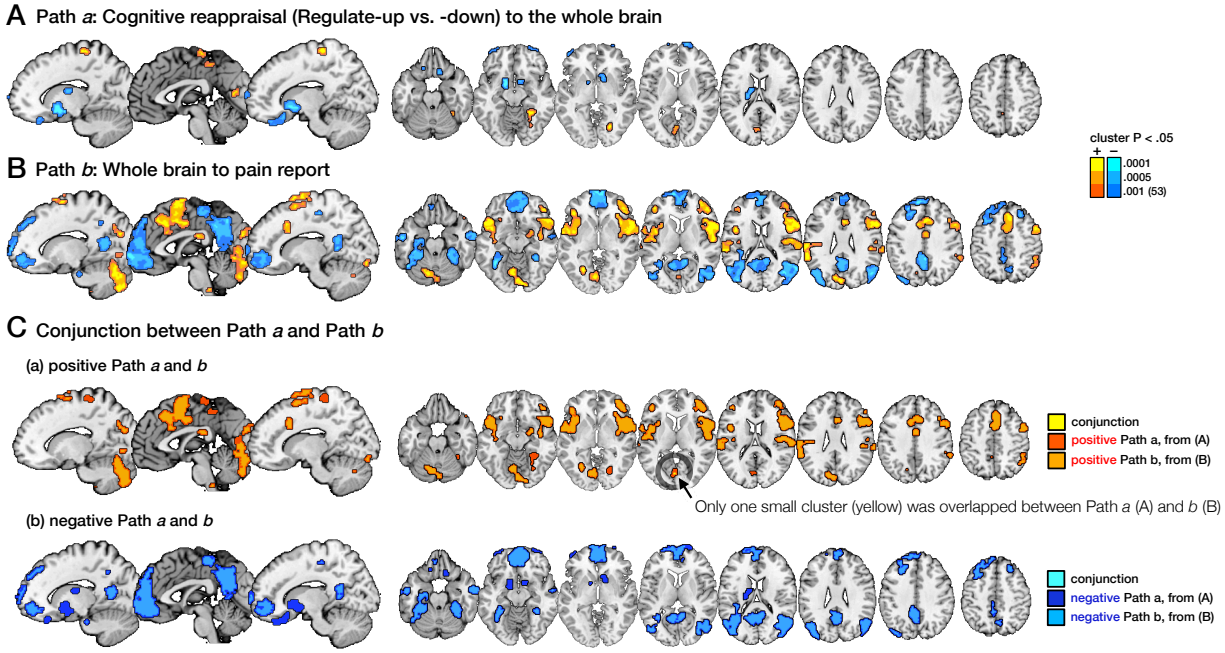


Figure 10. Path *a*, *b* and conjunction of the mediation analysis results of the relationship between cognitive reappraisal and pain rating in the whole-brain search. (A) Significant brain regions in the Path *a*, where cognitive reappraisal (Regulate-up vs. -down) was the predictor, and brain voxel activity was the outcome. **(B)** Significant brain regions in the Path *b*, where brain voxel activity was the predictor, and pain report was the outcome. The colored regions were significant at cluster-extent based threshold $p < .05$, family-wise error rate (FWER) corrected based on Monte-Carlo simulation. The legend indicates primary threshold levels and cluster extent sizes (in parentheses). **(C)** Conjunction brain maps between positive regions in Path *a* and Path *b* (C-a) and between negative regions in Path *a* and Path *b* (C-b), respectively. Yellow and cyan colors show the overlapped regions between Path *a* and Path *b*, but there was only one small cluster that was overlapped between Path *a* and Path *b*.

These null findings suggested the existence of additional mediating pathways between regions responsive to cognitive reappraisal and those associated with pain reports. To identify such pathways, we took an *a priori* approach based on recent findings that a pathway connecting NAc and the ventromedial prefrontal cortex (vmPFC) is implicated in aspects of pain processing (Apkarian et al., 2009; Baliki et al., 2010) and in the transition to chronic pain (Baliki et al., 2012). We identified *a priori* regions-of-interest (ROIs) in NAc and vmPFC based on Baliki et al. (2012), and tested whether a pathway from NAc to vmPFC mediated cognitive effects on pain in a three-path multilevel mediation analysis. NAc-vmPFC connectivity was a significant, positive mediator of the relationship between cognitive reappraisal and pain rating, with each link of the pathway from Reappraisal (Regulate-up vs. -down) → NAc → vmPFC → Pain Report significant (Figure 11A). Reversing the direction of the mediation, i.e., Reappraisal → vmPFC → NAc → Pain Report, yielded non-significant results, in keeping with recent work suggesting that NAc is more closely associated with cognitive reappraisal than vmPFC (Wager et al., 2008).

To identify additional mediators that may have been missed in the ROI analysis, we conducted whole-brain searches using multilevel three-path mediation analysis, in which three variables (cognitive reappraisal instruction, pain reports, and either NAc or vmPFC) were specified *a priori*, and a voxel-wise search was conducted for mediators (Figures 11B and 11C). We first identified brain mediators of the relationship between the Reappraisal-NAc connection and pain reports. VMPFC was the only significant brain mediator that survived multiple-comparison correction (Figure 11B and Table 2). We also searched for brain mediators of the relationship between Reappraisal and the vmPFC-pain report connection. NAc was the only significant mediator (Figure 11C and Table 2).

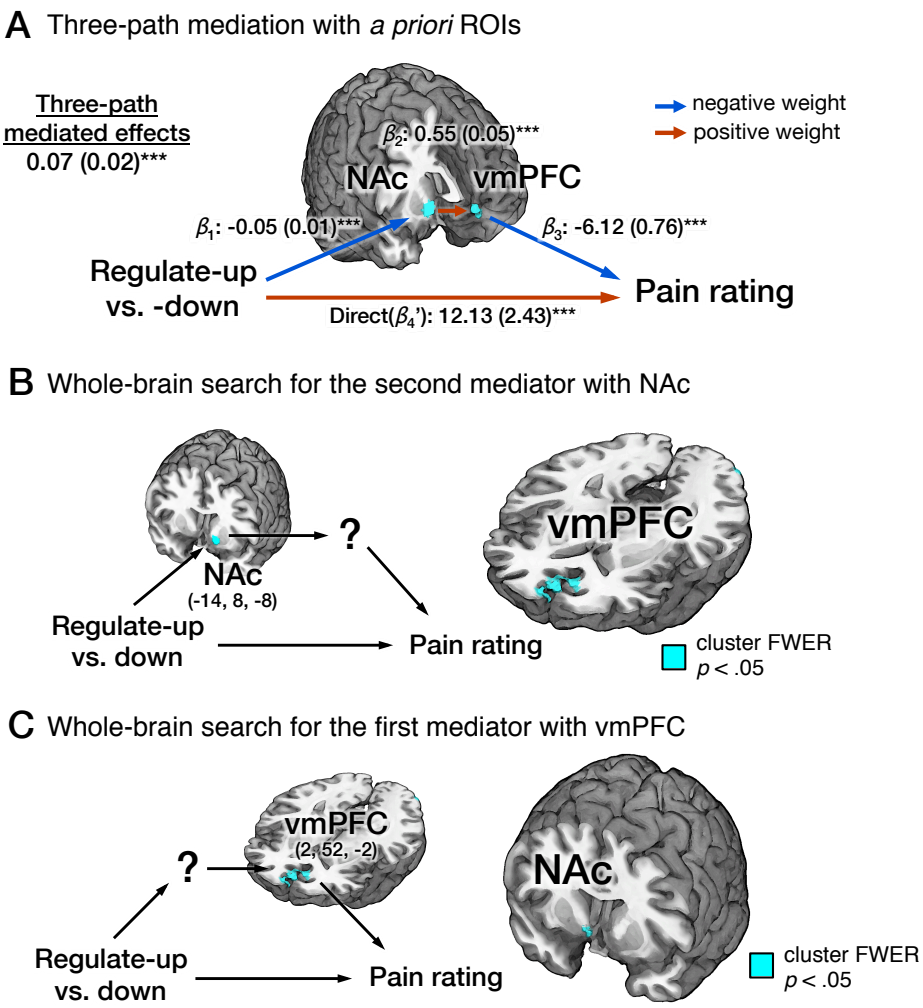


Figure 11. NAc-vmPFC connectivity mediates the effects of cognitive reappraisal on pain rating. (A) Multilevel three-path mediation analysis with two *a priori* ROIs, the nucleus accumbens (NAc; MNI: 10, 12, -8) and the ventromedial prefrontal cortex (vmPFC; MNI: 2, 52, -2) from (Baliki et al., 2012). Stimulus intensity and the neurologic pain signature (NPS) response were included as covariates. The lines are labeled with path coefficients, and standard errors are shown in parentheses (for more details about three-path mediation analyses, see the supplementary material). (B) Whole-brain three-path mediation analysis with NAc (MNI: -14, 8, -8), which is from the GLM results shown in Figure 7A, as the first mediator. VMPFC (MNI: 2, 52, -2) was the only significant second brain mediator from the whole-brain search ($p < .05$, family-wise error rate corrected based on cluster extent, with a primary threshold of $p < .001$). (C) Whole-brain three-path mediation analysis with vmPFC (MNI: 2, 52, -2), which is from the results shown in Figure 11B. Right NAc (MNI: 8, 8, -6) was the only significant second brain mediator from the whole-brain search. For more details of path coefficients, see Table 2. ***$p < 0.001$, two-tailed.

Table 2. Path coefficients for whole-brain three-path mediation analyses

		Regulate-up vs. -down (X)→M1				M1→M2				M2→Pain rating (Y)				X→M1→M2→Y			
Brain Mediators		β_1	SE	Z	P	β_2	SE	Z	P	β_3	SE	Z	P	$\beta_1\beta_2\beta_3$	SE	Z	P
(a) M1: Nucleus Accumbens (MNI: -14, 8, -8)																	
M2	vmPFC	-0.057	0.014	-4.33	0.000	0.215	0.040	3.96	0.000	-6.220	0.891	-3.97	0.000	0.030	0.008	3.37	0.001
(b) M2: ventromedial prefrontal cortex (MNI: 2, 52, -2)																	
M1	NAc	-0.049	0.013	-3.79	0.000	0.324	0.040	3.76	0.000	-5.908	0.799	-3.89	0.000	0.042	0.008	3.49	0.000

Note. The results of two whole-brain three-path mediation analyses are presented. In both mediation models, X was a regressor for the contrast of Regulate-up (1) vs. Passive experience (0) vs. Regulate-down (-1). (a) In the first mediation model, the left nucleus accumbens (NAc) (MNI coordinate: -14, 8, -8), which was a brain region significantly associated with Regulate-up vs. -down instructions (Fig 2A), was entered as the first mediator (M1), and we searched for significant second mediators (M2) of the relationship between the regulation (X)-NAc (M1) connection and Pain rating (Y) in the whole-brain. The result showed the ventromedial prefrontal cortex (vmPFC, mm center = 2, 52, -2) was the only significant second mediator. (b) In the second mediation model, vmPFC that was the significant second mediator from the first whole-brain three-path mediation analysis was entered as the second mediator (M2), and we searched for significant first mediators (M1) of the relationship between regulation (X) and the vmPFC (M2)-Pain rating (Y) connection. The result showed right NAc (mm center = 8, 8, -6) was the only significant first mediator. All results were thresholded at $p < .05$, family-wise error rate (FWER) corrected based on cluster extent, with a primary threshold of $p < .001$. The size of cluster extent for FWER correction was estimated based on Monte Carlo simulation ($k > 17$ and 18 for [a] and [b], respectively). M1, the first mediator; M2, the second mediator.

CHAPTER IV

DISCUSSION

In summary, we found strong evidence that pain is influenced by both noxious stimulus intensity and cognitive reappraisal, but that they are mediated by different brain systems. The effects of stimulus intensity were mediated by the NPS, an *a priori* pattern shown to be diagnostic of physical pain (as opposed to other salient, affective conditions) in individual participants. Conversely, the effects of cognitive reappraisal were mediated by a NAc-vmPFC pathway previously shown to be important for emotion and valuation in a range of contexts (Baliki et al., 2012; Rangel et al., 2008; Roy et al., 2012; Tom et al., 2007; Wager et al., 2008), but which did not respond to changes in noxious stimulus intensity.

One interpretation of these results is that cognitive reappraisal does not impact pain, but rather induces a cognitive decision bias. However, the weight of extant evidence argues for a more nuanced view. The vmPFC, rather than regions associated with the NPS, correlates with spontaneous, clinical pain (Baliki et al., 2006), and the exact NAc-vmPFC pathway we tested was recently shown to predict the transition from acute to chronic back pain (Baliki et al., 2012; Hashmi et al., 2013), demonstrating a functional role in pain. NAc activity has been shown to increase with pain relief (Baliki et al., 2010; Navratilova et al., 2012), and NAc and vmPFC have been implicated in placebo analgesia (Schweinhardt et al., 2009). In addition, both regions have been shown to be important predictors of changes in appetitive and self-regulatory behaviors in the real world (Demos et al., 2012; Falk et al., 2010), and nearby subgenual cingulate has become a major target for effecting long-term changes in depression (Ressler and Mayberg, 2007). These findings suggest that medial prefrontal-NAc pathways play a fundamental role in the behavioral value of pain and other affective events. Thus, it is likely that our NAc-vmPFC

connectivity findings do not simply reflect decision bias, but rather reflect more fundamental evaluative processes that play a role in the construction of pain experience.

The identification of separate pathways mediating sensory and cognitive modulation of pain provides a step towards identification of neurophysiological components of pain. Pain is widely thought to arise from interactions among sensory, affective, and evaluative processes (Fields, 2004; Tracey, 2010), but the different systems involved in these various components have been difficult to identify. Our results suggest that the NPS (including lateral somatosensory and medial limbic regions) encodes a signal that is closely tied to nociceptive pain, whereas the NAc-vmPFC pathway and related networks encode information about the affective value of sensory input in context. Both are potentially important for the overall experience of pain, with different manipulations affecting each. Thus, the findings provide a new window into the different ways that both psychological and pharmacological interventions may work to relieve it. Treatments that impact the two systems we identified may have different long-term consequences for the persistence and quality of pain and other affective states.

Beyond pain itself, our findings suggest that it is possible to identify more precisely the nature of what is regulated in emotion regulation studies. Cognitive reappraisal may not simply reduce the strength of 'emotional responses'—rather, emotion likely arises from interactions among multiple processes, some more closely tied to sensory events, and others linked to valuation and decision processes that are nonetheless central to emotional experience. Our findings suggest that the latter class of processes, rather than the former, may be most strongly impacted by cognitive reappraisal.

BIBLIOGRAPHY

Apkarian, A.V., Baliki, M.N., and Geha, P.Y. (2009). Towards a theory of chronic pain. Prog Neurobiol 87, 81-97.

Atlas, L.Y., Bolger, N., Lindquist, M.A., and Wager, T.D. (2010). Brain mediators of predictive cue effects on perceived pain. J Neurosci 30, 12964-12977.

Baliki, M.N., Chialvo, D.R., Geha, P.Y., Levy, R.M., Harden, R.N., Parrish, T.B., and Apkarian, A.V. (2006). Chronic pain and the emotional brain: specific brain activity associated with spontaneous fluctuations of intensity of chronic back pain. J Neurosci 26, 12165-12173.

Baliki, M.N., Geha, P.Y., Fields, H.L., and Apkarian, A.V. (2010). Predicting value of pain and analgesia: nucleus accumbens response to noxious stimuli changes in the presence of chronic pain. Neuron 66, 149-160.

Baliki, M.N., Petre, B., Torbey, S., Herrmann, K.M., Huang, L., Schnitzer, T.J., Fields, H.L., and Apkarian, A.V. (2012). Corticostriatal functional connectivity predicts transition to chronic back pain. Nat Neurosci 15, 1117-1119.

Baron, R.M., and Kenny, D.A. (1986). The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 51, 1173-1182.

Buhle, J.T., Silvers, J.A., Wager, T.D., Lopez, R., Onyemekwu, C., Kober, H., Weber, J., and Ochsner, K.N. (2013). Cognitive Reappraisal of Emotion: A Meta-Analysis of Human Neuroimaging Studies. Cereb Cortex.

Coghill, R.C., Sang, C.N., Maisog, J.M., and Iadarola, M.J. (1999). Pain intensity processing within the human brain: a bilateral, distributed mechanism. J Neurophysiol 82, 1934-1943.

Davis, M., Walker, D.L., and Myers, K.M. (2003). Role of the amygdala in fear extinction measured with potentiated startle. Ann N Y Acad Sci 985, 218-232.

Demos, K.E., Heatherton, T.F., and Kelley, W.M. (2012). Individual differences in nucleus accumbens activity to food and sexual images predict weight gain and sexual behavior. J Neurosci 32, 5549-5552.

Efron, B., and Tibshirani, R. (1993). An Introduction to the Bootstrap (Chapman & Hall/CRC).

Falk, E.B., Berkman, E.T., Mann, T., Harrison, B., and Lieberman, M.D. (2010). Predicting persuasion-induced behavior change from the brain. J Neurosci 30, 8421-8424.

Fernandez, E., and Turk, D.C. (1989). The utility of cognitive coping strategies for altering pain perception: a meta-analysis. Pain 38, 123-135.

Fields, H. (2004). State-dependent opioid control of pain. Nat Rev Neurosci 5, 565-575.

Forman, S.D., Cohen, J.D., Fitzgerald, M., Eddy, W.F., Mintun, M.A., and Noll, D.C. (1995). Improved Assessment of Significant Activation in Functional Magnetic-Resonance-Imaging (Fmri) - Use of a Cluster-Size Threshold. Magnet Reson Med 33, 636-647.

Gross, J.J., and John, O.P. (2003). Individual differences in two emotion regulation processes: implications for affect, relationships, and well-being. J Pers Soc Psychol 85, 348-362.

Gross, J.J., and Munoz, R.F. (1995). Emotion Regulation and Mental-Health. Clin Psychol-Sci Pr 2, 151-164.

Hashmi, J.A., Baliki, M.N., Huang, L., Baria, A.T., Torbey, S., Hermann, K.M., Schnitzer, T.J., and Apkarian, A.V. (2013). Shape shifting pain: chronification of back pain shifts brain representation from nociceptive to emotional circuits. Brain 136, 2751-2768.

Kenny, D.A., Korchmaros, J.D., and Bolger, N. (2003). Lower level mediation in multilevel models. Psychological methods 8, 115-128.

Koyama, T., McHaffie, J.G., Laurienti, P.J., and Coghill, R.C. (2003). The single-epoch fMRI design: validation of a simplified paradigm for the collection of subjective ratings. NeuroImage 19, 976-987.

Lawrence, J.M., Hoeft, F., Sheau, K.E., and Mackey, S.C. (2011). Strategy-dependent dissociation of the neural correlates involved in pain modulation. Anesthesiology 115, 844-851.

Leknes, S., and Tracey, I. (2008). A common neurobiology for pain and pleasure. Nat Rev Neurosci 9, 314-320.

MacKinnon, D.P., Lockwood, C.M., Hoffman, J.M., West, S.G., and Sheets, V. (2002). A comparison of methods to test mediation and other intervening variable effects. Psychological methods 7, 83-104.

Metcalfe, J., and Mischel, W. (1999). A hot/cool-system analysis of delay of gratification: dynamics of willpower. Psychol Rev 106, 3-19.

Navratilova, E., Xie, J.Y., Okun, A., Qu, C., Eyde, N., Ci, S., Ossipov, M.H., King, T., Fields, H.L., and Porreca, F. (2012). Pain relief produces negative reinforcement through activation of mesolimbic reward-valuation circuitry. Proc Natl Acad Sci U S A 109, 20709-20713.

Ochsner, K.N., Silvers, J.A., and Buhle, J.T. (2012). Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. Ann N Y Acad Sci 1251, E1-24.

Paton, J.J., Belova, M.A., Morrison, S.E., and Salzman, C.D. (2006). The primate amygdala represents the positive and negative value of visual stimuli during learning. Nature 439, 865-870.

Poldrack, R.A. (2011). Inferring mental states from neuroimaging data: from reverse inference to large-scale decoding. Neuron 72, 692-697.

Rainville, P., Hofbauer, R.K., Paus, T., Duncan, G.H., Bushnell, M.C., and Price, D.D. (1999). Cerebral mechanisms of hypnotic induction and suggestion. J Cogn Neurosci 11, 110-125.

Rangel, A., Camerer, C., and Montague, P.R. (2008). A framework for studying the neurobiology of value-based decision making. Nat Rev Neurosci 9, 545-556.

Ressler, K.J., and Mayberg, H.S. (2007). Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nat Neurosci 10, 1116-1124.

Rissman, J., Gazzaley, A., and D'Esposito, M. (2004). Measuring functional connectivity during distinct stages of a cognitive task. NeuroImage 23, 752-763.

Roy, M., Shohamy, D., and Wager, T.D. (2012). Ventromedial prefrontal-subcortical systems and the generation of affective meaning. Trends Cogn Sci 16, 147-156.

Schweinhardt, P., Seminowicz, D.A., Jaeger, E., Duncan, G.H., and Bushnell, M.C. (2009). The anatomy of the mesolimbic reward system: a link between personality and the placebo analgesic response. J Neurosci 29, 4882-4887.

Shrout, P.E., and Bolger, N. (2002). Mediation in experimental and nonexperimental studies: new procedures and recommendations. Psychological methods 7, 422-445.

Sobel, M.E. (1982). Asymptotic confidence intervals for indirect effects in structural equation models. (Washington, DC: American Sociological Association).

Taylor, A.B., MacKinnon, D.P., and Tein, J.-Y. (2007). Tests of the three-path mediated effect. Organizational Research Methods 11, 241-269.

Tom, S.M., Fox, C.R., Trepel, C., and Poldrack, R.A. (2007). The neural basis of loss aversion in decision-making under risk. Science 315, 515-518.

Tracey, I. (2010). Getting the pain you expect: mechanisms of placebo, nocebo and reappraisal effects in humans. Nat Med 16, 1277-1283.

Urry, H.L., van Reekum, C.M., Johnstone, T., and Davidson, R.J. (2009). Individual differences in some (but not all) medial prefrontal regions reflect cognitive demand while regulating unpleasant emotion. NeuroImage 47, 852-863.

Wager, T.D., Atlas, L.Y., Lindquist, M.A., Roy, M., Woo, C.W., and Kross, E. (2013). An fMRI-based neurologic signature of physical pain. N Engl J Med 368, 1388-1397.

Wager, T.D., Davidson, M.L., Hughes, B.L., Lindquist, M.A., and Ochsner, K.N. (2008). Prefrontal-subcortical pathways mediating successful emotion regulation. Neuron 59, 1037-1050.

Wager, T.D., Keller, M.C., Lacey, S.C., and Jonides, J. (2005). Increased sensitivity in neuroimaging analyses using robust regression. NeuroImage 26, 99-113.

Wager, T.D., Rilling, J.K., Smith, E.E., Sokolik, A., Casey, K.L., Davidson, R.J., Kosslyn, S.M., Rose, R.M., and Cohen, J.D. (2004). Placebo-induced changes in FMRI in the anticipation and experience of pain. Science 303, 1162-1167.

Wager, T.D., van Ast, V.A., Hughes, B.L., Davidson, M.L., Lindquist, M.A., and Ochsner, K.N. (2009). Brain mediators of cardiovascular responses to social threat, part II: Prefrontal-subcortical pathways and relationship with anxiety. NeuroImage 47, 836-851.

Whalen, P.J., Kagan, J., Cook, R.G., Davis, F.C., Kim, H., Polis, S., McLaren, D.G., Somerville, L.H., McLean, A.A., Maxwell, J.S., and Johnstone, T. (2004). Human amygdala responsivity to masked fearful eye whites. Science 306, 2061.

Yarkoni, T., Poldrack, R.A., Nichols, T.E., Van Essen, D.C., and Wager, T.D. (2011). Large-scale automated synthesis of human functional neuroimaging data. Nat Methods 8, 665-670.

APPENDIX

A. SUPPLEMENTAL METHODS

Participants

Thirty-three healthy, right-handed participants completed the study (M_{age} = 27.9 ± 9.0 years, 22 females). The sample consisted of 39% Caucasian, 33% Asian, 12% Hispanic, and 15% African American participants. All participants provided informed consent. The study was approved by the Columbia University Institutional Review Board. Preliminary eligibility was assessed with a general health questionnaire, a pain safety screening form, and an fMRI safety screening form. Participants reported no history of psychiatric, neurological, or pain disorders.

Materials and Procedures

Thermal stimulation and pain ratings. Thermal stimulation was delivered to the volar surface of the left inner forearm applied using a TSA-II Neurosensory Analyzer (Medoc Ltd., Chapel Hill, NC) with a 16 mm Peltier thermode end-plate. The stimulation was delivered on two spots located on the middle forearm that alternated between runs. Each stimulus lasted 12.5 seconds, with 3-second ramp-up and 2-second ramp-down periods and 7.5 seconds at target temperature. Six levels of temperature were administered to the participants (level 1: 44.3°C, level 2: 45.3°C, level 3: 46.3°C, level 4: 47.3°C, level 5: 48.3°C, level 6: 49.3°C). After each stimulation, participants indicated whether the stimulation was painful or not. Then, depending on their answers, they rated the warmth (no sensation at all to very warm but not yet painful) or pain (no pain to worst imaginable pain) elicited by the stimulation by moving a cursor on a 100 points scale. In all analyses, both scales were then collapsed into a common intensity continuum spanning from 0 (no sensation) to 200 (worst pain imaginable), with pain threshold at 100.

fMRI task design (Figure 2). FMRI images were acquired during 9 functional runs. Runs #1, 2, 4, 5, 6, 8, and 9 were "Passive experience" runs. Among those, Run #1, 2, 4, 8, and 9 comprised 11 stimulations from level 1 (44.3°C) to 5 (48.3°C), for a total of 55 stimulations over the 5 runs (11 times each temperature). Transitional frequencies between temperature levels were counterbalanced over the 55 stimulations so that each temperature level was preceded twice by each of the five temperatures, and each of the five "Passive experience" runs started with a different temperature. Runs #1, 4 and 9 began with temperature level 1, 3 or 5; Runs #2 and 8 always started with levels 4 and 2, respectively. Different presentation orders were generated for each participant. During run #5 and 6, the temperatures presented in runs #4 and #8 were all increased by one degree, so that the five levels of temperature presented in these runs spanned from level 2 (45.3°C) to level 6 (49.3°C).

Run #3 and 7 were "Regulation" runs. Participants were asked to cognitively "increase" (Regulate-up) or "decrease" (Regulate-down) pain intensity. Instructions for *Regulate-up* and *down* are as following:

> *Instruction for Regulate-up:* During this scan, we are going to ask you to try to imagine as hard as you can that the thermal stimulations are more painful than they are. Try to focus on how unpleasant the pain is, for instance, how strongly you would like to remove your arm from it. Pay attention to the burning, stinging and shooting sensations. You can use your mind to turn up the dial of the pain, much like turning up the volume dial on a stereo. As you feel the pain rise in intensity, imagine it rising faster and faster and going higher and higher. Picture your skin being held up against a glowing hot metal or fire. Think of how disturbing it is to be burned, and visualize your skin sizzling, melting and bubbling as a result of the intense heat.

Instruction for Regulate-down: During this scan, we are going to ask you to try to imagine as hard as you can that the thermal stimulations are less painful than they are. Focus on the part of the sensation that is pleasantly warm, like a blanket on a cold day. You can use your mind to turn down the dial of your pain sensation, much like turning down the volume dial on a stereo. As you feel the stimulation rise, let it numb your arm, so any pain you feel simply fades away. Imagine your skin is very cool, from being outside, and think of how good the stimulation feels as it warms you up.

The order of the two conditions was counterbalanced across subjects. These two "Regulation" runs comprised 10 randomly presented stimulations (twice each of the first 5 levels; same order for Regulate-up and -down condition).

Every run started with a baseline period during which a fixation cross was presented for 18 seconds. Then 10 or 11 trials were administered depending of the type of run. Each trial started with a 12.5-second long thermal stimulation, followed by a 4.5 to 8.5 seconds long pre-rating period. During both stimulation and pre-rating periods, the fixation cross remained on the screen. After the pre-rating period, instructions to "press left if not painful" and "press right if painful" were displayed on the left and right halves of the screen for 4 seconds.

Participants gave their answers by pressing on the left or right button of an fMRI-compatible response box. Then, participants had 7 seconds to rate the intensity of the warmth or painful sensation by moving a cursor along a rating scale displayed in the center of the screen by using the trackball of the response box, and clicking on one of the button once to enter their decision. The numerical value associated with the position of the cursor on the scale (0 to 100) was also displayed in the middle of the screen, immediately below the scale. Finally, the fixation

cross was presented once more at the end of the rating period for a 5- to 9- seconds long inter-trial interval.

Behavioral analysis

We analyzed the behavioral data using a multi-level generalized linear model analysis, implemented with custom code written in Matlab (MathWorks, Natick, MA). The outcome variable was pain reports for each trial. Within subject predictors at the first level of the model included cognitive reappraisal conditions (Regulate-up vs. Passive experience vs. Regulate-down were coded as 1, 0, and -1), stimulus intensity (i.e., temperature), and their interaction. A between-subject covariate included the order of the direction of the regulation runs (Regulate-up first vs. Regulate-down first were coded as 1 and -1). Because the two ”Regulation” runs did not have the highest level of heat intensity, 49.3°C, we displayed results only for 5 levels of stimulus intensity in Figures 2C and 2D. However all levels of heat intensity were included in analyses.

FMRI acquisition and preprocessing

Imaging acquisition. Whole-brain fMRI data were acquired on a 3T Philips Achieva TX scanner at Columbia University’s Program for Imaging in Cognitive Science (PICS). Structural images were acquired using high-resolution T1 spoiled gradient recall images (SPGR) for anatomical localization and warping to a standard space. Functional images were acquired with an echo-planar imaging sequence (TR = 2000 ms, TE = 20 ms, field of view = 224 mm, 64×64 matrix, 3×3×3 mm voxels, 42 interleaved slices, parallel imaging, SENSE factor 1.5). The Passive experience runs that had 11 stimulations (#1,2,4,5,6,8,9) lasted 6 minutes and 58 seconds (209 TRs), and the Regulation runs that only had 10 trials lasted 6 minutes and 22 seconds (191 TRs). Stimulus presentation and behavioral data acquisition were controlled using E-Prime software (PST Inc.).

Preprocessing. Prior to preprocessing, global outlier time points (i.e. "spikes" in BOLD signal) were identified by computing both the mean and the standard deviation (across voxels) of values for each image for all slices. Mahalanobis distances for the matrix of slice-wise mean and standard deviation values (concatenated) x functional volumes (time) were computed, and any values with a significant χ^2 value (corrected for multiple comparisons based on the more stringent of either false discovery rate or Bonferroni methods) were considered outliers (less than 1% of images were outliers). The output of this procedure was later used as a covariate of non-interest in the first level models.

Functional images were slice-acquisition-timing and motion corrected using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Structural T1-weighted images were coregistered to the first functional image for each subject using an iterative procedure of automated registration using mutual information coregistration in SPM8 and manual adjustment of the automated algorithm's starting point until the automated procedure provided satisfactory alignment. Structural images were normalized to MNI space using SPM8, interpolated to 2×2×2 mm voxels, and smoothed using a 6mm full-width at half maximum Gaussian kernel.

FMRI Analysis

First-level analysis and robust regression. First-level general linear model (GLM) analyses were conducted in SPM8. The first 4 volumes of each run were discarded, and the 9 runs were concatenated for each subject. Boxcar regressors, convolved with the canonical hemodynamic response function, were constructed to model periods for the 12.5-sec thermal stimulation (6 levels) and 11-sec rating periods. We included three regressors that are parametric modulation of cognitive reappraisal, stimulus intensity (temperature), and their interaction, analogous to the behavioral analysis.

The fixation cross epoch was used as an implicit baseline. A high-pass filter of 180 seconds was used. Other regressors of non-interest (nuisance variables) included a) "dummy" regressors coding for each run (intercept for each run); b) linear drift across time within each run; c) the 6 estimated head movement parameters (x, y, z, roll, pitch, and yaw), their mean-zeroed squares, their derivatives, and squared derivative for each run (total 24 columns); d) indicator vectors for outlier time points identified based on their multivariate distance from the other images in the sample (see above); e) indicator vectors for the first two images in each run; f) signals from white matter and ventricle.

Second-level analyses (group) were conducted using robust regression (Wager et al., 2005) with cognitive regulation strength and/or pain sensitivity as second-level covariates. All results were thresholded at $P < .05$, family-wise error rate (FWER) corrected based on cluster extent with primary threshold of $P < .001$, $P < .0005$, or $P < .00001$, two-tailed. The cluster extents for FWER correction were estimated based on Monte Carlo simulation (10,000 iterations) with 3dClustSim of AFNI (http://afni.nimh.nih.gov/) using the estimated intrinsic smoothness (Forman et al., 1995). For the purpose of display, we pruned the results using two additional higher levels of voxel-wise threshold.

Single trial analysis. We employed the single trial, or "single-epoch", design and analysis approach. 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. (2004) .

Similar to the parametric modulation model, boxcar regressors, convolved with the canonical hemodynamic response function, were constructed to model periods for the 12.5-sec thermal stimulation (6 levels) and 11-sec rating periods. Then, we included a trial-specific regressor for each trial, as well as several types of nuisance covariates that are identical to above.

One important consideration in the single trial analysis is that trial estimates could 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 collinearity with nuisance regressors) were calculated, and any trials with VIFs that exceeded 2.5 were excluded from the following analyses. The average number of excluded trials was 9.55 (SD = 4.13) per subject. The single-trial beta images were used in mediation analyses (see below) and ROI analyses.

Pattern expression analysis. In order to calculate the strength of expression of the neurologic pain signature pattern, we calculated the NPS response by taking the cross-product of a vectorized activation image ($\vec{\beta}_{map}$) with the NPS pattern ($\vec{w}_{map}$), i.e., $\vec{\beta}_{map}^{T}\vec{w}_{map}$, yielding a continuous scalar value. For mediation and other analyses, we used the NPS response calculated from the single-trial beta images.

Multilevel mediation analysis

Multi-level two-path mediation analysis. The multi-level mediation analyses based on a standard 3-variable path model (Baron and Kenny, 1986) were performed using the Mediation Toolbox (http://wagerlab.colorado.edu/tools) (Wager et al., 2008; Wager et al., 2009). The mediation analysis tests whether a covariance between two variables (X and Y) can be explained by a third variable (M). A significant mediator is one whose inclusion as an intermediate variable in a path model of the effects of X on Y significantly affects the slope of the X – Y relationship;

that is, the difference (*c – c'*) is statistically significant. Brain regions that are mediators are candidates for links in functional pathways that relate brain activity in multiple regions to behavior and other outcomes.

In the current study, we used stimulus intensity or cognitive regulation (Regulate-up vs. Regulate-down) for each trial as the "X" variable and pain reports for each trial as the "Y" variable. Thus, the X-Y relationship (Path *c*) is the linear association between stimulus intensity or regulation and pain reports.

More formally, the 3-variable mediation test can be basically captured in a system of three equations:

$$y = cx + e_y \quad (1)$$

$$m = ax + e_m \quad (2)$$

$$y = bm + c'x + e'_y \quad (3)$$

Here *y*, *x*, and *m* are *n* (trial) × 1 data vectors for each subject containing the outcome (*y*, the reported pain), the predictor (*x*, stimulus intensity or regulation), and data from a candidate mediating voxel, a cluster, or the NPS response (*m*, activity in single-trial beta images). e_y , e_m , and e'_y vectors denote residual error for the outcome and mediator controlling for *x* and the outcome controlling for *x* and *m*, respectively. Path *a* is the estimated linear change in *m* per unit change in *x*. Path *b* is the slope of the mediator-outcome relationship controlling for *x*. The Paths *c* and *c'* are as described above. Statistical tests on Paths *a* and *b* coefficients assess the significance of each relationship. In addition, a statistical test of *(c – c')* can be performed by testing the significance of the product of the path coefficients of Path *a*×*b*.

Based on this first-level mediation model, we conducted multi-level mediation analysis, which is designed for explaining both within-subjects and between-subjects variations in the

same model by treating the participant as a random effect (for the details of the method, see (Wager et al., 2009)). This analysis can provide information about brain-behavior relationships at two levels. The first level accounts for the relationships between dynamic variations across time (within individual participants) in stimulus intensity or regulation (X), brain activity (M), and pain reports (Y). The second level tests for consistency across individuals, allowing population inference, and accounts for known sources of variations in individual pathway strength (i.e., person-level moderators) (Kenny et al., 2003). Whole brain multi-level mediation analysis tests the mediation effect at each voxel (for more details, see Atlas et al., 2010; Wager et al., 2009).

We used bootstrapping for significance testing. Bootstrap tests (Efron and Tibshirani, 1993) have been shown to be a useful way to assess mediation in small samples (Efron and Tibshirani, 1993; Shrout and Bolger, 2002). Bootstrapping provides a more accurate and generally more sensitive test for assessing the magnitude of indirect (Path $a \times b$) effects than the Sobel test (Sobel, 1982), which assumes a normal distribution of Path $a \times b$ estimates. Even if Paths a and b estimates may both be normally distributed, the Path $a \times b$ product is not expected to be normally distributed (MacKinnon et al., 2002). We estimated distributions of subject-level path coefficients by randomly sampling with replacement 10,000 observations (rows) from the matrix of [a b c' c ($a \times b$)] path coefficients. Two-tailed p-values were calculated from the bootstrap confidence interval.

In order to test whether the NPS response mediated the relationship between cognitive regulation and pain report, we conducted two multilevel mediation analyses (Figure 5). As explained above, X was stimulus intensity (Figure 5A) or cognitive regulation (Regulate-up vs. Regulate-down; Figure 5B), Y was pain reports, and M was the NPS responses that were calculated from single-trial beta images. In the mediation model with stimulus intensity as X,

cognitive regulation was included as a covariate, and in the model with cognitive regulation as X, stimulus intensity was included as a covariate.

In addition, we conducted two whole-brain searches with multi-level mediation analysis to identify brain mediators of the effects of stimulus intensity and cognitive regulation on pain (Figure 9). Covariates were included in the same way as above.

Multi-level three-path mediation analysis. The three-path mediation analysis can assess relationships among stimulus intensity or cognitive regulation (X), two different brain mediators (M1 and M2), and pain report (Y). The analysis is based on a three-path mediation model suggested by Taylor et al. (2007).

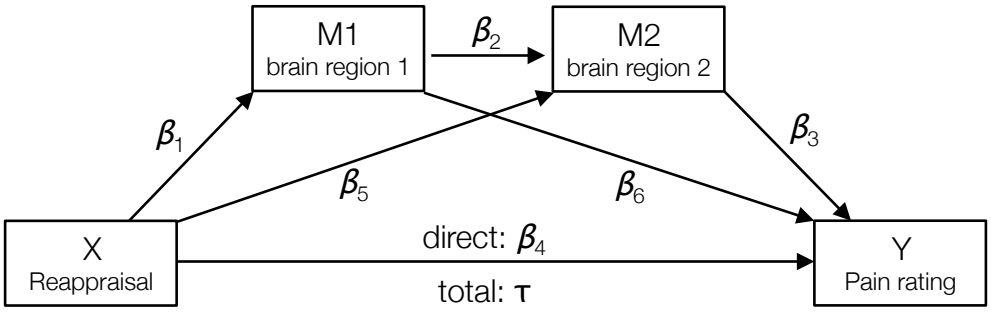
Adopting the notational convention of Taylor et al. (2007), the three-path mediation model can be captured in a system of the following four equations and a diagram:

$$y = \tau x + e_y \tag{4}$$

$$m_1 = \beta_1 x + e_{m_1} \tag{5}$$

$$m_2 = \beta_2 m_1 + \beta_5 x + e_{m_2} \tag{6}$$

$$y = \beta_4 x + \beta_3 m_2 + \beta_6 m_1 + e'_y \tag{7}$$



Here, we are interested in the effects mediated by both mediators ($\beta_1\beta_2\beta_3$). We used two different criteria of testing for the three-path mediation effects, and only if variables met both

criteria, they were considered to be significant mediator: 1) the joint significance test (each of the three paths (i.e., $\beta_1, \beta_2, \beta_3$) should be significant (MacKinnon et al., 2002)), and 2) the product-of-coefficients test using bootstrap test (the product of coefficients, $\beta_1\beta_2\beta_3$ [its sample estimate is $b_1b_2b_3$], should be significantly nonzero). These two criteria were shown to be better than other methods in terms of type I error, power, and coverage (Taylor et al., 2007).

The multi-level implementation is same with the two-path mediation analysis (for the details, see Wager et al., 2009) except for the calculation of the variance of the mediated path ($b_1b_2b_3$). In the two-path mediation analysis, we used the following equation from Kenny, Korchmaros, & Bolger (2003) :

$$\sigma_{ab}^2 = b^2\sigma_a^2 + a^2\sigma_b^2 + \sigma_a^2\sigma_b^2 \quad (8)$$

Here, *a* and *b* is path estimate for path a and b. In the three-path mediation analysis, we used the multivariate delta estimator using the following equation from Taylor et al. (2007) :

$$\sigma_{b_1b_2b_3}^2 = b_1^2b_2^2\sigma_{b_3}^2 + b_1^2b_3^2\sigma_{b_2}^2 + b_2^2b_3^2\sigma_{b_1}^2 \quad (9)$$

This variance estimate was used in Empirical Bayes estimation procedure for second-level bootstrapping of the path coefficients (Wager et al., 2009).

For the whole brain search using three-path multi-level mediation analysis, the three-path multi-level mediation analysis was carried out at each voxel given X, one of mediator (M1 or M2), and Y.

To identify potential pathways connecting cognitive regulation and reported pain, we used two *a priori* ROIs (the nucleus accumbens [NAc] and the ventromedial prefrontal cortex [vmPFC]) as the first and second mediators (Figure 11A). The ROI coordinates were from Baliki et al. (Baliki et al., 2012). For the ROI values, we averaged activity across voxels within a sphere

(r = 6mm) around the ROI centers. In order to control for nociception-related brain activity, stimulus intensity and the NPS response were included as covariates.

In addition to the ROI analysis, we conducted whole-brain searches using multilevel three-path mediation analysis, where three variables (cognitive regulation, pain reports, and one brain region) were specified *a priori*, and a voxel-wise search was conducted for mediators. For the first whole-brain search (Figure 11B), left NAc (MNI, -14, 8, -8) from the robust regression results for the Regulate-up vs. Regulate-down contrast (Figure 7A) was used as the first mediator (M1). For the second whole-brain search (Figure 11C), vmPFC (MNI, 2, 52, -2) from the first whole-brain search was used as the second mediator (M2). Consistent with the ROI analysis, stimulus intensity and the NPS response were included as covariates.

B. STIMULUS INTENSITY-RELATED BRAIN ACTIVITY

Regions	MNI coordinate			Max statistics			No. of Voxels	Volume (mm^3)
	x	y	z	T	Z	-log (min p value)		
Positive								
Operculum (R)	50	-6	10	10.01	6.69	10.96	3625	29000
Operculum (L)	-54	-8	10	12.36	7.44	13.30	1375	11000
aINS (L)	-34	6	8	5.53	4.60	5.67	15	120
mINS (L)	-38	-6	-8	7.74	5.77	8.40	113	904
	-36	0	16	5.22	4.41	5.29	3	24
SMA	2	10	46	10.70	6.93	11.68	2461	19688
Cerebellum (R)	32	-58	-32	7.26	5.54	7.83	131	1048
Cerebellum (L)	-24	-58	-28	9.80	6.62	10.73	946	7568
Thalamus (R)	12	-14	0	7.11	5.47	7.65	185	1480
Thalamus (L)	-10	-14	0	7.08	5.45	7.60	83	664
Caudate (R)	16	6	6	6.49	5.15	6.88	68	544
MFG (R)	44	44	4	8.62	6.15	9.42	57	456
PAG	0	-26	-4	6.13	4.95	6.43	51	408
Amygdala (R)	20	0	-14	6.14	4.95	6.44	33	264
Postcentral gyrus (R)	24	-44	66	5.54	4.61	5.69	29	232
LG (L)	-2	-66	0	5.54	4.61	5.69	11	88
	-12	-72	0	5.60	4.64	5.76	10	80
Putamen (R)	26	-4	0	5.67	4.68	5.85	7	56
Cingulate	-4	-10	36	5.37	4.50	5.47	6	48
MFG (L)	-42	38	24	5.22	4.41	5.29	4	32
MFG (R)	36	50	16	5.95	4.85	6.20	3	24
Negative								
Postcentral gyrus (L)	-38	-24	52	8.24	5.99	8.99	511	4088
VMPFC	0	60	-8	6.38	5.09	6.74	317	2536
	6	32	-12	5.66	4.68	5.84	38	304
MTG (R)	50	-62	18	6.49	5.15	6.88	163	1304
	58	-8	-16	6.82	5.32	7.29	62	496
PHG (L)	-26	-20	-24	8.24	5.99	8.99	246	1992
PHG (R)	28	-16	-26	6.39	5.10	6.76	102	816
Precuneus/PCC	6	-52	38	5.69	4.69	5.87	89	712
	4	-62	22	5.84	4.78	6.06	41	328
	-6	-54	8	5.35	4.49	5.45	5	40
FP	0	68	20	6.19	4.98	6.50	195	1560
Cerebellum (R)	30	-40	-24	5.88	4.81	6.12	30	240
OG (L)	-42	-70	-6	5.29	4.45	5.37	6	48
OG (R)	38	-68	20	7.12	5.47	7.65	7	56
AG (L)	-42	-70	28	6.02	4.89	6.29	101	808
MTG (L)	-56	-8	-20	9.59	6.54	10.51	87	696
Paracentral gyrus	2	-30	58	5.69	4.69	5.87	26	208
STG (R)	64	-28	4	6.85	5.34	7.33	15	120
MPFC	-10	54	40	5.72	4.71	5.91	7	56

Note. The reported regions were significantly associated with by stimulus intensity (parametric modulation). The results were significant at $p < .05$, family-wise error rate (FWER) corrected based on cluster extent ($k > 3$), with a primary threshold of $p < .00001$. The size of cluster extent for FWER correction was estimated based on Monte Carlo simulation. AG, angula gyrus; FP, frontal pole; INS, insula; LG, lingual gyrus; MFG, middle frontal gyrus; MPFC, medial prefrontal cortex; MTG, middle temporal gyrus; OG, occipital gyrus; PCC, posterior cingulate cortex; PAG, peryaqueductal gray; PHG, parahippocampal gyrus; PCC, posterior cingulate cortex; SMA, supplementary motor area; SMG, supramarginal gyrus; STG, superior temporal gyrus; VMPFC, ventromedial prefrontal cortex.

C. COGNITIVE REAPPRAISAL-INDUCED BRAIN ACTIVITY

Regions	MNI coordinate			Max statistics			No. of Voxels	Volume (mm^3)
	x	y	z	T	Z	-log (min p value)		
Regulate-up vs. -down: positive								
Paracentral gyrus	10	-28	70	6.29	5.04	6.63	183	1464
Regulate-up vs. -down: negative								
Caudate (L)	-18	-16	18	5.88	4.80	6.11	88	704
NAc (L)	-14	8	-8	5.64	4.67	5.81	84	672
Regulation vs. Passive experience: positive								
SMA	4	-2	58	6.00	4.87	6.26	108	864
IFJ (L)	-50	2	48	5.03	4.28	5.04	106	848
IFJ (R)	48	0	52	5.43	4.54	5.54	105	840
Regulation vs. Passive experience: negative								
SPL (R)	34	-74	46	4.71	4.08	4.64	85	680

Note. The reported regions were significantly associated with Regulate-up vs. Regulate-down and Regulation vs. Passive experience instructions (parametric modulation). The results were significant at $p < .05$, family-wise error rate (FWER) corrected based on cluster extent ($k > 84$), with a primary threshold of $p < .0005$. The size of cluster extent for FWER correction was estimated based on Monte Carlo simulation. IFJ, inferior frontal junction; NAc, nucleus accumbens; SMA, supplementary motor area; SPL, superior parietal lobe.