NEURAL MECHANISMS SUPPORTING COGNITIVE INFLUENCES ON SUPRASPINAL NOCICEPTIVE PROCESSING: INSIGHTS FROM BRAIN LESIONS

by

Christopher Jenn Starr

A Dissertation Submitted to the Graduate Faculty of WAKE FOREST UNIVERSITY GRADUATE SCHOOL OF ARTS AND SCIENCES in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in

NEUROBIOLOGY AND ANATOMY

May 2009
Winston-Salem, North Carolina

Approved by:
Robert C. Coghill, Ph.D., Advisor
Department of Neurobiology and Anatomy

Examining Committee:
Lumy Sawaki, M.D., Ph.D., Chairman
Department of Neurology

John G. McHaffie, Ph.D.
Department of Neurobiology and Anatomy

Emilio Salinas, Ph.D.
Department of Neurobiology and Anatomy

Robert A. Kraft, Ph.D.
Department of Radiology
ACKNOWLEDGMENTS

In my education and training, I have been guided and helped by many wonderful faculty members. I would like to take this opportunity to acknowledge the members of my committee, Dr. Sawaki, Dr. Kraft, Dr. Salinas, and Dr. McHaffie. They have, in many ways, contributed to my graduate work and professional development. Thank you for your kindness and support. Without a doubt, my academic training has been most memorable due to my advisor Dr. Coghill. His perceptive mentorship is the kind of guidance I can only wish to provide for my future students. Over the years, not only has he taught me how to think and write like a good researcher, but he also has become a good friend to me, for which I’m very grateful. Thank you for looking out for me. I also want to thank all my colleagues in Dr. Coghill’s lab who both contributed to my work and made my graduate school experience something I will always miss and look back to.

I cannot even begin to imagine where I would be today without all the support and love from my friends and family. First and foremost, I would like to thank my parents. My mother, Jenny Starr, who has given me encouragements through many difficult times and imparted on me countless invaluable life lessons. And although I lost my father when I was still a young child, my life has been blessed with love and happiness because of her. My stepfather, Suwit Sridee, who has always been supportive and proud of every little step that I have taken in my journey. My sister, Cassie, who has provided me with companionship and support from the time we were toddlers. My aunt and uncle Nongluck and Vichien Charaslertrungsi, and my grandmother Sukanya Kositsawat who, although thousands of miles away at the opposite end of the globe, have continued to unconditionally show love and support for me and my family. Also, I want to take this moment to reminisce the memory of my father and grandfather, Vichai Charaslertrungsi and Chana Kositsawat. Although they are no longer with us, the short time that I spent with them in my childhood continues to inspire me and gives me strength to aspire for the better and brighter future. Lastly, I want to thank all my family members and friends, whose names are too numerous to be mentioned here, for being there for me and believing in me. Thank you.

*I dedicate this thesis work to my family.*
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<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>CES-D</td>
<td>Center for Epidemiological Studies - Depression Scale</td>
</tr>
<tr>
<td>CPSP</td>
<td>central post-stroke pain</td>
</tr>
<tr>
<td>D1</td>
<td>dopamine receptor D1</td>
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<tr>
<td>D2</td>
<td>dopamine receptor D2</td>
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<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
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<td>DTI</td>
<td>diffusion tensor imaging</td>
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<td>EEG</td>
<td>electroencephalogram</td>
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<td>FEF</td>
<td>frontal eye field</td>
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<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
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<tr>
<td>GPe</td>
<td>globus pallidus externus</td>
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<tr>
<td>GPi</td>
<td>globus pallidus internus</td>
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<tr>
<td>IPL</td>
<td>inferior parietal lobule</td>
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<tr>
<td>MCA</td>
<td>middle cerebral artery</td>
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<td>MD</td>
<td>mediodorsal nucleus</td>
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<tr>
<td>MEG</td>
<td>magnetoencephalogram</td>
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<tr>
<td>MFG</td>
<td>middle frontal gyrus</td>
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<tr>
<td>OP</td>
<td>operculum</td>
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<tr>
<td>PAG</td>
<td>periaqueductal gray</td>
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<tr>
<td>PANAS-X</td>
<td>Positive and Negative Affect Scale – Expanded form</td>
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<tr>
<td>PCA</td>
<td>principal component analysis</td>
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<td>PCC</td>
<td>posterior cingulate cortex</td>
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<tr>
<td>PO</td>
<td>parietal operculum</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
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<tr>
<td>RVM</td>
<td>rostroventromedial medulla</td>
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<tr>
<td>SI</td>
<td>primary somatosensory cortex</td>
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<tr>
<td>SII</td>
<td>secondary somatosensory cortex</td>
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<tr>
<td>SMA</td>
<td>supplementary motor area</td>
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<tr>
<td>SNC</td>
<td>substantia nigra pars compacta</td>
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<tr>
<td>SNR</td>
<td>substantia nigra pars reticulata</td>
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<tr>
<td>SN/VT</td>
<td>substantia nigra/ventral tegmental area</td>
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<tr>
<td>STAI</td>
<td>State and Trait Anxiety Inventory</td>
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<tr>
<td>STN</td>
<td>subthalamic nucleus</td>
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<td>VAS</td>
<td>visual analog scale</td>
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<td>VA</td>
<td>ventral anterior nucleus</td>
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<td>VL</td>
<td>ventral lateral nucleus</td>
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<td>VMPFC</td>
<td>ventromedial prefrontal cortex</td>
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<td>VPI</td>
<td>ventral posterior inferior nucleus</td>
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<td>VPL</td>
<td>ventral posterior lateral nucleus</td>
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<tr>
<td>VPM</td>
<td>ventral posterior medial nucleus</td>
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The subjective experience of pain is truly unique since the percept of a noxious stimulus can vary greatly depending on the context in which the sensory event takes place. Even within the same context, different internal cognitive information related to previous experience, affective state, and attention unique to each individual may importantly influence how a nociceptive stimulus will be experienced. Although attempts have been made to elucidate the mechanisms by which cognitive information influences the pain processing, most studies describe these influences in terms of the facilitation and attenuation of incoming sensory nociceptive information via descending pain control systems. However, multiple lines of evidence suggest that neural mechanisms operating at supraspinal level may allow cognitive information to directly influence cortical processing of nociceptive information. In contrast to studies that show attenuation or facilitation of incoming sensory information from the spinal cord, these supraspinal neural mechanisms may directly influence how nociceptive information is processed at the cortical level. In the present investigations, we show clear data to
provide useful insights into the neural mechanisms that can support cognitive influences on supraspinal nociceptive processing. We found that a large portion of interindividual variations in pain sensitivity can be accounted for by variations in various psychological factors that are processed at the supraspinal level. Furthermore, the neural circuitries associated with the insula and the putamen can, via different routes of action, utilize various cognitive and contextual information unique to each individual from brain areas involved in memory, attention, and affect to influence cortical processing of nociceptive information. Specifically, these mechanisms may involve the direct modulation of various nociceptive processing brain areas as well as the selection of appropriate cortical networks to process the incoming nociceptive information in a context-relevant manner. The proposed neural mechanisms allow the organism to appropriately respond and attend to the stimuli that are more behaviorally relevant while avoiding unnecessary alarm response to noxious stimuli that are of less potential danger. Accordingly, these dynamic interactions may be important in shaping a complete subjective experience of pain and may contribute to the large interindividual differences in pain experience.
INDIVIDUAL DIFFERENCES IN PAIN EXPERIENCE

According to the International Association for the Study of Pain, “pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” Large interindividual variations exist in pain (Coghill et al., 2003; Nielsen et al., 2008). Since a true subjective experience of pain varies significantly from one individual to the next, the pain experience is truly unique to an individual. In a clinical setting, pain ratings and analgesic requirements greatly vary among patients depending on clinical syndromes, types of surgery, and a variety of other factors (Jamison et al., 1993; Price, 1999; Benrud-Larson and Wegener, 2000; Lovatsis et al., 2007). Accordingly, providing effective pain management has proven to be a difficult task.

Many studies have attempted to predict this interindividual variation in pain by measuring a variety of independent variables and using them as predictors. Blood pressure, outcome expectation, age, gender, and experimental pain ratings have all been used as predictors with some success (Scott et al., 1983; Jamison et al., 1993; Macintyre and Jarvis, 1996; Sheffield et al., 2000; Bisgaard et al., 2001; Wise et al., 2002; Granot et al., 2003; Kalkman et al., 2003; Logan et al., 2003; Staud et al., 2003; Staud et al., 2004; Hsu et al., 2005; Pan et al., 2006). Anxiety scores, for example, have been shown to correlate positively with pain ratings and have good predictive value for total analgesics requirement (Scott et al., 1983; Rhudy and Meagher, 2000; Keogh and Mansoor, 2001;
Klain et al., 2001; Maggirias and Locker, 2002; Harden et al., 2003; Kalkman et al., 2003; Logan et al., 2003; Granot and Lavee, 2005; Hsu et al., 2005; Pan et al., 2006; Colloca and Benedetti, 2007). Postoperative pain ratings correlate significantly with experimental pain ratings prior to the surgery (Granot et al., 2003; Hsu et al., 2005; Pan et al., 2006). Blood pressure and age are negatively correlated with pain ratings and analgesic usage (Kalkman et al., 2003; Pan et al., 2006). Clinical studies also show that emotional states and attitudes of patients can also have an effect on pain associated with chronic diseases (Benrud-Larson and Wegener, 2000; Haythornthwaite and Benrud-Larson, 2000; Schanberg et al., 2000). For instance, catastrophizing about pain and clinical depression can negatively impact the pain experienced by patients (Haythornthwaite and Benrud-Larson, 2000; Auerbach et al., 2001; Sullivan et al., 2001; Dickens et al., 2003; Jones et al., 2003; Edwards et al., 2004; Gracely et al., 2004; Bar et al., 2005; Granot and Lavee, 2005; Alschuler et al., 2008; Graff-Guerrero et al., 2008).

Similarly, in an experimental setting, manipulation of affect can alter pain ratings. Manipulations that have a positive effect on mood or emotional state, such as pleasant music, pleasant pictures, and humorous films, generally reduce pain perception (Cogan et al., 1987; Zelman et al., 1991; Good, 1996; Weisenberg, 1998; Weisenberg et al., 1998; de Wied and Verbaten, 2001; Meagher et al., 2001; Villemure and Bushnell, 2002). Conversely, experimental manipulations that have a negative effect on mood and emotions have been shown to increase pain (Zelman et al., 1991; Weisenberg et al., 1998; de Wied and Verbaten, 2001; Meagher et al., 2001; Villemure and Bushnell, 2002).

More interestingly, interindividual differences in pain sensitivity seen in psychophysics can also be detected with functional brain imaging in nociceptive
processing brain areas (Coghill et al., 2003). In a study by Coghill et al., highly sensitive normal individuals exhibited greater brain activation in pain-related brain areas including anterior cingulate cortex (ACC), insula, and secondary somatosensory cortex (SII) than less sensitive normal individuals (Coghill et al., 2003). Thus, processing of nociceptive information at the supraspinal level may underlie these interindividual differences in pain experience. Even within an individual, pain ratings can vary significantly from one time to the next (Rosier et al., 2002). Therefore, pain experience is shaped by contexts, previous experience, and a variety of cognitive factors unique to each individual and setting. Moreover, when patients in vegetative states receive noxious stimulation, brain activation is only detected in the thalamus and SI, but not other brain areas (Faymonville et al., 2003). However, a noxious stimulus given to normal subjects activates a wide range of brain areas including SII, SI, insula, ACC, and basal ganglia (Coghill et al., 1994; Peyron et al., 2000; Coghill et al., 2001). Thus, the pain experience is more complex than a detection of the simple relay of sensory information to the primary somatosensory area. A complete subjective pain experience engages many brain areas involved in different aspects of nociceptive and cognitive processing. Therefore, the interaction of these brain areas and the integration of cognitive information unique to an individual during pain likely shapes how one experiences pain and may underlie the individual differences in pain experience.
SUPRASPINAL PROCESSING OF NOCICEPTIVE INFORMATION

Pain is a unique sensory experience that is subserved by a variety of brain areas and processed in a highly distributed and parallel fashion (Coghill et al., 1994; Peyron et al., 2000; Coghill et al., 2001). Different brain areas are believed to be responsible for different aspects of nociceptive processing (Price, 1999). These include sensory-discriminative, emotional-affective, and motivational-cognitive aspects of nociceptive processing.

**Sensory-discriminative dimension**

Sensory-discriminative aspect of nociceptive processing refers to the sensory aspects of pain such as its location or its intensity (Price, 1999). Areas such as the primary somatosensory cortex (SI) and secondary somatosensory cortex (SII) have traditionally been suggested to be involved in the sensory-discriminative aspects of nociceptive processing (Price, 1999). SI, in particular, has been suggested to be involved in the localization of a noxious stimulus due to its topographic organization and the small receptive fields of its neurons (Huffman and Krubitzer, 2001). In addition, nociceptive neurons in SI can also alter firing discharge based on the intensity of the noxious stimulus, suggesting that they may have the ability to encode stimulus intensity (Kenshalo and Isensee, 1983). Nevertheless, under 50% of imaging studies have reported detectable SI activation during noxious stimulation (Peyron et al., 2000). Reasons such as attention to the stimulus, stimulus size, and stimulus intensity have all been attributed to this, however the results remain inconclusive (Peyron et al., 2000). On the other hand, SII is consistently bilaterally activated in pain studies. This is congruent with the known
anatomical studies of the bilaterality of SII receptive fields (Robinson and Burton, 1980b; Dong et al., 1989). In addition, a number of studies have noted that the strength of responses in this region increases as the intensity of the noxious stimulus increases, suggesting this area likely plays an important role in coding stimulus intensity (Dong et al., 1989; Coghill et al., 1999; Peyron et al., 1999; Peyron et al., 2000). In addition, both somatosensory areas receive direct inputs from nociceptive neurons in the VPL, VPM, and VPI nuclei of the thalamus (Friedman and Murray, 1986; Darian-Smith et al., 1993; Stevens et al., 1993).

Moreover, recent neuroimaging studies investigating brain mechanisms supporting two-point spatial and intensity discrimination of pain have shown that large networks of brain areas may be actively involved in such processes (Oshiro et al., 2007; Oshiro et al., 2008). Specifically, prefrontal cortex, ACC, posterior parietal cortex, caudate, and thalamus were engaged during a spatial discrimination of pain task (Oshiro et al., 2007), while the prefrontal cortex, insula, and ACC were engaged during intensity discrimination (Oshiro et al., 2008). These results suggest that each aspect of nociceptive processing may actually occur in a fairly distributed fashion with multiple brain areas involved. Interestingly, brain areas that are not traditionally thought of as related to sensory-discriminative processing such as the ACC and prefrontal cortex may also contribute importantly to these processes. In addition, the overlap seen in brain areas that are involved in both discrimination processes suggest that each brain region may be involved in multiple aspects of pain processing. Taken together, these findings indicate that assigning one specific function to a given brain area may not be appropriate.
Emotional-affective dimension

Emotional-affective aspect of pain refers to the negative connotations and unpleasant qualities associated with the noxious stimuli (Price, 1999). Areas closely linked to the limbic system such as the anterior cingulate cortex (ACC) and the insula have been associated with this aspect of nociceptive processing. Both ACC and the insula have large bilateral receptive fields (Robinson and Burton, 1980b, a; Sikes and Vogt, 1992; Schneider et al., 1993; Hutchison et al., 1999; Zhang et al., 1999). Both brain areas are anatomically connected to the amygdala and hippocampus and receive direct nociceptive inputs from medial and intralaminar nuclei of the thalamus including parafascicular and centromedian and mediodorsal nuclei (Mufson et al., 1981; Mesulam and Mufson, 1982a; Mufson and Mesulam, 1984; Vogt and Pandya, 1987; Vogt et al., 1987; Saunders et al., 1988).

Activation of the ACC is well correlated with pain unpleasantness ratings of noxious stimuli (Rainville et al., 1997; Rainville et al., 1999; Price, 2000; Rainville, 2002). In addition, skillful hypnotic manipulations of pain affect can alter ACC activation, resulting in corresponding increase or decrease in pain unpleasantness ratings without affecting pain intensity ratings (Rainville et al., 1997; Rainville et al., 1999). Moreover, cingulotomy for intractable pain in chronic pain patients can significantly reduce the unpleasant qualities of pain while minimally affecting its sensory aspects (e.g. detection and location) (Davis et al., 1994; Richter et al., 2004; Greenspan et al., 2008).

The insula may also be important in the affective processing of pain since patients with insular lesions are reported to have pain asymbolia, a condition which is best described as inappropriate emotional and affective response to a noxious stimulus despite
patients’ intact ability to detect the noxious stimulus (Berthier et al., 1988). Presumably, connections between nociceptive processing areas, including SII and SI, and limbic areas such as amygdala were disrupted due to insular lesions. Thus, incoming nociceptive sensory information may not be coupled with the appropriate emotion and affect generated by the limbic system. Similarly, the insula has been viewed as the interoceptive cortex responsible for generating subjective feelings of self, based on incoming lamina I homeostatic afferents conveying pain and temperature information (Craig, 2002). Thus, various subjective feelings including pain unpleasantness are thought to derive from incoming inputs from lamina I. Nevertheless, lesions of the posterior insula and parietal operculum have been shown to raise pain thresholds (Greenspan and Winfield, 1992; Schmahmann and Leifer, 1992; Greenspan et al., 1999). In addition, activation of the insula also positively correlates with that of pain intensity ratings suggesting that it may code for pain intensity (Derbyshire et al., 1997; Coghill et al., 1999). These findings suggest that the insula likely plays an important role in both sensory-discriminative and emotional-affective aspects of pain.

**Motivational-cognitive dimension**

Motivational-cognitive aspect of pain reflects higher-level cognitive processes including understanding of the potential significance, future consequences, and the meaning of pain (Price, 1999). Consistent with its role in goal-directed behavior and executive function, the prefrontal cortex has been suggested to be involved in this aspect of nociceptive processing. Patients undergoing prefrontal lobotomy can recognize a nociceptive stimulus as noxious but are emotionally indifferent and show few affective
reactions (Freeman and Watts, 1948; King et al., 1950). In addition, they often have problems appreciating the significance and meaning of the stimulus and show little concern about its negative implications in terms of damage to the body or threat to life. This suggests that prefrontal lobotomy disrupted the ongoing cognitive evaluations related to the long-term implications of having a persistent pain condition (Freeman and Watts, 1948; King et al., 1950; Price, 1999).

The prefrontal cortex activation detected during the period of expectation prior to placebo administration suggests that this area may be partly responsible for generating the context for pain relief that may underlie placebo analgesia (Wager et al., 2004). More recently, multiple lines of evidence suggest that this area may act as the cognitive pain control center (Wiech et al., 2008). In an experiment where subjective cognitive control over pain is manipulated, subjects were given a device that when pressed would stop the noxious stimulus (Salomons et al., 2004; Wiech et al., 2006; Salomons et al., 2007). Even though the device was only illusory, the perceived control over one’s pain significantly lowered pain ratings to the same noxious stimulus delivered without the device. Furthermore, this perceived control is accompanied by greater activation of the prefrontal cortex, suggesting that cognitive influences from this brain area contribute importantly to pain processing (Salomons et al., 2004; Wiech et al., 2006; Salomons et al., 2007). In addition, cognitive pain control strategies such as self-distraction (thought suppression) and reappraisal (reinterpretation of the current stimulus in a more positive manner) showed that activation within the prefrontal cortex were effective in reducing pain anticipatory anxiety (Kalisch et al., 2006; Wiech et al., 2006). Furthermore, in an experimental model of chronic pain, the prefrontal cortex is differentially activated
during allodynia when compared to stimulation of normal skin (Iadarola et al., 1998; Baron et al., 1999; Lorenz et al., 2002; Apkarian et al., 2004; Seifert and Maihofner, 2007), suggesting that cognitive components play an important part in pathological pain states. Activation of the prefrontal cortex during heat allodynia has also been negatively correlated with pain ratings as well as ACC activation and midbrain-medial thalamic functional connectivity, indicating that the prefrontal cortex may play an important role in modulating these structures to ‘keep pain out of the mind’ (Lorenz et al., 2003). The importance of the prefrontal cortex in cognitive pain control mechanisms is also substantiated by the finding of decreased prefrontal areas’ gray matter density in chronic pain patients (Apkarian et al., 2004). These results suggest that, in addition to its role in working memory and attention-related processes (Posner et al., 1980; Corbetta et al., 1993; Knudsen, 2007; Corbetta et al., 2008), the prefrontal cortex also plays an important role in motivational-cognitive aspect of pain.

**Other aspects of nociceptive processing**

In addition to the previously mentioned brain areas’ contribution to nociceptive processing, other brain areas including the putamen, cerebellum, supplementary motor area (SMA), and inferior parietal lobule (IPL) have also been consistently activated in pain studies (Coghill et al., 1994; Peyron et al., 2000; Coghill et al., 2001). Their roles in pain processing have been extrapolated based on their known functions in other processes unrelated to pain (Peyron et al., 2000). For example, putamen, cerebellum, and SMA are suggested to be important in motor-related processes during pain including movement inhibition and escape planning during pain (Peyron et al., 2000). Conversely, IPL has
been suggested to be important in processes related to spatial attention during pain (Peyron et al., 2000). However, the exact roles of these structures in nociceptive processing remain poorly understood.

**COGNITIVE INFLUENCES ON NOCICEPTIVE PROCESSING**

Multiple lines of evidence suggest that the pain experience can be greatly shaped by higher-level cognitive information (Price et al., 1999; Wiech et al., 2008). Most studies investigating the cognitive influences on pain perception have looked at how manipulation of attention, expectation, and placebo can influence the pain experience since these higher-level processes rely extensively on cognitive and contextual information. Studies examining the effects of cognitive influences on pain perception have shown that the effects of attention, expectation, and placebo can significantly alter both pain perception as seen in psychophysical pain ratings as well as patterns and strength of detectible brain activation in pain-related brain areas (Ploghaus et al., 1999; Petrovic et al., 2000; Sawamoto et al., 2000; Bantick et al., 2002; Petrovic et al., 2002; Valet et al., 2004; Wager et al., 2004; Koyama et al., 2005; Bingel et al., 2006; Dunckley et al., 2007; Quevedo and Coghill, 2007; Wiech et al., 2008)

**Attention**

The intrusive nature of the pain experience makes a nociceptive stimulus effective in capturing and maintaining attention. A variety of brain areas including the frontal, parietal, and cingulate areas have been suggested to be involved in attention-
related processes in many sensory modalities, especially visuospatial attention (Corbetta et al., 1993; Corbetta et al., 2008). Many brain areas such as the ACC, prefrontal cortex, and posterior parietal areas are commonly activated in both pain and attention-related processes, suggesting that they may be important in processes related to attention during pain (Nobre et al., 1997; Coghill et al., 1999; Peyron et al., 1999; Petrovic et al., 2000; Nobre, 2001; Bantick et al., 2002; Coghill et al., 2003).

Multiple lines of evidence have shown that attention can powerfully modulate how nociceptive information is processed and how pain is experienced (Bushnell et al., 1985; Petrovic et al., 2000; Frankenstein et al., 2001; Bantick et al., 2002; Tracey et al., 2002; Villemure and Bushnell, 2002; Lorenz et al., 2003; Valet et al., 2004; Dunckley et al., 2007; Quevedo and Coghill, 2007; Wiech et al., 2008). Many of these studies look at the effect of distraction on pain and pain-related brain activation. These studies were able to show that distracting subjects from pain by using cognitively demanding tasks such as Stroop tasks can significantly reduce pain ratings and pain-related brain activation in areas like SI, SII, thalamus, and the insula (Petrovic et al., 2000; Frankenstein et al., 2001; Bantick et al., 2002; Tracey et al., 2002; Valet et al., 2004; Dunckley et al., 2007). In these distraction studies, increased brain activation was noted in the ACC, prefrontal cortex, and the periaqueductal gray (PAG) (Petrovic et al., 2000; Bantick et al., 2002; Valet et al., 2004; Dunckley et al., 2007; Wiech et al., 2008).

Furthermore, distraction has been shown to increase the functional connectivity of ACC and prefrontal cortex with subcortical structures involved in pain modulation such as the PAG (Valet et al., 2004). Moreover, direct anatomical connection between the two structures also exist (Hadjipavlou et al., 2006). Given that these brain areas are known to
be involved in the descending control system via opioid-mediated analgesia that acts at the level of the dorsal horn (Mayer and Price, 1976; Basbaum and Fields, 1978; Fields and Basbaum, 1978, 1999; Gebhart, 2004), it has been suggested that distraction may modulate pain via opioid-sensitive descending modulatory pathway initiated by the ACC (Tracey et al., 2002; Valet et al., 2004; Dunckley et al., 2007).

In another study by Quevedo and Coghill (2007), pain perception was assessed during divided and directed attention tasks with two noxious stimuli presented simultaneously. Subjects were instructed to either divide their attention between the two stimuli or to direct their attention towards one of the stimuli to provide pain ratings. Interestingly, when attention was divided, the same noxious stimulus was rated as significantly less painful when compared to stimulus rated during directed attention. The authors concluded that top-down attention may modulate the spatial integration of nociceptive information at the level of dorsal horn via altering receptive fields and sensitivity of nociceptive neurons (Quevedo and Coghill, 2007).

In addition to top-down influences converging at the level of the spinal cord, attentional modulation of nociceptive processing may also involve higher-order cortico-cortical interactions such as those seen in other modalities. In the visual system, for example, covert (internally directed) attention towards a particular point in space can significantly enhance the sensitivity of visual neurons in the corresponding visual field even without eye saccade. Areas including the FEF, DLPFC, ACC, and IPL have been suggested to be an important source of top-down signals to initiate increased sensitivity in these brain areas (Posner et al., 1980; Mesulam, 1981; Corbetta et al., 1991; Corbetta
et al., 1993; Nobre et al., 1997; Mesulam, 1999; Nobre, 2001; Knudsen, 2007; Corbetta et al., 2008).

It is conceivable that similar attentional control systems may be engaged during pain. A network of fronto-parietal and cingulate brain areas including the ACC, DLPFC, and IPL may similarly mediate top-down attentional influences on nociceptive processing brain areas to influence how nociceptive information is processed. In fact, Lorenz et al. (2003) have shown that activity within the dorsolateral prefrontal cortex (DLPFC) may negatively modulate activity of the insula and ACC, ultimately resulting in decreased pain ratings. Furthermore, direct attention during painful stimulus yields an increase in functional coupling between various brain regions involved in pain processing as measured by MEG and EEG, suggesting that cortico-cortical interactions are enhanced during attention on pain (Ohara et al., 2006; Hauck et al., 2007; Ohara et al., 2008). Although this evidence suggest that attentional modulation of nociceptive processing can occur via cortico-cortical interactions, little is known about how these interactions occur and what brain areas may support these interactions.

**Expectation and anticipatory anxiety**

**Certain expectation**

Expectation allows the organism to prepare for impending stimuli by providing a mental representation of the stimuli based on prior knowledge stored in memory (Koyama et al., 2005). The two types of expectations (certain and uncertain) for aversive stimuli studies differ based on the degree of certainty associated with the nature of the impending stimulus. As opposed to uncertain expectation studies where the nature of the
impending stimulus is ambiguous (may or may not be noxious), the nature of the impending stimulus in certain expectation studies is presumed to be surely noxious. This type of experiment is usually carried out by presenting a cue signaling the impending noxious stimulus to the subjects followed by an expectation time period, then followed by the noxious stimulus itself. By examining patterns of brain activation during the expectation time period between the cue and the noxious stimuli, brain areas active during certain expectation may be determined.

Many studies have found brain regions including the ACC, insula, and the prefrontal cortex to be activated during the expectation time period (Chua et al., 1999; Ploghaus et al., 1999; Sawamoto et al., 2000; Koyama et al., 2005). In addition, in a study where subjects expect increased pain, an identical stimulus with a cue for higher pain generated elevated pain ratings and enhanced pain-related brain activation (Sawamoto et al., 2000). Conversely, when subjects receiving an intensely painful stimulus were falsely given an auditory cue signaling a moderately painful stimulus, they rated the intensely painful stimulus as significantly less intense when compared to the correctly signaled intensely painful stimulus (Koyama et al., 2005). In both studies, changes in pain ratings were also accompanied by corresponding alterations of pain-related brain activation in ACC, insula, SII, and other nociceptive processing brain areas (Sawamoto et al., 2000; Koyama et al., 2005).

Regions of expectation-related activation within the ACC, insula, and prefrontal cortex also greatly overlap with regions activated during painful stimulation itself, suggesting that these highly interconnected brain areas may be capable of processing both internal and external mental representations of the noxious stimuli (Mesulam and
Mufson, 1982a; Mufson and Mesulam, 1982; Friedman et al., 1986; Vogt and Pandya, 1987; Ploghaus et al., 1999; Koyama et al., 2005). Since expectations are formed from previous experiences stored in memory and current contexts associated with the impending stimulus, brain areas associated with memory retrieval such as the amygdala and hippocampus may play an important role (Porrino et al., 1981; Murray and Mishkin, 1983; Saunders et al., 1988; Henson et al., 1999; Anderson and Phelps, 2001; Smith et al., 2004). During the expectation period, cognitive information related to previous experience stored in memory may be retrieved from the amygdala and hippocampus and flow to ACC, insula, and prefrontal cortex where a mental representation of the impending stimulus is formed (Mufson et al., 1981; Friedman et al., 1986; Vogt and Pandya, 1987; Koyama et al., 2005). This expectation-related information may be transferred to connected nociceptive processing brain areas to modulate nociceptive processing of afferent sensory information (Koyama et al., 2005). These processes prime the brain by minimizing computational complexity while increasing speed and accuracy of afferent processing (Posner et al., 1980; Bushnell et al., 1985; Koyama et al., 2005). Nevertheless, if the expectations are to be able to prepare the organism for the impending stimulus, then the brain must learn about the validity of pain expectation by determining if expectations are either confirmed or violated by the incoming nociceptive input (Ploghaus et al., 2000; Wiech et al., 2008). In a study by Ploghaus et al. (2000), the hippocampus was differentially activated when subjects unexpectedly received painful stimulus but actually expected a nonpainful stimulus. Thus, the hippocampus may play a crucial role in detecting a mismatch between expectations and incoming sensory information, and may facilitate learning and updating of expectation of aversive stimuli.
These results suggest that cognitive information including expectation formed from prior knowledge of the stimulus may importantly shape the pain experience by providing top-down signals to bias nociceptive processing.

**Uncertain expectation and anticipatory anxiety**

Contrary to certain expectations, uncertain expectations (uncertain about the nature of the impending stimulus) can induce a significant amount of anticipatory anxiety and may be associated with different patterns of brain activation. Anticipatory anxiety associated with uncertainty of the impending stimulus has been shown to increase pain sensitivity across studies (Cornwall and Donderi, 1988; Rhudy and Meagher, 2000; Ploghaus et al., 2001). Rhudy and Meagher (2000), for example, have shown that induced anticipatory anxiety can generate a significant decrease in pain thresholds. Cornwall and Donderi (1988) similarly showed that experimentally induced anxiety can raise pain ratings, when compared with similar noxious stimuli that were not associated with anticipatory anxiety.

Imaging studies investigating uncertain expectation and anticipatory anxiety often examine brain activation during the expectation time period between the presentation of an uncertain cue and the presentation of the actual stimulus (Reiman et al., 1989; Drevets et al., 1995; Hsieh et al., 1999; Simpson et al., 2001). In this case, the subjects were informed that the cue may either be follow by an innocuous (nonpainful) stimulus or a noxious stimulus. Uncertain expectation-related brain activation was identified within SI, ventromedial prefrontal cortex (VMPFC), ACC, and insula (Hsieh et al., 1999; Porro et al., 2002). In addition, SI deactivation in the surrounding regions
corresponding to skin sites different from stimulation site were also noted (Drevets et al., 1995). The contrast of regions of SI activation surrounded by regions of deactivation likely represents top-down attentional influences to enhance the sensitivity of the respective area while inhibiting the surroundings (Porro et al., 2002).

The VMPFC activation is slightly more difficult to interpret. This area is tonically active and may be involved in monitoring the environment and surroundings as a part of a default mode network (Greicius et al., 2008). However, it is deactivated during painful stimulation and directed attention (Simpson et al., 2001). It is well connected with the amygdala and the hippocampus and may receive cognitive information related to the context of the impending stimulus (Ploghaus et al., 2000; Ploghaus et al., 2003). Furthermore, this area may also be important in autonomic arousal related to anticipatory anxiety since patients with VMPFC lesions failed to show autonomic arousal during high uncertainty or risk tasks when compared to healthy volunteers (Bechara et al., 1996). In fact, Simpson et. al (2001) have shown that subjects exhibiting greater anticipatory anxiety actually displayed lesser deactivation of the VMPFC, indicating that the default network may be actively monitoring and preparing for the uncertainty. Accordingly, VMPFC activation seen in uncertain expectation may represent a vigilant, alert state that prepares subjects for the uncertain nature of the impending event (Ploghaus et al., 2003).

In addition to SI and VMPFC, areas such as the ACC and insula seen activated during certain expectation are also activated during uncertain expectation (Sawamoto et al., 2000; Ploghaus et al., 2001; Ploghaus et al., 2003). Thus, these brain areas may similarly provide a mental representation of the impending stimulus and facilitate modulation of nociceptive processing by expectation-related information. Subjects
perceived noxious stimuli that is associated with anticipatory anxiety as more painful and exhibited greater activation of the ACC and the insula than similar noxious stimuli that are not associated with such contexts (Sawamoto et al., 2000; Ploghaus et al., 2001). Such increases in pain ratings and pain-related brain activation may arise from an amplification signal generated by the hippocampus (Ploghaus et al., 2001). In fact, Ploghaus et al. showed that hippocampus responded differentially to identical noxious stimuli, depending on whether the perceived pain intensity was enhanced by anticipatory anxiety (Ploghaus et al., 2001). In addition, activity within the hippocampus also reliably predicted the increased activation of the insula and ACC during subsequent painful stimulation. Thus, Ploghaus et al. concluded that the hippocampus may resolve aversive behavioral conflict (uncertain expectation) by sending amplification signals to the neural representation of the aversive event to bias the organism toward a behavior that is adaptive to the worst possible outcome.

**Placebo**

Placebo analgesia is a phenomenon best described as a potent reduction in pain following administration of an inert substance associated with contexts for pain relief (i.e. suggestion that the medication brings potent pain relief in a number of patients). In some instances, the effects of placebo analgesia rival that of weak opioid analgesics (Price et al., 2008).

In pain studies, areas including the ACC, insula, prefrontal cortex, and PAG are consistently activated during placebo analgesia (Petrovic et al., 2002; Zubieta et al., 2005;
Placebo analgesia has been shown to produce significant decreases in both psychophysical pain ratings as well as pain-related brain activation in areas such as the ACC, insula, and thalamus (Petrovic et al., 2002; Wager et al., 2004; Bingel et al., 2006). Thus, placebo effects likely result from modulation of nociceptive processing by contextual information, and not simply response bias (Price et al., 2008). Furthermore, placebo analgesic effects can be blocked by naloxone, a mu-opioid antagonist, suggesting that placebo analgesia may partly be mediated via opioidergic pathways and the descending modulatory system (Amanzio and Benedetti, 1999; Benedetti et al., 1999).

Areas including the PAG and rostroventral medulla (RVM) are known to play an important role in the descending modulatory control of pain (Mayer and Price, 1976; Basbaum and Fields, 1978; Fields and Basbaum, 1999). Brain areas such as the ACC and the amygdala are known to project strongly to PAG, which subsequently projects to the RVM (Fields and Basbaum, 1999; Hadjipavlou et al., 2006). These areas have opioidergic and non-opioidergic descending modulatory projections that act at the dorsal horn at the level of the spinal cord where they can either inhibit or facilitate incoming nociceptive information (Mayer and Price, 1976; Basbaum and Fields, 1978; Fields and Basbaum, 1999).

Both opioid and placebo analgesia activate the same region within the ACC. In addition, placebo-induced ACC activation has been shown to correlate significantly with PAG activation, suggesting that ACC may directly modulate the PAG during placebo analgesia (Petrovic et al., 2002). Moreover, functional connectivity between the ACC and PAG increased significantly during placebo analgesia, but not during pain itself.
(Bingel et al., 2006). Therefore, ACC has been suggested to be the cortical source that recruits the opioid-dependent descending pain modulatory system, linking the placebo effect with endogenous pain control.

Although placebo analgesia has often been described in terms of activation of the descending controls system, cortico-cortical interactions at the supraspinal level are likely equally important. Craggs et. al (2007) showed that interactions of brain areas including ACC, DLPFC, and insula activity during placebo analgesia are likely important for the initiation and the maintenance of placebo analgesia. Insula activity, for instance, was shown to be negatively correlated with that of ACC during placebo analgesia. In addition, Wager et. al (2004) also suggested that if placebo effects can only be described in terms of attenuation of nociceptive sensory transmission due to descending controls that act at the spinal level, there should be equal reductions of pain activation in all brain areas involved in nociceptive processing. However, pain activations were only reduced in a few brain regions. Furthermore, different brain areas displayed different activation time courses throughout the duration of the placebo analgesia (Wager et al., 2004). Areas including the prefrontal cortex, ACC, and PAG showed early response and likely represent the opioidergic, descending modulatory pathway while areas including contralateral thalamus and insula showed decreases only after more prolonged pain. Furthermore, prefrontal activation has also been observed prior to the noxious stimulation during placebo, indicating that expectation of pain relief may engage brain mechanisms that underlie the placebo effect (Wager et al., 2004). These results suggest that although the placebo effect may partly be mediated by the attenuation of transfer of nociceptive sensory information from the spinal cord by activation of the descending control, a major
portion of the placebo effect may be mediated centrally by changes in specific pain regions at the cortical level.

Taken together, these findings indicate that the placebo effect involves higher-order processes related to cognitive pain modulation. Moreover, brain areas important for other cognitive modulation of pain such as those activated during expectation and higher-level cognitive pain control including the insula and prefrontal cortex (Wager et al., 2004; Koyama et al., 2005; Wiech et al., 2005; Kalisch et al., 2006; Wiech et al., 2006; Wiech et al., 2008) are also likely to play an important role in placebo analgesia. These brain areas may generate an internal mental image of the placebo pain relief and mediate their influence via cortico-cortical interactions with other nociceptive processing brain areas that may or may not be involved in descending control to contribute to placebo analgesia.

SUPRASPINAL MECHANISMS SUPPORTING COGNITIVE INFLUENCES ON NOCICEPTIVE PROCESSING

Brain areas that can play an important part in integrating cognitive information with nociceptive information during pain must be connected to a variety of cognitive and nociceptive processing brain areas. This allows information about expectation, previous experience, memory, and attention to be integrated with incoming nociceptive information. Although the insula and the putamen differed substantially in their known functions, both are well connected with various brain areas involved in both cognitive and nociceptive processing. Accordingly, the neural circuitries involving the insula and
putamen may provide the neural substrates to support brain mechanisms that can facilitate cognitive influences on supraspinal nociceptive processing.

**Potential roles of the insula**

The insula is a multimodal area that is anatomically connected to various brain areas involved in olfactory, gustatory, somatosensory, limbic, motor, autonomic, and other association functions (Mufson et al., 1981; Mesulam and Mufson, 1982a; Mufson and Mesulam, 1982; Friedman and Murray, 1986; Friedman et al., 1986; Schneider et al., 1993). More specifically, the insula is divided into anterior and posterior subdivisions, which differed substantially in their cytoarchitecture and anatomical connections (Mufson et al., 1981; Mesulam and Mufson, 1982a; Mufson and Mesulam, 1982; Friedman and Murray, 1986; Friedman et al., 1986). While the anterior insula is made up of agranular and dysgranular sectors, the posterior insula is made up mostly of granular sector (Mesulam and Mufson, 1982b). The anterior insula is reciprocally connected with the olfactory cortex, gustatory cortex, and limbic areas such as parahippocampal gyrus, amygdala, and ACC while the posterior insula is connected to SI, SII, auditory areas, and motor areas (Mufson et al., 1981; Mesulam and Mufson, 1982a; Mufson and Mesulam, 1982; Friedman and Murray, 1986; Friedman et al., 1986). Accordingly, the insula has been suggested to be important in many sensory systems such as taste, smell, and other homeostatic maintenance functions (Oppenheimer et al., 1996; Faurion et al., 1999; Small et al., 1999; Colivicchi et al., 2004). This extensive connectivity makes the insula well positioned to serve as a path for somatosensory information to reach the limbic system (Friedman et al., 1986). Accordingly, the insula has been suggested to be
important for assigning appropriate subjective feelings to various somatosensations including temperature, pain, hunger, and thirst (Craig, 2002). However, the exact role that this structure plays in pain remains poorly understood.

To date, few studies have investigated the integrity of pain perception following lesions of the insula. In one study done in a group of patients undergoing surgery for epilepsy, the neurosurgeon performed intracerebral stimulation using transopercular electrode to evoke sensory response in these awake patients (Mazzola et al., 2006). Stimulation of the insular cortex was found to evoke various sensory experiences including pain and others such as gustatory, olfactory, and auditory (Mazzola et al., 2006). In another study, patients with lesions affecting the posterior insula and parietal operculum, but not anterior insula, had elevated heat pain thresholds (Greenspan and Winfield, 1992; Greenspan et al., 1999). Moreover, limited psychophysical studies of patients with insular lesions exhibited pain asymbolia, a condition where patients can still recognize noxious stimuli as painful but exhibit inappropriate affective responses and have difficulty in appraising the meaning and significance of such stimuli (Berthier et al., 1988). Presumably, the lesions disrupt the links between nociceptive sensory information and limbic areas such as the amygdala and ACC. Thus, the insula may also play an important role in the construction of pain affect. More recently, the insula has been viewed as the interoceptive cortex responsible for generating subjective feelings of self based on incoming homeostatic afferents conveying information such as thirst, sensual touch, pain, temperature, and hunger (Craig, 2002).

The insula is bilaterally activated during a unilateral noxious stimulation (Coghill et al., 1994; Coghill et al., 1999; Peyron et al., 2000). In fact, it is one of the most
consistently activated brain area during pain and has been suggested to play an important role in nociceptive processing (Coghill et al., 1994; Coghill et al., 1999; Peyron et al., 2000). Furthermore, the insula may play an important role in intensity coding as insular activation has been correlated with noxious stimuli of increasing intensities (Derbyshire et al., 1997; Coghill et al., 1999). Moreover, it is rich in opioid receptors and has connections to brain regions involved in the descending pain control system such as the ACC and PAG (Mayer and Price, 1976; Basbaum and Fields, 1978; Fields and Basbaum, 1999; Baumgartner et al., 2006). Additionally, it has large bilateral receptive fields and receives direct nociceptive input from mediodorsal, ventral posterior inferior, and centromedian nuclei of the thalamus (Mufson and Mesulam, 1984; Friedman and Murray, 1986).

The extensive connectivity of the insula allows nociceptive information to be sequentially transferred from SI and SII, and posterior parietal cortex to the posterior insula, then to the anterior insula, then onto the ACC, prefrontal cortex, amygdala, and hippocampus (Mufson et al., 1981; Mesulam and Mufson, 1982a; Mufson and Mesulam, 1982; Friedman and Murray, 1986; Friedman et al., 1986). Reciprocal connections may also allow information related to affect, working memory, attention, and previous experience to be transferred in the opposite direction to be integrated with nociceptive information within the insula. Activation of the insula during placebo, opioid analgesia, expectation, and hypnosis suggests that it may be important in both anti-nociceptive as well as pro-nociceptive processes (Petrovic et al., 2002; Lorenz et al., 2003; Derbyshire et al., 2004; Koyama et al., 2005; Zubieta et al., 2005; Kong et al., 2006; Craggs et al., 2007; Kong et al., 2007) and underscores the importance of this brain area in using
cognitive and contextual information to influence nociceptive processing. In addition, activity in the anterior insula can also modulate activation of the prefrontal cortex and anterior cingulate cortex in a task or situation-dependent fashion (Craggs et al., 2007; Sridharan et al., 2008). These findings suggest that the insula may be well positioned to utilize incoming cognitive information to modulate connected brain areas involved in processing of sensory-discriminative, affective, and cognitive-evaluative components of pain (Mufson et al., 1981; Mesulam and Mufson, 1982a; Mufson and Mesulam, 1982; Friedman and Murray, 1986; Friedman et al., 1986). Conversely, the insula may play an important role in pain by providing context-relevant modulation of nociceptive processing brain areas to shape the pain experience.

**Potential roles of the putamen**

Putamen activation during pain has often been suggested as a motor-related response to pain (Coghill et al., 1999; Bingel et al., 2004) since the basal ganglia has been traditionally viewed as an area primarily responsible for movement-related tasks (Albin et al., 1989; Alexander et al., 1990; Chudler and Dong, 1995). The putamen is often activated during noxious stimulation and has nociceptive neurons that respond to noxious stimuli of graded intensities (Chudler, 1998). Furthermore, it has also been shown to contain high density of opioid receptors, suggesting that this region may play an important role in nociception (Chudler and Dong, 1995; Sprenger et al., 2005; Baumgartner et al., 2006). Additionally, variations in activity of striatal dopamine D2 receptor have been shown to significantly correlate with pain ratings and modulation of pain (Hagelberg et al., 2002; Hagelberg et al., 2004; Pertovaara et al., 2004; Scott et al.,
Moreover, patients suffering from chronic pain syndromes including fibromyalgia, atypical facial pain, and burning mouth syndrome have been shown to have reduced D2 dopaminergic activity (Hagelberg et al., 2003b; Hagelberg et al., 2003a; Wood et al., 2007). However, to date, the exact role of the putamen in pain processing remains poorly understood.

The putamen, together with the caudate nucleus, makes up the striatum and serves as one of the major input nuclei to the basal ganglia (Alexander et al., 1990; Parent and Hazrati, 1995; Middleton and Strick, 2000). The striatum receives direct glutamatergic afferent inputs from the cerebral cortex and limbic structures, as well as intralaminar and midline nuclei of the thalamus (Alexander et al., 1990; Parent and Hazrati, 1995; Gerfen and Wilson, 1996; Groenewegen et al., 1999; Mengual et al., 1999; Middleton and Strick, 2000; Van der Werf et al., 2002) (Figure 1). In addition, the putamen also receives modulatory dopaminergic inputs from the substantia nigra pars compacta (SNc) in the midbrain (Gerfen and Wilson, 1996). The striatum then relays signals, via direct and indirect routes and, to its output nuclei, namely, the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) (Gerfen and Wilson, 1996) (Figure 1). These output nuclei project to the thalamus and, via the thalamus, to target cortical and limbic regions from which the basal ganglia input originated completing the loop (Alexander et al., 1990; Parent and Hazrati, 1995; Middleton and Strick, 2000; McHaffie et al., 2005).

In the direct pathway, the excitatory glutamatergic inputs from the cortex activate the striatum (Figure 1). This leads to an increase in activity of GABAAergic (inhibitory) neurons that send projections from the striatum to the GPi and SNr (Albin et al., 1989;
Gerfen and Wilson, 1996). This suppresses the activities of tonically-active inhibitory GABAergic neurons that send projections to the thalamus. Since these thalamic nuclei send excitatory projection back to respective cortical areas where the information originated to complete the loop (Parent and Hazrati, 1995), the activation of the direct pathway leads to increased cortical activity by disinhibition of specific thalamic nuclei (Chevalier and Deniau, 1990).

In the indirect pathway, excitatory input to the striatum leads to increased inhibition of the external segment of the globus pallidus (GPe) (Albin et al., 1989; Gerfen and Wilson, 1996)(Figure 1). Since GPe has GABAergic projections to subthalamic nucleus (STN), this results in an increase in STN activity via disinhibition. Since the STN has excitatory (glutamatergic) projections to GPi and SNr, which, in turn send inhibitory GABAergic projections to the thalamus, activation of this pathway leads to suppressed cortical activity by inhibition of the thalamus.

In addition, the activity of both direct and indirect pathways can be powerfully modulated by dopamine (Albin et al., 1989; Gerfen and Wilson, 1996). Most striatal dopamine inputs are derived from nigrostriatal projections originating from SNc in the midbrain. The nigrostriatal dopamine acts at either the striatal D1 receptor to activate the direct pathway, or the striatal D2 receptor to activate the indirect pathway (Albin et al., 1989; Gerfen and Wilson, 1996)(Figure 1).

Although, we will limit most of our discussion to include only the cortico-basal ganglia-thalamo-cortical loops, it is important to note that parallel subcortical loops also exist (McHaffie et al., 2005). These loops have a thalamic relay on the input link of the circuit, rather than on the return link of the circuit as seen in the cortico-basal ganglia-
thalamo-cortical loops (McHaffie et al., 2005). Since midline and intralaminar nuclei of the thalamus are well connected to the striatum, midbrain and hindbrain structures that send projections to these two thalamic nuclei may send information to the basal ganglia via this thalamic relay. These brain areas include the superior and inferior colliculi, periaqueductal grey, pedunculopontine nucleus, cuneiform area and parabrachial complex, and various pontine and medullary reticular nuclei (Erro et al., 1999; Krout and Loewy, 2000a, b; Krout et al., 2001; Krout et al., 2002). Correspondingly, a direct return link to each of these midbrain and hindbrain structures from GPi and SNr (Schneider, 1986; Yasui et al., 1991; Deniau and Chevalier, 1992; Redgrave et al., 1992; Takada et al., 1994; Erro et al., 1999; Kirouac et al., 2004; Takakusaki et al., 2004) complete the subcortico-thalamo-basal ganglia-subcortical loops (McHaffie et al., 2005).

This architecture allows the putamen, as part of the striatum, to be able to influence various cortical and subcortical activities (Figure 1). More specifically, this neural configuration allows the putamen to engage large areas of the cerebral cortex to respond in a task specific manner via differentially exerting its influence on the GPi and SNr to modulate varying levels of disinhibition of the thalamus (Chevalier and Deniau, 1990; Parent and Hazrati, 1995).

Although these cortico-basal ganglia-thalamo-cortical loops are largely parallel and segregated, there are multiple lines of evidence that show that the integration and interaction of information in these parallel loops likely occurs. For example, medium spiny projection neurons within the striatum can also serve as the main integrating elements of the basal ganglia since they receive large numbers of projections from both the cerebral cortex and intrinsic basal ganglia neurons (Parent and Hazrati, 1995).
Cortico-striatal terminal arborizations from a variety of brain areas, including the prefrontal cortex, ACC, FEF, hippocampus, and posterior parietal cortex, overlap extensively as they converge on single striatal projection neurons (Haber, 2003; Haber et al., 2006; Calzavara et al., 2007). These brain areas are involved in processes related to attention, memory, and affect (Murray and Mishkin, 1983; Rainville et al., 1997; Price, 2000; Smith et al., 2004; Knudsen, 2007; Corbetta et al., 2008; Marschner et al., 2008). This pattern of overlapping projections may be seen at many levels of the basal ganglia relay structures as information from large area of the cerebral cortex is passed progressively into smaller relay structures within the basal ganglia (Gimenez-Amaya et al., 1995; Groenewegen et al., 1999; Haber et al., 2000). In addition, striatal projections to output nuclei may diverge, as GPi and SNr both receive information that originated from the same cortical area. Given that these two output nuclei project to two different locations on thalamic nuclei, some thalamo-cortical projections from one information loop may terminate at the origination of the cortico-striatal projection of a different information loop (Joel and Weiner, 1994). This neural configuration is intrinsic to the relationship between the basal ganglia and the neocortex and can facilitate the convergence and confluence of various information from many functional domains. This allows the putamen, as part of the striatum, to be well positioned to integrate information from many brain regions.

In addition, multiple lines of evidence suggest that the putamen may be important in using contextual cues in task performance (Jaeger et al., 1993; Koski et al., 1999; Coull et al., 2000). Koski et. al, for example, showed that the ratio of number of correctly signaled cues used to direct attention was directly correlated with activity of the
putamen (Koski et al., 1999), while Coull et. al showed that endogenous (top-down) attention activated the putamen and a network of brain areas (Coull et al., 2000). Similarly, previous experiment using single neuron recording showed that neurons in the putamen were differentially activated during the delay period between cue presentation and the task performance (Jaeger et al., 1993, 1995). Moreover, the putamen has been suggested to be important in sustaining prolonged salience of a noxious stimulus by maintaining the sustained engagement of prefrontal, cingulate, and parietal areas during pain (Downar et al., 2003). Taken together, these findings suggest that the role of putamen in nociceptive processing may involve the integration of various cognitive information and utilizing its neural resources to determine which brain networks are engaged during the inflow of afferent nociceptive information.
Figure 1. Cortico-basal ganglia-thalamo-cortical loop. The striatum receives afferent inputs from the cerebral cortex, limbic structures, and the thalamus. The striatum then relays signals, via direct and indirect pathways and, to its output nuclei, namely, the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). These output nuclei project to the thalamus and, via the thalamus, to target cortical and limbic regions from which the basal ganglia input originated completing the loop. In addition, the activity of both direct and indirect pathways can be powerfully modulated by nigrostriatal dopaminergic projections originating from SNC in the midbrain. The nigrostriatal dopamine acts at either the striatal D1 receptor to activate the direct pathway, or the striatal D2 receptor to activate the indirect pathway.
Cortico-basal ganglia-thalamo-cortical loop
AIMS OF THE PRESENT INVESTIGATION

The present manuscript will explore the neural mechanisms by which cognitive information influences supraspinal nociceptive processing. In the second chapter, we will examine the relationship between interindividual variations in pain sensitivity and various cognitive and psychological factors to determine if nociceptive processing and pain experience can be shaped by various cognitive influences. Next, we will investigate the potential roles of the insular cortex and the putamen in providing the neural substrates for facilitating these cognitive influences at the supraspinal level. The third chapter will examine if the bidirectional reciprocal connections of the insular cortex with various cognitive and nociceptive processing brain areas can provide a framework for this brain region to integrate and utilize cognitive information to modulate brain areas involved in various aspects of nociceptive processing. The fourth chapter will determine if the neural circuitry involving the putamen and the basal ganglia can integrate various cognitive and context information as well as utilize its cortico-basal ganglia-thalamo-cortical-loop architectural configuration to selectively engage cortical networks to process nociceptive information in a context-relevant manner. The contributions from these studies can provide a better understanding of the supraspinal mechanisms of pain processing and shed some light on the neural mechanisms that allow cognitive information unique to each individual to shape how nociceptive information is differentially processed and how pain is experienced.


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Chapter 2

MULTIFACTORIAL PREDICTORS OF EXPERIMENTAL THERMAL PAIN

Christopher J. Starr, Timothy T. Houle, and Robert C. Coghill

The following manuscript is in preparation for submission. Stylistic variations are due to the requirements of the journal. Christopher Starr designed the paradigm, collected data, and prepared the manuscript. Dr. Robert Coghill acted in an advisory and editorial capacity.
Abstract

Although large interindividual differences in pain exist, the underlying factors that contribute to these variations remain poorly understood. Consequently, being able to accurately explain variability in pain ratings in term of its contributing factors could provide insights into the better understanding of individual differences in pain experience. In the present investigation, we show that a significant portion of the variability in experimental heat pain ratings may be predicted using simple quantitative sensory testing and a series of psychological questionnaires including State Trait and Anxiety Inventory (STAI), Center for Epidemiologic Studies – Depression Scale (CES-D), and Positive and Negative Affect Schedule – Expanded form (PANAS-X). Using factor analysis to identify a combination of meaningful predictors, our multifactorial model can reliably predict a significant amount of the variability in heat pain sensitivity ratings ($r^2 = 0.537$, $p=0.027$). Out of the five composite predictors for heat pain sensitivity, two variables, negative mood and cool detection, significantly carried the majority of the weight in this model ($\beta = -0.469$, $p=0.033$ and $\beta = -0.591$, $p=0.006$, respectively). Moreover, individual variables including heat pain thresholds and self-assessment of pain sensitivity were found to be poor predictors of heat pain sensitivity ($r^2=0.0332$; $p=0.4292$; $r^2=0.043867$; $p=0.3622$, respectively). Taken together, these results suggest that a variety of factors underlie individual differences in pain experience, and that a reliable model for predicting pain should be constructed from a combination of these factors.
Introduction

A complete subjective experience of pain is uniquely personal and varies significantly from one individual to the next (Coghill et al., 2003; Nielsen et al., 2008). Twin studies indicate that a large portion of interindividual variations in pain experience cannot be accounted for by genetic factors alone. Thus, other factors including environmental, psychological, and cognitive factors unique to each individual may play an important role in shaping one’s pain experience (MacGregor et al., 1997; Norbury et al., 2007; Nielsen et al., 2008). However, little is known about factors that contribute to interindividual differences in pain sensitivity. For this reason, providing care for patients in pain has proven to be a difficult and intricate task, and many patients report being unsatisfied with pain relief and care that they have received (Liu and Wu, 2007; Lovatsis et al., 2007).

To date, many studies have attempted to predict pain experienced by postoperative patients using various predictors ranging from pain thresholds, anxiety scores, blood pressure, and age (Jamison et al., 1993; Macintyre and Jarvis, 1996; Wise et al., 2002; Logan et al., 2003; Edwards et al., 2004). Most find statistically reliable, but weak correlations between these predictors and important outcome variables. Nevertheless, across studies, suprathreshold experimental pain ratings have been shown to be highly correlated with clinical pain intensity ratings, analgesic use, and other important outcome variables (Granot et al., 2003; Werner et al., 2004; Hsu et al., 2005; Pan et al., 2006). Granot et al. (2003), for instance, reported 48°C suprathreshold noxious thermal stimulation as being useful in predicting postoperative pain intensity rating during both rest and activity ($r=0.434-0.527; p<0.01$), while pain thresholds were not.
More recently, many studies have suggested that psychological factors can also significantly influence one’s subjective pain experience (Maggirias and Locker, 2002; Logan et al., 2003; Staud et al., 2003; Staud et al., 2004; Granot and Lavee, 2005; Hsu et al., 2005; Pan et al., 2006). For instance, in a postcesarean pain study by Pan et al. (2006), a significant portion of the variability in total analgesic requirement can be explained by variability in STAI (State Trait Anxiety Inventory) scores \( r^2 = 0.22, p<0.01 \). Furthermore, multiple lines of evidence indicate that emotional states and attitudes of patients can have a profound impact on pain associated with chronic diseases (Benrud-Larson and Wegener, 2000; Haythornthwaite and Benrud-Larson, 2000; Schanberg et al., 2000). However, the exact contribution of various psychological factors including anxiety, depression, and personality to interindividual variations in pain sensitivity remains poorly understood. In addition, although variables such as one’s self-assessment of pain sensitivity and pain thresholds have often been used to predict an individual’s pain sensitivity, the reliability of these variables as predictors are yet to be established.

Since experimental pain ratings of suprathreshold noxious stimuli may account for much of variability of clinical pain intensity ratings, analgesic use, and other important outcome variables (Granot et al., 2003; Hsu et al., 2005; Pan et al., 2006), being able to identify reliable predictors of experimental pain ratings may provide useful insights to better understand the factors that may be important in contributing to individual differences in pain experience. In order to investigate these questions and generate reliable models that can be used to predict experimental pain ratings, we performed detailed quantitative sensory testing and psychological assessments on a group of healthy volunteers.
Methods

Subjects

Twenty-one healthy volunteers (eleven male and ten female), 21–38 years old (mean 26.7), participated in this study. All subjects gave informed consent acknowledging that they understood: (1) that they would experience experimental painful stimuli, (2) that all methods and procedures were clearly explained, and (3) that they were free to withdraw from the experiment at any time without prejudice. All procedures were approved by the Institutional Review Board of Wake Forest University School of Medicine.

Assessment of psychological factors

Prior to quantitative sensory testing and thermal stimulation, subjects were asked to rate their self-assessment of pain sensitivity using a Visual Analog Scale (VAS). The scale has ‘not at all sensitive to pain’ anchored on one end and ‘extremely sensitive to pain’ on the other. Subsequently, they were asked to complete the State Trait and Anxiety Inventory (STAI), Center for Epidemiologic Studies – Depression Scale (CES-D), and Positive and Negative Affect Schedule – Expanded form (PANAS-X). STAI is a questionnaire that assesses trait (20 questions) and state (20 questions) anxiety. CES-D is a 20-item self-report scale designed to measure presence and severity of depressive symptoms (Radloff, 1977). Both STAI and CES-D have been used in many pain studies to assess anxiety and depression, respectively (Harden et al., 2003; Jones et al., 2003; Kalkman et al., 2003; Schuler et al., 2004; Pan et al., 2006; Alschuler et al., 2008; Young
Casey et al., 2008). PANAS-X is a 60-items questionnaire designed to measure different inner states and emotions. The PANAS-X has two higher dimensions that assess negative and positive affect, and another dimension for specific affects that is divided into basic negative emotions, basic positive emotions, and other affective states. Among the 60 words presented, ten are in the negative affectivity and ten are in positive affectivity dimensions. Each word is rated on a scale from one to five, as to whether the word fits the habitual or current state of the individual (Watson and Clark, 1991). In this study, the habitual state was requested.

_Psychophysical data collection_

_Heat pain stimulation_

Subjects rated pain using a 15-cm plastic visual analog scale (VAS) that has been widely used to assess pain because of ease of use while providing quantifiable measurements of pain intensity and pain unpleasantness (Parisian Novelty Co., Chicago, IL; (Price et al., 1994)). The minimum was anchored with ‘No pain sensation’ or ‘Not at all unpleasant’, while the maximum was anchored with ‘Most intense pain imaginable’ or ‘Most unpleasant imaginable’. Using an audio analogy, subjects were instructed to distinguish between pain intensity and pain unpleasantness (Price et al., 1989). All thermal stimuli were applied to their nondoninant ventral forearm via a 16×16-mm² peltier device (Medoc TSA II, Ramat Yishai, Israel) secured with a Velcro strap. Baseline temperature was maintained at 35°C, and stimulus temperatures were delivered with rise and fall rates of 6°C/s and were feedback controlled. During a training session, subjects rated 32 noxious heat stimuli applied to their non-dominant ventral forearm (35,
Cold pain stimulation

During cold pain stimulation, thermal stimuli were delivered to the ventral surface of the non-dominant forearm via a 32×32-mm² peltier device (Medoc TSA II, Ramat Yishai, Israel). Subjects provided post-stimulus pain intensity and pain unpleasantness VAS ratings of eighteen stimuli of five different temperatures (35, 20, 15, 10, 5 or 0°C) delivered at 5 s duration in a pseudo-random fashion on the non-dominant ventral forearm. To minimize sensitization habituation or hyperalgesia, all trials were separated by a minimum of 30 s and were performed on previously unstimulated sites of the skin (Pedersen and Kehlet, 1998a, b).
Quantitative testing of sensory thresholds

Thermal thresholds

Heat pain threshold, cold pain threshold, warm detection threshold, and innocuous cool detection threshold were determined by the method of limits. For each of the four modalities of interest, the 32×32-mm² thermode was applied to the non-dominant ventral forearm. For warm detection and heat pain thresholds, the temperature was increased at 1°C/s from 35 to 50°C. Subjects were then asked to indicate either the transition point at which the baseline temperature transitions into a warm sensation (warm detection) or when nonpainful warm sensation changed into a painful heat sensation (heat pain) by pressing a button. For innocuous cool and cold pain thresholds, temperature was decreased at 1°C/s from 35 to 0°C. Subjects were subsequently asked to indicate the transition point at which the baseline temperature changed into a cool sensation (innocuous cool) or when nonpainful cool sensation transitions into a painful cold sensation (cold pain). For each of the modalities measured, the test was repeated successively six times and the mean threshold temperature was calculated. To minimize sensitization habituation or hyperalgesia, all trials were separated by a minimum of 30 s and were performed on previously unstimulated sites of the skin (Pedersen and Kehlet, 1998a, b).

Cold pain tolerance

After the thermal stimulation, subjects were requested to immerse their dominant hand in a container of ice-saturated water (approximately 1-2°C) up to the level of the wrist until the pain became intolerable (Jones et al., 1988; Sindrup et al., 1993; Petersen-
Felix et al., 1994; Naef et al., 2003). Time from the start of the immersion of the hand until withdrawal were recorded.

Statistical analysis

Statistical analyses were conducted using SPSS software version 13.0 (SPSS Inc., Chicago, IL). Descriptive statistics were calculated for all variables (i.e., mean, SD, and range) as appropriate. For all analyses, \( \alpha \) was set at 0.05 for statistical significance.

To determine if predictors and outcomes could be reduced into meaningful subsets based on their relationship to each other, principal component factor analysis with varimax rotation was used (Pan et al., 2006). Factor analysis often is used as a data reduction technique or as a method to mathematically identify meaningful subgroups of items. Care was taken to create a solution of item groups (factors) that were very similar to each other, but relatively uncorrelated. Because factor analysis is typically conducted on much larger samples than that of the current exploratory study, a minimally acceptable factor loading of 0.80 or more was used to better ensure the stability of identified factors (Pan et al., 2006). Predictors were combined to form factor scores by summing the individual items in the factor. Outcomes were also combined to form factor scores by summing the individual items in the factor. In addition, some independent variables that accounted for a large portion of the variability in the data set, but did not exhibit covariation with other independent variables were also used independently as factors. Reliability estimates of the factors were conducted using Cronbach \( \alpha \). Next, multiple regression analyses were used to examine if a group of generated predictors (factors) could be used in conjunction to reliably predict each outcome factor. Finally, Pearson
correlations were used to determine if individual predictors of heat pain threshold and self-assessment of pain sensitivity can reliably predict heat pain sensitivity.
Results

Predictors assessment

The complete details for predictor and outcome variables are shown in table 1. The mean (SD) heat and cold pain thresholds were 47.157 (1.590)°C and 8.544 (8.085)°C, respectively. The mean (SD) warm and cool detection thresholds were 36.849 (0.6795)°C and 31.913 (1.283)°C, respectively. The mean self-assessment of pain sensitivity was 3.195 (1.455). The mean state and trait anxiety scores were 33.333 (6.983) and 37.762 (9.731), respectively. The mean negative and positive affect dimensions of PANAS-X were 15.714 (5.849) and 34.238 (4.878), respectively. The mean CES-D score was 7.619 (4.822). It is important to note that none of our subjects had depression scores that would fall in the range of clinical depression (greater than or equal to 21 on CES-D).

Outcomes assessment

The mean (SD) VAS pain intensity and unpleasantness ratings for 49°C noxious heat stimuli were 2.322 (1.466) and 1.413 (1.022), respectively (Table 1). The mean (SD) VAS pain intensity and unpleasantness ratings for 0°C noxious cold stimuli were 1.452 (1.559) and 0.960 (1.471), respectively. The mean (SD) cold pain tolerance duration was 80.05 (100.694) seconds. It is important to note that there is a large interindividual variability in the range of pain ratings provided by the subjects. This finding is consistent with those of other experimental pain studies that also report large
interindividual variations in pain ratings and highlights the large interindividual differences that exist in the pain experience (Rosier et al., 2002; Coghill et al., 2003).
| Table 1. Descriptive Statistics of Main Predictor Variables and Outcome Variables |
|---------------------------------|-------------|---------|--------|
| **n** | **Range** | **Mean** | **SD**  |
| **Predictor variables** | | | |
| Sensory modalities threshold | | | |
| Cool detection threshold (°C) | 21 | 27.417 – 33.3 | 31.913 | 1.283 |
| Cold pain threshold (°C) | 21 | 0.433 - 28.35 | 8.544 | 8.085 |
| Warm detection threshold (°C) | 21 | 36 – 38.5 | 36.849 | 0.6795 |
| Heat pain threshold (°C) | 21 | 42.633 – 49.433 | 47.157 | 1.590 |
| Psychological factors assessment | | | |
| Self-assessment of pain sensitivity (VAS) | 21 | 1 – 5.7 | 3.195 | 1.455 |
| STAI | | | |
| State anxiety | 21 | 20 – 46 | 33.333 | 6.938 |
| Trait anxiety | 21 | 25 – 55 | 37.762 | 9.731 |
| Total anxiety# | 21 | 47-96 | 71.095 | 13.141 |
| CES-D score | 21 | 1 – 20 | 7.619 | 4.822 |
| PANAS-X | | | |
| Negative affect | 21 | 10 – 32 | 15.714 | 5.849 |
| Positive affect | 21 | 21 – 43 | 34.238 | 4.878 |
| Total affectΦ | 21 | 4 – 33 | 18.524 | 6.392 |
| Outcome variables | | | |
| Noxious stimuli VAS ratings | | | |
| Intensity rating at 0 °C | 21 | 0.133 – 7.267 | 1.452 | 1.559 |
| Unpleasantness rating at 0 °C | 21 | 0 – 6.567 | 0.960 | 1.471 |
| Intensity rating at 49 °C | 21 | 0.4 – 6.167 | 2.322 | 1.466 |
| Unpleasantness rating at 49 °C | 21 | 0 - 3.2 | 1.413 | 1.022 |
| Cold tolerance (seconds) | 19 | 9.93 – 440.53 | 80.05 | 100.694 |
| Coefficient of intensity* | 21 | 0.802 – 8.074 | 4.136 | 1.926 |
| Intercept of intensity* | 21 | -20.003 to (-1.398) | -10.453 | 4.714 |
| Coefficient of unpleasantness& | 21 | -0.5 -12.596 | 4.650 | 3.415 |
| Intercept of unpleasantness& | 21 | -32.372 – 0.725 | -12.26 | 8.486 |

# Sum of state anxiety and trait anxiety, Φ Positive affect minus negative affect, *Variables derived from generating temperature vs. heat pain intensity ratings stimulus-response curve , & Variables derived from generating temperature vs. heat pain unpleasantness ratings stimulus-response curve.
Predictive model using factor analysis

The analysis resulted in five newly formed predictive factors (groups) that accounted for 90% of the total observed variance in the predictors (Table 2). The analysis also yielded five newly formed outcome factors (groups) that accounted for 90% of the total observed variances in outcomes (Table 3). The analysis allows independent variables that exhibited substantial covariation to be combined to form a single factor (thus taking up less room in the regression model). Each factor was then given a name consistent with the several independent measured component variables (Table 2 and 3). For example, negative affect score, depression score, and anxiety scores exhibited substantial covariation and were combined to form a single predictor factor - negative mood (Table 2). Similarly, the outcome variables heat pain intensity ratings and heat pain unpleasantness ratings also exhibited a great deal of covariation and were combined to form the heat pain sensitivity outcome factor (Table 3). These predictor factors were then used in conjunction to generate multiple regression models for predicting each of the composite outcome factors.
<table>
<thead>
<tr>
<th>Factor Number</th>
<th>Factor Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 1</td>
<td>Thresholds difference</td>
<td>This represents the difference between heat pain and cold pain thresholds</td>
</tr>
<tr>
<td>Factor 2</td>
<td>Warm detection</td>
<td>Threshold temperature for detecting warm sensation</td>
</tr>
<tr>
<td>Factor 3</td>
<td>Cool detection</td>
<td>Threshold temperature for detecting cool sensation</td>
</tr>
<tr>
<td>Factor 4</td>
<td>Negative mood</td>
<td>This consists of the sum of CES-D, negative affect dimension of PANAS-X, and STAI anxiety scores</td>
</tr>
<tr>
<td>Factor 5</td>
<td>Pain positivity</td>
<td>This consists of the sum of positive affect dimension of PANAS-X and self-assessment of pain sensitivity scores</td>
</tr>
</tbody>
</table>
Table 3. The Five Outcome Factors (Groups) and Their Corresponding Component Outcome Variables

<table>
<thead>
<tr>
<th>Factor Number</th>
<th>Factor Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor 1</td>
<td>Heat pain sensitivity</td>
<td>This consists of the sum of mean intensity and mean unpleasantness VAS rating of 49°C noxious heat stimuli</td>
</tr>
<tr>
<td>Factor 2</td>
<td>Cold pain sensitivity</td>
<td>This consists of the sum of mean intensity and mean unpleasantness VAS rating of noxious cold stimuli</td>
</tr>
<tr>
<td>Factor 3</td>
<td>Heat pain intensity stimulus-response</td>
<td>This represents the difference between coefficient and intercept of heat pain intensity stimulus-response curve</td>
</tr>
<tr>
<td>Factor 4</td>
<td>Heat pain unpleasantness stimulus-response</td>
<td>This represents the difference between coefficient and intercept of heat pain unpleasantness stimulus-response curve</td>
</tr>
<tr>
<td>Factor 5</td>
<td>Cold tolerance</td>
<td>This represents the time duration (seconds) in which subjects were able to keep their hand submerged in ice/water mixture.</td>
</tr>
</tbody>
</table>
Final predictive model with multiple regression analysis

Final predictive models with multiple regression analysis for each composite outcome factor are shown in table 4. Each of the models was generated using five composite predictive factors (negative mood, pain thresholds, pain positivity, cool detection, and warm detection). Out of the five multiple regression models generated, only models for heat pain and cold pain sensitivity reached statistical significance (Table 4). However, since the model for cold pain sensitivity ($r^2 = 0.614$, $p=0.008$) was largely driven by an outlier, it was excluded from our findings. The five composite predictors provided the best predictive model for heat pain sensitivity ($r^2 = 0.537$, $p=0.027$)(Fig. 1 and Table 5). Out of the five composite predictors, negative mood and cool detection significantly carried a majority of the weight in this model ($\beta = -0.469$, $p=0.033$ and $\beta = -0.591$, $p=0.006$, respectively)(Table 5). Thus, these factors may contribute importantly to the individual differences in pain experience during experimental heat pain. The models generated for cold tolerance, heat pain intensity stimulus-response, and heat pain unpleasantness stimulus-response were not statistically significant, although there appears to be a trend towards significance for heat pain intensity stimulus-response ($r^2 = 0.442, F=2.372$, $p=0.089$) and heat pain unpleasantness stimulus-response ($r^2 = 0.410, F=2.087$, $p=0.124$) models.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>F</th>
<th>P Value</th>
<th>r²</th>
<th>Adjusted r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat Pain Sensitivity</td>
<td>3.483</td>
<td>0.027</td>
<td>0.537</td>
<td>0.383</td>
</tr>
<tr>
<td>Cold Pain Sensitivity *</td>
<td>4.766</td>
<td>0.008</td>
<td>0.614</td>
<td>0.485</td>
</tr>
<tr>
<td>Heat Pain Intensity Stimulus</td>
<td>2.372</td>
<td>0.089</td>
<td>0.442</td>
<td>0.255</td>
</tr>
<tr>
<td>Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat Pain Unpleasantness Stimulus</td>
<td>2.087</td>
<td>0.124</td>
<td>0.410</td>
<td>0.214</td>
</tr>
<tr>
<td>Response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold Tolerance</td>
<td>0.374</td>
<td>0.857</td>
<td>0.135</td>
<td>-0.226</td>
</tr>
</tbody>
</table>

* Model significance influenced by an outlier. After the outlier was removed, the model was not significant.

F = F statistic, which evaluates the model; r² = variance in outcome accounted for by the predictors.
Table 5. Multiple Regression Analyses for Heat Pain Sensitivity Outcome Factor (Final Model)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictors</th>
<th>b</th>
<th>β</th>
<th>t</th>
<th>P Value</th>
<th>F</th>
<th>P Value</th>
<th>$r^2$</th>
<th>Adjusted $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat pain sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative mood</td>
<td>-0.054</td>
<td>-0.469</td>
<td>-2.355</td>
<td>0.033</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain thresholds</td>
<td>-0.044</td>
<td>-0.163</td>
<td>-0.843</td>
<td>0.413</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain positivity</td>
<td>0.048</td>
<td>0.109</td>
<td>0.603</td>
<td>0.555</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cool detection</td>
<td>-1.113</td>
<td>-0.591</td>
<td>-3.166</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm detection</td>
<td>-0.160</td>
<td>-0.045</td>
<td>-0.243</td>
<td>0.811</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b = unstandardized regression coefficient; β = standardized regression coefficient; F = F statistic, which evaluates the model; $r^2$ = variance in outcome accounted for by the predictors; t = t statistic, which evaluates the predictor.
Figure 1. Multifactorial model of heat pain sensitivity. The multifactorial model of heat pain sensitivity can account for a significant portion of the variability in actual heat pain sensitivity ratings ($r^2=0.537; p=0.027$). The graph shows heat pain sensitivity ratings as predicted by our multifactorial model (regression line) vs. subjects’ actual heat pain sensitivity ratings.
Pearson correlations between heat pain sensitivity and pain thresholds and self-assessment of pain sensitivity

In addition, we found that heat pain thresholds accounted for a very small variability in heat pain sensitivity ($r^2=0.043867; p=0.3622$)(fig. 2A). Self-assessment of pain sensitivity also did not account for a significant portion of variability in heat pain sensitivity ($r^2=0.0332; p=0.4292$)(fig. 2B). These results suggest that both heat pain threshold and self-assessment of pain sensitivity are not reliable predictors of pain experience at suprathreshold noxious temperature.
Figure 2. Heat pain thresholds and self-assessment of pain sensitivity as predictors of heat pain sensitivity. Variability in heat pain thresholds cannot reliably explain variability in heat pain sensitivity ratings ($r^2=0.044; p=0.3622$). (A). Similarly, variability in self-assessment of one’s own pain sensitivity poorly predicts variability in one’s heat pain sensitivity ratings ($r^2=0.033; p=0.4292$) (B).
Discussion

The subjective experience of pain is a uniquely individual and personal sensory experience that involves much more than a simple relay of incoming nociceptive information. To date, the factors that contribute to interindividual differences in the pain experience remain poorly understood. In the present investigation, we show that self-assessment of pain sensitivity and pain thresholds were found to be poor predictors of an individual’s pain sensitivity ($r^2=0.0332, p=0.4292, r^2=0.043867, p=0.3622$, respectively). However, factor analysis with a combination of meaningful predictors can produce a model with significant improvements over single independent variables in predicting heat pain sensitivity ($r^2 = 0.537, p=0.027$)(Fig. 1 and Table 5). Out of the five composite predictors for heat pain sensitivity, negative mood and cool detection significantly carried a majority of the weight in this model ($\beta = -0.469, p=0.033$ and $\beta = -0.591, p=0.006$, respectively). The finding that acute heat pain sensitivity cannot be easily predicted by any single variable is consistent with evidence that pain is a complex subjective experience that is constructed from a variety of factors unique to each individual.

Psychological factors and pain sensitivity

Previously, many studies have suggested that the pain experience may be greatly influenced by many psychological factors, including anxiety and emotional state (Maggirias and Locker, 2002; Logan et al., 2003; Staud et al., 2003; Staud et al., 2004; Granot and Lavee, 2005; Hsu et al., 2005; Pan et al., 2006). The prevalence of depression in the chronic pain patient population suggests that pain experience and psychological state such as mood may be closely linked (Geisser et al., 2000; Sullivan et
al., 2001; Turk and Okifuji, 2002). Similarly, catastrophizing about pain and clinical depression have been shown to negatively affect the pain experienced by patients (Haythornthwaite and Benrud-Larson, 2000; Auerbach et al., 2001; Sullivan et al., 2001; Dickens et al., 2003; Jones et al., 2003; Edwards et al., 2004; Gracely et al., 2004; Bar et al., 2005; Granot and Lavee, 2005; Alschuler et al., 2008; Graff-Guerrero et al., 2008). However, most of these studies were done in clinical populations (e.g. clinically depressed patients or patients undergoing major surgical procedures). Moreover, many studies done in non-clinical settings that reported increased pain sensitivity from anxiety actually tested the effects of induced anticipatory anxiety of an impending event on pain as opposed to the effects of baseline anxiety levels on pain (Cornwall and Donderi, 1988; Rhudy and Meagher, 2000; Ploghaus et al., 2001). In present investigation, negative mood (sum of negative affect, depression, and anxiety scores) was inversely correlated with heat pain sensitivity ($\beta = -0.469$, p=0.033)(Table 5). In addition, since none of our subjects met the criteria for clinical depression, these findings likely reflect how a combination of negative psychological factors and emotional state can influence nociceptive processing in general population in a non-clinical setting. Thus, even in the normal healthy subjects, a combination of psychological test scores may provide useful information for predicting one’s pain sensitivity. It is possible that the observed inverse relationship between negative mood and pain sensitivity may reflect the contribution of brain areas involved in processing cognitive information in priming anti-nociceptive systems through top-down modulation. However, the exact underlying mechanisms and neural correlates behind this observed effect are not yet known and require further investigation.
Predicting one's own pain sensitivity

Self-assessment of pain sensitivity is often used as supplemental information in determining analgesic/anesthetic requirements of patients before an acute surgical procedure. For example, patients who believe that they are highly sensitive to pain may strongly prefer conscious sedation over a local anesthetic block when undergoing 3rd molar tooth extraction. However, self-assessments of pain sensitivity have previously been shown to be poor predictors of relatively complex correlates of actual pain sensitivity such as acute pain tolerance, temporal summation of pain, and clinical pain (Robinson et al., 2004; Edwards and Fillingim, 2007). Consistent with these findings, we found that an insignificant portion of the variability in experimental heat pain sensitivity was accounted for the variability in self-assessment of pain sensitivity ($r^2=0.043867$; $p=0.3622$)(Fig. 2). Taken together, these findings suggest that obtaining self-assessments of pain sensitivity may not provide practically useful information for pain management.

Relationship of thermal thresholds and pain sensitivity

Pain thresholds, together with the slope of the stimulus-response curve, form the fundamental elements of psychophysical equations that predict the magnitude of pain evoked by a given stimulus. Accordingly, individual variations in pain thresholds would be expected to predict a substantial portion of the variability in pain sensitivity. Studies of post-operative pain, however, have produced mixed results on the ability of pain thresholds to predict pain. One study suggests that both resting and evoked pain can be predicted to some extent by pain thresholds (Pan et al., 2006) while other studies support
no such relationship (Granot et al., 2003). However, these studies necessarily use a threshold stimulus modality that is distinct from that of the post-operative pain. Accordingly, the mismatch between the threshold and the suprathreshold stimulus modalities may contribute to the variability of the findings. In the present study, even when the modality of the threshold stimulus is the same as the modality of the suprathreshold stimulus, we found that no significant amount of variability in experimental heat pain sensitivity was accounted for by the variability in heat pain thresholds ($r^2=0.0332; p=0.4292$)(Fig. 2). Furthermore, since it seems intuitive to assume that subjects with lower pain thresholds may exhibit higher pain ratings at suprathreshold noxious temperatures, studies of experimental pain have frequently used subjects’ pain thresholds to determine appropriate suprathreshold noxious temperatures (Lautenbacher et al., 1995; Huber et al., 2006). The present findings raise substantial questions about the validity of this strategy.

In contrast to pain thresholds, cool detection threshold was also a major predictor of heat pain sensitivity in our model. Cool stimuli have been shown to inhibit pain (Gammon and Starr, 1941; Bini et al., 1984), and cool-sensitive primary afferent nerve fibers have been reported to also respond to painful heat stimuli (Dubner et al., 1975; Long, 1977). This is evident by the burning pain sensation following the unmasking of the effects of cool sensitive primary afferents during thermal grill illusion (Craig and Bushnell, 1994). Moreover, patients suffering from central post-stroke pain often suffer from cold allodynia, a burning pain sensation evoked by innocuous cool stimuli (Andersen et al., 1995; Vestergaard et al., 1995; Greenspan et al., 2004; Bowsher, 2005; Kim et al., 2007). Although, the inverse correlation between cool detection and heat pain
sensitivity seen in our model \( (\beta = -0.591, p=0.006) \) (Table 5) may suggest that cool-sensitive afferents may play a role in anti-nociception during noxious stimulation, it is unclear how cool detection could account for a significant proportion of variability in pain ratings. Thus, the exact roles that cool-sensitive afferents play in heat pain modulation require further investigation. Nonetheless, measurement of cool detection threshold can provide useful predictive information about experimental heat pain sensitivity.

*Prediction of pain with multifactorial models*

Even though the sample size in the present investigation was relatively small, statistical significance in predictive power and correlations were observed. Our model, generated from relatively simple and brief questionnaires and sensory testing, accounted for over half of the variability observed in suprathreshold noxious pain ratings \( (r^2 = 0.537, p=0.027) \) (Fig. 1 and Table 5). These results suggest that a multifactorial model can provide a more accurate and reliable method to predict an individual’s thermal pain sensitivity than a model constructed from a single independent variable. Additionally, since suprathreshold heat pain stimuli ratings have been reported to have good predictive value because they closely mimic the clinical pain experience (Granot et al., 2003; Werner et al., 2004; Pan et al., 2006), our model may potentially be beneficial in screening for subjects that may suffer from severe postoperative pain and need additional pain management. Nevertheless, a significant part of the variability in pain ratings cannot be explained by our existing multifactorial predictive model. In addition, our study only attempts to explain variability in experimental pain. Thus, applying these
results to predict treatments for clinical pain should be done with caution. For example, individual variation in analgesic requirements is predicted by a different set of factors than post-operative pain (Pan et al., 2006). Clinical pain is a complex experience influenced by many factors and is impossible to be simulated in a laboratory setting. However, the refinement and eventual application of multifactorial predictive models of experimental pain, clinical pain, and analgesic requirements holds the potential to significantly improve the quality of care provided to these patients.

Acknowledgements

This study was supported by NIH R01 NS39426 and DA20168.
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Chapter 3

ROLES OF THE INSULAR CORTEX IN THE MODULATION OF PAIN:
INSIGHTS FROM BRAIN LESIONS

Christopher J. Starr, Lumy Sawaki, George F. Wittenberg, Jonathan H. Burdette,
Yoshitetsu Oshiro, Alexandre S. Quevedo, and Robert C. Coghill

The following manuscript is accepted for publication and is currently in press in the
Journal of Neuroscience (expected in March 2009). Stylistic variations are due to the
requirements of the journal. Christopher Starr designed the paradigm, collected data, and
prepared the manuscript. Dr. Robert Coghill acted in an advisory and editorial capacity.
Abstract

Subjective sensory experiences are constructed by the integration of afferent sensory information with information about the uniquely personal internal cognitive state. The insular cortex is anatomically positioned to serve as one potential interface between afferent processing mechanisms and more cognitively-oriented modulatory systems. However, the role of the insular cortex in such modulatory processes remain poorly understood. Two individuals with extensive lesions to the insula were examined to better understand the contribution of this brain region to the generation of subjective sensory experiences. Despite substantial differences in the extent of the damage to the insular cortex, three findings were common to both individuals. First, both subjects had substantially higher pain intensity ratings of acute experimental noxious stimuli than age-matched control subjects. Second, when pain-related activation of the primary somatosensory cortex was examined during left and right-sided stimulation, both individuals exhibited dramatically elevated activity of the primary somatosensory cortex ipsilateral to the lesioned insula in relation to healthy control subjects. Finally, both individuals retained the ability to evaluate pain despite substantial insular damage and no evidence of detectible insular activity. Taken together, these results indicate that the insula may be importantly involved in tuning cortical regions to appropriately utilize prior cognitive information during afferent processing. Finally, these data suggest that a subjectively available experience of pain can be instantiated by brain mechanisms that do not require the insular cortex.
**Introduction**

The insular cortex is often bilaterally activated during noxious somatosensory stimulation and has been suggested to play an important role in pain processing (Coghill et al., 1994; Coghill et al., 1999). Afferent nociceptive information can be transmitted rostrally from SII to the posterior insula and then to the anterior insula. The reciprocal connections of the insula with the prefrontal cortex, ACC, amygdala, parahippocampal gyrus and SII can allow afferent nociceptive information to be integrated with information related to working memory, affect, and attention (Mufson et al., 1981; Mesulam and Mufson, 1982; Mufson and Mesulam, 1982; Friedman and Murray, 1986; Friedman et al., 1986).

Activation of the insular cortex has been correlated with the intensity of noxious stimulation, suggesting that this structure may play a role in pain intensity coding (Derbyshire et al., 1997; Coghill et al., 1999). Consistent with this role, psychophysical data indicate that patients with lesions affecting the posterior insula and parietal operculum, but not the anterior insula, exhibit elevated heat pain thresholds (Greenspan and Winfield, 1992; Greenspan et al., 1999). However, when tested with suprathreshold noxious stimuli, patients with insular lesions exhibit more complex alterations in their experience of pain. They exhibit pain asymbolia, a condition where individuals can recognize noxious stimuli as painful but exhibit inappropriate affective responses and have difficulty in appraising the meaning and significance of such stimuli (Berthier et al., 1988).
The extensive connectivity of the insula suggests that it may play a complex, multi-faceted role in pain. For example, activation of the insula during placebo, opioid analgesia, expectation, and hypnosis suggests that it may be important in both anti-nociceptive as well as pro-nociceptive processes (Petrovic et al., 2002; Lorenz et al., 2003; Derbyshire et al., 2004; Koyama et al., 2005; Zubieta et al., 2005; Kong et al., 2006; Craggs et al., 2007; Kong et al., 2007). Activity in the anterior insula can also modulate activation of the prefrontal cortex and anterior cingulate cortex in a task or situation-dependent fashion (Craggs et al., 2007; Sridharan et al., 2008). These findings suggest that the insula may be well positioned to utilize cognitive information to modulate connected brain areas involved in processing of sensory-discriminative, affective, and cognitive-evaluative components of pain. Accordingly, lesions of the insula would be expected to lead to a complex pattern of altered experiences of pain evoked by suprathreshold noxious stimuli. Such changes could even include increases in pain sensitivity due to a loss of modulation of brain areas involved in various aspects of nociceptive processing.

To date, few studies have investigated the integrity of pain perception and alteration in pain-related brain activation following damage to the insula. To investigate the role that the insula plays in pain processing and modulation, sensory testing and fMRI experiments were used to examine two patients who suffered from large left middle cerebral artery (MCA) ischemic strokes with lesions encompassing the insular cortex.
Methods

Subjects

Two male stroke patients (age 53 and 59) were recruited to participate in the study. Both patients suffered from large left MCA strokes with lesions encompassing the insular cortex. A group of 14 healthy older adults was recruited to assess differences in pain perception, sensory thresholds, and pattern of brain activations in lesioned subjects. There were eight females and six males (age, 46–75 years; mean, 59 years). Out of the fourteen subjects, thirteen participated in the imaging session. One female participant did not participate in the brain imaging session due to claustrophobia. They underwent the same experimental protocols as the stroke patients. All study participants gave written, informed consent acknowledging that (1) they would experience experimental painful stimuli, (2) all methods and procedures were clearly explained, and (3) they were free to withdraw from the experiment at any time without prejudice. All of the procedures were approved by the Institutional Review Board of Wake Forest University School of Medicine.

Psychophysical data collection

During the psychophysical training session, subjects practiced rating 32 noxious heat stimuli (35, 43–49°C, 5 s duration) using a visual analog scale (VAS). The VAS is a 15-cm plastic sliding scale device widely used to assess pain because of ease of use while providing quantifiable measurements of pain intensity and pain unpleasantness (Parisian Novelty Co., Chicago, IL; (Price et al., 1994)). The minimum was anchored with ‘No pain sensation’ or ‘Not at all unpleasant’, while the maximum was anchored with ‘Most
intense pain imaginable’ or ‘Most unpleasant imaginable’. Using an audio analogy, subjects were instructed to distinguish between pain intensity and pain unpleasantness (Price et al., 1989). The stimuli were applied to their dorsal calves via a 16×16-mm² peltier device (Medoc TSA II, Ramat Yishai, Israel) secured with a Velcro strap. Baseline temperature was maintained at 35°C, and stimulus temperatures were delivered with rise and fall rates of 6°C/s and were feedback controlled. These data are not reported further.

Next, subjects provided post-stimulus pain intensity and pain unpleasantness VAS ratings of eighteen graded noxious heat stimuli of three different temperatures (35, 45, or 50°C) delivered at 5 s duration in a pseudo-random fashion on each calf. To minimize sensitization, habituation or hyperalgesia, all trials were separated by a minimum of 30 s and were performed on previously unstimulated sites of the skin (Pedersen and Kehlet, 1998a, b).

Finally, subjects received identical stimulus paradigms that would be used during the functional magnetic resonance imaging (fMRI) session to familiarize them with the temporal sequence of stimuli within a series to minimize variations in cognitive components such as expectation and anxiety.

Quantitative testing of sensory thresholds

Tactile thresholds

To quantitatively assess tactile thresholds and deficits of areas that may be affected by the lesions, von Frey filaments were used to examine the ventral forearms and dorsal calves bilaterally using the methods of constant stimuli. Minimum force (in Newtons) required for subjects to consistently (>~75%) detect touch for each of the areas
was recorded. Each body area was tested a variable number of times as the threshold was successively approximated with different von Frey filaments.

**Thermal thresholds**

Heat pain threshold, cold pain threshold, warm detection threshold, and innocuous cool detection threshold of both right and left calves were determined by the method of limits. For each of the four modalities of interest, the 32×32-mm² thermode was applied to the dorsal calf. For warm detection and heat pain thresholds, the temperature was increased at 1°C/s from 35 to 50°C. Subjects were then asked to indicate either the transition point at which the baseline temperature transitions into a warm sensation (warm detection) or when nonpainful warm sensation changed into a painful heat sensation (heat pain) by pressing a button. For innocuous cool and cold pain thresholds, temperature was decreased at 1°C/s from 35 to 0°C. Subjects were subsequently asked to indicate the transition point at which the baseline temperature changed into a cool sensation (innocuous cool) or when nonpainful cool sensation transitions into a painful cold sensation (cold pain). For each of the modalities measured, the test was repeated successively six times and the mean threshold temperature was calculated. To minimize sensitization, habituation or hyperalgesia, all trials were separated by a minimum of 30 s and were performed on previously unstimulated skin sites (Pedersen and Kehlet, 1998a, b).
Functional imaging

The fMRI session consisted of 8 series (4 during stimulation of the unaffected side, 4 during stimulation of the affected side, alternating between sides). Long duration noxious stimuli were delivered using a block design (49°C, 30 s on 30 s off, 5 cycles) with baseline temperature of 35°C. At the end of each fMRI series, the subjects were asked to provide overall pain intensity and unpleasantness VAS ratings. The dorsal calves were selected as sites of stimulation because the primary somatosensory cortex (SI) leg representations were far removed from the areas affected by the strokes. This ensures that results seen are due to lesions affecting the insular cortex, and not SI.

Image acquisition and image processing

fMRI data were acquired on a 1.5T General Electric Twin-Speed LX Scanner with a birdcage quadrature head coil (General Electric Medical Systems, Milwaukee, WI). For functional imaging, blood oxygenation level-dependent images of the entire brain were acquired continuously by using single-shot echoplanar imaging [echo time (TE), 40 ms; repetition time (TR), 2 s; 28 x 5-mm-thick slices; in-plane resolution, 3.72 x 3.75 mm; flip angle, 90°; no slice gap] (Ogawa et al., 1990). Each fMRI series consisted of 165 volumes and lasted 350 seconds long with 20 seconds equilibration time at the beginning of each series. During each fMRI acquisition series, subjects were requested to close their eyes. High-resolution structural scans were acquired using a three-dimensional (3D) spoiled gradient-echo (3D inversion spoiled gradient-recalled acquisition in a steady state) sequence (inversion time, 600 ms; TR, 9.1 ms; flip angle,
20°; TE, 1.98 ms; matrix, 256 x 196; section thickness, 1.5 mm with no gap between sections; 124 sections; in-plane resolution, 0.9375 x 0.9375 mm; field of view, 24 cm).

The functional image analysis package FSL [Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (Center for FMRIB, University of Oxford, Oxford, UK)] was used for image processing and statistical analysis. The functional data were movement corrected, spatially smoothed by 5 mm full-width at half-maximum (FWHM) with a 3D isotropic Gaussian kernel, and temporally filtered by a nonlinear high-pass filter with a cutoff period of 90 s. Each functional image was scaled by its mean global intensity (intensity normalization). Next, each subject's functional images were registered to their structural data using a seven-parameter linear 3D transformation and transformed into standard stereotaxic space (as defined by the Montreal Neurological Institute) using a 12-parameter linear 3D transformation (Talairach and Tournoux, 1988; Jenkinson et al., 2002). Using animated time series, visual inspection confirmed that spatial transformations during registration and movement correction were successfully accomplished.

Statistical analysis of psychophysical data

To examine the effects of insular lesions on pain, we compared pain intensity and unpleasantness ratings between affected and unaffected sides within each patient using both one-factor and two-factor analyses of variance (ANOVA) (JMP software; SAS Institute, Cary, NC). To determine if lesioned subjects still retained the capacity to evaluate pain intensity evoked by graded noxious stimuli (35, 45, 50°C, 5 s), two-factor within-subjects ANOVAs were used to examine the effects of body side and stimulus
temperature on pain intensity and unpleasantness ratings within each patient. For long duration noxious stimulation during fMRI (49°C, 30 s on 30 s off, 5 cycles), we used separate ANOVAs (affected and unaffected sides) to determine if lesioned patients exhibited different sensitivity than healthy control subjects. Since the small number of lesioned subjects (N=2) complicates within-group variance estimates, we also used descriptive statistics using 90\textsuperscript{th} and 10\textsuperscript{th} percentile range of controls data as additional criteria to determine the significance of sensory alterations displayed by each patient.

**Statistical analysis of regional signal changes within the brain**

Pain-related activations were examined using simple boxcar functions. The regressor was convolved with a gamma-variate model of the hemodynamic response (delay, 6 s; SD, 3 s) and its temporal derivative and was temporally filtered with the same parameters as the fMRI data. For each individual, fixed effects general linear modeling (GLM) analyses were used to identify brain activation associated with the modeled hemodynamic response function (HRF) (Woolrich et al., 2001) while random effects analyses were used to assess activation across individuals. $Z$ (Gaussianised T/F) statistic images were thresholded using clusters determined by $z>2.3$ and a (corrected) cluster significance threshold of $p<0.05$ (Worsley et al., 1992).

**Region of interest (ROI) analysis**

To increase statistical power to detect activations within the insular cortex, an insula region of interest was used in the analysis to reduce the number of voxels examined to determine if ipsilateral insular activation (contralateral insular activation not
possible due to the extent of the lesions) had occurred when the affected (right) body side was stimulated. The mask was generated from activation data of stimulation of the unaffected (left) side. Additionally, to determine whether there were any differential activations of SI between stimulation of the affected and unaffected sides that may explain altered pain processing, we also examined SI using an ROI analysis.

In the patients, the left SI ROI mask was generated from activation data during stimulation of affected (right) side. This mask was then used to examine the activation within the left SI ROI during stimulation of the affected (right) side. However, no detectible right SI activation was noted during stimulation of the unaffected (left) side. Therefore, to examine right SI activity during the stimulation of the unaffected (left) side, the left SI ROI mask generated from stimulation of the affected (right) side was flipped left-to-right. In the controls, the left and right SI ROI masks were generated from activation data during stimulation of the right and left body sides, respectively. Next, we calculated the left SI/right SI ROI activation ratio. One-factor ANOVA was then used to determine the effects of lesions on this ratio. In addition, patients’ data were only considered significantly different from controls if they fell outside of the 90th and 10th percentile range of control subjects' data.
Results

Patients

Radiologic findings

Patient 1: There were no acute intracranial abnormalities (Fig. 1). There had been a remote segmental left middle cerebral artery infarction with encephalomalacia of the anterior left temporal lobe and portions of the insular cortex including the posterior insula. There was also an infarction involving the left putamen (Fig. 1). These left middle cerebral artery branch infarctions led to brain volume loss as well as associated *ex vacuo* dilation of the left lateral ventricle and Wallerian degeneration extending into the left cerebral peduncle and pons. Patient 1’s lesions involved large portions of the insula, parts of SII, basal ganglia, and white matter (Fig. 1). Minor chronic small vessel ischemic changes were also present in the deep white matter. At the time of the experiment, the time after stroke was 12 years (Table 1).

Patient 2: There were no acute intracranial abnormalities (Fig. 1). There was a large remote left middle cerebral artery infarction with associated *ex vacuo* dilation of the left lateral ventricle and Wallerian degeneration extending through the internal capsule and into the left cerebral peduncle and left pons. Along the medial margin of this infarction were areas of increased signal on T1-weighted images, likely due to cortical laminar necrosis. Patient 2’s lesions were more extensive than patient 1’s and involved large portions of the insula and SII as well as parts of basal ganglia and white matter (Fig. 1). Diffuse brain volume loss was noted. Note was also made of abnormal increased signal in the distal left internal carotid artery, consistent with severely diminished flow versus complete occlusion of the left internal carotid artery. At the time of the
experiment, time after stroke was 1 year (Table 1). It should be noted that patient 2 also suffered from hemorrhagic transformation following his ischemic stroke.
Figure 1. High resolution T1 images showing the extent of the lesions. Both patients had large left middle cerebral artery (MCA) ischemic strokes with lesions encompassing the insular cortex. Patient 1’s lesions involved large portions of the insula, parts of second somatosensory cortex (SII), basal ganglia, and white matter. Patient 2’s lesions were more extensive than patient 1’s and involved large portions of the insula and SII, parts of basal ganglia and white matter. Patient 2 also suffered from hemorrhagic transformation of the ischemic stroke.
Table 1. Clinical features and tactile thresholds of patients. Patient 1 did not display any tactile deficits compared to the controls. Patient 2’s thresholds were slightly higher than those of the controls with thresholds of affected (right) side being slightly higher than those of the unaffected body side.
Table 1. Clinical features and tactile thresholds of patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age at onset</th>
<th>Age at testing</th>
<th>Time after stroke (yrs)</th>
<th>Tactile thresholds ± SEM (Newtons)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left arm</td>
</tr>
<tr>
<td>Patient 1</td>
<td>M</td>
<td>41</td>
<td>53</td>
<td>12</td>
<td>1.65</td>
</tr>
<tr>
<td>Patient 2</td>
<td>M</td>
<td>58</td>
<td>59</td>
<td>1</td>
<td>4.08</td>
</tr>
<tr>
<td>Control</td>
<td>6M</td>
<td>N/A</td>
<td>59 (mean)</td>
<td>N/A</td>
<td>3.15 ±0.17</td>
</tr>
</tbody>
</table>
Neurologic findings and tactile thresholds

Patient 1: Sensory examination was normal throughout position and vibration. von Frey testing showed that the patient did not display tactile deficits in any areas tested when compared to those of controls (Table 1). There was residual right hemiparesis: biceps strength was 4/5, wrist flexion and finger flexion were 3+/5 on the Medical Research Council (MRC) scale. For the right leg, strengths of hip flexors, knee flexors and extensors were 4+/5. There was no aphasia or impaired cognition. No central post-stroke pain (CPSP) was noted. No tremor or spasticity was noted.

Patient 2: There was mild decreased sensation to touch and pin prick of the right face, arm, and leg. von Frey testing showed that although the patient displayed some tactile deficits, these were relatively mild suggesting that the patient’s perception of touch was largely intact (Table 1). There was distal weakness involving the right upper extremity. For right upper extremity strength, finger extension was 4-/5, finger flexion was 4+/5 and proximal strength, including the deltoids, was 4+/5. Leg strength was 5/5. There was no aphasia or impaired cognition. No CPSP was noted. No tremor or spasticity was noted.

Thermal thresholds

Patient 1: The innocuous cool detection thresholds of both affected and unaffected sides were roughly symmetric and were in the range of those of the controls (Fig. 2). The cold pain threshold of the affected (right) body side was slightly lower than that of the unaffected side. However, thresholds of both sides were in the range of those of the controls. The innocuous warm detection threshold of the affected side was slightly
higher than that of the unaffected side. The heat pain threshold of the unaffected side was markedly lower than that of the affected side as well as of the controls (Fig. 2). In fact, although the heat pain threshold of the affected side was normal, the heat pain threshold of the unaffected side was in the range of the innocuous warm detection threshold, and is consistent with allodynia.

Patient 2: The innocuous cool detection threshold of the affected side was markedly lower than that of the unaffected side and was in the range of cold pain threshold of the controls (Fig. 2). For cold pain, patient 2 did not report feeling any pain even at 0°C on both affected and unaffected sides. For innocuous warm detection, the threshold of the unaffected side was normal, but the threshold of affected (right) side was markedly elevated and was in the range of heat pain threshold. Additionally, it is important to note that both innocuous warm and cool detection thresholds in this patient were asymmetric between affected and unaffected sides (Fig. 2). In contrast, heat pain thresholds of both affected and unaffected sides were normal and symmetric.

These results (Fig. 2 and Table 1) suggest that the lesions likely affected brain areas more involved in temperature sensations than tactile sensations, consistent with the role of the insular cortex in temperature sensation (Craig et al., 2000).
Figure 2. Thermal thresholds (means±SEM). Disturbances in temperature sensations seen in patients are consistent with lesions to the insula. *Patient 1:* Although the heat pain threshold of the affected side was normal, the threshold of the unaffected side was in the range of the innocuous warm detection threshold, and is consistent with allodynia. 

*Patient 2:* The innocuous cool detection threshold of the affected side was markedly lower than that of the unaffected side and controls, and was in the range of the cold pain threshold. In addition, patient reported feeling no cold pain even at 0°C (*). The innocuous warm detection threshold of the affected side was markedly elevated when compared to the unaffected side and controls, and was in the range of the heat pain threshold. Both innocuous warm and cool detection thresholds were asymmetric between sides. For each patient, only the average of 6 measurements per thermal threshold was recorded, so standard error of the mean could not be calculated.
Patients retain the ability to discriminate pain intensity despite lesions

Both patients were able to evaluate brief noxious stimuli of graded intensities (35, 45, 50°C, 5 s) applied to the dorsal calf on affected and unaffected sides. Both patients exhibited monotonic increases in VAS ratings of pain intensity and unpleasantness as stimulus temperature increased (patient 1: pain intensity: $F(2,10)=133.3144$, $p<0.0001$, pain unpleasantness: $F(2,10)=30.7348$, $p<0.0001$; patient 2: pain intensity: $F(2,10)=172.0429$, $p<0.0001$, pain unpleasantness: $F(2,10)=106.7061$, $p<0.0001$)(Fig. 3). No significant effect of body side was observed (patient 1: pain intensity: $F(1,5)=3.7824$, $p=0.1094$, pain unpleasantness: $F(1,5)=0.4547$, $p=0.5300$; patient 2: pain intensity: $F(1,5)=4.7348$, $p=0.0815$, pain unpleasantness: $F(1,5)=1.4650$, $p=0.2802$)(Fig. 3). These results suggest that patients’ ability to discriminate pain intensity still remained intact despite large unilateral lesions of the insula. During graded noxious stimulation of the controls, subjects exhibited monotonic increases in VAS ratings of pain intensity and unpleasantness as stimulus temperature increased (intensity: $F(2,12)=34.6503$, $p<0.0001$; unpleasantness: $F(2,12)=19.1711$, $p=0.0002$)(Fig. 3). There was no significant main effect of body sides on pain intensity and unpleasantness ratings (intensity: $F(1,13)=3.3841$, $p=0.0888$; unpleasantness: $F(1,13)=0.0013$, $p=0.9714$)(Fig. 3).
Figure 3. Pain intensity and unpleasantness VAS ratings during the graded noxious stimulation (means±SEM). Both patients retained ability to discriminate noxious stimuli of graded intensities. Both exhibited monotonic increases in VAS ratings of pain intensity and unpleasantness as stimulus temperature increased.
Elevated pain ratings in patients with insular lesions

During long duration noxious stimulation (30 s on, 30 s off, 5 cycles, 49°C) during the fMRI session, both patients exhibited significantly higher VAS ratings of pain intensity of the affected (right) body side as compared to those of the control group (F(1,13)=7.3720, p=0.0177)(Fig. 4A). Interestingly, both patients’ pain unpleasantness ratings of the affected body side did not significantly differ from those of the control group, although patient 2 displayed a trend towards elevated pain unpleasantness on the affected side (F(1,13)=2.3825, p=0.1467)(Fig. 4B). Both patients’ pain intensity and unpleasantness ratings of the unaffected (left) body side did not significantly differ from those of the control group (pain intensity: F(1,13)=3.0652, p=0.1035; pain unpleasantness: F(1,13)=0.0950, p=0.7629)(Fig. 4), although patient 1’s pain intensity ratings during both short and long duration stimulation of unaffected (left) body side were higher than any of the individual control subjects (Fig. 3 and 4A).

Each patient responded very differently during long duration noxious stimulation. Patient 1 showed markedly elevated pain intensity ratings across both body sides. In fact, patient 1’s pain intensity ratings of both body sides were higher than any of the individual control subjects (Fig. 4A). More interestingly, despite significantly elevated pain intensity ratings, patient 1 exhibited relatively normal pain unpleasantness ratings across both body sides (Fig. 4B). These results suggest that an altered pain affect processing and a disconnect between the two dimensions of pain may have occurred. Patient 2, on the other hand, displayed elevated pain intensity and unpleasantness ratings on the affected without much disparity between the two dimensions of pain (Fig. 4). In fact,
both pain intensity and unpleasantness ratings of the affected body side of patient 2 were higher than any of the individual control subjects. Nevertheless, although both patients responded differently, elevated pain ratings and hypersensitivity, in general, were noted in both patients.
Figure 4. Pain intensity and unpleasantness VAS ratings during the long duration noxious stimulation (means±SEM). The solid squares indicate individual control’s data. The solid triangles indicate 90th percentile of the control’s data. Both patients exhibited significantly elevated pain intensity ratings as compared to those of the control group (A). However, the effect of lesion on pain unpleasantness was not statistically significant, although patient 2 displayed a trend towards elevated pain unpleasantness on the affected side (B). Additionally, each patient responded differently. While patient 2 had asymmetric pain ratings between sides without much disparity between the two pain dimensions, patient 1 had elevated pain intensity ratings across sides with normal pain unpleasantness.
Asymmetry of pain ratings between affected and unaffected body sides

To determine if there are significant differences in pain ratings between the two sides, we examined pain intensity and pain unpleasantness ratings between affected and unaffected sides within each patient. During long duration noxious stimulation, patient 1’s pain intensity and unpleasantness ratings did not significantly differ between affected and unaffected sides (pain intensity: F(1,3)=3.9452, p=0.1412; pain unpleasantness: F(1,3)=0.60, p=0.4950)(Fig. 4). In patient 2, both pain intensity and unpleasantness ratings were significantly greater on the affected side than they were on the unaffected side (pain intensity: F(1,3)=11.4165, p=0.0431; pain unpleasantness: F(1,3)=13.15491, p=0.0361)(Fig. 4). In the control group, there was no difference in pain intensity and unpleasantness ratings of long duration noxious stimuli applied to left and right sides (intensity: F(1,12) = 2.8813, p =0.1154; unpleasantness: F(1,12) = 0.0053, p=0.9429)(Fig. 4). This suggests that although some disturbances in pain and temperature sensations were shared by both patients, other aspects of pain processing may be differentially altered in each patient due to differences in their lesions and other factors unique to each individual.

Activations of the insula and other pain-related activations

During long duration noxious stimulation (49°C, 30 s on 30 s off, 5 cycles) of the dorsal calf of the unaffected (left) body side in both patients, pain-induced brain activations were identified within the ACC, supplementary motor area (SMA), SII, insula, dorsolateral prefrontal cortex (DLPFC) and cerebellum (Fig. 5 and Table 2).
However, it is important to note that although patient 1’s ACC activation was largely within the right hemisphere, patient 2’s ACC activation appears to be largely within the left hemisphere. No thalamic activations were detected in either patient. Stimulation of the left side in controls activated ACC, SMA, SII, insula, SI, thalamus, and cerebellum (Fig. 5 and Table 2). These patterns of activations are consistent with normal brain activations during pain (Coghill et al., 1994; Peyron et al., 2000; Coghill et al., 2001; Oshiro et al., 2007). In the control group, insular activations were detected bilaterally. In the patient group, since the left insula was heavily damaged, only the right insula could have been activated (Fig. 5 and Table 2). Upon stimulation of the unaffected (left) body side, contralateral (right) insular activation was detected in both patients.

During painful stimulation of the affected (right) body side in patient 1, pain-induced brain activations were identified within the SMA, SI, SII, DLPFC, the frontal operculum, and the cerebellum (Fig. 5 and Table 2). In patient 2, stimulation of the right (affected) leg activated SI, SII, SMA, cerebellum, ACC and the contralateral (left) thalamus (Fig. 5 and Table 2). It is interesting to note that patient 2 displayed a robust activation of the contralateral (left) thalamus, although no ipsilateral thalamic activation was detected. Nevertheless, neither patient displayed any ipsilateral (right) insular activation (Fig. 5 and Table 2). In both patients only the ipsilateral (right) insula could be activated due to damage to the contralateral (left) insula. During stimulation of the right dorsal calf of the control group, pain activations detected were similar to those during stimulation of the left leg and included bilateral insular activation (Fig. 5 and Table 2). In addition, insular ROI analysis was used to examine the possibility of subthreshold activation of the insula in patients. ROI analysis of the insula showed that stimulation of
the unaffected body side in both patients produced a detectable contralateral insular
activation within the insular ROI while stimulation of the affected body side still did not
produce any detectable activation within the ipsilateral insula. This suggests that insular
activation may not be necessary for a conscious experience of pain, and that contralateral
insular activation appears to be necessary in order to produce ipsilateral insular
activation.
Figure 5. Pain-related activation of the insula. Stimulation of the unaffected (left) leg in patients activated similar brain areas as in controls. However, in the patients only the right (contralateral) insula was activated since the left insula was damaged by the lesions. In the controls, insular activation was detected bilaterally. In contrast, painful stimulation of the affected (right) leg in patients did not produce any detectable right (ipsilateral) insular activation. This suggests that insular activation may not be necessary for a conscious experience of pain, and that contralateral insular activation appears to be necessary in order to produce ipsilateral insular activation. Structures named at the bottom are for both controls and patients. VMPFC, ventromedial prefrontal cortex; DLPFC, dorsolateral prefrontal cortex; PCC, posterior cingulate cortex; dACC, dorsal anterior cingulate cortex; ACC/SMA, anterior cingulate cortex/supplementary motor area.
Table 2. Pain-related brain activations. Peak Z scores for the controls were obtained from group analysis. Cluster sizes and peak locations are listed within parentheses as the number of voxels and x,y,z coordinates (in millimeters) according to standard stereotaxic space. IPL, inferior parietal lobule; SPL, superior parietal lobule; -, no statistically reliable change.
<table>
<thead>
<tr>
<th>Region</th>
<th>Patient 1’s side of stimulation</th>
<th>Patient 2’s side of stimulation</th>
<th>Controls’ side of stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L [90x115]</td>
<td>R [90x115]</td>
<td>L [90x115]</td>
</tr>
<tr>
<td><strong>Cerebellum</strong></td>
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<td></td>
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<tr>
<td>Right</td>
<td>(-)</td>
<td>3.62 (240; 50, -76,16)</td>
<td>4.73 (2254; 26, -62, -28)</td>
</tr>
<tr>
<td>Left</td>
<td>3.9 (408; -38,-60, -36)</td>
<td>4.39 (342; -16,-72, -40)</td>
<td>4.02 (2254; -48, -64, -24)</td>
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<tr>
<td><strong>Thalamus</strong></td>
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<td>Right</td>
<td>(-)</td>
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<tr>
<td>Left</td>
<td>(-)</td>
<td>(-)</td>
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<tr>
<td><strong>Globus pallidus/ Putamen</strong></td>
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<td></td>
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<tr>
<td>Right</td>
<td>(-)</td>
<td>(-)</td>
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<tr>
<td>Left</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
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<tr>
<td><strong>Anterior insula</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>6.48 (2003; 42,28,6)</td>
<td>(-)</td>
<td>7.64 (1286; 34,22,6)</td>
</tr>
<tr>
<td>Left</td>
<td>(-)</td>
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<tr>
<td><strong>Posterior insula</strong></td>
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<td>Right</td>
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<td>Left</td>
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<tr>
<td><strong>Prefrontal cortex</strong></td>
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<tr>
<td>Right</td>
<td>3.949 (2003; 44,42,2)</td>
<td>3.7 (435; 52,46,0)</td>
<td>4.22 (555; 42,52,0)</td>
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<td>(-)</td>
<td>2.61 (188; -38,54,12)</td>
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</tr>
<tr>
<td>Right</td>
<td>2.52 (169; 12,48)</td>
<td>3.463 (239; 61,10,56)</td>
<td>(-)</td>
</tr>
<tr>
<td>Left</td>
<td>(-)</td>
<td>(-)</td>
<td>4.32 (216; -2,6,38)</td>
</tr>
<tr>
<td><strong>IPL/SPL</strong></td>
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<tr>
<td>Right</td>
<td>(-)</td>
<td>(-)</td>
<td>3.06 (417; 64,-36,36)</td>
</tr>
<tr>
<td>Left</td>
<td>(-)</td>
<td>4.54 (417; -22,-54,64)</td>
<td>(-)</td>
</tr>
<tr>
<td><strong>SI (leg)</strong></td>
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<td>3.38 (417; -16,-38,66)</td>
<td>(-)</td>
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<tr>
<td><strong>SH</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Right</td>
<td>4.71 (2003; 68,-12,18)</td>
<td>3.12 (255; 52,-10,10)</td>
<td>5.44 (417; 66,-24,22)</td>
</tr>
<tr>
<td>Left</td>
<td>4.22 (151; -60,-24,18)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td><strong>Supplementary Motor Area</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>3.48 (169; 6,6,58)</td>
<td>3.74 (239; 6,6,60)</td>
<td>(-)</td>
</tr>
<tr>
<td>Left</td>
<td>3.81 (192; -12,-22,62)</td>
<td>4.096 (218; -4,12,40)</td>
<td>6.542 (11952; -6,-32,68)</td>
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</tbody>
</table>
| <deactivation>         | -3.89 (1280; 8,-403 (1152; -6,-42 (1175; -4,60,092; 2,-4,53 (27536; -6,-4029 (14209; 6,-115)}
<table>
<thead>
<tr>
<th>Cingulate Cortex</th>
<th>70,22</th>
<th>10,56,18</th>
<th>2,58,28</th>
<th>38,26</th>
<th>58,16</th>
<th>52,30</th>
</tr>
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<tbody>
<tr>
<td>VMPFC</td>
<td>-5.33 (2123; -6,60,10)</td>
<td>(-)</td>
<td>-4.62 (898; 8,54,18)</td>
<td>-3.708 (9488; 14,64,16)</td>
<td>-4.036 (27536; -12,58,10)</td>
<td>-4.53 (8370; 2,56,-6)</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>-4.29 (257; -50,-72,8)</td>
<td>-4.79 (1152; 6,-62,20)</td>
<td>(-)</td>
<td>(-)</td>
<td>-3.907 (27536; 40,-74,28)</td>
<td>-4.118 (14209; -28,-78,30)</td>
</tr>
</tbody>
</table>
Differences in SI activation between lesioned subjects and healthy controls

During stimulation of the unaffected (left) body side in both patients, there were no detectable SI activations (Fig. 6 and Table 2). In contrast, stimulation of the affected (right) body side produced robust contralateral SI activations in both lesioned subjects (Fig. 6 and Table 2). It appears that SI activations in patients only became sufficiently large to be reliably detected during stimulation of the affected body side when insular activation was not present (Fig. 5, 6 and Table 2). Stimulation of the either side in controls generated reliable contralateral SI activation.

SI ROI analysis was used to determine whether these differences in contralateral SI activation between stimulation of affected and unaffected sides were significantly different from healthy controls. Both patients’ left/right SI ROI activation ratios were significantly greater than those of the controls (F(1,13)=12.5121, p=0.0036)(Fig. 7). In fact, both patients’ ratios were greater than any of the individual control subjects (Fig. 7). These results suggest that in the absence of the insula, brain areas such as SI may be recruited to help with the processing of nociceptive information.
Figure 6. SI activation during pain. Stimulation of the unaffected (left) side did not generate any SI activation in both patients. Interestingly, stimulation of the affected (right) body side produced robust contralateral SI activations in both patients. It appears that SI activations in patients may only be reliably detected during stimulation of the affected side when insular activation was not present (Fig. 5). These results suggest SI may be recruited to help with processing of nociceptive information following insular damage.
Figure 7. Left/right SI ROI activation ratio. The solid squares indicate individual control’s data. The solid triangles indicate 90th percentile of the control’s data. In both patients, the left/right SI ROI activation ratios were significantly greater than those of the controls. Thus, contralateral SI activation during stimulation of the affected (right) side relative to contralateral SI activation during stimulation of the unaffected (left) side was significantly greater in patients than the controls. This suggests that in the absence of insula, brain areas such as SI may be recruited to help with the processing of nociceptive information.
Recruitment of DLPFC activation in lesioned subjects

Right DLPFC activations during painful stimulation were detected in both patients (Fig. 8 and Table 2). Painful stimulation of the unaffected (left) body side activated the right DLPFC in both patient 1 and patient 2 but not in the controls (Fig. 8 and Table 2). However, stimulation of the affected (right) body side activated the right DLPFC in patient 1, but not in patient 2 or controls (Fig. 8 and Table 2). Although DLPFC activation has been reported during spatial discrimination of pain (Oshiro et al., 2007), it was not detected in the controls in this investigation. Right DLPFC activations seen in our patients may suggest increased burden on the remaining neural networks to process nociceptive information in the face of insular damage.
Figure 8. DLPFC activation during pain. Right DLPFC activations during painful stimulation were detected in both patients, but not in the controls. Painful stimulation of the unaffected (left) side activated the right DLPFC in both patients but not in the controls. However, stimulation of the affected (right) body side activated the right DLPFC in patient 1, but not in patient 2 and controls. Right DLPFC activations may represent recruitment of additional brain areas to help with processing nociceptive information in the face of insular damage. DLPFC, dorsolateral prefrontal cortex.
**Discussion**

Although the insular cortex has been frequently shown to be activated during the processing of pain, the specific roles that this structure can play in the generation of a pain experience remain poorly characterized. Stimulus-response studies suggest that it may be positively related to the perceived magnitude of pain and, accordingly, may be involved in sensory-discriminative processing (Derbyshire et al., 1997; Coghill et al., 1999). However, both patients retained the ability to provide ratings of graded pain intensity during noxious stimulation of their affected side despite having large areas of the insular cortex damaged by strokes and exhibiting no detectible activity in either the contralateral or ipsilateral insular cortex. In sharp contrast to the diminished experience of pain that would be predicted from previous studies of insular lesions, both patients exhibited significantly increased ratings of pain intensity. When taken together with the insular activation detected in studies that evoke analgesia by either pharmacological or psychological manipulations, this suggests that the insula may instead play a complex, modulatory role in the processing of nociceptive information (Petrovic et al., 2002; Lorenz et al., 2003; Derbyshire et al., 2004; Koyama et al., 2005; Zubieta et al., 2005; Kong et al., 2006; Craggs et al., 2007; Kong et al., 2007). Moreover, during stimulation of the affected sides, both lesion patients exhibited consistently greater activation of SI than during stimulation of the unaffected side (relative to healthy controls) that may reflect a recruitment of additional SI activity to compensate for the loss of the contribution of the insula to sensory processing. These findings indicate that insular activation, although frequently observed in studies of pain, may not be necessary to elicit
a conscious pain experience. Thus, subjective awareness of pain intensity can be realized via multiple, distinct patterns of brain activity.

**Specificity of insular lesions**

Systematic studies of patients with brain lesions affecting the insular cortex have been rarely performed due to the difficulty of recruiting individuals who are MRI compatible, free from central post-stroke pain, and do not exhibit aphasia. Furthermore, the interpretation of lesion studies is complicated by the uniquely individual patterns of damage. However, when commonalities between individuals are related to overlapping regions of damage, substantial insights into the function of the damaged region can be derived. In the present investigation, the lesions affected large portions of the insular cortex with patient 2’s lesions being more extensive than patient 1’s. Despite the differences in the extent of each individual's lesion, both individuals consistently reported abnormally high sensitivity to pain and exhibited marked increases in the activity of the left primary somatosensory cortex (ipsilateral to the lesioned insula). These common findings in both patients further imply that damaged brain areas common to both patients may be responsible.

Although both patients’ lesions involved structures outside the insular cortex such as the basal ganglia and white matter tracts, the disturbances in temperature sensations that both patients exhibited are consistent with the damage to the insula since this structure is thought to be involved in the processing of temperature sensations (Craig et al., 2000). Neither patient displayed motor symptoms such as spasticity or tremors that would be characteristic of basal ganglia lesions. In fact, ongoing studies show reduced
pain sensitivity in patients with basal ganglia lesions in contrast to the increased pain sensitivity observed in the present investigation (unpublished observations).

We also selected the lower legs as sites of stimulation because the SI leg representations were far removed from brain areas affected by the strokes. Mild tactile deficits in our patients suggest that SI and thalamo-cortical projections to SI were minimally affected by the strokes since lesions affecting SI can significantly disrupt tactile sensation (Knecht et al., 1996). Furthermore, stimulation of the affected leg in both patients generated pronounced activation of SI. These findings suggest that the psychophysical differences seen in our study were primarily due to lesions affecting the insula, and did not result from either direct damage to SI or from disruption of thalamo-cortical projections to SI.

Although lesions affected some portions of SII and posterior insula, we did not see hypoalgesia, as expected of SII and posterior insular lesions (Greenspan and Winfield, 1992; Greenspan et al., 1999). Thus, these results may likely be due to damage that also involves the anterior insula. Although the functions of posterior and anterior insula differ due to their distinct anatomical connections, both divisions are extensively interconnected (Mesulam and Mufson, 1982; Mufson and Mesulam, 1982; Friedman et al., 1986). Thus, damage to either division may disrupt the transfer of information and the function of both divisions of the insula. Nevertheless, ascribing the alterations in pain sensitivity to the anterior insula should be done with caution since portions of both anterior and posterior insula were damaged.
Altered sensory processing and insular lesions

To date, little is known about how insular lesions alter the processing of suprathreshold noxious stimuli. Previous studies using sensory testing indicate that pain thresholds are elevated following lesions involving the posterior insula and parietal operculum, but not the anterior insula (Greenspan and Winfield, 1992; Greenspan et al., 1999). During testing with suprathreshold stimuli, individuals with insular lesions have been reported to exhibit pain asymbolia - a reduced appreciation of the meaning and significance of noxious stimuli while retaining the capacity to identify such stimuli as painful (Berthier et al., 1988). Although the partial dissociation of pain intensity and unpleasantness observed in patient 1 may be consistent with reports of pain asymbolia, the view that insular lesions preferentially alter cognitive/emotional appreciation of pain may be oversimplified. In response to long duration noxious stimuli, both lesion subjects in the present investigation exhibited pain sensitivity that was substantially higher than age-matched control subjects, but neither had ratings of pain unpleasantness that were significantly below normal.

In contrast to other studies that show strong insular activation without SI activation (Derbyshire et al., 1994; Tolle et al., 1999; Peyron et al., 2000), the absence of insular contribution to nociceptive processing appears to be associated with a substantial increase in SI activation during nociceptive stimulation. Thus, SI and its associated networks may be recruited to help with processing of nociceptive information following insular damage. Additionally, right DLPFC activation noted in our patients may represent processes related to the evaluation of nociceptive information, reflecting increased demands on the remaining neural networks following insular damage. These
findings suggest that there are multiple ways that the brain can process nociceptive information and instantiate an experience of pain.

Top-down signal modulation and elevation of pain ratings following insular lesions

Both patients exhibited increased pain ratings relative to those of normal controls. Such increases in pain sensitivity may be indicative of loss of descending inhibitory control, since the insular cortex is connected to regions such as the ACC and DLPFC (Mesulam and Mufson, 1982; Mufson and Mesulam, 1982) that are associated with activation of the PAG (Lorenz et al., 2003; Gebhart, 2004; Ohara et al., 2005; Kong et al., 2007; Tracey and Mantyh, 2007). However, converging lines of evidence from expectation, hypnosis, and placebo studies suggest that the insula may play a role in pain modulation by tuning the responsiveness of other brain areas via cortico-cortical interactions (Petrovic et al., 2002; Lorenz et al., 2003; Derbyshire et al., 2004; Koyama et al., 2005; Zubieta et al., 2005; Kong et al., 2006; Craggs et al., 2007; Kong et al., 2007). Complex cognitive information related to mood, previous experience, expectation, and emotion may flow from various brain networks involving the amygdala, hippocampus, ACC and prefrontal cortex to the anterior insula to be integrated with nociceptive information (Mufson et al., 1981; Mesulam and Mufson, 1982; Mufson and Mesulam, 1982; Friedman and Murray, 1986; Friedman et al., 1986). In the environmental context of an experimental setting, the insula may encode a safety signal that tunes afferent sensory processing mechanisms to reflect the prior information that the noxious stimulus is safe and well-controlled and will not result in long-term damage. These interactions may contribute importantly to modulation of pain affect and pain intensity processing in a
context-relevant fashion. This ability to use internal knowledge to modulate the gain of incoming information through top-down signal modulation may be disrupted by insular lesions. As a result, pain ratings were elevated.

Conclusion

The insula is well positioned to both send and receive information from areas important in sensory processing as well as memory retrieval, attention, and affect (Mufson et al., 1981; Mesulam and Mufson, 1982; Mufson and Mesulam, 1982; Friedman and Murray, 1986; Friedman et al., 1986). This dynamic, bidirectional interactive network provides a substrate for the insula to integrate higher-level internal cognitive information with incoming afferent sensory information. Moreover, the insula may selectively gate nociceptive information at the cortical level to modulate varying levels of appreciation of the stimulus. Through utilizing complex cognitive information to provide modulation of cognitive-evaluative, affective, and sensory discriminative dimensions of pain the insula contributes to the construction of a unique signature/fingerprint of pain experience for each individual.

Acknowledgements

This study was supported by NIH R01 NS39426. The authors thank FMRIB Image Analysis Group, Oxford University for the FSL analysis software and Stephania Jordan for her help in collecting data for the experiment.
REFERENCES


Chapter 4

ROLES OF THE PUTAMEN IN THE PAIN EXPERIENCE: INSIGHTS FROM FUNCTIONAL AND STRUCTURAL CONNECTIVITY AND BRAIN LESIONS


The following manuscript has been submitted. Stylistic variations are due to the requirements of the journal. Christopher Starr designed the paradigm, collected data, and prepared the manuscript. Dr. Robert Coghill acted in an advisory and editorial capacity.
Abstract

Pain is an intrinsically intrusive experience that readily overshadows competing cognitive demands. One factor that may contribute to the prepotent nature of pain is the ability of afferent nociceptive information to engage a complex network of cerebral cortical regions. Using MRI to assess structural and functional connectivity in healthy subjects, we show that the putamen is connected with the cerebral cortical regions with involvement in attention, affect, and somatosensory processing. The integration of such cognitive information with incoming nociceptive input within the putamen may importantly contribute to the selective engagement of situation-appropriate cortical networks. Consistent with this role, patients with lesions of the putamen exhibited selectively reduced pain sensitivity and diminished cortical activation.
Introduction

The putamen, together with the caudate nucleus, makes up the striatum, a major site of cortical and subcortical input into the basal ganglia (Alexander et al., 1990). Although the putamen is frequently activated during pain (Jones et al., 1991; Coghill et al., 1999; Peyron et al., 2000; Bingel et al., 2004), its significance has often been assumed as simply indicative of motor-related processing (Jones et al., 1991; Coghill et al., 1999; Bingel et al., 2004) given the long standing view of the basal ganglia as critical structures associated with movement (Albin et al., 1989; Alexander et al., 1990). However, both the putamen and caudate nuclei contain high densities of opioid receptors and have nociceptive neurons that respond to noxious stimuli of graded intensities (Chudler and Dong, 1995; Chudler, 1998; Koyama et al., 2000; Sprenger et al., 2005; Baumgartner et al., 2006). Additionally, striatal dopamine D2 receptor activity has been correlated with individual variability in pain, pain modulation, and many chronic pain syndromes (Hagelberg et al., 2002; Hagelberg et al., 2003b; Hagelberg et al., 2003a; Hagelberg et al., 2004; Pertovaara et al., 2004; Scott et al., 2006; Wood et al., 2007). Thus, the basal ganglia may play an important role in the processing and/or modulation of pain. To date, the functional significance of the putamen in pain processing remains poorly understood.

The striatum receives direct afferents from the cerebral cortex, limbic structures and the thalamus (principally the midline and intralaminar nuclei), in addition to modulatory dopaminergic inputs from the substantia nigra pars compacta (SNC) in the midbrain (Alexander et al., 1990; Parent and Hazrati, 1995; Gerfen and Wilson, 1996; Groenewegen et al., 1999; Mengual et al., 1999; Van der Werf et al., 2002). The striatum
then relays signals, via direct and indirect routes, to the principal output nuclei, namely, the internal globus pallidus (GPi) and the substantia nigra pars reticulata (SNr) (Smith et al., 1998). The output nuclei project directly to anterior regions of the thalamus. Thalamic outputs then target cortical and limbic regions from which the basal ganglia input originated forming cortico-basal ganglia–thalamo-cortical loops (Alexander et al., 1990; Parent and Hazrati, 1995; Middleton and Strick, 2000). Although these loops are largely parallel and segregated, there is evidence for interactions and convergence between the main projection lines both within the basal ganglia and between external structures (Joel and Weiner, 1994; Groenewegen et al., 1999; Haber, 2003; McHaffie et al., 2005). This organization allows the putamen, as part of the striatum, to influence cortical processing of information in a task-specific manner via differentially exerting its influence on GPi/SNr to modulate varying levels of disinhibition of the thalamus (Parent and Hazrati, 1995).

Consistent with this anatomic organization and the influence of the basal ganglia on widespread regions of the cerebral cortex, converging lines of evidence suggest that the basal ganglia may be involved in many high-level processes unrelated to motor tasks (Schultz et al., 1993; Chudler and Dong, 1995; Downar et al., 2003; Scott et al., 2006; Balleine et al., 2007). For instance, patients suffering from diseases affecting the neurotransmitters pathways of the basal ganglia as in Parkinson’s disease often display cognitive changes in response selection and decision-making (Seiss and Praamstra, 2004; Frank et al., 2007). Accordingly, the neural circuitry involving the putamen has been suggested to contribute importantly to decision-making and selection of behaviorally-relevant tasks by enabling engagement of specific sets of cortical networks needed to
accomplish the task (Redgrave et al., 1999; Balleine et al., 2007). In the case of pain, activity within the putamen and basal ganglia may influence which brain networks are engaged during the inflow of afferent nociceptive information.

The identification of the functional and structural relationship between the putamen and both cortical and subcortical regions activated during pain may provide insights into understanding the role that this structure plays in the construction of a complete subjective experience of pain. To investigate the role that the putamen and its neural correlates play in pain processing, sensory testing, fMRI, functional connectivity, and diffusion tensor imaging (DTI) were used to examine a group of healthy normals and patients with lesions affecting the putamen.
Methods

Subjects

Fourteen normal healthy adults were recruited to participate in the study. There were eight females and six males (age, 46–75 years; mean, 59 years). Out of the fourteen subjects, thirteen participated in the imaging session. One female participant did not participate in the brain imaging session due to claustrophobia. In addition, to examine how brain lesions affecting the putamen may alter pain perception, sensory thresholds, and pattern of brain activations, a group of nine stroke patients, seven male and two females, (age, 46-71; mean, 59 years) were also recruited to participate in the study. All patients suffered from left ischemic strokes with lesions encompassing the putamen (Fig. 1 and Supplementary Table 1). All subjects underwent the same experimental protocols. All study participants gave written, informed consent acknowledging that (1) they would experience experimental painful stimuli, (2) all methods and procedures were clearly explained, and (3) they were free to withdraw from the experiment at any time without prejudice. All of the procedures were approved by the Institutional Review Board of Wake Forest University School of Medicine.

Psychophysical data collection

Subjects rated pain using a 15-cm plastic visual analog scale (VAS) that has been widely used to assess pain because of ease of use while providing quantifiable measurements of pain intensity and pain unpleasantness (Parisian Novelty Co., Chicago, IL; (Price et al., 1994)). The minimum was anchored with ‘No pain sensation’ or ‘Not at all unpleasant’, while the maximum was anchored with ‘Most intense pain imaginable’ or
‘Most unpleasant imaginable’. Using an audio analogy, subjects were instructed to distinguish between pain intensity and pain unpleasantness (Price et al., 1989). All thermal stimuli used for suprathreshold stimulation were applied to the posterior aspect of the lower leg via a 16×16-mm² peltier device (Medoc TSA II, Ramat Yishai, Israel) secured with a Velcro strap. Baseline temperature was maintained at 35°C, and stimulus temperatures were delivered with rise and fall rates of 6°C/s and were feedback controlled. During a psychophysical training session, subjects rated 32 noxious heat stimuli (35, 43–49°C, 5 s duration) using the VAS in order to gain experience rating pain. These data are not reported further.

Next, subjects provided post-stimulus pain intensity and pain unpleasantness VAS ratings of eighteen graded noxious heat stimuli of three different temperatures (35, 45, or 50°C) delivered at 5 s duration in a pseudo-random fashion on each leg. To minimize sensitization, habituation or hyperalgesia, all trials were separated by a minimum of 30 s and were performed on previously unstimulated sites of the skin (Pedersen and Kehlet, 1998b, a).

Finally, subjects received identical stimulus paradigms that would be used during the functional magnetic resonance imaging (fMRI) session to familiarize them with the temporal sequence of stimuli within a series to minimize variations in cognitive components such as expectation and anxiety.
Quantitative testing of sensory thresholds

Tactile thresholds

To quantitatively assess tactile thresholds of the healthy normals and deficits of areas that may be affected by the lesions in patients, von Frey filaments were used to examine the ventral forearms and dorsal calves bilaterally using the method of constant stimuli. Minimum force (in Newtons) required for subjects to consistently (>~75%) detect touch for each of the areas was recorded. Each body area was tested a variable number of times as the threshold was successively approximated with different von Frey filaments.

Thermal thresholds

Heat pain threshold, cold pain threshold, warm detection threshold, and innocuous cool detection threshold of both right and left calves were determined by the method of limits. For each of the four modalities of interest, the 32×32-mm² thermode was applied to the dorsal calf. For warm detection and heat pain thresholds, the temperature was increased at 1°C/s from 35 to 50°C. Subjects were then asked to indicate either the transition point at which the baseline temperature transitions into a warm sensation (warm detection) or when nonpainful warm sensation changed into a painful heat sensation (heat pain) by pressing a button. For innocuous cool and cold pain thresholds, temperature was decreased at 1°C/s from 35 to 0°C. Subjects were subsequently asked to indicate the transition point at which the baseline temperature changed into a cool sensation (innocuous cool) or when nonpainful cool sensation transitions into a painful
cold sensation (cold pain). For each of the modalities measured, the test was repeated successively six times, and the mean threshold temperature was calculated. To minimize sensitization, habituation or hyperalgesia, all trials were separated by a minimum of 30 s and were performed on previously unstimulated skin sites (Pedersen and Kehlet, 1998b, a).

**Functional imaging**

The fMRI session consisted of 8 series [4 during stimulation of the left (unaffected in lesion patients) side, 4 during stimulation of the right (affected in lesion patients) side, alternating between sides]. Long duration noxious stimuli were delivered using a block design (49°C, 30 s off 30 s on, 5 cycles) with baseline temperature of 35°C. At the end of each fMRI series, the subjects were asked to provide overall pain intensity and unpleasantness VAS ratings.

**Image acquisition and image processing**

fMRI data were acquired on a 1.5T General Electric Twin-Speed LX Scanner with a birdcage quadrature head coil (General Electric Medical Systems, Milwaukee, WI). For functional imaging, blood oxygenation level-dependent images of the entire brain were acquired continuously by using single-shot echoplanar imaging [echo time (TE), 40 ms; repetition time (TR), 2 s; 28 x 5-mm-thick slices; in-plane resolution, 3.72 x 3.75 mm; flip angle, 90°; no slice gap] (Ogawa et al., 1990). Each fMRI series consisted of 165 volumes and lasted 350 s long with 20 s equilibration time at the beginning of each series. During each fMRI acquisition series, subjects were requested to close their
eyes. High-resolution structural scans were acquired using a three-dimensional (3D) spoiled gradient-echo (3D inversion spoiled gradient-recalled acquisition in a steady state) sequence (SPGR, inversion time, 600 ms; TR, 9.1 ms; flip angle, 20°; TE, 1.98 ms; matrix, 256 x 196; slice thickness, 1.5 mm with no gap between sections; 124 sections; in-plane resolution, 0.9375 x 0.9375 mm; field of view (FOV), 24 cm). In addition, to help visualize the extent of the lesion, high-resolution fast spin echo (FSE) images (TR, 4200 ms; flip angle, 90°; TE, 85 ms; matrix, 256 x 256; slice thickness, 5 mm with no gap between slices; 26 slices; in-plane resolution, 0.85937 x 0.85938 mm; FOV, 22 cm;) and high-resolution axial fluid-attenuated inversion recovery (FLAIR) (inversion time, 2000 ms; TR, 8000 ms; flip angle, 90°; TE, 120 ms; matrix, 256 x 256; slice thickness, 5 mm with no gap between slices; 26 slices; in-plane resolution, 0.85937 x 0.85938 mm; FOV, 22 cm) were also acquired.

The functional image analysis package FSL [Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (Center for FMRIB, University of Oxford, Oxford, UK)] was used for image processing and statistical analysis. The functional data were movement corrected, spatially smoothed by 5 mm full-width at half-maximum (FWHM) with a 3D isotropic Gaussian kernel, and temporally filtered by a nonlinear high-pass filter with a cutoff period of 90 s. Each functional image was scaled by its mean global intensity (intensity normalization). Next, each subject's functional images were registered to their structural data using a seven-parameter linear 3D transformation and transformed into standard stereotaxic space (as defined by the Montreal Neurological Institute) using a 12-parameter linear 3D transformation (Talairach and Tournoux, 1988; Jenkinson et al., 2002). Using animated time series,
visual inspection confirmed that spatial transformations during registration and movement correction were successfully accomplished.

**Diffusion tensor image (DTI) acquisition**

To minimize the imaging distortion associated with eddy currents, DTI data was acquired using a dual spin-echo single shot echo-planar imaging (EPI) (Reese et al., 2003). The diffusion-weighted images were acquired along 25 isotropically distributed directions with a b-value of 1000 s/mm$^2$ and a voxel size of 0.9375 $\times$ 0.9375 mm (TR, 11000 ms; TE, 79.3 ms; 128 x 128 matrix; slice thickness, 3 mm with no gap between sections; 45 sections; field of view, 24 cm).

**Statistical analysis of psychophysical data**

To examine the effects of putamen lesions on pain, we compared pain intensity and unpleasantness ratings between left (unaffected) and right (affected) sides using analyses of variance (ANOVA) (JMP software; SAS Institute, Cary, NC). To determine if lesioned subjects exhibited different pain sensitivity during brief graded noxious stimuli (35, 45, 50°C, 5 s), three-factor ANOVAs were used to examine the effects of body side, stimulus temperature, and lesion on pain intensity and unpleasantness ratings. For long duration noxious stimulation during fMRI (49°C, 30 s on 30 s off, 5 cycles), we used two-factor ANOVAs to determine if lesioned patients exhibited different sensitivity than healthy normal subjects. In addition, three-factor ANOVAs were used to examine the effects of body sides, body location (arm vs. leg), and lesion on tactile thresholds,
while two-factor ANOVAs were used to examine the effects of body side and lesion on thermal thresholds.

Lesion volume characterization

In order to better understand the relationship between sensory deficits and lesion volume and location, each patient's lesion was identified in a semi-automated fashion. Segmentation of each patient’s lesion was performed manually using automated tissue class segmentation (FSL FAST software) as guide for the visualization of lesions. Each patient’s high-resolution structural images (SPGR, FSE, FLAIR) were used as inputs for multi-channel automated tissue class segmentation with four classes of tissues (gray matter, white matter, cerebrospinal fluid, and damaged tissue) specified as outputs of the segmentation (Zhang et al., 2001). Then, the lesion mask for each patient was manually drawn using segmentation outputs and structural images to help with the visualization of the affected region. These masks were then registered to standard space and summed across individuals to generate a lesion location frequency map. Next, both lesion volume (in mm$^3$) and % difference in pain intensity ratings between body sides for each patient $\left[\frac{(\text{VAS left}-\text{VAS right})}{\text{VAS left}}\right] \times 100$ were log transformed. The relationship between these log transformed values was then assessed by regression analysis.

Statistical analysis of regional signal changes within the brain

Pain-related activations were examined using simple boxcar functions. The regressor was convolved with a gamma-variate model of the hemodynamic response (delay, 6 s; SD, 3 s) and its temporal derivative and was temporally filtered with the same
parameters as the fMRI data. For each individual, fixed effects general linear modeling (GLM) analyses were used to identify brain activation associated with the modeled hemodynamic response function (HRF) (Woolrich et al., 2001) while random effects analyses were used to assess activation across individuals. In addition, contrasts comparison between patients vs. healthy subjects brain activations were done using fixed effects analyses to determine which brain areas exhibited significantly greater activation in each group. Z (Gaussianised T/F) statistic images were thresholded using clusters determined by \( z > 2.3 \) and a (corrected) cluster significance threshold of \( p < 0.05 \) (Worsley et al., 1992).

**Seed masks for functional and structural connectivity**

In order to analyze the functional and structural connectivity of regions of the putamen activated during pain, seed masks were first generated. This was accomplished by binarizing both contralateral and ipsilateral regions of the putamen activated during both left and right-sided noxious stimulation in healthy normal subjects. Thus, four putamen seed masks were generated: 1) left putamen during stimulation of left body side, 2) right putamen during stimulation of left body side, 3) left putamen during stimulation of right body side, 4) right putamen during stimulation of right body side.

**Statistical analysis of functional connectivity**

The functional connectivity of areas of the putamen activated during pain was determined by regression analyses using a mean time course of activity from each of the four putamen seed masks. These analyses produced four individual subject-level maps of
all correlated voxels for each time series. The time course of white matter (WM) activity was employed as a regressor of no interest (Di Martino et al., 2008). In order to identify the WM, we used a segmented MNI 152 brain atlas (Di Martino et al., 2008). The segmented WM image was thresholded to ensure 75% tissue type probability then applied to each individual's time series to generate a WM mean time course. For each individual, fixed effects general linear modeling (GLM) analyses were used to identify functionally correlated brain areas while random effects analyses were used to assess functional connectivity across individuals. Z statistic images were thresholded using clusters determined by $z>2.3$ and a (corrected) cluster significance threshold of $p<0.05$ (Worsley et al., 1992).

Statistical analysis of probabilistic tractography

To determine the structural connectivity of regions of the putamen activated during pain, probabilistic tractography was performed on DTI data as described previously (Behrens et al., 2003a; Johansen-Berg et al., 2004; Johansen-Berg et al., 2005). Probabilistic tractography utilizes the anisotropic diffusion of water in white matter. Since water diffuses along the direction of the white matter tract without crossing the myelin sheaths, the orientation of diffusion can provide useful insights into the orientation of white matter fibers. The orientation of maximal diffusion is called the principal diffusion direction (PDD). Probability density function (PDF) for the uncertainty of the PDD can be computed using Bayes’ rule based on the parameters given in the data (Behrens et al., 2003a). Then, Markov Chain Monte Carlo sampling can be used to identify possible tract directions based on the PDFs. Multiple sampling of the
dataset produces connectivity distributions, i.e., the possible tracts that could occur from a particular region of the brain. This technique allows us to examine the directions and connections of each brain region (Hadjipavlou et al., 2006).

Probabilistic tractography provides quantification of the likelihood of connectivity between the seed mask and other brain areas. An image generated by probabilistic tractography shows the tract path between the seed and other connected brain areas. The difference between outputs of the different sample sizes converged with higher sampling rates. A sample size of 10,000 was determined to be sufficiently large for connectivity analyses and tract image generation (Behrens et al., 2003a; Hadjipavlou et al., 2006). The quantification of tract pathways was performed in native diffusion space for each subject with masks in MNI space, registered to diffusion space. For each of the thirteen normal subjects, probabilistic tractography was used to determine which brain areas were anatomically connected to each of the four putamen seed masks. Four tract images (one for each of the four putamen seed masks) were generated for each individual. The generation of tract images was completed in MNI space for each subject to minimize any potential registration error. The resulting tract images were then binarized and summed across group to generate a frequency map for each seed mask. This frequency map shows brain areas that were anatomically connected with each of the seed masks across individuals. This allowed overlay of individual images to show where in the brain across the group the individual subjects had found analogous paths (Hadjipavlou et al., 2006).
Results

Neurologic findings and tactile thresholds of patients with putamen lesions

Nine ischemic stroke patients, seven males and two females, (age, 46-71; mean, 59 years) with lesions affecting the left putamen participated in the study (Fig. 1 and Supplementary Table 1). Subjects were tested from 2 weeks to 15 years (mean time: 4 years) after their stroke. Sensory examinations were normal throughout position and vibration, although there were mild decreases in touch on the right side in some patients (Supplementary Table 1). These were well within the range of normal variability of the healthy normal subjects. Furthermore, von Frey testing revealed that the tactile thresholds of patients were not significantly different from healthy normal subjects (group: F(1,21)=0.9857, p=0.3321; body sides: F(1,21)=1.2059, p=0.2846; group * body sides interaction: F=1.7092, p=0.2052), although in both groups, the arms were more sensitive than the legs (body location: F(1,21)=12.1694, p=0.0022; group * body location interaction: F(1,21)=0.0022, p=0.9631)(Fig. 2A). Motor testing revealed that most patients exhibited a degree of motor deficits on the right (affected) side, including weakness of right upper and/or lower extremities (Supplementary Table 1). These findings are characteristic of lesions involving the putamen and the basal ganglia and indicate that patients’ sense of touch was minimally affected by the stroke. In all patients, there was no aphasia or impaired cognition, no central post-stroke pain (CPSP), and no signs of hemineglect.
Figure 1. High resolution structural MRI images showing the extent of the lesions in each patient. All nine patients had ischemic strokes with lesions encompassing the left putamen. Slice location is given as mm above the AC-PC line in standard space in the z-axis.
Supplementary Table 1. Clinical features and tactile thresholds of patients. Most patients exhibited some degree of motor deficits with subjectively mild sensory deficits characteristic of lesions involving the putamen and the basal ganglia. In all patients, there was no aphasia or impaired cognition, no central post-stroke pain (CPSP), and no signs of hemineglect. MRC, Medical Research Council; LUE, left upper extremity; RUE, right upper extremity; LLE, left lower extremity; RLE, right lower extremity.
<table>
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<tr>
<th>Patient</th>
<th>Sex/Age at onset/age at testing</th>
<th>Time after stroke (yrs)</th>
<th>Tactile thresholds ± SEM (Newtons)</th>
<th>Neurologic exam</th>
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<td></td>
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<td>Right arm</td>
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<tr>
<td>1</td>
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strength 4/5, wrist flexion and finger flexion 3+/5. For the right leg, strengths of hip flexors, knee flexors and extensors were 4+/5.

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<tr>
<th>Patients</th>
<th></th>
<th>6M 3F/ 53/59 (mean)</th>
<th>4.89 ±1.77</th>
<th>2.76 ±0.33</th>
<th>2.90 ±0.37</th>
<th>3.27 ±0.37</th>
<th>3.32 ±0.37</th>
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</thead>
<tbody>
<tr>
<td>Normals</td>
<td></td>
<td>6M 8F / 59 (mean)</td>
<td>N/A</td>
<td>3.15 ±0.17</td>
<td>3.12 ±0.16</td>
<td>3.61 ±0.16</td>
<td>3.62 ±0.15</td>
</tr>
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</table>
**Thermal thresholds**

In patients, cool detection thresholds on both the left and right sides were in the range of those of the healthy normals (Fig. 2B). There was no main effect of group or sides (group: F(1,16)=0.9267, p=0.3501; body sides: F(1,16)=2.6339; p=0.1241) nor was there a group*body side interaction (F(1,16)=2.1180, p=0.1649).

Cold pain thresholds of both sides of patients were significantly lower than those of the healthy normal subjects (group: F(1,15)=12.8012, p=0.0027)(Fig. 2B). In addition, there was no statistically reliable side-to-side difference in cold pain thresholds of patients when compared to healthy normal subjects (group * body sides interaction: F(1,15)=0.6393, p=0.4364), nor was there a significant main effect of stimulation side (body sides: F(1,15)= 0.0707, p=0.7939). This suggests that putamen lesions may be unique in disrupting cold pain sensations bilaterally (Fig. 2B).

Patients had higher warmth detection thresholds on their affected sides than did healthy normal subjects (group * body sides interaction: F(1,17)=4.6821, p=0.0450)(Fig. 2B). There was a significant main effect of side of stimulation on innocuous warm detection thresholds (body sides: F(1,17)=8.1487, p=0.0110); however, there was no main effect of group (group: F(1,17)=0.4665, p=0.5038).

The left (unaffected side) and right (affected side) heat pain thresholds of patients appear completely normal and symmetric (Fig. 2B). There were no significant main effects of group and sides and no detectable side-to-side difference in patients when compared to healthy normals (group: F(1,17)=0.2043, p=0.6570; body sides: F(1,17)=0.3743, p=0.5488; group * body side interaction: F(1,17)=0.0133, p=0.9095).
These results (Fig. 2B) suggest that putamen lesions can disrupt cold pain thresholds and warm detection thresholds while leaving other temperature sensations intact. Thus, patients’ ability to experience and provide pain ratings for noxious heat stimulus was likely minimally affected by the lesions.
Figure 2. Tactile and thermal thresholds (means±SEM). Tactile threshold of patients with putamen lesions were not significantly different from those of healthy controls (A). Patients with putamen lesions displayed some disturbances in cold pain thresholds and warm detection thresholds while exhibiting normal heat pain and innocuous cool detection thresholds (B). Cool detection thresholds on both the left and right sides of patients were in the range of those of the healthy normals. However, cold pain thresholds of patients were significantly lower than those of the healthy normal subjects on both sides (p=0.0027). In addition, patients had higher warmth detection thresholds on their affected sides than did healthy control subjects (p=0.045). The left (unaffected) and right (affected) heat pain thresholds of patients appear completely normal and symmetric.

LUE, left upper extremity; RUE, right upper extremity; LLE, left lower extremity; RLE, right lower extremity. (*) denotes statistical significance (p<0.05).
Short-duration pain ratings of patients with putamen lesions

Patients were able to evaluate brief noxious stimuli of graded intensities (35, 45, 50°C, 5 s) applied to the posterior aspect of the lower legs on both left (unaffected) and right (affected) sides (Fig. 3). Both patients and healthy subjects exhibited monotonic increases in VAS ratings of pain intensity and unpleasantness as stimulus temperature increased (pain intensity: F(1,21) = 79.3310, p<0.0001; pain unpleasantness: F(1,21)=52.6866, p<0.0001). There were no significant differences between patients and healthy normals in pain intensity and unpleasantness ratings as the stimulus temperature increased (temperature * group interaction: pain intensity: F(1,21)=1.3894, p=0.2517; pain unpleasantness: F(1,21)=0.2545, p=0.6192)(Fig. 3). However, patients exhibited significantly lower VAS ratings of pain intensity during stimulation of the right (affected) side than those of left (unaffected) side when compared to healthy normals (body sides * group interaction: pain intensity F(1,21)=8.5972, p=0.0080)(Fig. 3A). Although side-to-side differences in pain unpleasantness ratings between the patients and healthy subjects did not reach statistical significance, there was a strong trend towards a difference (body sides * group interaction: pain unpleasantness: F(1,21)=4.2445, p=0.0520)(Fig. 3B). There was no significant main effect of body side and group on pain intensity and pain unpleasantness, although the effect of sides on pain unpleasantness ratings showed a strong trend (sides: pain intensity: F(1,21)=1.1271 p=0.3005; pain unpleasantness: F(1,21)=4.0633, p=0.0568; group: pain intensity: F(1,21)=0.0376, p=0.8481; pain unpleasantness: F(1,21)=0.0290, p=0.8664)(Fig. 3). These results suggest that putamen lesions may result in decreased pain sensitivity to brief graded noxious stimuli on the right (affected) body side when compared to healthy normal subjects.
Figure 3. Pain intensity and unpleasantness VAS ratings during the graded noxious stimulation (means±SEM). Patients retained the ability to discriminate noxious stimuli of graded intensities applied to the posterior aspect of the lower legs on both left (unaffected) and right (affected) sides. However, patients exhibited significantly lower VAS ratings of pain intensity during stimulation of the right (affected) side than those of left (unaffected) side when compared to healthy normals (p=0.008). Although side-to-side differences in pain unpleasantness ratings between the patients and healthy subjects did not reach statistical significance, there was a strong trend towards a difference (p=0.052). (*) denotes statistical significance (p<0.05).
A. GRADED NOXIOUS HEAT PAIN INTENSITY RATINGS

B. GRADED NOXIOUS HEAT PAIN UNPLEASANTNESS RATINGS
Long-duration pain ratings of patients with putamen lesions

During long duration noxious stimulation (30 s off, 30 s on, 5 cycles, 49°C) in the fMRI session, patients exhibited significantly lower VAS ratings of pain intensity during stimulation of the right (affected) side than those of the left (unaffected) side when compared to healthy normals (body sides * group interaction: pain intensity: F(1,20)=9.5462, p=0.0058)(Fig. 4). Although, side-to-side differences of pain unpleasantness ratings between patients and healthy normals did not reach statistical significance, there was a strong trend towards a difference (body sides * group interaction: pain unpleasantness: F(1,20)=3.4957, p=0.0762)(Fig. 4). There were no significant main effects of body sides on pain intensity and unpleasantness ratings, although the effect of sides on pain intensity ratings showed a strong trend (pain intensity: F(1,21)=3.8843, p=0.0627; pain unpleasantness: F(1,21)=0.4540, p=0.5081).

Similarly, there were no significant main effects of group on pain intensity and unpleasantness ratings, but the effect of group on pain unpleasantness ratings showed a strong trend (pain intensity: F(1,20)=1.3840, p=0.2532; pain unpleasantness: F(1,20)=3.4373, p=0.0786)(Fig. 4). These results suggest that unilateral lesions of the putamen may decrease pain sensitivity to both brief and prolonged noxious stimulation on the right (affected) body side when compared to healthy normal subjects.
Figure 4. Pain intensity and unpleasantness VAS ratings during long duration noxious stimulation (means±SEM). Putamen lesions produced decreased pain sensitivity to long duration noxious stimuli on the right (affected) body side in patients when compared to healthy normal subjects. Patients exhibited significantly lower VAS ratings of pain intensity during stimulation of the right (affected) side than those of the left (unaffected) side when compared to healthy normals (p=0.0058). Although, side-to-side differences of pain unpleasantness ratings between patients and healthy normals did not reach statistical significance, there was a strong trend towards a difference (p=0.0762). (*) denotes statistical significance (p<0.05).
Lesion volume and difference in pain ratings between sides

The lesions in the patients were centered in the left putamen with a few subjects showing larger, more extensive lesions (Fig 5, Fig 1). Patients with smaller, focal lesions of the putamen exhibited greater differences in pain intensity ratings between sides whereas patients with larger, more extensive lesions exhibited smaller differences in pain ratings between sides. Thus, it appears that focal lesions specific to the putamen may be effective in producing unilateral disruption of nociceptive processing. In addition, there was no statistically significant relationship between time after stroke and differences in pain intensity ratings between sides (p=0.440).
Figure 5. Focal lesions specific to the putamen are effective in producing large differences in pain ratings between sides in patients. Voxels in color represent the number of patients with lesions at a given locus (left panel). The patients’ lesions were concentrated within the left putamen (outlined in black) with a few subjects showing larger, more extensive lesions. The difference in pain intensity ratings between sides is inversely logarithmically related to lesion volume (right panel).
Putamen and other pain-related brain activation in healthy normal subjects

During long duration noxious stimulation (49°C, 30 s off 30 s on, 5 cycles) of the lower leg of the left side in healthy normals, brain activation was identified within the anterior cingulate cortex (ACC), supplementary motor area (SMA), secondary somatosensory cortex (SII), insula, SI, frontal operculum, thalamus, putamen, and cerebellum (Fig. 6 and Supplementary Table 2). Stimulation of the right lower leg produced similar patterns of brain activation (Fig. 6 and Supplementary Table 2). This pattern of brain activation is consistent with normal brain activation during pain (Coghill et al., 1994; Peyron et al., 2000; Coghill et al., 2001; Oshiro et al., 2007).

While putamen activation was detected bilaterally during stimulation of each body side individually, the patterns and degree of ipsilateral and contralateral putamen activation differed substantially (Fig. 6, Fig. 7A, and Supplementary Table 2). During stimulation of the left side, left (ipsilateral) putamen activation volume was 400 mm$^3$ and centered at (-28, 0, 2) in standard space, whereas right (contralateral) putamen activation volume was 720 mm$^3$ and centered at (28, 1, 5)(Fig. 7A). During stimulation of the right side, right (ipsilateral) putamen activation volume was 912 mm$^3$ and centered at (28, 3, 2), while left (contralateral) putamen activation volume was 1168 mm$^3$ and centered at (-27, 1, 2). Regardless of side of stimulation, however, the ipsilateral putamen activation appears to be smaller when compared to that of contralateral putamen (Fig. 7A and Fig. 6). Furthermore, both contralateral and ipsilateral putamen activation during stimulation of the right side appear to be more pronounced than those during stimulation of the left body side, suggesting that some asymmetry of activity of the putamen during pain may exist (Fig. 7A).
Additionally, during painful stimulation of either body side, activation was identified within anterior areas of the thalamus that have a high probability of having connections with the prefrontal cortex according to the Oxford Thalamic Connectivity Atlas (Behrens et al., 2003b; Behrens et al., 2003a)(Fig. 6 and Supplementary Table 2). Although correlating the thalamic activation loci to specific thalamic nuclei is difficult, these activated regions appear to occur in areas consistent with the medial dorsal (MD), ventral lateral (VL), and ventral anterior (VA) nuclei of the thalamus (Tailarach and Tournoux, 1988; Lancaster et al., 2000; Lancaster et al., 2007) (Fig. 6 and Supplementary Table 2).
Figure 6. Pain-related brain activation in normals and patients. During long duration noxious stimulation of either body side in the healthy normals, brain activation was identified within the ACC, SMA, SII, insula, SI, frontal operculum, thalamus, putamen, and cerebellum (left side of figure). Putamen activation was detected bilaterally during stimulation of either body side. Additionally, activation was identified within anterior areas of the thalamus during painful stimulation of either body side. By contrast, during painful stimulation of the unaffected (left) body side in patients, pain-induced brain activation was identified within the ACC, insula, frontal operculum, SMA, and cerebellum (right side of figure). However, no SII, thalamic or putamen activation was observed. Stimulation of the right (affected) leg in patients activated the insula and frontal operculum. In general, brain activation during stimulation of the affected (right) side was less pronounced than that during stimulation of the unaffected (left) side in patients. In addition, pain-related brain activation during stimulation of either side in patients is less robust than that of healthy subjects. VMPFC, ventromedial prefrontal cortex; DLPFC, dorsolateral prefrontal cortex; PCC, posterior cingulate cortex; ACC, anterior cingulate cortex; SMA, supplementary motor area; OP, operculum; IPL, inferior parietal lobule.
Figure 7. Putamen activation and functional connectivity. The patterns of ipsilateral and contralateral putamen activation differed substantially regardless of side of stimulation (A). The ipsilateral putamen activation appears to be smaller when compared to that of contralateral putamen activation. These four putamen activation loci were used as seeds for functional connectivity analyses. Functional correlation analyses revealed that regardless of differences in patterns, shape, and location of the four seeds used, the majority of functionally connected brain areas identified overlap substantially (B). Color scale on the right denotes the number of seeds showing functional connection with that brain region. Functionally connected brain areas identified included: 1) nociceptive processing areas including ACC, insula, thalamus, and SII, 2) attention-related areas including DLPFC, MFG, FEF, ACC, IPL, and SMA, 3) memory processing areas including the amygdala and hippocampus. In addition, SN/VTA area in the midbrain was also found to be functionally connected with the putamen and its associated brain network.
Supplementary Table 2. Pain-related activation. Peak Z scores were obtained from group analysis. Cluster sizes and peak locations are listed within parentheses as the number of voxels and x,y,z coordinates (in millimeters) according to standard stereotaxic space. IPL, inferior parietal lobule; VMPFC, ventromedial prefrontal cortex; -, no statistically reliable change.
Supplementary Table 2. Pain-related activation.

<table>
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<th>Region</th>
<th>Normals' side of stimulation</th>
<th>Patients' side of stimulation</th>
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<td>R</td>
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<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>3.717 (9128; 6, -84, -34)</td>
<td>4.17 (5021; 20, -88, -34)</td>
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<td>4.089 (9128; -30, -70, -26)</td>
<td>4.03 (5021; -26, -76, -30)</td>
</tr>
<tr>
<td>Putamen</td>
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<td>3.245 (5963; 18, -2, -8)</td>
</tr>
<tr>
<td>Left</td>
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<td>4.47 (5963; 36, 26, 0)</td>
</tr>
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<td>Left</td>
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<td>4.08 (5963; -34, 12, -2)</td>
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<td>(-)</td>
<td>3.132 (5963; -32, -18, 8)</td>
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<tr>
<td>Left</td>
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<td>(-)</td>
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<tr>
<td>Anterior Cingulate Cortex</td>
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<td>3.254 (1774; -6, 2, 38)</td>
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<tr>
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<td>4.06 (2245; 4, -34, 74)</td>
<td>4.03 (1774; -10, -24, 72)</td>
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<tr>
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<td>Thalamic nuclei</td>
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<td>deactivation</td>
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<td>-4.029 (14209; 6, -52, 30)</td>
</tr>
<tr>
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<td>Coordinates</td>
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<td>--------------</td>
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<td>Occipital lobe</td>
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<td></td>
<td>-4.118</td>
<td>(14209; -28,-78,30)</td>
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<td>-3.54</td>
<td>(1430; -42,-66,22)</td>
</tr>
</tbody>
</table>


**Pain-related brain activation in patients with putamen lesions**

During painful stimulation of the unaffected (left) body side, pain-induced brain activation was identified within the ACC, insula, frontal operculum, supplementary motor area (SMA), and cerebellum (Fig. 6 and Supplementary Table 2). However, in contrast to that seen in normal subjects, no SII, thalamic or putamen activation was observed. Stimulation of the right (affected) leg activated the insula and frontal operculum (Fig. 6 and Supplementary Table 2). In general, brain activation during stimulation of the affected (right) side was less robust than that during stimulation of the unaffected (left) side. Compared to stimulation of the unaffected side, there was no detectable ACC activation during stimulation of the affected side (Fig. 6 and Supplementary Table 2). This may be attributable to reduced pain experienced by the patients during stimulation of their affected side. When compared to healthy normals, patients exhibited lesser activation in a number of areas involved in nociceptive processing including SII, SI, ACC, insula, thalamus, and putamen during stimulation of both affected and unaffected sides (results not shown). These findings indicate that while the psychophysical differences were only observed unilaterally, unilateral lesions of the putamen may produce a generalized, diffuse pattern of altered brain activity that is manifested bilaterally (Fig. 3, Fig. 4, and Fig. 6).

**Functional connectivity of the putamen**

Functional correlation analyses were performed on each of the four putamen seed masks (two for each side of stimulation, one ipsilateral, one contralateral) in our healthy normal subjects in order to identify which brain areas had activity that covaried with that
of the putamen (Fig. 7). These analyses revealed that largely overlapping brain areas exhibited activity which was correlated with each of the four putamen seed masks, suggesting that a similar brain network may be associated with both contralateral and ipsilateral regions of the putamen activated during pain (Fig. 7 and Supplementary Table 3).

Functionally connected brain areas identified included: 1) nociceptive processing areas including ACC, insula, thalamus, and SII, 2) attention-related areas including dorsolateral prefrontal cortex (DLPFC), middle frontal gyrus (MFG), frontal eye field (FEF), ACC, IPL, and SMA, 3) memory processing areas including the amygdala and hippocampus (Fig. 7B and Supplementary Table 3). Additionally, functional connections were also identified in the midbrain region. Although correlating these functionally connected midbrain areas to specific loci is difficult, these functionally connected regions appear to occur in areas consistent with the substantia nigra/ventral tegmental area (SN/VTA) (Fig. 7B and Supplementary Table 3) (Talairach and Tournoux, 1988; Lancaster et al., 2000; Lancaster et al., 2007). Moreover, most brain areas that were functionally connected with the either the left or right putamen exhibited correlated activity bilaterally (Fig. 7B and Supplementary Table 3). The functional connections between SII, insula, thalamus, amygdala, hippocampus, and SN/VTA and the putamen were all bilateral, with the ACC, SMA, and FEF exhibiting correlated activity along the midline (Fig. 7B and Supplementary Table 3). However, both the left and right putamen were functionally connected with right lateralized portions of IPL, MFG, and DLPFC (Fig. 7B and Supplementary Table 3). These right lateralized brain areas have been
suggested to play an important role in processes related to visuospatial attention (Corbetta et al., 1993; Mesulam, 1999; Knudsen, 2007; Corbetta et al., 2008).

Although some functionally connected brain areas identified in correlation analyses were similar to those activated during noxious stimulation, areas such as DLPFC, MFG, and FEF were unique to correlation analyses (Fig. 7B, Fig. 6, Supplementary Table 2 and Supplementary Table 3). Furthermore, areas including SI and cerebellum previously identified in pain-induced brain activation were not identified in our functional correlation analyses (Fig. 7B, Fig. 6, Supplementary Table 2 and Supplementary Table 3). Thus, these functional connectivity analyses were effective in identifying brain areas that exhibited activity which was related to that of the putamen but did not necessarily exhibit significant co-variation with the timecourse of noxious stimulus-related activity.
Supplementary Table 3. Functional correlations. Peak Z scores were obtained from group analysis. Peak locations are listed within parentheses as x,y,z coordinates (in millimeters) according to standard stereotaxic space. IPL, inferior parietal lobule; -, no statistically reliable change.
# Supplementary Table 3: Functional correlations.

<table>
<thead>
<tr>
<th>Region</th>
<th>Putamen mask left side stimulation</th>
<th>Putamen mask right side stimulation</th>
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<tr>
<td></td>
<td>L.</td>
<td>R.</td>
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<td></td>
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<td></td>
<td>(-)</td>
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</tr>
<tr>
<td>Cerebellum</td>
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<td>(-)</td>
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<tr>
<td>Right</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Left</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>5.09(12,-20,-2)</td>
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<td>5.153(16,-14,-2)</td>
<td>4.313(24,-22,2)</td>
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<td>4.963(-8,-22,-2)</td>
<td>4.827(-8,-22,0)</td>
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<td></td>
<td>5.366(-12,-20,-2)</td>
<td>5.045(-16,-10,4)</td>
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<td>3.286(42,-48,50)</td>
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<tr>
<td>SI (leg)</td>
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</tr>
<tr>
<td>SII</td>
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<td>(-)</td>
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<td>Amygdala</td>
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<td>20)</td>
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<tr>
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<td>2.82(-24,-4,-16)</td>
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</table>
Comparison of structural and functional connectivity

Tractography is a useful tool in identifying the anatomical framework that may support functional connections in a neural network. Together, a combination of DTI and functional connectivity may provide useful insights into understanding the dynamics of an identified brain network. Most of the brain regions exhibiting functional connectivity with the putamen were also shown to be connected to the putamen via tractography (Fig. 8 and Fig. 7B). Areas such as insula, SMA, ACC, FEF, thalamus, MFG, amygdala, hippocampus, and SN/VTA were all shown to be structurally connected to regions of the putamen activated during pain in all four seed masks (Fig. 8). However, in contrast to the bilateral patterns of functional connectivity, most of the connections identified via tractography were limited to the hemisphere ipsilateral to the portion of the putamen used for the seed mask (Fig. 8). For example, the anatomical connections of the putamen with the insula were only ipsilateral, while functional correlation analyses suggest that information from the contralateral insula may also reach the putamen indirectly (Fig. 8 and Fig. 7B). Additionally, areas such as SII and DLPFC, identified in the functional correlation analyses, were not shown to be connected to the putamen via tractography (Fig. 8). Thus, inputs from these brain areas may reach the putamen indirectly. Moreover, brain areas including the cerebellum and SI were neither functionally nor structurally connected to the putamen despite being activated during pain.
Figure 8. Comparison of structural and functional connectivity of the putamen. Each of the four panels separated by white vertical lines displays functional (Correlation) and structural (DTI) connections of each of four seeds. Functional and structural connections are placed side-by-side within each panel for comparison. Similarities/differences between functional and structural connectivity derived from one seed are reproducible across all four seeds. Most of the brain regions exhibiting functional connectivity with the putamen were also shown to be structurally connected to the putamen via tractography. Areas such as insula, SMA, ACC, FEF, thalamus, MFG, amygdala, hippocampus, and SN/VTA were all shown to be structurally connected to regions of the putamen activated during pain. However, in contrast to the bilateral patterns of functional connectivity, most of the connections identified via tractography were limited to the hemisphere ipsilateral to the portion of the putamen used for the seed mask. Please note that the significance color bar in functional connectivity refers to Z-score while it denotes the number of subjects displaying corresponding structural connections in the DTI.
Discussion

Pain is an intrinsically intrusive experience that readily wins out over other competing cognitive demands and conveys highly salient information relevant to the status of the organism (Price, 1999). To date, the neural mechanisms that allow incoming nociceptive information to capture attention and to appropriately engage brain networks to generate a subjectively available experience of pain remain poorly understood. However, the present data provide multiple, converging lines of evidence that indicate that the basal ganglia, and specifically, circuits that involve the putamen, may contribute to processes that allow nociceptive information to capture the conscious experience via the selection of appropriate brain networks. In the present investigation, bilateral regions of the putamen activated during pain were shown to be functionally connected to a variety of brain areas that are active during pain but that also play important roles in attention, affect, and memory. Many of these functional connections are consistent with anatomical connections as demonstrated by tractography as well as previous anatomical studies of the putamen in nonhuman primates (Russchen et al., 1985; Smith and Parent, 1986; Alexander and Crutcher, 1990; Yeterian and Pandya, 1991, 1993; Parent and Hazrati, 1995; Chikama et al., 1997; Haber et al., 2006; Calzavara et al., 2007; Staempfli et al., 2008). Moreover, patients with lesions of the putamen exhibited decreased pain sensitivity. Taken together, these results suggest that the putamen and its associated brain networks may play a more unique role in the construction of the pain experience than previously thought.
Integration of cognitive information within the putamen and basal ganglia

The striatum, composed of the caudate nucleus and putamen, serves as major input structure to the basal ganglia and is at the center of the cortico-basal ganglia–thalamo-cortical loop (Alexander and Crutcher, 1990; Parent and Hazrati, 1995). In this framework, information from many cortical, limbic, and thalamic areas access the striatum via segregated parallel channels (Alexander and Crutcher, 1990). In the present investigation, regions of the putamen activated during pain were functionally connected to a variety of brain areas involved in sensory and affective aspects of nociceptive processing such as SII, thalamus, insula and ACC. Also, brain areas associated with memory such as the amygdala and hippocampus (Murray and Mishkin, 1983; Coghill et al., 1994; Coghill et al., 1999; Ploghaus et al., 2001; Smith et al., 2004; Marschner et al., 2008) and brain areas involved in attention-related processes including the DLPFC, FEF, MFG, ACC, IPL, and SMA were functionally connected with regions of the putamen activated during pain (Posner et al., 1980; Mesulam, 1999; Peyron et al., 1999; Nobre, 2001; Knudsen, 2007; Corbetta et al., 2008).

Although these cortico-basal ganglia–thalamo-cortical loops are highly segregated, a degree of convergence and interaction between information within each loops, both within the basal ganglia and extrinsic structures, are facilitated by overlapping patterns of cortico-striatal terminal arborizations as well as diverging efferent projections of different parallel loops that overlap at each relay point of the pathway (striatum, output nuclei, thalamus, and cortex) (Joel and Weiner, 1994; Groenewegen et al., 1999; Haber, 2003; Haber et al., 2006; Calzavara et al., 2007). In addition, medium spiny projection neurons within the striatum can serve as the main integrating elements of the basal
ganglia since they receive large numbers of projections from both the cerebral cortex and intrinsic basal ganglia neurons (Parent and Hazrati, 1995). This configuration allows the putamen, as part of the striatum, to be well positioned to integrate information from a wide array of regions of the cerebral cortex and can provide a cognitive context for information processing.

**Selection of cortical networks by the basal ganglia**

The putamen has inhibitory projections to the internal segment of the globus pallidus (GPI) and substantia nigra pars reticulata (SNr), which, in turn, have inhibitory projections to several thalamic nuclei including VL, VA, and MD. Consistent with this circuitry, putamen activation during pain was associated with activation in several thalamic regions consistent with these nuclei. These thalamic nuclei, in turn, project back to large areas of the cerebral cortex including frontal, parietal, insula, and cingulate areas (Mufson and Mesulam, 1984; Vogt et al., 1987; Alexander and Crutcher, 1990; Alexander et al., 1990; Parent and Hazrati, 1995; Middleton and Strick, 2000). Thus, the putamen can influence activity in large areas of the cerebral cortex via differentially exerting its influence on GPI and SNr to modulate varying levels of disinhibition of the thalamus. Furthermore, sustained activity of cortical networks including parietal, frontal, and cingulate regions during task performance is frequently accompanied by sustained activity within the putamen (Koski et al., 1999; Coull et al., 2000; Downar et al., 2003). Accordingly, the putamen may contribute importantly to the selection of behaviorally-relevant tasks by enabling engagement of specific sets of cortical networks needed to accomplish the task (Redgrave et al., 1999; Balleine et al., 2007). In the case of pain, the
putamen may have the neural resources to contribute to the determination of which brain networks are engaged during the inflow of afferent nociceptive information.

**Biasing of selection by afferent nociceptive information**

Incoming nociceptive inputs may access the basal ganglia circuitry relatively directly via relays in the thalamus since the midline and intralaminar thalamic nuclei are connected to the putamen (Mengual et al., 1999; Van der Werf et al., 2002) and serve as major sites of terminations of spinal nociceptive afferents (Willis, 1985). In addition, SN/VTA activity also correlated significantly with activity of regions of the putamen activated during pain. The SNc has relatively direct connections with the parabrachial nucleus in the midbrain (Schneider, 1986; Vankova et al., 1992), which receives afferent nociceptive information from the spinal cord (Bernard and Besson, 1990; Craig, 1995; Klop et al., 2005). These findings suggest that the SNc may be in a position to utilize nociceptive information to modulate striatal activities and efficiency of cortico-striatal terminals. These nigrostriatal dopaminergic modulatory projections, together with nociceptive input conveyed by thalamostriatal projections from the intralaminar/midline thalamus, are well positioned to bias the selection mechanism in the putamen in order to allow incoming nociceptive information to outcompete ongoing cognitive processes. This circuitry may contribute substantially to the cognitive intrusiveness of pain.

**Disruption of selection by lesions of the putamen**

Lesions of the putamen may affect the engagement of appropriate brain networks that are normally responsible for processing nociceptive information during pain.
Consistent with this postulate, patients with putamen lesions exhibited significantly lower pain ratings when compared to those of healthy subjects. Nevertheless, pain ratings and pain-related brain activation within ACC and insula were not completely abolished. Thus, regardless of putamen lesions, some aspects of nociceptive information can still be processed, albeit with diminished ability to fully recruit cortical activity.

It is also important to note that reduced pain sensitivity of patients was not simply due to nonspecific decreases in somatosensory capabilities following the stroke since their heat pain thresholds and tactile thresholds were virtually normal when compared to those of the healthy subjects. In addition, the results seen in this study are likely specific to lesions of the putamen and not due to other structures affected by the lesion since patients with the largest lesions actually exhibited the smallest difference in pain intensity ratings between sides whereas the smallest, most focal putamen lesions produced greatest difference between sides (Fig. 5). Such results are unlikely to be attributable to decreased attention due to neglect since patients did not show any signs of hemineglect during neurological examination. Moreover, patients’ lesions were located in the left hemisphere as opposed to the right, which is more involved in spatial processing (Vuilleumier et al., 2001; Karnath et al., 2004).

**Conclusion**

Putamen activation is often noted during painful stimulation, however its contribution to the experience of pain has been poorly understood. The present data provide multiple lines of evidence that suggest that the putamen is involved in pain-related processes that extend far beyond motor-related responses. Although pain is an
intrinsically intrusive experience and highly salient, the significance and behavioral relevance of nociceptive sensory input relies heavily upon the cognitive context in which the noxious stimulus occurs (Price, 1999). Regions of the putamen activated during pain are functionally and structurally connected with a variety of brain areas involved in memory, attention, and nociceptive processing. Accordingly, the interaction and the integration of cognitive and nociceptive information within the putamen may be important in determining the selection of brain networks that are engaged during pain. Furthermore, lesions of the putamen can produce a significant disruption in this process resulting in reduced pain sensitivity. Thus, the putamen and associated neural networks may contribute importantly to determining the behavioral relevance and saliency of incoming nociceptive information based not only on the strength of the sensory input, but also its interaction with other external environmental and internal cognitive information unique to each individual.

Acknowledgements

This study was supported by NIH R01 NS39426. The authors thank FMRIB Image Analysis Group, Oxford University for the FSL analysis software and Stephania Jordan for her help in collecting data for the experiment.
REFERENCES


Chapter 5

DISCUSSION

INTERINDIVIDUAL DIFFERENCES IN PAIN

A complete subjective experience of pain is a truly, unique personal experience. Two similar noxious stimuli may elicit very different responses in two individuals. Although it may be mild to one individual, the same stimulus can be very painful to another individual. Thus, large interindividual differences in pain experience exist. These differences in pain ratings are also accompanied by changes in brain activation during pain, suggesting that these differences in individual pain ratings were not due to altered recall processes, but reflect real changes in neural mechanisms (Coghill et al., 2003). These individual differences make pain assessments difficult and, consequently, hard to effectively manage (Benrud-Larson and Wegener, 2000; Lovatsis et al., 2007). Many studies have used various physical traits, including age, blood pressure, and gender to try to assess these individual differences in pain (Macintyre and Jarvis, 1996; Wise et al., 2002; Kalkman et al., 2003; Logan et al., 2003; Granot et al., 2004). Most found that these physical differences can account for a very small portion of interindividual variation in pain ratings. Consistent with these studies, twin studies have shown that a large amount of variability remains unaccounted for, thus other factors unrelated to genetic differences may be responsible (MacGregor et al., 1997; Norbury et al., 2007; Nielsen et al., 2008).

In experiment 1 (chapter 2), although all subjects received a 49°C suprathreshold noxious stimulus, the ratings of the stimulus differed substantially from one individual to
the next. While these variations could conceivably be due to differences in individual’s heat pain thresholds, heat pain threshold was not a reliable predictor of heat pain sensitivity. Instead, a significant portion of the variability of interindividual differences in pain ratings was reliably predicted by a model generated from a combination of predictor factors unique to each individual. These results indicate that an individual’s pain experience cannot be predicted from one’s sensory thresholds and genetic differences, suggesting that other factors unique to each individual must be taken into account. Thus, a combination of psychological factors including anxiety level, personality, and depression level can reliably predict a significant portion of variability in experimental pain ratings, indicating that cognitive factors may contribute importantly to interindividual differences in pain.

Many important brain areas including amygdala, hippocampus, ACC, and the prefrontal cortex are known to be involved in various cognitive processes. For example, the amygdala and hippocampus have been shown to be activated during memory recall tasks (Murray and Mishkin, 1983; Ploghaus et al., 2001; Smith et al., 2004; Marschner et al., 2008). ACC and amygdala are important in affective processing (Murray and Mishkin, 1983; Rainville et al., 1997; Price, 2000; Ploghaus et al., 2001; Smith et al., 2004; Marschner et al., 2008). Similarly, anxiety and depression have been shown to influence brain activation within the amygdala and prefrontal cortex (Gracely et al., 2004; Giesecke et al., 2005; Kalisch et al., 2006; Bar et al., 2007; Bishop, 2007). Moreover, the prefrontal cortex is also differentially activated during pain experience with long-term significance and implications such as clinical pain as well as higher-level cognitive pain control such as reappraisal and thought suppression (Bechara et al., 1996; Baron et al.,
1999; Henson et al., 1999; Simpson et al., 2001; Lorenz et al., 2003; Wiech et al., 2006; Salomons et al., 2007). Taken together with results from other studies that show influences of expectation, hypnosis, and placebo on nociceptive processing (Sawamoto et al., 2000; Petrovic et al., 2002; Faymonville et al., 2003; Ploghaus et al., 2003; Derbyshire et al., 2004; Wager et al., 2004; Koyama et al., 2005; Zubieta et al., 2005; Craggs et al., 2007), brain areas that can access this stored cognitive information and utilize it to influence the processing of current nociceptive information play an important role in shaping how pain is experienced. Thus, individual differences in pain sensitivity and how one experiences pain are greatly influenced by a variety of cognitive factors unique to each individual that cannot be simply attributed to genetic and physical differences.

MODULATION OF NOCICEPTIVE PROCESSING BY COGNITIVE INFORMATION

Top-down modulation by cognitive information

Many studies have shown that contextual and cognitive information can importantly shape one’s perception of a sensory stimulus. In studies of visuospatial attention, correctly-signaled visual cues can improve the accuracy and search time (Corbetta et al., 1993; Pessoa et al., 2003; Knudsen, 2007; Corbetta et al., 2008). This top-down modulation involves activities in brain areas involved in attentional control, including DLPFC, ACC, FEF and IPL. These brain areas are important in utilizing contextual cues to provide top-down signals to modulate the sensitivity of cortical visual sensory processing areas via cortico-cortical interactions. This cognitively driven
attention (endogenous or covert) process can enhance the sensitivity of visual neurons in the corresponding visual field even before an eye saccade is made to detect a visual stimulus (Corbetta et al., 1993; Pessoa et al., 2003; Knudsen, 2007; Corbetta et al., 2008).

Cognitive influences on nociceptive processing can also importantly shape how pain is experienced. For example, a pessimistic outlook about one’s health condition and catastrophizing about one’s pain can negatively impact the pain experience and suffering in patients with chronic illnesses (Geisser et al., 2000; Haythornthwaite and Benrud-Larson, 2000; Auerbach et al., 2001; Jones et al., 2003; Giesecke et al., 2005). In a study where the context of pain relief is manipulated, results differed substantially depending on the situation. One such example is from open vs. hidden injection placebo studies. In the open condition, delivery of potent analgesics is apparent to the patients (e.g., seeing the nurse inject analgesics in intravenous line). On the contrary, in the hidden condition, the analgesics are delivered without the patients being aware of when or how much analgesics were delivered. Interestingly, patients who were aware of analgesics injections (open) reported more pain relief and require fewer analgesics than patients who were not aware of delivered analgesics (hidden) (Levine and Gordon, 1984; Amanzio et al., 2001; Benedetti et al., 2003; Colloca et al., 2004; Price et al., 2008). In keeping with this notion, other studies have shown that previous experience with, and certain expectations of, the success of a particular treatment for pain relief can significantly influence the effectiveness and the outcome of the current treatment in providing pain relief (Price et al., 1999; De Pascalis et al., 2002; Price et al., 2008). These findings suggest that cognitive information can importantly shape how nociceptive information is
processed, possibly via neural mechanisms involving top-down modulation and cortico-cortical interactions.

**Neural mechanisms supporting cognitive modulation of pain**

Most studies examining the effects of cognitive influences on nociceptive processing examine how various cognitive manipulations such as attention, expectation, and placebo influence nociceptive processing (Bushnell et al., 1985; Bantick et al., 2002; Tracey et al., 2002; Ploghaus et al., 2003; Valet et al., 2004; Wager et al., 2004; Koyama et al., 2005; Petrovic et al., 2005; Dunckley et al., 2007; Quevedo and Coghill, 2007). Across studies, cognitive modulation of nociceptive processing has been shown to occur at both spinal and supraspinal levels.

*Cognitive influences via spinal interactions*

Attention, for instance, has been suggested to modulate spatial integration of nociceptive processing and receptive field of neurons at the level of spinal cord (Bushnell et al., 1985; Quevedo and Coghill, 2007). However, although most studies describe cognitive top-down influences on nociceptive processing as originating from the cortex, these influences inevitably converge at the brainstem via opioid-dependent pathways. Placebo studies have described cognitive modulation in terms of activation of a cortical network, including the ACC, insula, and prefrontal cortex, ultimately converging at the ACC which then acts on the PAG to modulate descending control systems (Price and Barrell, 2000; Petrovic et al., 2002; Ploghaus et al., 2003; Wager et al., 2004; Zubieta et al., 2005; Bingel et al., 2006; Price et al., 2008; Wiech et al., 2008). Similarly, most
studies of attention reported that ACC plays a major role in activating the descending inhibitory control system during distraction to attenuate incoming sensory signals, ultimately resulting in decreased pain ratings (Frankenstein et al., 2001; Bantick et al., 2002; Tracey et al., 2002; Dunckley et al., 2007; Wiech et al., 2008). These results suggest that one way cognitive information can influence nociceptive processing is via influencing the degree of modulation of the PAG and, subsequently, the RVM, which sends modulatory projections to the dorsal horn of the spinal cord to attenuate or facilitate nociceptive sensory transmission.

*Cognitive influences via supraspinal interactions*

Nevertheless, the importance of cortico-cortical interactions in cognitive pain modulation has long been suspected (Wiech et al., 2008). More recent studies have shown that the effects of hypnosis, expectation, and placebo on pain result from cortico-cortical interactions that can powerfully modulate the processing of nociceptive information (Faymonville et al., 2003; Koyama et al., 2005; Craggs et al., 2007). For example, cortico-cortical interactions involving the insula and other structures including ACC, DLPFC, and SII are important in the initiation and the maintenance of placebo analgesia (Craggs et al., 2007). Activity of the insula was found to be negatively correlated with that of ACC and positively correlated with that of DLPFC during placebo analgesia.

Conversely, the insular cortex is also activated during imagined and hypnotically induced pain (Faymonville et al., 2003; Derbyshire et al., 2004). Moreover, the modulatory effects of hypnotic pain control are thought to involve interactions of ACC
with a network of brain areas including the insula and prefrontal cortex (Faymonville et al., 2003). Consistent with this notion, Wager et. al suggested that selective patterns and timecourses of reduction in pain-related brain activation during placebo, as opposed to simple generalized attenuation of brain activation, indicate that a major portion of the placebo effect may be mediated centrally by changes in specific pain regions at the cortical level (Wager et al., 2004).

In addition, studies examining the effects of expectation on pain have shown that a network of expectation-related brain areas, including the insula, significantly overlap with those of pain-related brain areas (Ploghaus et al., 1999; Sawamoto et al., 2000; Koyama et al., 2005). Accordingly, these expectation-related brain areas are ideally positioned to potently modulate activities of nociceptive processing brain areas during pain via top-down cortico-cortical modulation (Koyama et al., 2005). Similarly, studies looking at perceived control over one’s pain and higher-level cognitive pain control strategies show that prefrontal cortex activation was directly associated with decreased activation in many nociceptive processing brain areas (Salomons et al., 2004; Kalisch et al., 2006; Wiech et al., 2006; Salomons et al., 2007). In these studies, both pain ratings and activation within nociceptive processing areas were potently modulated by expectation.

**Establishing the roles of the insular cortex in the modulation of pain**

In experiment 2 (chapter 3), we provide multiple lines of evidence to support the role of the insular cortex in serving as one important potential interface between the internal cognitive state and nociceptive processing by integrating cognitive information
and providing top-down modulations of connected brain areas to influence nociceptive processing.

Traditionally, the insular cortex has been suggested to be important in assigning an appropriate affective response to a nociceptive stimulus by providing a link for the “cortico-limbic” pathway to transfer information from somatosensory to limbic areas (Friedman et al., 1986; Berthier et al., 1988). For example, somatosensory information from SII, SI, and posterior parietal cortex can flow from these brain areas to the posterior insula, then to anterior insula, and then onto limbic areas such as the ACC, amygdala, and hippocampus (Friedman et al., 1986). It is this transfer of somatosensory information to the limbic areas that is thought to be responsible for generating the affective dimension of pain experience (Berthier et al., 1988). According to this view, lesions of the insula would be expected to produce pain asymbolia, the lack of an appropriate affective response during painful stimulus (Weinstein et al., 1955; Berthier et al., 1988). However, we found that pain unpleasantness ratings of patients with insular lesions were not significantly lower compared to those of controls. Thus, in addition to its role in pain affect, the insular cortex may also be involved in other aspects of nociceptive processing.

Additionally, this brain area is an important “interoceptive” cortex critical for the experience of pain and other feelings associated with physiological and homeostatic changes (Craig, 2002, 2003a, b). According to this model, primary homeostatic afferents emerging from lamina I of the spinal cord carry important homeostatic information such as pain and temperature to the thalamus then to the insular cortex. The feelings and subjective awareness associated with the physiological self are thought to rely on the interpretation of this information by the insular cortex. The feeling of anxiety, for
example, may be the result of the interpretation of afferent homeostatic input conveying information such as increased respiratory rate, racing heart beat, and decreased gastrointestinal motility. Accordingly, damage to this structure would be expected to abolish pain sensations. In sharp contrast to this view of the insula, we found that patients with lesions of the insula can still experience pain and retain ability to discriminate noxious stimuli of graded intensities. In fact, patients with insular lesions exhibited increased pain sensitivity when compared to age-matched control subjects. In the absence of any detectible insular activation during painful stimulation, these results suggest that the insular cortex is not necessary to instantiate a complete subjective experience of pain. Moreover, these findings indicate a more modulatory role of the insular cortex in pain processing rather than a node critical for the pain experience. Taken together with other studies of various cognitive manipulations of pain including placebo, expectation, hypnosis, and attention that show insular activation (Ploghaus et al., 1999; Petrovic et al., 2002; Faymonville et al., 2003; Derbyshire et al., 2004; Wager et al., 2004; Koyama et al., 2005; Craggs et al., 2007), our results suggest that the insula may be importantly involved in using contextual information to influence the processing of nociceptive sensory information.

**Integrating and utilizing cognitive information to modulate nociceptive processing**

The modulatory role of the insula is supported by its bidirectional, reciprocal connections with cognitive and nociceptive processing brain areas including the amygdala, hippocampus, prefrontal cortex, ACC, SII, and thalamus (Mufson et al., 1981; Mesulam and Mufson, 1982; Mufson and Mesulam, 1982, 1984; Friedman et al., 1986).
This configuration allows the insula access to stored cognitive information as well as to exert its influence on connected brain areas. For example, the insula may access emotional memory and previous experience stored in the amygdala and hippocampus and utilizes this information to influence activity of the connected nociceptive processing brain areas. Accordingly, insula is well positioned to integrate cognitive information as well as to actively modulate connected nociceptive processing brain areas in a context-dependent fashion. Moreover, recent findings have suggested that activity within the anterior insula may actively modulate activities of other brain regions in a task-related manner (Sridharan et al., 2008), including its interaction with ACC and the prefrontal cortex during placebo analgesia (Craggs et al., 2007). This suggests that the insula is well suited to influence the activity of nociceptive processing brain areas to shape how pain is experienced. Thus, appropriate top-down modulation of SII and thalamus by the insula may influence how the sensory-discriminative aspect of pain is perceived, while modulation of the ACC may influence the affective component of pain. The observed increases in pain sensitivity suggest that insular lesions disrupt patients’ ability to use cognitive information, including the safety context associated with the experiment, to appropriately modulate nociceptive processing brain areas involved in affective and sensory processing areas of pain such as SII, ACC, and thalamus (Christianson et al., 2008).

Because the insula is involved with areas that are actively engaged in the descending control such as ACC and PAG, it is possible that the increased pain sensitivity noted may be due in part to increased nociceptive output from the spinal cord (Fields and Basbaum, 1978; Mesulam and Mufson, 1982; Mufson and Mesulam, 1982;
Fields and Basbaum, 1999; Price, 1999). Such an effect would lead to equal alterations in both pain dimensions. However, a unique disconnect between the two pain dimensions seen in one patient suggests that higher-level dissociations between different cortical areas involved in processing various aspects of pain might have occurred. Moreover, disruptions of insular modulation of descending control system interactions would also lead to an unspecific increase in brain activity in all nociceptive processing areas reflective of increased nociceptive sensory output from the spinal cord. Conversely, the lack of insular contributions to nociceptive processing in lesioned patients produces a selective and differential increase in activity of only few cortical areas, namely SI and DLPFC. This suggests that the modulatory role of the insula in nociceptive processing likely occurs at the cortical level.

Additionally, despite the recruitment of other brain areas to help with nociceptive processing, activity within these brain areas may not be sufficient to fully compensate for the roles of insula during nociceptive processing as evident by the increased pain sensitivity seen in both patients. Apparently, SI does not have the neural resources and configuration to fully compensate for the role of the insula in nociceptive processing. For example, SI does not have direct reciprocal connections to brain areas involved in cognitive processing such as the prefrontal cortex, amygdala, hippocampus, and ACC like the insula (Mufson et al., 1981; Mesulam and Mufson, 1982; Mufson and Mesulam, 1982, 1984; Friedman et al., 1986). Accordingly, SI may not be able to sufficiently fulfill the cognitive modulatory roles of the insula.

In conclusion, the insula and associated brain areas contribute importantly to the processes involved in the integration of cognitive information from various brain areas by
providing appropriate top-down cortico-cortical modulation of nociceptive processing brain areas in a context-dependent fashion. This modulatory role of the insula may partly underlie how contextual information can influence our pain perception in both clinical and non-clinical settings.

**SELECTIVE ENGAGEMENT OF NOCICEPTIVE PROCESSING BY COGNITIVE INFORMATION**

The highly intrusive nature of the pain experience makes a nociceptive stimulus hard to ignore. Few other stimuli are more intrusive and demand such an immediate behavioral response than pain (Price, 1999). Pain engages widely distributed regions of cortex (Coghill et al., 1994; Coghill et al., 1999; Peyron et al., 2000). This brain network includes ACC, insula, SII, SI, SMA, and thalamus involved in attention, affect, and somatosensory processing (Coghill et al., 1994; Coghill et al., 1999; Peyron et al., 2000). These results suggest that pain carries highly salient information effective in engaging response from a large network of brain areas to process incoming nociceptive information (Price, 1999). Nevertheless, the strength of behavioral response and the degree of intrusiveness that a painful stimulus commands depend greatly on the contexts in which the sensory event takes place (Price, 1999).

For example, an intense noxious event that has been associated with bad consequences in the past may evoke a greater cortical response than one without such a context. Thus the pain experience depends on many other factors unrelated to the physical magnitude of the sensory input. In the case of a bee-sting, for instance, the brain may be able to utilize contextual cues such as buzzing sound, sight of a bee, and previous
experience of a bee-sting to influence the magnitude of the brain’s response. Accordingly, the pain associated with the bee-sting may be felt as more intrusive than a noxious stimulus of similar intensity since it has previously been associated with previous bad consequences. This suggests that the behavioral relevance and saliency of incoming nociceptive information may be determined prior to the full engagement of cortical response during inflow of such sensory information. Accordingly, an underlying mechanism analogous to those of action selection suggested for motor system likely exists for pain processing.

Although the putamen activation during pain has often been noted, its roles and contribution to nociceptive processing remain poorly understood and confined to motor-related response to the noxious stimuli (Alexander et al., 1990; Coghill et al., 1999; Peyron et al., 2000; Bingel et al., 2004). However, multiple lines of evidence suggest that the neural circuitry involving the putamen may contribute importantly to the processes involved in integrating a variety of cognitive information to provide salience and behavioral relevance to incoming nociceptive sensory input and appropriately engage selective cortical networks to process incoming nociceptive information during pain.

**Integration and selection architecture within the putamen and the basal ganglia**

Many anatomical frameworks intrinsic to the basal ganglia can facilitate the integration of information from many functional domains. The striatum receives incoming afferents from a variety of cortical, limbic, and thalamic areas (Willis, 1985; Alexander and Crutcher, 1990; Parent and Hazrati, 1995; Mengual et al., 1999; Van der Werf et al., 2002) involved in attention, memory, affect, and nociceptive processing.
These brain areas send overlapping projections converging on single striatal projections neurons (Haber, 2003; Haber et al., 2006; Calzavara et al., 2007). Within the basal ganglia, patterns of overlapping projections may be seen at each relay point as information from widespread region of cortex are passed progressively onto smaller basal ganglia structures (Gimenez-Amaya et al., 1995; Groenewegen et al., 1999; Haber et al., 2000). In addition, striatal projections to output nuclei may diverge, as GPi and SNr both receive information that originated from the same cortical area. Given that these two output nuclei project to two different locations on thalamic nuclei, some thalamo-cortical projections from one information loop may terminate at the origination of the cortico-striatal projection of a different information loop (Joel and Weiner, 1994). This neural configuration is intrinsic to the relationship between the basal ganglia and the neocortex and facilitate the convergence and confluence of various information from many functional domains. This allows the putamen, as part of the striatum, to be well positioned to integrate cognitive information from many brain regions.

In addition to playing a role in integration, many lines of evidence suggest that the basal ganglia circuitry also have the anatomical resources and configuration that may facilitate the interaction of information in parallel loops (Joel and Weiner, 1994; Redgrave et al., 1999). Such interaction may be important in determining the salience of an incoming signal (Joel and Weiner, 1994; Redgrave et al., 1999). For example, excitatory glutamatergic inputs onto an individual spiny neuron in the striatum must be sufficiently depolarizing to generate action potentials (Gerfen and Wilson, 1996), thus serving to initially filter out weak competing signals. In addition, there are local inhibitory collaterals between striatal spiny neurons as well as longer range inhibitory
interneurons that may cause highly activated striatal elements to suppress activity in more weakly activated channels (Plenz, 2003; Tepper and Bolam, 2004). Finally, at the level of output nuclei of the basal ganglia, local inhibitory collaterals between output nuclei neurons also may enhance the contrast between the inhibited and non-inhibited channels (Mailly et al., 2003). Taken together, the neural configuration of the basal ganglia may facilitate the integration and interaction of various cognitive information from many brain areas. These processes may be important in providing context for determining the saliency of the incoming signals and selection of most behaviorally relevant tasks when there are multiple competing tasks for limited neural resources (Redgrave et al., 1999).

**Utilizing cognitive information to influence nociceptive processing**

In experiment 3 (chapter 4), regions of the putamen activated during pain were functionally connected to various brain areas including: 1) attention-related brain areas including FEF, DLPFC, ACC, IPL, and MFG; 2) emotion and memory-related brain areas including the amygdala and hippocampus; 3) Nociceptive processing brain areas including the ACC, thalamus, and SII. Together with the integrative and interactive anatomical framework described above, the neural circuitry involving the putamen and the basal ganglia may play an important role in integrating a variety of cognitive and contextual information related to memory, previous experience, affect, and attention as well as higher-level cognitive information. The interactions of these various types of information may be important in determining the behavioral relevance of the incoming sensory information.
Consistent with this notion, many studies have shown that the putamen is important in using cue-related information to influence execution of a task (Jaeger et al., 1993; Koski et al., 1999). Similarly, a network of brain areas including SMA, putamen, and thalamus were important in initiating endogenous (cognitively driven) attention shifts (Coull et al., 2000), implying that the top-down influence of cognitive information on a network of brain areas may rely on activity within the putamen. Moreover, studies have shown that lesions of the striatum can affect the ability to use contextual cues in performing tasks (Amalric et al., 1995; Devan et al., 1999; Chang et al., 2006). Thus, the putamen may play an important role in utilizing cognitive information to influence cortical processing of sensory information. In addition, sustained putamen activity has also been noted during prolonged noxious stimulation (Downar et al., 2003), and during sustained cortical activity in various brain areas including parietal, frontal, and cingulate regions during task performance (Koski et al., 1999; Coull et al., 2000; Downar et al., 2003). Taken together with the suggested roles of the functionally connected brain areas identified in the study in various higher-level cognitive processes such as hypnosis, expectation and placebo (Sawamoto et al., 2000; Petrovic et al., 2002; Faymonville et al., 2003; Ploghaus et al., 2003; Derbyshire et al., 2004; Wager et al., 2004; Koyama et al., 2005; Zubieta et al., 2005; Craggs et al., 2007), the neural circuitry involving the putamen may play an important role in utilizing cognitive information to influence information processing at the cortical level.
Selection through disinhibition

In the selection for action model, the striatum can differentially exert its influence on the output nuclei of the basal ganglia, GPi and SNr, to modulate varying levels of disinhibition of the specific thalamic nuclei (Chevalier and Deniau, 1990; Parent and Hazrati, 1995) (Figure 1). Since these thalamic nuclei project back to the cerebral cortex (Figure 1), the basal ganglia can influence cortical activity in a task-related manner. In experiment 3 (chapter 4), we found that putamen activation during pain was associated with activation in several thalamic regions consistent with nuclei such as VA, VL, and MD. These nuclei receive input from the output nuclei of the basal ganglia, GPi and SNr, and project to large areas of the cerebral cortex including frontal, parietal, insula, and cingulate areas (Mufson and Mesulam, 1984; Vogt et al., 1987; Alexander and Crutcher, 1990; Alexander et al., 1990; Parent and Hazrati, 1995; Middleton and Strick, 2000) (Figure 1). Taken together with the functional connections of the putamen with various cognitive processing brain areas, this configuration allows the neural circuitry involving the putamen to utilize internal cognitive information to provide selective engagement of appropriate cortical networks to process sensory inputs during inflow of nociceptive information. Moreover, the degree and strength of cortical response elicited by the incoming nociceptive information may be importantly shaped by the behavioral-relevance of the incoming signal determined by context and other cognitive features.
Figure 1. Architecture of the basal ganglia supporting cognitive influences on cortical activity. The striatum receive input from various cognitive processing brain areas. In addition, the striatum can differentially exert its influence on the output nuclei of the basal ganglia, GPi and SNr, to modulate varying levels of disinhibition of the specific thalamic nuclei. These thalamic nuclei including VA, VL, and MD project to large areas of the cerebral cortex including frontal, parietal, insula, and cingulate areas. This allows the basal ganglia to utilize cognitive information to influence cortical activity.
**Strength and degree of cortical response**

The contribution of this neural circuitry involving the putamen may partly underlie detectible differences in pattern and strength of activation of nociceptive processing brain networks during different cognitive manipulations. During placebo and expectation for decreased pain, brain networks involved in nociceptive processing are actively engaged to a lesser degree as reflected by a diffuse, generalized reduction in brain activation as compared to similar noxious stimulation without such context (Sawamoto et al., 2000; Petrovic et al., 2002; Faymonville et al., 2003; Ploghaus et al., 2003; Derbyshire et al., 2004; Wager et al., 2004; Koyama et al., 2005; Zubieta et al., 2005; Craggs et al., 2007). Similarly, in studies where attention was not directed to the stimulus, pain ratings were decreased and accompanied by a generalized reduction in activation of pain-related brain networks (Frankenstein et al., 2001; Bantick et al., 2002; Tracey et al., 2002; Dunckley et al., 2007; Wiech et al., 2008).

In the clinical realm, for instance, a moderately intense abdominal pain may be perceived differently in two individuals depending on the context. If an individual, whose family member had previously passed away from colon cancer, or whose abdominal pain had preceded bad outcome in the past, pain may evoke a more potent behavioral response than it would otherwise. Thus, the brain may utilize various contextual cues to influence the degree and strength of the cortical response to incoming sensory information. Accordingly, pain that has higher behavioral relevance (e.g., signaling occurrence of cancer) may engage a more robust response than pain of similar magnitude without such a context. This neural mechanism can provide salience contrast.
for the more behaviorally relevant event to engage a more potent response than lower priority one, thereby optimizing survival benefits to the organism.

Accordingly, patients with putamen lesions may have alterations in the ability to integrate and utilize cognitive information to influence how incoming sensory information is processed. Moreover, given the role of the putamen and its associated brain networks in engaging large cortical response during pain, patients with putamen lesions may also fail to engage appropriate cortical networks to process incoming nociceptive information. Consistent with this notion, patients with putamen lesions exhibited diffuse, generalized reduction in brain activity during pain and exhibited decreased pain sensitivity. This suggests that cortical networks important for processing nociceptive information may not be fully engaged. Accordingly, incoming nociceptive information may not be fully processed and appreciated.

**Biasing selection mechanisms by nociceptive information**

Consistent with the known anatomical connections between intralaminar and midline thalamic nuclei with the striatum (Mengual et al., 1999; Van der Werf et al., 2002)(Figure 2), the putamen is both structurally and functionally connected to the thalamus. These thalamic nuclei are importantly involved in nociceptive processing and receive direct nociceptive input from the spinal cord (Willis, 1985)(Figure 2). This allows nociceptive information from the spinal cord to reach the basal ganglia circuitry relatively early and directly via fewer synapses (Figure 2). This pathway may represent a hardwired mechanism to ensure that afferent nociceptive information rapidly influences the selection architecture to interrupt ongoing cognitive processes.
In addition, the putamen and the striatum also receive modulatory nigrostriatal dopaminergic inputs from the SNc in the midbrain (Gerfen and Wilson, 1996)(Figure 2). Although midbrain dopaminergic activity has been viewed by some as purely related to reward-prediction error (the difference between actual reward and the predicted reward), other studies have shown that nigrostriatal dopaminergic release can also be evoked by highly salient, nonrewarding stimuli (Horvitz, 2000; Zink et al., 2003; Redgrave and Gurney, 2006; Zink et al., 2006). However, studies on dopaminergic activity during pain have shown conflicting results. While some have shown inhibition of nigrostriatal dopamine activity after onset of a noxious stimulus, others showed evoked dopamine release (Barasi, 1979; Hagelberg et al., 2002; Pruessner et al., 2004; Ungless, 2004; Ungless et al., 2004; Coizet et al., 2006; Scott et al., 2006; D'Ardenne et al., 2008; Leknes and Tracey, 2008). Nevertheless, recent studies on chronic pain patients with burning mouth, fibromyalgia, and atypical facial pain syndromes have shown that these patients have lower striatal D2 receptor activity when compared to healthy controls (Hagelberg et al., 2003a; Hagelberg et al., 2003b; Hagelberg et al., 2004; Wood et al., 2007). Presumably, alterations in striatal dopamine activity can significantly influence how nociceptive information is processed. Moreover, variations in nigrostriatal dopamine release during pain have also been shown to be associated with variations in sensory and affective ratings of painful stimuli (Hagelberg et al., 2002; Pertovaara et al., 2004; Scott et al., 2006).

In experiment 3 (chapter 4), SN/VTA activity correlated significantly with activity of regions of the putamen activated during pain. In addition, SNc has relatively direct connections with parabrachial nucleus in the midbrain (Schneider, 1986; Vankova
et al., 1992), which receives direct afferent nociceptive information from the spinal cord (Bernard and Besson, 1990; Craig, 1995; Klop et al., 2005)(Figure 2). Thus, the SNC may be in a position to use nociceptive information to modulate striatal activity and efficiency of cortico-striatal terminals via its nigrostriatal dopaminergic modulatory projections (Albin et al., 1989; Gerfen and Wilson, 1996)(Figure 2). These findings suggest that nigrostriatal dopaminergic activity can influence the activity of the neural circuitry involving the putamen to ensure the selection of appropriate brain networks during the inflow of afferent nociceptive information. These hardwired mechanisms may partly underlie the prepotent nature and the effectiveness of a painful stimulus to engage behavioral response and capture attention. The intrusiveness associated with pain may confer evolutionary benefit for survival, as it ensures that the potentially injured body site is attended to.

Although these mechanisms tend to bias the selection of nociceptive information to be processed, not all noxious stimuli elicit robust cortical responses and engage behavioral responses. For example, one may not be immediately aware of a cut or an injury until some time later. One possible explanation for this is that the incoming nociceptive information may not be salient enough to “override” the apparently more salient ongoing cognitive processes (e.g., fleeing from a potentially life-threatening situation). Another possible explanation is that the influences provided by cognitive information, which may operate at both conscious and subconscious levels, (i.e., attention directed to the stimulus, previous experience with the stimulus, and context surrounding the stimulus) may not convey sufficient behavioral relevance or salience to the incoming
sensory signal for it to win out in the competition among other ongoing cognitive processes.
Figure 2. Biasing selection by afferent nociceptive information from spinal cord. The striatum receive input from the intralaminar and midline thalamic nuclei. These thalamic nuclei are importantly involved in nociceptive processing and receive direct nociceptive input from the spinal cord. This allows nociceptive information from the spinal cord to reach the basal ganglia circuitry relatively early and directly via fewer synapses. In addition, the striatum also receive modulatory nigrostriatal dopaminergic inputs from the SNC in the midbrain. In addition, SNC has relatively direct connections with parabrachial nucleus in the midbrain, which receives afferent nociceptive information from the spinal cord. Thus, the SNC may utilize nociceptive information to modulate striatal activity and efficiency of cortico-striatal terminals via its nigrostriatal dopaminergic modulatory projections. These two pathways may represent hardwired mechanisms to ensure that afferent nociceptive information will enter the selection architecture to interrupt ongoing cognitive processes.
Modulation vs. selection

Although both putamen and insula receive large incoming afferents from brain areas involved in both cognitive and nociceptive processing, and utilize cognitive information to influence nociceptive processing, their routes of action differ substantially. The bidirectional anatomical framework of the insula, supported by its reciprocal anatomical connections with various brain areas (Mufson et al., 1981; Mesulam and Mufson, 1982; Mufson and Mesulam, 1982, 1984; Friedman et al., 1986), allows the insula to be in a position to directly modulate activity of each cortical area involved in various aspects of nociceptive processing. This is supported by the fact that insular lesions produced specific and differential increases in the activity of a few cortical areas accompanied by increased pain sensitivity. These changes potentially reflect a disruption of the transmission of an appropriate modulatory safety signal to nociceptive processing brain areas (Christianson et al., 2008).

In contrast, the flow of information from the cortex to the putamen is mostly unidirectional (Alexander et al., 1990; Parent and Hazrati, 1995). Thus, the putamen may not be able to directly modulate activity of each brain area. Nevertheless, the putamen has outgoing efferents to output nuclei of the basal ganglia, GPi and SNr, that can modulate thalamo-cortical flow of information. Since the thalamic output nuclei project to large areas of the cerebral cortex, the neural circuitry involving the putamen can engage a large network of brain areas to respond to a nociceptive stimulus in a context-relevant fashion via disinhibition of specific thalamic nuclei. Consistent with this role, lesions of the putamen produced diffuse, generalized reductions in pain-related brain activation in large areas of the cerebral cortex and decreased pain sensitivity. This likely
reflects disruptions in processes involved in selective engagement of appropriate cortical networks for the processing of nociceptive information.

**FINAL CONSIDERATIONS**

Cognitive information is important for a full appreciation of a nociceptive stimulus and for a complete subjective experience of pain. Accordingly, brain areas involved in integrating cognitive influences into a percept of the sensory stimulus must be able to utilize information related to attention, expectation, memory, and affect to influence how sensory nociceptive information is processed. We provide multiple lines of evidence to show that mechanisms including modulation and selection of cortical networks active during pain based on cognitive information and contexts may involve contributions from neural circuitry that include brain areas such as the insula and putamen. These processes allow organisms to appropriately respond to the threat of the stimulus, and, thus, can be very important for survival. Finally, these cognitive influences may explain why a complete subjective experience of pain varies from one individual to the next. Simply put, the contribution of an individual’s unique experiences in life may underlie individual differences in pain and significantly shape how nociceptive information is processed.
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CURRICULUM VITAE

Christopher Jenn Starr

533 Ivy Glen Drive – Winston-Salem, NC 27127
cstarr@wfubmc.edu

NAME: Christopher Jenn Starr

ADDRESS:

Residence: 533 Ivy Glen Drive
Winston-Salem, NC 27127
(859) 229-7125

Business: Department of Neurobiology and Anatomy
Wake Forest University School of Medicine
Medical Center Boulevard.
Winston-Salem, North Carolina 27157

PERSONAL INFORMATION:

Birthplace: Bangkok, Thailand.

Birth date: 08/05/1983

Citizenship: Thai and American

Marital status: Single

EDUCATION:

2004-2009 Wake Forest University
Winston-Salem, NC
School of Medicine – MD/PhD

2001-2004 University of Kentucky
Lexington, KY
College of Arts and Sciences – Biology – B.S.
EMPLOYMENT:

General Employment History

2002 Research Assistant
Department of Chemistry,
University of Kentucky

2002 Research Assistant
College of Pharmacy,
University of Kentucky

2002-2004 Research Assistant
Department of Neurobiology and Anatomy
University of Kentucky

Teaching Experience

2003 Teaching Assistant
Department of Chemistry,
University of Kentucky,

PROFESSIONAL MEMBERSHIPS:

American Pain Society
International Association for the Study of Pain
Organization for Human Brain Mapping
Society for Neuroscience

HONORS AND AWARDS:

2001 University of Kentucky Academic Excellence
Merit Scholarship

2001 1st Place Winner, American Chemical Society
(ACS) Chemistry Competition, University of
Kentucky
2001 Department of Chemistry, University of Kentucky Merit Scholarship

2001 – 2004 Kentucky Educational Excellence Scholarship

2001 – 2004 Dean’s List, University of Kentucky

2004 Graduate with Honors in Biology, University of Kentucky

2004 Summa Cum Laude, University of Kentucky

2004 Wake Forest University School of Medicine MD/PhD Fellowship

2007 American Pain Society Young Investigator Travel Award, Washington D.C.

2008 Travel Award to attend the International Association for the Study of Pain meeting in Glasgow, Scotland

2008 Scholarship to attend “How to Combine Clinical and Research Careers in Neuroscience,” Washington, D.C., sponsored by AUPN, ANA, NIH, and NINDS

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