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Neural Mechanisms of the Subjective Experience of Pain.

Turning on the Alarm: The Neural Mechanisms of the Transition from Innocuous to Painful Sensation.

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Abstract

The experience of pain occurs when the level of a stimulus is sufficient to elicit a marked affective response, putatively to warn the organism of potential danger and motivate appropriate behavioral responses. Understanding the biological mechanisms of the transition from innocuous to painful levels of sensation is essential to understanding pain perception as well as clinical conditions characterized by abnormal relationships between stimulation and pain response. Thus, the primary objective of this study was to characterize the neural response associated with this transition and the correspondence between that response and subjective reports of pain.

Towards this goal, this study examined BOLD response profiles across a range of temperatures spanning the pain threshold. 14 healthy adults underwent functional magnetic resonance imaging (fMRI) while a range of thermal stimuli (44-49°C) were applied. BOLD responses showed a sigmoidal profile along the range of temperatures in a network of brain regions including insula and mid-cingulate, as well as a number of regions associated with motor responses including ventral lateral nuclei of the thalamus, globus pallidus and premotor cortex. A sigmoid function fit to the BOLD responses in these regions explained up to 85% of the variance in individual pain ratings, and yielded an estimate of the temperature of steepest transition from non-painful to painful heat that was nearly identical to that generated by subjective ratings.

These results demonstrate a precise characterization of the relationship between objective levels of stimulation, resulting neural activation, and subjective experience of pain and provide direct evidence for a neural mechanism supporting the nonlinear transition from innocuous to painful levels along the sensory continuum.

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1. Introduction

The International Association for the Study of Pain has defined pain as "an unpleasant sensory *and* emotional experience associated with actual or potential tissue damage…." (Merskey & Bogduk, 1994). This definition highlights the fact that pain is not a simple product of sensory input but involves an interaction between sensory and affective responses. One way to understand the mechanisms of this critical mind-body interaction is to examine the transition from innocuous to painful levels of sensory input.

The "pain threshold" is commonly understood to be a discrete level of sensory input, above which a marked affective response is elicited, putatively to warn the organism of potential danger and motivate appropriate behavioral responses. Large inter-individual differences have been observed in the level of stimulation required to reach this threshold (Coghill, McHaffie, & Yen, 2003; Petrie, 1978). Furthermore, characterization of the pain threshold as a "probability function" rather than a discrete event (Gracely, 1999) suggests that individuals may also differ in the degree to which the threshold can be reliably found at a discrete point along a continuum of stimulus intensity. Instead, many individuals show a more gradual transition from non-painful to painful subjective experience along the sensory continuum, with the steepness of the transition varying from one individual to another. The aim of the present research, then, was to examine individual differences in neural responses corresponding to these changes in subjective experience.

Understanding these individual differences is both clinically and scientifically relevant. Examining how

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the brain generates an aversive affective response to peripheral signals is critical to elucidating emotion's function as a warning or "alarm" system. Of particular interest clinically is the fact that this system may be overly sensitive, resulting in "false alarms" or pain experiences that do not, in fact, signal danger. Many chronic pain disorders are characterized by oversensitivity to sensory input (i.e allodynia or hyperalgesia). Thus, understanding the biological mechanisms of the transition from innocuous to painful levels of sensation will help us understand not only the neural response to danger but potentially conditions like chronic pain which are characterized by pain that does not provide any adaptively salient information.

The primary objective of this study was to provide a precise characterization of the relationship between objective levels of stimulation, neural activation, and subjective report of pain, particularly at the transition between innocuous and pain-inducing temperatures. One difficulty in characterizing this relationship is the confound between individual differences in pain sensitivity and pain labeling behavior (Petrie, 1978). Psychophysical techniques such as method of levels which are used to measure individuals' pain thresholds are reliant on self-report. Additionally, previous studies (Bornhovd et al., 2002; Buchel et al., 2002) identifying regions of the brain selectively responding to supra-threshold levels of stimulation have also relied on categories based upon subjective pain ratings. It is therefore difficult to know whether individual differences in the self-reported transition from innocuous to painful stimulation reflect subjective experience or merely idiosyncratic reporting behavior. Indeed, the ability or propensity of subjects to consistently report a given stimulus as painful or not (a categorical decision) does not even necessarily map reliably onto their ratings of pain or stimulus intensity when using a non-categorical, interval measure. One way to address this problem is to seek convergence between multiple measures of pain, for example by examining neural responses to a range of stimuli

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that span expected pain thresholds. An example of such an approach (Timmermann et al., 2001) fit different response curves to magnetoencephalography (MEG)-derived neural responses in SI and SII to 4 magnitudes of laser pain stimuli. They found differences in the shape of response profiles in SI versus SII, though the experimental design and use of MEG precluded testing for response profile differences across a wider range of brain regions and to a more continuous range of applied stimuli. In this study, we modeled BOLD response profiles along a continuum of applied temperatures using whole-brain voxelwise non-linear curve fitting techniques that are sensitive to responses like pain, which may occur either in an "all-or-none" fashion or in a graded fashion, rather than as a consistent function of temperature. As neural activation was modeled independently of self-report, we were then able to examine the convergent validity of categorical reports of pain, subjective ratings of pain and neural response profiles across all of the brain regions involved in pain perception.

2. Materials and Methods

2.1 Subjects

20 right-handed subjects were recruited. Subjects were excluded if they were pregnant, claustrophobic or had a present psychiatric or chronic pain disorder or significant history of such disorders. They were screened for neurological disorders or other medical conditions that could affect pain sensitivity or regular use of drugs such as opioids or NSAIDS that could alter pain perception. Subjects were also excluded based on subjective testing (n=3, see below) or for incomplete data (n=3), leaving fourteen subjects (8 female) ranging in age from 21 to 37 years old (mean 26.1) in the present analysis.

2.2 Thermal Stimulus Equipment

A thermal stimulator (TSA-II, Medoc Advanced Medical Systems) was used to generate the heat

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stimuli. A 30*30mm MRI-compatible peltier device was attached to the volar surface of the left forearm, 10cm from the wrist.. Application of heat was controlled through interfacing with a PC running EPrime software (Psychology Software Tools, Inc.).

2.3 Determination Of Stimulus Range

Prior to performing the study task, testing was conducted to determine the range of temperatures to be used for each subject during the subsequent experimental task. Stimulation began at 32 ^{o}C and increased by 0.7 ^{o}C/ second. Subjects were asked to stop the stimulation by pressing a button when their pain reached an 8 on an 11-point numeric rating scale (NRS), with 0 representing "no-pain" and 10 representing "the worst pain imaginable". This was repeated 10 times, with a 30 second break between each presentation. To ensure that the range of temperatures used in the experiment contained temperatures that the subject found painful, the maximum temperature used in the experiment was the mean of the final five trials plus one degree Celsius. The maximum temperature was constrained due to ethical and safety considerations to be 49 ^{o}C, so a degree could not be added to subjects whose mean temperature was 49 ^{o}C. For the subsequent experimental task, 6 temperatures spaced 1 ^{o}C apart were used with the upper limit determined as described. Based on these individually determined criteria, all but 3 subjects chose either 48 ^{o}C or 49 ^{o}C. The 3 subjects who chose lower temperatures were excluded, as this number was insufficient to provide a statistically reliable sample of persons with lower pain thresholds. Thus due to homogeneity of temperatures chosen by subjects as "8 out of 10", all subjects included in this analysis received the same range of temperatures (44-49 ^{o}C).

2.4 Experimental Task

We used fMRI to examine the neural response to painful and non-painful thermal stimulation. Subjects

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received three presentations of each of six consecutive temperatures in pseudo-random order (i.e. a random order determined prior to the experimental session with the constraint that there was no order-temperature confound) to avoid possible confounding effects of adaptation or sensitization (Davis, Johnstone, Mazzulla, Oler, & Whalen, 2010). Stimulation stayed at destination temperature for 5 seconds (plus ramp times approximately 1.5 seconds each way, at $10^{o}C/s$).

10 +/-2 seconds after each stimulus, subjects were instructed to rate the preceding stimulus on two rating screens (each 5 seconds in length, with 1 second in between). Both were 11-point Likert scales ranging from 0-10. The first measured "warmth" (0="no sensation", 10="most intense warmth imaginable"). Subjects were instructed that in this condition, they would essentially be rating the temperature of the stimulus. On the second rating screen they were asked to rate the degree to which the stimulus was painful (0="no pain", 10="worst pain imaginable"). They were also instructed to press a button when the stimulus was experienced as painful. There was another interval of 10 +/-2 seconds prior to the next stimulus presentation, so that the total length of the interstimulus interval was 31 seconds.

2.5 FMRI Aquisition Details

Images were acquired on a General Electric (Fairfield, CT) SIGNA 3.0 tesla high-speed imaging device with a quadrature head coil. Functional images consisted of 30 interleaved 4mm sagittal T2*-weighted gradient echo, echo-planar imaging (EPI) slices covering the entire brain (1 mm interslice gap; 64 x 64 in-plane resolution; 240 mm field of view (FOV); 2000 ms repetition time; 30 ms echo time (TE); 60° flip angle; 185 image volumes per run). Functional images were collected in 2 runs of 6 minutes and 20 seconds each. Immediately following acquisition of functional images, a three-dimensional T1-weighted inversion recovery fast gradient echo high resolution anatomical image was acquired (256 x

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256 in-plane resolution; 240 mm FOV; 124 x 1.2 mm axial slices).

<u>2.6 Analyses</u>

Analysis of button press and subjective ratings was carried out in SPSS. For each subject, the button

presses were used to determine a subject-specific categorical pain threshold, the minimum temperature

at which subjects pressed the button to indicate pain more often than not. Percent button presses for

each temperature were also calculated across subjects. The mean ratings on the two subjective scales

were calculated for each temperature and subject.

Analysis of FMRI data was performed with Analysis of Functional NeuroImages (AFNI) software

(Cox, 1996). After discarding the first 5 images collected for each of the runs during reconstruction, the

images were time corrected for slice acquisition order, and motion corrected registering all the time

points to the last time point of the last run. Single-subject GLMs were computed to estimate the BOLD

response for each of the temperature conditions, using a set of 5 sine-basis functions to model the

BOLD response to each temperature over a window of 20 seconds starting at temperature onset.

Predictors were included to model BOLD responses to subject button presses and the subjective rating

screen, thus avoiding any confounding of rating-related activation with responses to the stimulation

itself. Six predictors (3 translation, 3 rotation) based upon estimated motion were also included to

model possible variance due to motion (Johnstone et al., 2006). Voxelwise maps of estimated BOLD

responses to each temperature were then converted to percent signal change by averaging the response

amplitude over the period 6 to 16 seconds post-stimulus onset (corresponding to the peak of the BOLD

response), dividing by the baseline signal and multiplying by 100. The percent signal change maps for

all temperatures were then concatenated to produce a series of 6 BOLD response magnitudes, which

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were blurred with a 6mm full width at half maximum (FWHM) Gaussian spatial filter.

To flexibly model a step-like BOLD response profile over the six temperatures, we used the AFNI program 3dNLfim to iteratively fit a 4-parameter sigmoid function to the series of BOLD responses. This technique was designed to allow us to independently i) fit a sigmoid to the BOLD responses and ii) test to see if that sigmoid predicted pain ratings. Crucially, sigmoidal fitting was done purely on the basis of the magnitude of the BOLD response across different temperatures and was in no way dependent on the pain ratings. The sigmoid function is given by the following formula:

QuickTime™ and a
decompressor
are needed to see this picture.

y is the BOLD response magnitude, a_0 represents the baseline, a_1 is a scaling factor, a_2 determines the steepness of the step in the sigmoid function, and a_3 determines the temperature (t) at which the sigmoid shows maximum steepness. The function therefore allows the flexible fitting of BOLD responses that differ (either across brain regions or across individuals) in the extent to which they show step-like, as opposed to linear, profiles across temperatures. Of the fitted parameters, the steepness (a_2) parameter is of most interest in the current study, since it determines the degree to which BOLD response shows a non-linear, stepped response to thermal stimuli. The a_3 parameter can also be used as an estimate of the threshold temperature, in that it describes the temperature at which the sigmoid shows maximum steepness (though note that this does not imply that a discrete threshold actually exists, since the maximum steepness might not be very steep). For comparison with a basic linear temperature response model, we separately fit a linear model to the same series of 6 BOLD response magnitudes. For both models, a voxelwise goodness of fit statistic was also computed, R^2, which indicates the variance in BOLD response across different temperatures explained by the non-constant

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component of each model.

Resulting parameter images were spatially transformed to Talairach space prior to group-level analysis. We used a two-step procedure for group-level voxelwise analyses:

1. To identify brain regions that show at least some sensitivity to thermal stimulation, the percent signal change in response to the 49°C temperature condition was entered into a one-sample t-test to determine those regions of the brain that showed a response to at least the highest temperature. These regions were further restricted to those in which the BOLD response to 49°C was significantly greater than to 44°C, indicating some degree of temperature sensitivity. A mask was created using the resulting statistical map, which was used to constrain the search space for subsequent analyses comparing linear to non-linear temperature responses.

2. The R^2 statistic from the sigmoid function was compared to the R^2 statistic from the linear function in a voxelwise paired t-test to identify voxels where the sigmoid model explained significantly more variance than the linear model.

All voxelwise analyses were corrected for multiple comparisons using cluster-extent thresholding based on Monte Carlo simulation, to give a corrected $p < 0.05$ (we also used the FSL program 'randomise' to perform non-parametric multiple comparison correction using permutation testing on maximum cluster sizes (Hayasaka & Nichols, 2003), which yielded the exact same set of significant clusters, with almost identical cluster sizes as when using Monte Carlo simulation).

To test the degree to which brain activation correlated with subjective ratings of warmth and pain, for all significant clusters the cluster mean percent signal change and sigmoid model fit values were extracted for each subject for each temperature. Each of these sets of values was then entered into two regressions for each subject to predict that subject's warmth and pain ratings respectively. From this

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analysis it was possible to determine the proportion of variance (the regression R^2) in an individual's

warmth and pain ratings that could be accounted for by that subject's raw percent signal change values

versus sigmoid fit values in each significant cluster.

3. Results

3.1 Pain threshold button presses

Individual subjects' pain thresholds were first categorically estimated based on the averaged button

press response for each temperature. One participant indicated temperatures of 44C, 45C, 48C and 49C

to be painful, but not 46C or 47C. The same subject rated pain at 44C as 0 or 1, which might indicate

that this subject's button presses were not reliable. The remaining subjects' button presses were more

consistent, with all temperatures above a subject-specific threshold temperature being indicated as

painful. Individual categorical subject-specific pain thresholds for these subjects ranged from 46C-49C

(M=48C, S.D.=1C). Percent of the time that the same subjects indicated pain ranged from 2% at 44C to

93% at 49C (see Table 1). Based on button presses, the temperature at which pain would be indicated

50% of the time would be expected to occur somewhere between 47C (29%) and 48C (60%).

3.2 Subjective ratings

Both warmth ratings and pain ratings differed significantly across the six temperatures ($F(5,60) = 63$, p

< 0.001 and $F(5,65) = 71$, $p < 0.001$ respectively; see Table 1), with mean pain ratings showing an

abrupt rise from 3.6 at 47C to 5.3 at 48C, consistent with the button press data (see Table 1). The pain

rating at this transition is consistent with mean heat pain ratings of 5 at pain thresholds determined by

method of limits in a prior study specifically examining the correspondence between pain ratings and

thresholds (Kelly, Cook, & Backonja, 2005). Differences between these ratings and those collected

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prior to the fMRI session to select the range of applied temperatures fell well within the test-retest range found in previous studies using a similar procedure (Yarnitsky, Sprecher, Zaslansky, & Hemli, 1995). Corresponding to the homogeneous temperature ranges obtained in selecting subjects for the study, the temperature at which the pain ratings showed the steepest increase showed a narrow range from 46.5^{o}C to 48.5^{o}C, averaging 47.7^{o}C across subjects. Although the sample was homogeneous with respect to this measure of subjective pain threshold, there was substantial variability in the shape of the subjective rating profiles across the different temperatures, with some subjects showing a more gradual increase (e.g. a maximum rise of only 1 point on the pain rating scale per ^{o}C), while others showed a more abrupt increase at a given temperature (e.g. a maximum rise of over 5 points on the scale per ^{o}C). Comparison of the shape of individual subjectively reported pain profiles with corresponding neural responses was the focus of the subsequent fMRI analysis.

3.3 Brain imaging data

Mixed-model analysis of goodness of fit parameters revealed a number of clusters in which the sigmoid function explained significantly more variance in temperature-related BOLD activation than the linear function (see Table 2; Figure 1). Across all these clusters the sigmoid a_2 parameter was significantly greater than zero ($F(1,13) = 12.5$, $p = 0.004$), indicating a significant non-linear steepness in BOLD responses across temperatures. There were no significant differences in a_2 across clusters ($F(1,13) < 1$). Clusters in thalamus, inferior parietal lobule extending to parietal operculum (SII), mid-cingulate, and bilateral insula have all previously been identified as components of a distributed pain processing network (Craig, 2003; Farrell, Laird, & Egan, 2005). Sigmoidal shaped MEG response profiles to pain stimuli have previously been found in SII (Timmermann et al., 2001). In prior research (Bornhovd et al., 2002) activation in the anterior insula was found to vary as a function of pain intensity, but not sub-

threshold temperature. The area of mid-cingulate identified in this study overlaps the cingulate region found by Buchel et al. (2002) to activate in response to thermal pain. A large cluster in the midbrain included substantia nigra, red nucleus and periaqueductal gray (PAG). The sigmoid fit was also associated with activation of clusters involved in planning and instantiation of motor responses, including the basal ganglia and premotor cortex.

In the current study, a more extended network of brain regions was found to show a sigmoidal response profile than in similar studies that used apriori planned contrasts (Bornhovd et al., 2002; Buchel et al., 2002) possibly reflecting greater statistical sensitivity when using the nonlinear function fitting approach. In particular, fitting a sigmoid allows for variation in the steepness and temperature location of the sigmoidal step, both across different brain regions and different subjects. Thus brain regions whose response profile could not be fit with a specific a priori planned contrast, but that might covary with subjectively experienced pain can be effectively modeled using the more flexible approach adopted here.

3.4 Correspondence between brain activation and warmth and pain ratings

We next tested the extent to which the sigmoid-fitted neural response profiles corresponded to the individual profiles of subjectively experienced pain across the different applied temperatures. While it is possible to summarise each participant's profile with a single value (e.g. "threshold" temperature, mean response gradient) and then carry out a between-subjects correlation of such values from BOLD responses and subjective reports, this approach results in a loss of relevant information about the shape of each individual's response across temperatures. Instead, we used linear regressions run separately on the data from each subject to determine the proportion of variance (the regression R^2) in an

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individual's warmth and pain ratings that could be accounted for by that subject's raw percent signal change values versus sigmoid fit values in each significant cluster. This analysis tests the degree to which the shape of an individual's BOLD response profile over the six temperatures, or the equivalent sigmoid fit profile, corresponds to the shape of the subjective response profile for the same subject.

The sigmoid fit explained significantly more of the variance in pain ratings than did the directly measured percent signal change BOLD signal ($F(1,13) = 110$, $p < 0.001$; see Table 3 and Figure 2). This was the case for all clusters except the posterior cingulate for which the difference was not significant (all clusters: $t(13) > 2.7$, $p < 0.016$, except posterior cingulate: $t(13) = 0.96$, $p = 0.36$). The sigmoid fit also explained significantly more of the variance in warmth ratings than did the percent signal change BOLD signal ($F(1,14) = 58$, $p < 0.001$). Similarly, this was the case for all clusters except the posterior cingulate for which the difference was not significant (all clusters: $t(12) > 2.2$, $p < 0.05$, except mid-cingulate: $t(12) = 1.1$, $p = 0.29$). The temperature of steepest sigmoid slope (the delay parameter in the sigmoid model) averaged 47.6°C, as compared to the temperature of steepest increase in subjective pain ratings, which was 47.7°C. Thus both the shape of the response profile as modeled by a sigmoid function, as well as the temperature of steepest sigmoid slope, showed tight correspondence with the subjective pain ratings. The only region where sigmoid-fitted brain activation explained more variance in pain ratings than warmth ratings was left anterior insula ($F(1,12) = 6.1$, $p = 0.03$; all other clusters $F < 1$).

4. Discussion

Pain is a sensory and affective experience that serves a critical adaptive function, inspiring the

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organism to enact protective behaviors. While it is often a response to sensory input, it does not have a direct, linear relationship to such input. Rather, individuals report a transition where sensory input goes from being relatively innocuous to painful at a particular temperature or range of temperatures (commonly referred to as the pain threshold).

In order to examine the neural mechanisms underlying this transition, we measured BOLD responses to thermal stimuli varying from 44°C to 49°C, and fit the BOLD response profile with a non-linear function designed to capture neural activation reflecting perceptual as distinct from sensory processing. We predicted that neural regions supporting the perception of pain, as opposed to the stimulus property of heat, would show a non-linear, sigmoidal-shaped increase in BOLD response magnitude across temperatures. The sigmoid function has been extensively used to model thresholded responses in different types of nonlinear systems including neuronal responses to pain (Neugebauer & Li, 2003), and prior data suggested a sigmoidal shape in both BOLD responses to thermal stimuli (Timmermann et al., 2001), as well as corresponding subjective reports (Bornhovd et al., 2002; Buchel et al., 2002).

Consistent with this prediction, the sigmoid fit was found to account for significantly more of the variance in the BOLD signal than a more simple linear function in a number of brain regions previously associated with the neural pain response. Activations of the insula and dorsal anterior cingulate in the present study are consistent with previously reported activation of these regions in neuroimaging studies of pain (Farrell et al., 2005; Peyron, Laurent, & Garcia-Larrea, 2000) and, more specifically, studies examining the transition from innocuous to painful sensation (Bornhovd et al., 2002; Buchel et al., 2002; though see Oertel et al., n.d.). The insula has been implicated in the integration of incoming information on the state of the body and corresponding subjective states (Craig, 2009) as well as with estimation of the magnitude of pain intensity (Baliki, Geha, & Apkarian, 2009;

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Coghill, Sang, Maisog, & Iadarola, 1999); but see also Moayedi & Weissman-Fogel (2009). Cingulate

has been implicated in facilitation of pain-related motor responses (Shackman et al., 2011). Both are

thought to be regions where cognitive and emotional information relevant to pain are processed and

integrated with sensory information (Brooks & Tracey, 2007; Ploner, Lee, Wiech, Bingel, & Tracey,

2011; Wiech et al., 2010), reinforcing the multi-faceted nature of the pain experience.

As mentioned previously, activation of dorsal cingulate and both anterior and posterior regions of

insula are consistent with similar activations in studies examining the transition from innocuous to

painful sensation (Bornhovd et al., 2002; Buchel et al., 2002). The present study found a more

extensive network of activations, however.

Of particular note is the preponderance of activations in regions associated with motor responses. The

observed thalamic activations were in the area of the ventral lateral nucleus, known to be a projection

site for spinothalamic afferents commonly associated with pain and thermal information (Craig &

Dostrovsky, 2001). While this region receives nociceptive input, it also receives projections from motor

regions including the globus pallidus (Borsook, 2007), which was significantly associated with the

transition from innocuous to painful sensation in this study. Ventral lateral nuclei also project to the

premotor cortex, consistent with activations observed in that region. Within this context, it is

noteworthy that the periacquductal gray which was also activated has not only been associated with

descending modulation of pain (Fields & Basbaum, 1999), but has been implicated in the instantiation

of fight or flight responses (Bandler & Keay, 1996). Taken together these findings strongly

demonstrate the prioritization of neural processing associated with preparation for action as stimuli

move from being innocuous to affectively salient. Studies examining neural activation associated with

perceived intensity of pain (Coghill et al., 1999; Derbyshire et al., 1997) have observed similar patterns

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of activation, leading to a question for future study, namely which of the activations associated with the transition from innocuous to painful sensation in the present study increase with graded increases in temperature above the pain threshold.

A key finding of this study is the corroboration of measures of self-reported pain by sigmoid-fitted BOLD response profiles in a network of pain processing regions derived totally independently from the self-reports. We found that sigmoid-fitted activation in these neural regions accounted for up to 85% of the variance in individual warmth and pain ratings (significantly more than the raw BOLD response magnitudes). Furthermore, the point of inflection in the fit neural response profile showed tight correspondence to the temperature at which subjects reported maximum increases in their subjective experience of pain and the point at which button presses indicating pain became consistent.

The fact that the neural and self-report data corroborate each other confirms that the observed shift in activation levels corresponds to the perceptual switch from innocuous to painful levels of stimulation. Importantly, it also provides a further validation of self-reports of pain (Coghill et al., 2003). This convergent validity is particularly important given variability observed across individuals in the degree to which a given point on the stimulus continuum reliably divides innocuous from painful experiences (Gracely, 1999). While such variance could be disregarded as idiosyncratic pain reporting behavior rather than a true reflection of perceptual experience, the correspondence between the profiles of sigmoid fitted brain activation in a distinct set of brain regions and subjective ratings strongly supports the notion that individuals differ not only in the level of stimulation required to consistently elicit pain, but the degree to which this threshold is a discrete point rather than a graded range of temperatures. One central feature of pain that remains to be thoroughly tested is the extent to which such transitions from innocuous to painful experience vary across individuals and with the experimental or clinical

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context. Our pain ratings indicate that at least in the current experimental context there is great individual variability, not necessarily in the temperature at which individuals show the steepest rise in pain ratings, but in the steepness of the rise. In addition, there is variability in the extent to which participants rate low temperatures as 0 or 1 on an interval pain scale, despite indicating with a categorical decision that such temperatures are not painful. This would suggest that the idea of a single well-defined temperature at which a stimulus becomes painful is not always justified. One of the motivations of the current study was to determine whether such variability in pain rating slopes could be accounted for by corresponding differences in BOLD response profiles. Our results show this to be the case. A strength of our analysis approach is that the formula used for non-linear curve fitting provides separate variables for the point of maximum inflection in the curve (which can be used as an index of the pain threshold) and slope of the curve (corresponding to the degree that the pain threshold is a discrete point or occurs over a range of temperatures for a given individual). By allowing for quantification of these variables, this methodology may provide clinically important insight into the mechanisms underlying individual differences in pain sensitivity. It also provides a means for determining the profile of pain in different experimental contexts and with different groups of individuals.

We note that although a sigmoid function provides flexibility in this regard, it still imposes theoretically-motivated constraints on the broad class of response profiles that will be fit. We further restricted our sigmoidal analysis to voxels that showed an increase from the lowest to the highest temperatures. It is quite possible that other networks in the brain that support different aspects of pain processing have a response profile that cannot be modeled with a sigmoid. For example, regions showing a uniform response across temperatures (which might be encoding the presence of a nociceptive stimulus), a decrease across temperatures (perhaps reflecting a switch away from

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background processing as is often the case with the "default" network) or other types of response would not have been captured. In addition, a number of brain regions in the current study showed no obvious plateau in the group average BOLD response at the highest temperatures, perhaps due to the limited range of painful temperatures available for use in the study (see Methods). In such cases, a polynomial could offer an equally good fit with fewer estimated parameters, though one would have to know that were the case for all individual subjects beforehand. Using a sigmoid function in this case allows a more flexible fit on the basis of fewer apriori assumptions at the expense of a single extra parameter to estimate. In sum, the function we describe here was not intended to give a comprehensive view of all the stages in pain processing, but rather was a way of testing a specific hypothesis in a focused manner. The general technique of using nonlinear fitting to response profiles could, however, be applied to test for other types of response depending on the theoretical question being asked.

One aspect of this study that was not explicitly addressed was the affective response of participants to the stimuli. Because participants were not aware of temperature they were about to experience on any given trial, it is possible that responses at lower temperatures included an element of relief and a reduction in anxiety when they realized the hottest stimulus was not being applied. Indeed, the neural processes by which ascending nociceptive signals are imbued with affective/hedonic salience is of vital importance to furthering our understanding of how the experience of pain varies across contexts, individuals, and the lifespan. The neural circuits that detect and assign affective salience to other sensory modalities such as vision (Ghashghaei, Hilgetag, & Barbas, 2007; Rolls, 2004) reflect a hierarchy of processing, in which early, fairly rudimentary and inflexible processes are modulated by later, more elaborate and flexible processes. Though this study did not directly address the neural process by which nociceptive signals become affectively salient, it complements studies of how this

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salience is modified under various cognitive and affective conditions (Ploner et al., 2011; Wiech et al., 2010). Such information is vital to our understanding of both pain and affective disorders, as well as their frequent comorbidity.

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Figure Legends

Figure 1. Regions showing significantly greater activation to 49°C than to 44°C and for which the sigmoid function explained significantly more of the variance in BOLD signal magnitude across different temperatures than did the linear function ($p < 0.05$ corrected). The image is colour coded by the sigmoid fit steepness parameter. Numbers above left of each image are z-coordinates.

Figure 2. Plot of BOLD responses, linear fits and sigmoid fits in right anterior insular, substantia nigra/red nucleus/PAG, medial frontal gyrus and mid-cingulate.

8. Table

Table 1. Percent of "pain" button responses and mean and standard deviation warmth and pain ratings for each temperature.

	temperature (ºC)					
	44	45	46	47	48	49
percent pain button presses	2	19	7	29	60	93
warmth rating	2.8 (1.2)	3.3 (1.1)	4.0 (1.5)	5.1 (1.4)	6.6 (1.3)	8.0 (1.5)
pain rating	1.3 (1.5)	1.1 (1.4)	1.8 (1.7)	3.6 (1.8)	5.3 (2.0)	7.4 (1.8)

8. Table

Table 2. Clusters for which the sigmoid function explained significantly more variance in temperature-related BOLD contrast than did the linear function. Sigmoid threshold temperature is the estimated a_3 parameter in the sigmoid function. Numbers in parentheses are standard deviations.

cluster location	Talairach coordinates			cluster size (2mm^3 voxels)	Sigmoid threshold temperature
right anterior insula (BA 13)	42	11	3	920	47.6 (0.5)
bilateral substantia nigra/red nucleus/PAG	−5	−14	−6	880	47.8 (0.6)
bilateral medial frontal gyrus (BA 6)	5	6	51	752	47.6 (0.6)
bilateral posterior cingulate (BA 23)	3	−29	26	264	47.5 (0.8)
left anterior insula (BA 13)	−31	14	3	240	47.7 (0.5)
right inferior parietal lobule (BA 40)	55	−41	29	208	47.4 (0.9)
bilateral mid-cingulate (BA 24)	3	−3	34	144	47.5 (0.7)
right lentiform nucleus/globus pallidus/putamen	16	4	0	120	47.8 (0.5)
right thalamus	17	−12	12	80	47.6 (0.8)
right parahippocampal gyrus (BA 27/28)	25	−27	−4	72	47.8 (0.9)
right posterior insula (BA 13)	43	−18	17	64	47.5 (0.6)
left thalamus	−16	−13	7	40	47.6 (1.0)
right middle temporal gyus	54	−32	−7	32	47.7 (0.9)

8. Table

Table 3. Percentage of variance in subjective ratings explained by the BOLD signal estimates and sigmoid function fit to the BOLD signal estimates. Values represent individual subject regression R^2 values averaged across all subjects.

Cluster location	R^2 warmth ratings		R^2 pain ratings	
	BOLD	sigmoid	BOLD	sigmoid
right anterior insula (BA 13)	.41	.73	.45	.72
bilateral substantia nigra/red nucleus/PAG	.60	.80	.57	.85
bilateral medial frontal gyrus (BA 6)	.57	.81	.50	.84
bilateral posterior cingulate (BA 23)	.51	.59	.59	.64
left anterior insula (BA 13)	.45	.62	.44	.71
right inferior parietal lobule (BA 40)	.49	.74	.47	.73
bilateral mid-cingulate (BA 24)	.54	.73	.62	.82
right lentiform nucleus/globus pallidus/putamen	.40	.78	.48	.74
right thalamus	.47	.73	.46	.76
right parahippocampal gyrus (BA 27/28)	.49	.70	.54	.72
right posterior insula (BA 13)	.51	.66	.50	.70
left thalamus	.36	.66	.37	.66
right middle temporal gyus	.51	.76	.56	.82
Mean across all clusters	.48	.72	.50	.75