Supplementary Methods

The present investigation employs the subjects and imaging data from our previous study (Zeidan *et al.*, 2011) in a new set of analyses designed to examine the brain mechanisms by which meditation reduces anxiety in the presence of innocuous thermal stimulation (neutral). A possible confound, then, is that brain activity corresponding to mindfulness meditation-related anxiety relief is potentially influenced by the noxious thermal heat MRI series that were interleaved with the neutral thermal stimuli series (Figure 1 in the main manuscript). However, the presence of pain scans provides an opportunity to assess the specificity of the brain networks that are involved in mindfulness meditation-related anxiety relief. Is the modulation of anxiety by meditation accomplished by brain mechanisms that are distinct from those that mediate meditation-induced pain relief, or do they share a common mechanism? In order to address these questions, we conducted regression and conjunction statistical analyses to determine if brain mechanisms supporting meditation-related anxiety relief are distinct from brain regions involved in meditation-related pain relief.

Multiple Regression Analyses to Identify Unique Anxiety Reduction-Related Brain Activity

A regression analysis was conducted for MRI series (MRI session 2) corresponding to innocuous (35°C) thermal stimulation and another separate regression analysis examined MRI series corresponding to noxious (49°C) thermal stimulation. For each regression analysis, statistical analysis of regional blood signal changes were performed within subjects across each respective time series using a first-level fixed effects general linear modeling approach (Woolrich *et al*., 2001) to identify differences between meditation and rest. Next, the percent change scores in pain intensity, pain unpleasantness, and SAI ratings were calculated,

demeaned and entered as covariates of interest in a random effects analysis in order to identify brain regions related to individual differences in pain intensity, pain unpleasantness and anxiety changes. All regressors were orthogonalized to each other and to the main effect of meditation to determine the unique variance in brain activity related to each regressor.

Conjunction Analyses to Identify Overlap between Pain and Anxiety Reduction-Related Brain Activity

In order to determine if the present findings are simply related to a general meditation effect and are not specific to anxiety modulation, we conducted conjunction analyses (z-score threshold = 2.3, p value threshold= .05) to identify the potential overlap in brain regions associated with meditation-related anxiety and pain relief in the presence of innocuous (neutral) thermal stimulation (35°C)(Nichols et al., 2005). To parallel the conjunction analysis of neutral thermal stimulation scans, we conducted a separate conjunction analysis to identify the potential significant overlap between brain regions associated with meditation-related anxiety and pain relief in the presence of noxious heat stimulation (49°C).

Supplementary Results

Brain Mechanisms supporting meditation-related anxiety relief are independent of brain regions corresponding to meditation-related pain relief

A regression analysis was conducted on regional brain activity in the presence of innocuous thermal stimulation comparing meditation to rest with the demeaned percent changes of pain intensity, pain unpleasantness, and SAI ratings entered as regressors. Greater reductions in pain intensity ratings were associated with greater meditation-related activation in the left anterior insula and the secondary somatosensory cortex (SII) [Supplementary Figure 1A (left panel)]. There was no significant activation in brain regions that corresponded with meditation-related reductions in pain unpleasantness ratings. However, greater pain unpleasantness ratings were associated with greater rest-related brain activation in the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC) [Supplementary Figure 1B (middle panel)]. Importantly, when controlling for the variability accounted for by meditation-related pain reductions, the current supplementary findings are consistent with our main manuscript findings (Figure 4 of the main manuscript). This regression analysis detected a highly similar set of brain regions involved in meditation-related anxiety relief as in the main manuscript. Regions positively associated with meditation-related anxiety reductions included the ventro-medial PFC (vmPFC), perigenual ACC (pgACC), ACC, superior frontal gyrus, bilateral anterior insula and SII [Supplementary Figure 1C (right panel]. Similarly, regions negatively associated with meditation-related anxiety relief included the posterior cingulate cortex and premotor cortex (Supplementary Figure 1C).

A regression analysis was conducted on regional brain activity in the presence of noxious thermal stimulation comparing meditation to rest using the demeaned percent changes

of pain intensity, pain unpleasantness, and SAI ratings. Greater meditation-related pain intensity rating reductions were associated with activation in the rostral ACC and bilateral anterior insula (Supplementary Figure 1A). In addition, greater reductions in pain unpleasantness ratings were associated with greater bilateral orbitofrontal cortex activation and thalamic deactivation (Supplementary Figure 1B). Activation in the ACC, mPFC, and occipital lobe was also negatively associated with pain unpleasantness ratings. After controlling for the variability of meditation-induced percent change in pain ratings, greater reductions in SAI ratings were related to greater activation in the occipital lobe, left anterior insula, and bilateral posterior insula/SII (Supplementary Figure 1C). Meditation-related increases in SAI ratings were negatively associated with activation in the PCC and the occipital lobe (Supplementary Figure 1C). These findings provide further evidence that the neural correlates of meditation-related anxiety relief are distinct from those involved with meditation-related changes in pain.

Absence of overlap in activation with associated with anxiety and pain relief

A conjunction analysis of data acquired during innocuous thermal stimulation revealed that brain activation reflecting meditation-related anxiety relief did not overlap significantly with brain activation associated with meditation-related pain relief (z-score threshold = 2.3, p value threshold= .05). A similar conjunction analysis revealed no significant overlap (z-score threshold = 2.3, p value threshold= .05) between brain regions involved with meditation-related anxiety and pain rating changes in the presence of noxious heat stimulation.

Supplementary Discussion

These supplementary analyses confirm that brain mechanisms supporting the modulation of anxiety by meditation are independent from those involved in meditation-related pain relief. Unique anxiety reduction–related activation was detected in neutral temperature scans after variability related to pain intensity and pain unpleasantness reductions had been accounted for. Similar unique anxiety reduction-related activation was also detected in scans involving noxious stimuli. Meditation-induced anxiety-related changes in brain activity were similar for both innocuous and noxious thermal stimulation in that they involved bilateral SII and left anterior insula.

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