Analogous Responses in the Nucleus Accumbens and Cingulate Cortex to Pain Onset (Aversion) and Offset (Relief) in Rats and Humans

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Running Head: Analogous responses to pain onset/offset in rats and humans

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ABSTRACT
In humans, functional magnetic resonance imaging (fMRI) activity in the anterior cingulate cortex (ACC) and the nucleus accumbens (NAc) appears to reflect affective and motivational aspects of pain. The responses of this reward-aversion circuit to relief of pain, however, have not been investigated in detail. Moreover, it is not clear whether brain processing of the affective qualities of pain in animals parallels the mechanisms observed in humans. Here, we analyzed fMRI BOLD activity separately in response to an onset (aversion) and offset (reward) of a noxious heat stimulus to a dorsal part of a limb in both humans and rats. We show that pain onset results in negative activity change in the NAc and pain offset produces positive activity change in the ACC and NAc. These changes were analogous in humans and rats, suggesting that translational studies of brain circuits modulated by pain are plausible and may offer an opportunity for mechanistic investigation of pain and pain relief.
INTRODUCTION

Substantial progress in our understanding of pain perception, processing and modulation in the brain has been achieved using neuroimaging techniques in humans. Pain is multidimensional with sensory, affective and motivational aspects that are processed in part by separate regions of the brain. Thus, the thalamus, primary and secondary sensory cortex and posterior insular cortex have consistently been implicated in sensory-discriminative processing (Bingel et al. 2004; Peyron et al. 1999), while the anterior cingulate cortex (ACC), anterior insula and prefrontal cortex are thought to be relevant to the cognitive-affective modulation of pain (Apkarian et al. 2005; Rainville et al. 1997). The mesolimbic reward circuit comprising projections from the midbrain ventral tegmental area to the nucleus accumbens (NAc) plays an important role in motivational aspects of pain (Geha et al. 2008; Oluigbo et al. 2012; Scott et al. 2006). A functional link between the NAc and the ACC in reward processing has been reported (Albrechet-Souza et al. 2012; Parkinson et al. 2000; Wacker et al. 2009) supporting the possibility that activity in these brain regions could reflect affective and motivational aspects of pain.

In contrast to human studies, investigations of the affective-motivational dimension of pain in animals, for example analyses of motivated behaviors and animal brain neuroimaging, have been scarce. Animal investigations may allow mechanistic studies that are not feasible in humans, including exploration of brain circuits relevant to pain as well as their modulation by analgesic treatments and drug side-effects. Whether affective processing of pain in animals could be analogous to the complex and unique human pain experience is not known. Increased cortical complexity in higher species is obvious and the limitations of animal models in studying affective components of pain must be acknowledged. Nevertheless, the basic homeostatic and reward brain circuits underlying motivated behavior are fundamental to survival and appear to be highly conserved between humans and rats (Craig 2003; Murray et al. 2011). Moreover, the imaging studies evaluating pain processing in rodents have produced remarkably consistent results and confirmed activation of the same brain regions commonly activated in human imaging of pain (Thompson and Bushnell 2012).

The ACC is engaged in ascending processing of nociceptive input as well as in regulation of the subjective feelings of pain unpleasantness. PET and fMRI imaging studies in humans have
repeatedly demonstrated activation of the ACC by acute nociceptive stimuli (Peyron et al. 2000). The activity in the ACC correlated with the subject’s ratings of pain unpleasantness (Rainville et al. 1997), supporting the role of the ACC in affective dimensions of pain. Consistent with this observation, lesioning the rACC in rats prevents formalin-induced conditioned place aversion (CPA) (Johansen et al. 2001) and blocks conditioned place preference (CPP) resulting from relief of ongoing pain (Qu et al. 2011). Because the ACC receives direct nociceptive inputs from the ascending spinothalamocortical pathway, these findings are consistent with the notion that nociceptive input to the ACC increases the activity in the region and this response in turn results in increased pain unpleasantness. The ACC receives connections from other cortical and subcortical regions including the prefrontal cortex, amygdala and the NAc (Cauda et al. 2011) that may modulate its activity. Thereby the ACC can integrate cognitive, emotional and motivational factors such as expectations, mood and anxiety to regulate the subjective pain experience. Additionally, the ACC activity has been linked to the subjective experience of pleasure for a variety of stimuli including odors, tastes and water in thirsty subjects (Wacker et al. 2009).

Relief of pain is rewarding in both humans (Grill and Coghill 2002; Leknes et al. 2011) and animals (Navratilova et al. 2012). We have previously shown that relief of ongoing post-surgical pain produces conditioned place preference in rats and activates mesolimbic reward circuit (Navratilova et al. 2012). In this study we analyzed in parallel in both rats and humans fMRI BOLD signals following an acute thermal noxious stimulation. Because the ACC and NAc both respond to aversive as well as rewarding (pleasant) stimuli, we hypothesized that the onset (aversive event) and offset (rewarding event) of an acute noxious stimulus would produce differential fMRI activations in these brain structures. In a phasic experiment of noxious heat in humans, we have previously demonstrated a change of valence of the functional MRI (fMRI) signal in the NAc (i.e., a negative BOLD response following the application of noxious heat and a positive BOLD response when the stimulation is terminated) (Becerra and Borsook 2008). Given the obvious differences in brains between humans and rodents, we have restricted this report to the ACC and NAC. Thus the objective of this study was (1) to investigate fMRI BOLD responses of the ACC and NAc to aversive and rewarding stimuli; and (2) to determine if the underlying circuitry for reward (pain offset) and aversion (pain onset) are similar across species.
The study followed NIH guidelines for the use of animals in research and was approved by the Institutional Animal Care and Use Committee. The human study was approved by the Institutional Review Board. Although it is not possible to match pain intensity experiences across species, we selected a temperature that seems to produce a moderate to strong pain stimulus: in humans we have ample experience that 46°C produces a pain intensity of 7/10—moderate to high pain. In rats, Baliki and colleagues (Baliki et al. 2005) have demonstrated on hot-plate experiments that above 48°C temperatures, rats indicate a significant pain response. Furthermore, to allow for more direct comparison between animal and human studies, the rat fMRI was performed in acclimated, trained, awake animals.

Humans:
Ten male, right-handed healthy controls were recruited for this study (age=40.5±10.2 (mean±stdev). Subjects were screened for mental health state and to be MRI safe.

Heat Stimulation: Humans underwent a painful fMRI scan using a TSA-2000 pain device (Medoc, Haifa, Israel). Three stimuli at 46 °C were delivered for 25 seconds with 30 seconds of neutral (32°C) temperature to the dorsum of the left hand with a 3x3 cm² Peltier thermode. Three stimuli were used for each subject (See Figure 1).

Imaging: Human imaging was acquired in a 3T Siemens Trio scanner (Siemens, Erlangen, Germany). An MPRAGE sequence was used to acquire structural images (TR/TE/Flip=25/5ms/35 FOV = 20 cm; slice thickness = 1.2 mm; in-plane resolution = 1.2 mm; matrix = 256 x 256). Functional scans were performed using a gradient echo, EPI sequence (TR/TE = 2.5s/30ms; slice thickness = 3 mm in-plane resolution 3.125 x 3.125mm), performed on a 41-slice oblique (perpendicular to the anterior-posterior commissure line) prescription.

Animals:
Twelve male Sprague-Dawley rats were used for this study with weight in the 300-350 g range. Rats were acclimated to the animal facilities for 24-48 hours after arrival. They were briefly anesthetized with 2% isoflurane and loaded into cradles similar to the one used for imaging and
placed in a box with speakers playing recorded scanner noises for 1 hour, rats were awake during the whole procedure. This procedure was repeated for 3 consecutive days. On the fourth day, the animals were scanned. For the imaging session, rats were briefly anesthetized with 2% isoflurane and secured on head-and-body holders. They were left to wake up for 30 minutes before the imaging session begun.

Heat Stimulation: A home-made small hollow copper block was positioned below the rat dorsum of the left hind paw. The block’s temperature was controlled by a circulating thermal bath. The temperature can be regulated from 0 to 50 °C (Becerra et al. 2011a). For these experiments, 5 stimuli 21 seconds long with variable interstimulus times (14-24 seconds) were used. A temperature of 48 °C was applied with resting temperatures of 32 °C (See Figure 1).

Imaging: Imaging data was acquired on a 4.7 T Brucker Scanner (Bruker Biospin, Billerica, MA). A multi-concentric dual-coil small animal restrainer (Insight NeuroImaging Systems) was used to perform the imaging studies. Imaging consisted of acquisition of anatomical scans and sensory functional scans. High-resolution anatomical scans were acquired using a RARE sequence with a FOV of 3 cm, 1 mm thick slices and 256x256 in-plane resolution. fMRI data was acquired utilizing an EPI sequence (TR/TE=3s/12ms, FOV 3.0 cm, 64x64 (0.46875 mm in-plane resolution), 15 1.5 mm axial slices) with 90 time points.

fMRI Analysis:
Details of fMRI data analysis have been published elsewhere (Becerra et al. 2011a; Upadhyay et al. 2010). Briefly, fsl tools (http://www.fmrib.ox.ac.uk/fsl) were utilized for preprocessing (brain extraction, motion correction, spike identification and spatial (humans 5mm; rats 0.6 mm) and temporal (100 s) filtering). Statistical analysis was carried out using a general linear model approach. The interest of this study was to detect changes associated with the onset and offset of the noxious stimulus. Accordingly, explanatory variables (EV) were created based on the temporal profile of the noxious stimuli. A rapid response to onset and a separate response to offset were modeled in addition to the heat response (Figure 1). These EVs were convoluted with the standard hemodynamic response (Becerra et al. 2011a; Upadhyay et al. 2010). To account for residual motion effects as well as potential spikes in the data; motion parameters and spikes were added as confounding EVs. Functional data was co-registered to an atlas.
(human atlas provided by fsl analysis tool package, rat MRI atlas developed in house and based
on a histological atlas (Becerra et al. 2011b). For registration, fsl linear registration tool was
used (flirt) with 12 DOF and tri-linear interpolation (Jenkinson et al. 2002). Human functional
data was interpolated to 2 mm³ voxels and rat data to 1mm slices with 0.1171875 mm in-plane
resolution (Becerra et al. 2011b).

Statistical results were grouped using a fixed-effects approach. For statistical inference,
statistical maps for onset and offset were thresholded using mixture-modeling approach
(implemented in our lab) that accounts for multiple comparisons as well as for potential
statistical model violations (Pendse et al. 2009), Briefly, mixture-modeling determines a series of
gaussians that model the null component and identifies non-null ones that can be ascribed to
activation and deactivation components, each voxel is classified and receives a probability that
belongs to a particular class (deactivation, null, activation). Voxels probabilities are thresholded
at 0.5 to determine the class they belong too. Classification maps are then used to determine
statistically significant activity. Group statistical maps were overlaid over a standard MRI brain
that has been co-register to a histological atlas (Becerra et al. 2011b) for NAC and ACC
identification. The present report concentrated in activation in accumbens and cingulate cortex
and all other brain activities have not been examined and/or removed from the data/figures.
RESULTS

No human scans were eliminated due to excessive motion artifacts. Two of the 12 rats were excluded as a result of movements during the scan (>0.5mm).

Humans

Group results for onset/offset activations in the anterior cingulate cortex in humans revealed no significant changes at the onset of the painful stimulus and significant activation was observed at the pain offset (pain relief) (Figure 2 and Table 1). In the nucleus accumbens the onset of the painful stimulus resulted in significant deactivation (negative change) of the fMRI signal while significant activation was observed to the onset of pain (Figure 2 and Table 1).

Rats

We have reported on a separate study brain activations to noxious heat in rats (Becerra et al. 2011b). We observed activity in sensory-discriminative, affective-emotional, descending, and autonomic pathways. Here, similar to humans, we did not observe any significant change in the activity of the ACC in rats at stimulus onset, however, the offset of a noxious thermal stimulus increased activation in the anterior cingulate/motor area. In the NAc, the onset of the noxious stimulus resulted in a decrease in fMRI signal response, which was most prominent in the core of the structure (Figure 2 and Table 1). Deactivation was also observed in cortical (sensory) and subcortical areas (caudate/putamen) with the onset of painful stimulus. fMRI response associated with noxious stimulus offset resulted in positive activation in an area of the NAc that corresponds to the core region (Figure 2 and Table 1).
DISCUSSION

In the current BOLD fMRI study we observed analogous brain activity changes to aversive (pain onset) and rewarding (pain offset) thermal stimuli in rats and humans. Specifically, we detected increased activation of the ACC at stimulus offset in both species. In the NAc we observed deactivation following the onset of noxious heat and activation upon cessation of the painful stimulus. We have previously demonstrated change of valence of the fMRI response in the NAc between pain onset and offset in humans (Becerra and Borsook 2008). This finding is confirmed here in a new cohort of experimental subjects and is additionally replicated in rats.

Parallel Activation of the Anterior Cingulate Cortex in Rats and Humans

The exact correspondence of brain structures across species is not always clear (Vogt and Paxinos 2012). The approach to identify similar structures has been based on function and cytoarchitecture (Vogt and Paxinos 2012). Accordingly, the rat cingulate is divided into an anterior (ACC), middle (MCC), and posterior or retrosplenial (RPL) cortices. The correspondence for the 3 divisions of the cingulate in rodents and humans has been established by Vogt and Paxinos. Our results indicate that activity in the cingulate corresponds to the anterior part for both species.

Pain-induced aversiveness is integrated, in part, within the rostral anterior cingulate cortex (rACC) (Tracey and Mantyh 2007). The ACC is also involved in endogenous pain control and descending pain modulation (Petrovic et al. 2002; Wager et al. 2004; Zubieta et al. 2005). Additionally, ACC activity is observed in response to positive stimuli (Wacker et al. 2009) suggesting a potential role in pleasantness of pain relief. Given the complexity of the ACC functions in pain processing, the overall response of the ACC during acute nociceptive stimulus may reflect (1) the initial aversive value of the stimulus, (2) the role of this structure in the behavioral, cognitive and emotional regulation of pain (Rainville 2002), or (3) the pleasant effects of pain relief. Most imaging studies have not evaluated pain onset and offset separately and the total response in acute evoked pain studies usually shows increased activation in the ACC to noxious stimuli (Apkarian et al. 2005; Peyron et al. 2000).

Our paradigm evaluated events related to immediate onset and immediate offset of pain. The temporal correlation of the predominant ACC response with the termination of the noxious stimulus supports the role of the ACC in pain relief and/or pain modulation. In our studies any
putative activity change at the pain onset was below the threshold, but this does not exclude a possible involvement of the ACC in the initial aversive response. Increased activation during evoked pain may also reflect that the ACC is integrated into a neural network that evaluates pain in the context of potentially threatening or ongoing stimuli (Lorenz and Casey 2005) and interoceptive processing (Taylor et al. 2009). Using analogous noxious stimulation to the dorsal part of a limb, we observed similar changes in the rACC activity in rat and man at these two times, supporting the notion that brain processing of the affective and motivational aspects of pain is conserved across mammalian species.

The NAc is the primary target of midbrain dopaminergic neurons (Ikemoto 2007) and plays a prominent role in reward-aversive behaviors (Carlezon and Thomas 2009; Deadwyler et al. 2004). Activation of dopaminergic neurotransmission in the NAc in response to primary food and liquid rewards and rewarding drugs has been well documented in both humans and animals (Becerra et al. 2006; Di Chiara et al. 2004; Roitman et al. 2008). In the past few years, research has implicated the region in pain related behaviors (Altier and Stewart 1999; Gear and Levine 2011; Magnusson and Martin 2002) and it has been shown that dopaminergic inputs from VTA to NAc can signal rewarding as well as aversive events (McCutcheon et al. 2012; Ungless et al. 2004).

Studies in rodents and humans of the ACC have implicated the structure in a number of processes involved in pain and analgesia. In preclinical studies, pharmacological manipulations such as microinjection of morphine into the ACC result in a naloxone reversible reduction in pain affect (LaGraize et al. 2006) or enhancement of pain facilitation when the region is stimulated (Calejesan et al. 2000). Studies have contributed to our understanding of the ACC in pain and emotional processing (Johansen et al. 2001). Mechanisms of activation and deactivation in the ACC in response to pain offset may involve activation of specific receptor inhibition or excitation (viz., glutamate and GABA receptors) populations (Ji and Neugebauer 2011). Lesions of the ACC reverse chronic pain in animal models (Qu et al. 2011) and humans (Yen et al. 2009). In human studies functional imaging has shown activation in the structure across most studies of experimental and clinical pain (Apkarian et al. 2005; Peyron et al. 2000). It should be noted that the structure is part of important neural networks (e.g., salience network (Borsook et al. 2013); that interacts with numerous other brain regions, the most prominent of which are the insula
and orbitofrontal cortex. The area is also involved in more generalized responses including aversive processing (Ortega et al. 2011) including conditioned place aversion (Minami 2009); pain relief (Navratilova et al. 2013) and placebo responses (Geuter et al. 2013). Differences in ACC in rats and humans include the presence of von Economo neurons in the human brain and other higher species (apes, whales) but not in rodents (Allman et al. 2011; Butti et al. 2009). Because of these specialized neuronal populations, potential differences in interpretation of awareness may be salient. Complex behaviors such as learning, context or interoceptive responses to aversive stimuli (Freeman et al. 1996; Seymour et al. 2005) that include discrimination of unpleasantness (Kulkarni et al. 2005), context inhibition (Talk et al. 2005) might not be present across species.

Parallel Activation of the Nucleus Accumbens in Rats and Humans

Regions of the ACC connect with limbic structures including the NAc (Baleydier and Mauguiere 1980; Powell and Leman 1976; Roberts et al. 2007). In rats, the anatomical location and segregation of the NAc into the core and shell is well defined (Paxinos 2004), but the structure is less well demarcated in humans (Cauda et al. 2011). In man, the NAc is located at the conjunction between the head of the caudate and the anterior portion of the putamen, laterally to the septum pellucidum (Groenewegen et al. 1999; Heimer et al. 1991). Together with the olfactory tubercle, the NAc forms the ventral striatum. In a prior evaluation of noxious heat activation in the NAc, based on the anatomical and potential functional differences in the core and shell, we suggested that these two sub-regions might differentially participate in aversion and reward (Aharon et al. 2006). Here we observe activation changes to the onset and offset of an aversive heat stimulus in the NAc core of the rat. Parallel activation in the human is located within what we may thus assume to be the core based on these functional results. Importantly, we observed analogous valence change in the NAc to the aversive (pain onset) and rewarding (pain offset) stimulus in both humans and rats. Thus, NAc activity in response to aversion and reward is conserved between species.

Conclusions

In conclusion this study investigated fMRI BOLD responses in the mesocorticolimbic circuit in response to onset and offset of an acute noxious thermal stimuli in parallel in humans and rats.
We show that the anterior cingulate cortex and the nucleus accumbens respond differently to
the onset and offset of pain suggesting a pivotal role of these brain regions in affective and
motivational processing of the hedonic value of pain and pain relief. Changes observed in
humans were replicated in rats. Thus the basic brain circuitry for processing pain aversiveness
and pleasure of pain relief appear conserved in mammals, indicating that preclinical studies of
affective aspect of pain and pain relief are likely translatable to humans and may provide new
ways for drug discovery.
**Figure Legends**

**Figure 1:** Analysis Model. The figure depicts the model of the thermal stimulus as well as the explanatory variable for the onset and offset of pain.

**Figure 2:** Human and Rat responses to the onset/offset of pain. Activation to the offset of pain was detected in the anterior cingulate in humans and rodents as well as in the nucleus Accumbens (NAc). The onset of pain induced a deactivation response in the brain that was detected in the NAc.

**Table 1:** Nucleus Accumbens and Cingulate Cortex Activations. Table indicates coordinates of maximum activation/deactivation in the Nucleus Accumbens (NAc) and cingulate cortex (ACC). Coordinates in humans refer to the standard MNI Atlas. Coordinates in rats correspond to the Rat Brain Atlas (Paxinos and Watson 2004).

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HUMAN

RAT

Heat Model
OFF Response
ON Response

Heat Model
OFF Response
ON Response
Pain Offset

ACC

Pain Onset +18 mm

Hum

+18 mm

Rat

ACC

+1.0 mm

2.3 -2.3

-7.5

NAc

+2.0 mm

7.5

2.3