Brain moderators supporting the relationship between depressive mood and pain
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Abstract
Pain and depressive mood commonly exhibit a comorbid relationship. Yet, the brain mechanisms that moderate the relationship between dysphoric mood and pain remain unknown. An exploratory analysis of functional magnetic resonance imaging, behavioral, and psychophysical data was collected from a previous study in 76 healthy, nondepressed, and pain-free individuals. Participants completed the Beck Depression Inventory-II (BDI), a measure of negative mood/depressive symptomology, and provided pain intensity and pain unpleasantness ratings in response to noxious heat (49°C) during perfusion-based, arterial spin–labeled functional magnetic resonance imaging. Moderation analyses were conducted to determine neural mechanisms involved in facilitating the hypothesized relationship between depressive mood and pain sensitivity. Higher BDI-II scores were positively associated with pain intensity (R² = 0.10; P = 0.006) and pain unpleasantness (R² = 0.12; P = 0.003) ratings. There was a high correlation between pain intensity and unpleasantness ratings (r = 0.94; P < 0.001); thus, brain moderation analyses were focused on pain intensity ratings. Individuals with higher levels of depressive mood exhibited heightened sensitivity to experimental pain. Greater activation in regions supporting the evaluation of pain (ventrolateral prefrontal cortex; anterior insula) and sensory-discrimination (secondary somatosensory cortex; posterior insula) moderated the relationship between higher BDI-II scores and pain intensity ratings. This study demonstrates that executive-level and sensory-discriminative brain mechanisms play a multimodal role in facilitating the bidirectional relationship between negative mood and pain.

Keywords: fMRI, Depression, Negative mood, Pain, Arterial spin labeling, Moderation

1. Introduction
The subjective experience of pain is driven, in part, by a host of factors predicated by affect, psychosocial context, and the capacity to regulate behavioral responses to nociceptive information. It is not surprising then, that negative mood significantly exacerbates behavioral and neural pain responses. The contiguity between pain and dysphoria is also directly translatable to the development of pathological and clinical conditions. That is, higher levels of depression predict greater chronic pain severity and pain symptomology.

In the presence of noxious stimulation in healthy individuals, negative affect is associated with (1) higher experimentally induced pain sensitivity, (2) attenuated pain thresholds, (3) lower pain tolerance, and (4) greater activation in brain regions that process fear (amygdala) and the evaluation of sensory processes (anterior insula). Together, these findings suggest that distressed mood sensitizes an individual’s attentional propensity towards arising sensory events. Others have postulated that depressive mood increases pain-related ruminations that may be more reflective of a generalized inability to self-regulate affective appraisals of noxious stimuli. Yet, the neural mechanisms supporting the facilitation of the interplay between depressive symptomatology and pain remain poorly characterized.

This study combined an optimized, arterial spin–labeled functional magnetic resonance imaging (fMRI) technique and psychophysical pain testing in healthy, nondepressed, and pain-free individuals to determine (1) whether higher levels of depressive mood are associated with increased behavioral pain responses and (2) the neural moderators supporting this postulated relationship. As used and validated in previous studies examining healthy individuals, the Beck Depression Inventory-II (Beck et al., 1996a) was used in this study as a measure of depressive mood. Alterations in the so-called Default Mode Network, a neural network characterized by oscillating activation between the medial prefrontal cortex (PFC) and posterior cingulate cortex (PCC), are associated with heightened negative mood and pain through exacerbated self-referential ruminative appraisals of ascending nociceptive signaling. Furthermore, negative mood during noxious heat is associated with higher pain reports and corresponding increases in pain-related lower level somatosensory activation. Novel, fMRI-based moderation analyses were used to test the hypotheses that the positive relationship between pain and negative mood covaries with greater activation in sensory...
discriminative brain regions (somatosensory/insula and thalamus)\textsuperscript{17} and brain regions supporting self-referential processes (medial PFC; PCC).\textsuperscript{19,37}

2. Methods and materials

2.1. Participants

These data were collected as part of a previously published data set examining brain mechanisms of meditation and placebo.\textsuperscript{80} Eighty-five healthy, pain-free, right-handed volunteers completed the first 2 sessions of the experiment (see “Study design” section). Magnetic resonance imaging-related artifacts compromised data from 9 subjects (defined below in the “Cerebral blood flow artifact detection procedures” section). Data from 76 participants (mean age = 27.0 ± 5 years; 40 women; 36 men; 57 = white, 8 = black, 5 = Asian, 5 = mixed race, and 1 = Hispanic) are presented here (Table 1). Exclusion criteria included individuals regularly taking psychotropic (antidepressants; anti-anxiety) or pain medications, and pregnant women. Wake Forest School of Medicine’s Institutional Review Board approved all study procedures. All subjects provided written, informed consent recognizing that they would experience painful heat stimuli, that all methods were clearly explained, and that they were free to withdraw from the study.

2.2. Stimuli

As described previously,\textsuperscript{64,79} a TSA-II device (Medoc) was used to deliver all thermal stimuli using a 16 × 16-mm thermal probe. The thermal probe was moved to a new stimulation site after each experimental series to reduce habituation. All stimulus temperatures were ±49°C.

2.3. Psychophysical assessment of pain

Pain intensity and unpleasantness ratings were assessed separately using a 15-cm plastic sliding visual analog scale (VAS).\textsuperscript{50} The minimum rating (0) was designated as “no pain sensation” or “not at all unpleasant,” whereas the maximum rating (10) was labeled as “most intense pain sensation imaginable” or “most unpleasant sensation imaginable,” respectively. There was a high correlation between pain intensity and pain unpleasantness ratings (r = 0.94; P < 0.001). Thus, due to the high collinearity between pain intensity and unpleasantness ratings and to better avoid unreliable and poorly reproducible parameter estimates,\textsuperscript{50} functional neuroimaging analyses were conducted on pain intensity ratings only.

2.4. Psychological outcomes

The BDI-II is a 21-item assessment using a 4-point Likert scale (0-3)\textsuperscript{9} with scores ranging from 0 to 63. Higher scores indicate greater levels of depressive symptomology/mood. In healthy participants, the Beck Depression Inventory-II (BDI-II) measures depressive symptomology,\textsuperscript{11,34,43,70} mood disturbance, negative affect, and depressive mood.\textsuperscript{42,60} The BDI-II exhibits high internal consistency (α = 0.91).\textsuperscript{9} As characterized previously and as used in general medical practice,\textsuperscript{9,11} normal depressive symptomology is associated with scores lower than 10, mild/minor depressive symptomology corresponded to scores equal to and/or greater than 10, and moderate to severe depression is associated with scores equal to and/or greater than 19. Importantly, there was a good range of BDI scores (0-18; mean = 2.92; SEM = 0.52), and all participants in this study exhibited BDI scores equal to or less than 18. Thus, all participants exhibited scores that were below the cutoff for clinically significant depression. The BDI-II was administered before session 1 to assess depressive mood (see “Study design” section).

2.5. Anatomical magnetic resonance imaging acquisition

Participants were scanned on a 3T Siemens Skyra scanner with a 32-channel head coil. High-resolution T1-weighted images were obtained using a MP-RAGE sequence: flip angle = 9°, TI = 900 ms, echo time = 2.95, repetition time = 2300 ms, pixel bandwidth = 240 Hz/pix, field of view = 25.6 × 24 cm, 192 slices, 1 mm isotropic spatial resolution, and GRAPPA factor of 2, scan time = 5 minutes. 12 seconds.

2.6. Functional magnetic resonance imaging acquisition

Four pseudocontinuous arterial spin labeling (PCASL) series\textsuperscript{53} were performed to acquire whole-brain cerebral blood flow (CBF) images: 2D single shot echo planar images (EPI), tagging duration = 1.8 seconds, postlabeling delay = 1.2 seconds, TI = 3 seconds, TE = 12 ms, TR = 4 seconds, flip angle = 90°, reps = 66, FOV = 22 × 22 cm, in-plane matrix size = 64 × 64, number of slices = 26, slice thickness = 5 mm with 1-mm slice gap, and scan time = 4 minutes 24 seconds. Background suppression was not used. The imaging slab covered the entire cerebellum and cerebrum, and the inferior edge of the imaging slab detected the bottom of the cerebellum. The tagging plane was set in a fixed position on the axial plane 2 cm below the imaging slab. Our PCASL sequence largely followed the recommended guidelines\textsuperscript{2} for the implementation of ASL for clinical applications, except for the employment of 3D GRASE acquisition and 2D EPI. Instead, we used a 2D EPI acquisition that is more suitable for functional measures due to lower sensitivity to motion artifacts.\textsuperscript{2} A single-shot EPI acquisition with GRAPPA factor of 2 was used.

2.7. Study design

2.7.1. Experimental session 1: psychophysical training

After providing written consent, participants completed the BDI-II. As previously conducted,\textsuperscript{64,79} participants underwent psychophysical training, where they were familiarized with 32, 5-second duration stimuli (35-49°C) on the ventral aspect of the left forearm and use of

| Table 1 |
|---------------------------------|-----------------|-----------------|
| Variable                        | Males (n = 36)  | Females (n = 40) | Combined (n = 76) |
| Age                            | 26.97 (0.71)    | 27.10 (0.90)    | 27.04 (0.58)     |
| BDI-II                         | 5.44 (0.75)     | 4.45 (0.72)     | 4.92 (0.52)      |
| Pain intensity                 | 4.49 (0.37)     | 5.00 (0.32)     | 4.76 (0.24)      |
| Pain unpleasantness           | 4.60 (0.41)     | 5.41 (0.35)     | 5.02 (0.27)      |

BDI-II, Beck Depression Inventory-II.
the VAS. The thermal probe was moved to a new location after each stimulus to reduce habituation/sensitization. Subjects were administered a 4-minute 24-second thermal stimulation series delivered to the back of the left lower leg that was identical to the heat paradigm used in the subsequent MRI session. This heat series consisted of 10 alternating 12-second plateaus of 49°C and 35°C.

2.7.2. Experimental session 2: magnetic resonance imaging session

On a separate day, participants reported to the Wake Forest MRI center and were positioned in the MRI scanner and placed their respective right calf on the thermal probe. During all MRI acquisition periods, participants were instructed to “stay still and keep eyes closed.” A structural MRI scan (~5 minutes) was acquired first. Next, 4 PCASL series were acquired (4 minutes 24 seconds each). The PCASL neutral series consisted of continual, innocuous (35°C) stimulation. The heat PCASL series included 10 alternating, 12-second plateaus of 49 and 35°C. Two heat and 2 neutral PCASL series were administered in an alternating fashion, and the order of administration was counterbalanced across participants. After each PCASL series, participants were instructed to provide VAS pain intensity and pain unpleasantness ratings “corresponding to the overall experience” of the respective PCASL series. The thermal probe was moved to a new location on the right calf after each PCASL series.

2.8. Statistical analysis of behavioral data

Behavioral data were analyzed using SPSS 19 software (IBM, Armonk, New York). As previously,79 pain intensity and unpleasantness ratings were analyzed separately. Bivariate correlational analyses examined the relationship between BDI and pain intensity and unpleasantness ratings, respectively.

2.9. Statistical analysis of neuroimaging data

2.9.1. Calculation of cerebral blood flow

Each 4D series of PCASL images was converted into a single CBF file. Alternating tag and control images were subtracted to generate perfusion-weighted series. Because of the motion-sensitive nature of PCASL, we filtered data with motion correction (degrees of freedom = 6) using FMRIB’s Linear Image Registration Tool 32 and removed individual perfusion-weighted images exhibiting gross perfusion fluctuations (CBF values = 2.25 SD above/below corresponding series mean) that may corrupt the final CBF map. 65 To reduce the influence of subject motion on CBF quantification, the PCASL time series data were filtered to remove individual perfusion-weighted images with higher motion parameters and perfusion fluctuations that corrupt the final CBF map. 65 The PCASL sequence included 66 perfusion-weighted volumes (images) per PCASL series per subject. Thus, across all 4 PCASL scans, there were a total of 264 images per subject. Of the 76 participants in this study, images were filtered out of 27 subjects. In total, there were 80 images removed across all 76 participants and their respective 20, 064 images. Thus, only 0.04% of the images were filtered out of the preprocessing stage. Long recovery time (3 TRs) after presaturation was used during the first volume of the PCASL data to allow for magnetization recovery. This volume was used to estimate the cerebral spinal fluid M0 value and to scale raw perfusion-weighted images into a quantitative CBF map according to the general kinetic model. 14 Global CBF was calculated by averaging the CBF of all voxels within the brain.

2.9.2. Cerebral blood flow artifact detection procedures

Careful visual inspections were first performed on perfusion-weighted images to identify gross MRI-related artifacts. Next, regional masks were created to sample CBF in the territories of the carotid and vertebral arteries to identify potential tagging failures. In addition, global CBF values were extracted to further characterize potential CBF artifacts. Cerebral blood flow images exhibiting low (<0.20 mL/100 g tissue/minutes) global/regional CBF values were subsequently characterized as anomalous 80 and could lead to inaccurate statistical maps 21.

2.9.3. Statistical analysis of regional signal changes within the brain

FSL’s (Functional Magnetic Resonance Imaging of the Brain [FMRIB] Software Library [Center for FMRIB Version 5.0, University of Oxford, Oxford, United Kingdom]) FMRIB Local Analysis of Mixed Effects (FLAME 1 + 2) was used for image processing and analysis. 80 Individual CBF volumes from each PCASL series were concatenated into one 4D volume for first-level analyses (4-volume series). Functional data were spatially smoothed with a 9-mm full-width at half-maximum 3D isotropic Gaussian kernel before standard processing within the FEAT module of FSL. Each CBF volume was scaled by its mean global intensity (intensity normalization) within the FEAT module of FSL to minimize confounds arising from global CBF fluctuations. Temporal filtering was not performed since each CBF volume in the series is temporally independent from adjacent CBF volumes. Functional images were registered to their respective structural space using a 6-parameter linear 3D transformation. Brain-extracted structural data were transformed into standard stereotactic space (as defined by Montreal Neurologic Institute) using a 12-parameter affine transformation followed by a nonlinear transformation (FNIRT; 10 × 10 × 10-mm resolution). 3,4,32 This nonlinear transformation was then applied to CBF data.

Statistical analysis of regional signal changes was performed on 4D concatenated CBF data (first-level analyses) using fixed-effects general linear modeling. 75 Activation across individuals was assessed using random-effects analyses. T/F statistic images were Gaussianized and thresholded using clusters determined by a z > 2.3. Corrected cluster significance threshold was set at P < 0.05. 77 This procedure ensures that the probability of false-positive findings was corrected for multiple comparisons. 76

2.9.4. Brain moderation analyses

A first-level analysis of variance was first performed for each participant to identify the main effect of pain (heat vs neutral stimulation). A second-level analysis was then performed across individuals to (1) identify significant mean effects corresponding to the main effect of pain and (2) brain moderators supporting the relationship between VAS pain intensity ratings and BDI-II scores. The interaction between mean-centered pain intensity ratings and BDI was characterized as the moderation term. 31 Mean-centered BDI and pain intensity ratings were entered as the first and second regressors, respectively. The moderation term between demeaned BDI and pain intensity ratings (BDI X INT) was modeled as the third regressor. Binary masks corresponding to significant brain activation maps from the first 2 regressors’ contrast images were created to extract mean intensity values (FSL’s Featquery tool) to verify that significant neural activation was significantly correlated with BDI or pain intensity ratings.
The SPSS PROCESS moderation/mediation macro was used to confirm the directionality of significant BDI X INT moderation effects. Binary masks corresponding to the significant brain moderation effects were created to extract mean intensity values for each participant (FSL’s Featquery tool). These mean intensity values were designated and entered as moderator (M) values in the confirmatory moderation analyses (SPSS PROCESS). We assessed the influence of mean intensity values (M) on the relationship between BDI (X) and pain intensity ratings (Y). Finally, we used the pick-a-point approach to identify the directionality of significant X × M moderation effects.

Specifically, the “pick-a-point” approach can also be referred to as an analysis of simple slopes or a spotlight analysis. It entails the most common approach to probing interaction effects and is used to delineate and explain the results of multiple regression with interactions (ie, moderation effects). The procedure involves selecting a value of the moderator (M) and then calculating the conditional effect of X on Y at the chosen value of M.

For our study, “M” is a quantitative variable that corresponds to brain activation levels (ie, mean intensity value) for each participant. When “M” is a quantitative variable, a common strategy when probing the moderation effect is to estimate the conditional effect of X (BDI) on Y (pain) when M (mean intensity value) is equal to the mean, 1 SD below the mean, and 1 SD above the mean. As such, the “points” chosen within the “pick-a-point” approach were delineated, by convention, at 3 values of the moderator (ie, 1 SD below the mean, the mean, and 1 SD above the mean). In this way, it can be ascertained whether BDI is related to pain ratings among those participants with “relatively low” (ie, 1 SD below the mean), “moderate” (ie, the mean), and “relatively high” (ie, 1 SD above the mean) brain activation.

In summary, the pick-a-point approach determines the conditional effect of X (BDI) on Y (pain intensity) at low (1 SD below the mean), average (mean), and high levels (1 SD above mean) of brain activation (M).

### 3. Results

#### 3.1. Behavioral results

**3.1.1. Dispositional negative mood is positively associated with pain ratings**

Higher BDI-II scores were associated with greater pain intensity (P = 0.01, r = 0.30; Figure S1, available at http://links.lww.com/PAIN/A790) and pain unpleasantness ratings (P = 0.006, r = 0.31; Figure S2, available at http://links.lww.com/PAIN/A790). Pain intensity and unpleasantness ratings were significantly correlated with each other (P < 0.001; r = 0.94). Demographic variables (ie, age and sex) did not significantly covary with pain intensity ratings or pain unpleasantness ratings (P's > 0.05) (Table 1).

#### 3.2. Brain moderation findings

**3.2.1. Noxious heat-induced brain activation**

When compared with 35°C stimulation, noxious (49°C) heat produced significant activation in the primary somatosensory cortex (SI) corresponding to the stimulation site, bilateral thalamus, cerebellum, anterior and midcingulate cortices, anterior/posterior insula, frontal operculum, secondary somatosensory cortex (SII), supplementary motor area (SMA), and inferior frontal gyrus. Significant deactivations were detected in the bilateral medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), and posterior cingulate cortex (PCC)/precuneus. (B) Lower pain intensity ratings associated with higher activation in the inferior parietal lobe.

**3.2.2. Pain intensity-related brain activation**

Between-subject differences in pain intensity ratings were negatively associated with supramarginal gyrus and angular gyrus activation (r = −0.42; P < 0.001; Fig. 1B; Table S1, available at http://links.lww.com/PAIN/A790). There were no significant positive correlations between neural activation and pain intensity ratings.
3.2.3. Brain moderators supporting the relationship between depressive mood and pain intensity

The relationship between BDI-II and pain intensity ratings (BDI X INT) was significantly moderated by activation in the contralateral SII, parietal and central operculum, posterior insula, and activation extending from the orbitofrontal cortex (OFC) to the ventrolateral PFC (vlPFC), and anterior insula (P < 0.001; Fig. 2; Tables S1 and S2, available at http://links.lww.com/PAIN/A790). The pick-a-point approach confirmed that high activation (i.e., 1 SD above mean neural activation) in these brain regions, t(72) = 3.64, P < 0.001, but not mean t(72) = 1.79, P = 0.08 or low t(72) = −0.17, P = 0.87 (i.e., 1 SD below mean brain activation) activation, moderated the positive relationship between BDI scores and pain intensity ratings (Fig. 2).

4. Discussion

The present findings demonstrate that the relationship between depressive mood and pain intensity ratings was driven by high activation (mean intensity values = 1 SD above mean activation) in brain mechanisms supporting somatosensory processing (contralateral SII, parietal–central operculum, and posterior insula) and cognitive-affective appraisals of nociceptive information (OFC; vlPFC; anterior insula) (Fig. 2). Our hypotheses were partially confirmed. That is, heightened somatosensory but not default mode network-based processing moderated the positive relationship between depressive mood and pain.

4.1. High ventrolateral prefrontal cortex, insular, and somatosensory activation moderated the positive relationship between Beck Depression Inventory-II and pain intensity ratings

Prefrontal, insular, and somatosensory regions are anatomically connected and well positioned to assimilate ascending nociceptive information into corresponding cognitive appraisals. High contralateral vlPFC, insular, SII, and anterior insula, parietal–central operculum activation moderated the positive relationship between BDI scores and pain intensity ratings (↑ pain + ↑ depressive symptoms; ↓ pain + ↓ depressive symptoms) (Fig. 2). This is fitting for a number of reasons. For one, the vlPFC and anterior insula play a multimodal role in modulating pain and affect. High contralateral SII, parietal operculum, and posterior insula activation also moderated the relationship between BDI-II and pain intensity ratings (Fig. 2). Activation in these neural regions is associated with facilitating pain-related attentional biases in depressed individuals. Surprisingly, higher somatosensory and insular activation also moderated low depression ratings and low pain scores. However, recent evidence demonstrates that this process potentially signifies the attention capturing nature of...
Appendix A. Supplemental digital content

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/A790.

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