Differential Activation of the Human Trigeminal Nuclear Complex by Noxious and Non-Noxious Orofacial Stimulation

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Abstract: There is good evidence from animal studies for segregation in the processing of non-nociceptive and nociceptive information within the trigeminal brainstem sensory nuclear complex. However, it remains unknown whether a similar segregation occurs in humans, and a recent tract tracing study suggests that this segregation may not exist. We used functional magnetic resonance imaging (fMRI) to define and compare activity patterns of the trigeminal brainstem nuclear complex during non-noxious and noxious cutaneous and non-noxious and noxious muscle orofacial stimulation in humans. We found that during cutaneous pain, signal intensity increased within the entire rostrocaudal extent of the spinal trigeminal nucleus (SpV), encompassing the ipsilateral oralis (SpVo), interpolaris (SpVi) and caudalis (SpVc) subdivisions. In contrast, muscle pain did not activate SpVi, but instead activated a discrete region of the ipsilateral SpVo and SpVc. Further, muscle noxious stimulation activated a region of the ipsilateral lateral pons in the region of the trigeminal principal sensory nucleus (Vp). Innocuous orofacial stimulation (lip brushing) also evoked a significant increase in signal intensity in the ipsilateral Vp; however, non-noxious muscle stimulation showed no increase in signal in this area. The data reveal that orofacial cutaneous and muscle nociceptive information and innocuous cutaneous stimulation are differentially represented within the trigeminal nuclear complex. It is well established that cutaneous and muscle noxious stimuli evoke different perceptual, behavioural and cardiovascular changes. We speculate that the differential activation evoked by cutaneous and muscle noxious stimuli within the trigeminal sensory complex may contribute to the neural basis for these differences. *Hum Brain Mapp* 30:3772–3782, 2009. © 2009 Wiley-Liss, Inc.

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INTRODUCTION

It is well established from animal investigations that nociceptive and non-nociceptive sensory information are differentially represented within the central nervous system [Sessle, 2000a]. In the spinal somatosensory system, for example, spinal nociceptive afferents terminate primarily in the dorsal horn of the spinal cord, while non-nociceptive spinal afferents terminate primarily in the dorsal column nuclei. In the trigeminal system,

nociceptive afferents from the face terminate primarily in the spinal trigeminal nucleus (SpV), particularly the sub-nucleus caudalis [Dubner and Ren, 2004; Sessle, 2000a], while non-nociceptive facial afferents terminate extensively throughout the trigeminal principal sensory nucleus (Vp) as well as the more rostral components of the SpV [Sessle, 2000].

Further, the termination patterns of spinal nociceptive afferents can be segregated according to the type of tissue from which they originate (e.g. cutaneous versus muscle) [Abrahams and Swett, 1986; Bandler et al., 2000; Lumb, 2002; Ohtori et al., 2000]. It has been suggested that this segregation of nociceptive afferents, and the differential representations of deep and superficial pain in higher brain centers, underlie the different psychophysical, behavioural and/or autonomic reactions evoked by painful stimuli arising from different body structures [Henderson et al., 2006]. Traditionally, the sub-nucleus caudalis has been considered to be the primary relay of nociceptive information, particularly cutaneous and mucosal, from the orofacial area [Dubner and Ren, 2004; Sessle, 2000a]. More recently, evidence has accumulated for nociceptive representations within more rostral components of SpV. In particular, the interpolaris/caudalis transition zone has been proposed as playing an important role in jaw muscle nociceptive processing [Ro and Capra, 1999; Wang et al., 2006]. Further, a recent rodent neural tracing investigation revealed that nociceptive afferents from the masseter not only terminate within the caudal regions of SpV but also can terminate within more rostral divisions of the trigeminal sensory complex, including Vp [Dessem et al., 2007]. This is surprising, given that Vp is traditionally thought to transmit primarily non-noxious discriminatory touch information.

It remains unknown whether there is a segregation of nociceptive inputs within the trigeminal system in humans. If indeed muscle nociceptive information is primarily processed in a specific region of the SpV that differs from the region processing cutaneous/mucosal nociceptive information, then this will afford the opportunity to characterize neurotransmitters and receptors within these regions. As nociceptive afferents are thought to play a significant role in the development and/or maintenance of some orofacial pain conditions, it may then be possible to selectively target these regions for relief of the persistent muscle pain that is a common symptom of temporomandibular disorders (TMD) [LeResche, 1997; Sessle, 2000b].

The aim of this investigation was to define and compare the patterns of fMRI signal intensity increases in the trigeminal nuclear complex during non-noxious cutaneous, noxious cutaneous and noxious muscle orofacial stimulation in humans. We hypothesize that both cutaneous and muscle orofacial noxious stimuli will evoke differential patterns of activity in the trigeminal brainstem nuclear complex.

METHODS

Subjects

Thirty healthy subjects (22 males) aged 19–52 years participated in this study. All procedures were carried out with the understanding and written informed consent of each subject. All procedures were approved by local institutional Human Research Ethics Committees (University of New South Wales, University of Sydney, Westmead Hospital) and were conducted in accordance with the conditions established by the Declaration of Helsinki.

Stimulus and MR Imaging

With subjects in a supine position, a fine plastic cannula (23 gauge), attached to a 1 ml syringe containing sterile hypertonic (4.5%) saline, was inserted deep into the central belly of the right masseter muscle; another was placed subcutaneously over the right masseter muscle. A continuous series of 130 gradient echo image sets using Blood Oxygen Level Dependent (BOLD) contrast was then collected using a 3 Tesla, Phillips Intera scanner (57 axial slices, TR = 4 s, TE = 30 ms, flip angle = 90°, FOV = 250 mm, raw voxel size = 1.95 × 1.62 × 3.3 mm thick). Following a 40 volume baseline, subjects received an intramuscular or subcutaneous bolus injection of hypertonic saline (0.3 ml). Subjects were not made aware of when the injection was to be administered, and the order in which it was to be presented. Each subject was instructed to press a button with their left thumb to indicate (i) when they felt the onset of pain, (ii) when the pain began to subside from its peak and (iii) when the pain had ceased. Immediately following each of the hypertonic-saline injection and while still inside the scanner, subjects were read a Modified Borg Scale for pain intensity (0 = infinitely small, 1 = minimal, 2 = mild, 3 = moderate, 4 = considerable, 5 = large, 7 = very large, 10 = extremely large/maximal), a Modified Borg Scale for pain unpleasantness (1 = Mild, 3 = discomforting, 5 = distressing, 7 = horrible, 10 = excruciating) [Borg et al., 1981] as well as a McGill Pain Questionnaire. Subjects were asked to rate the maximum pain intensity and unpleasantness as well as describe the sensory and affective qualities of the stimulus. After scanning, each subject was asked also to plot the profile of the pain intensity over time for each of the painful stimuli. Using this time plot, as well as the three time points (onset, maximum and cessation) indicated by the buzzer during each pain scanning period, a plot of pain intensity over time was created for each subject. These pain intensity time plots were then averaged across subjects to create a mean cutaneous and muscle pain intensity profile. A 3-D T1 weighted anatomical scan (voxel size = 0.8 × 0.8 × 0.8 mm) was also collected. In addition, 3 subjects returned for a subsequent scanning session a minimum of one month after their initial session to receive an injection of isotonic saline (0.9%, 0.3 ml) into the belly of the right

masseter muscle. The scanning protocol for this injection was identical to the two hypertonic saline injections.

In 12 subjects, we functionally defined the location of the trigeminal principal sensory nucleus in separate scanning sessions. Using a single fMRI series with the same parameters as those used during the first MRI session, a 10 volume baseline period was followed by a 10 volume period during which the right corner of the mouth was continuously brushed with a fine paint brush. This was repeated another 5 times for a total of 6 baseline and 6 brushing periods. We stimulated the lower lip instead of the skin overlying the masseter muscle because of the lip's high degree of sensory innervation and larger representation within areas involved in processing somatosensory information. In no subject was lip brushing described as painful.

MRI Analysis

Using SPM5 [Friston et al., 1995], functional image sets were motion corrected and only subjects with movement parameters less than 1 mm in the X, Y and Z planes were used for analysis. This resulted in the removal of 4 subject's images sets from the cutaneous pain analysis and 5 subject's images sets from the muscle pain analysis. In addition, for the cutaneous and muscle pain series, only image sets from those subjects that rated the pain intensity as 3 or greater were used for further analysis. Overall, 15 subject's image sets were used for the cutaneous pain analysis, 18 subject's images were used for the muscle pain analysis and 12 subject's images used for the lip brushing analysis. Using the SPM5 toolbox each individual's functional images were realigned and coregistered to their T1 anatomical image set. The brainstem was then isolated from the T1 image and normalized to the SPM5 brainstem template. The normalization parameters used in this process were then applied to the functional image sets. A mask of each individual's brainstem (without cerebellum) was created and this was applied to the normalized functional image sets. The brainstem fMRI image sets were then spatially (4 mm FWHM) and temporally smoothed (10 sec FWHM). The first 10 volumes were removed to allow for scanner equilibration.

We found that, although the onset of pain was similar in all subjects, the duration of pain varied considerably. To remove the confounding effects of this variability, for the cutaneous and muscle pain scans we restricted our fMRI analysis to the 30 volumes prior to and 30 volumes following each hypertonic saline injection (i.e. the period in which all subjects perceived considerable pain) (see Fig. 1). Significant changes in signal intensity were determined using a box-car model convolved with a hemodynamic delay. The potential confounding effect of slow scanner drift was removed by the use of a high pass temporal filter (cut-off 480 s). One-sample *t*-tests were performed to determine significant signal intensity increases

Figure 1.

Mean ($\pm$ S.E.M.) pain intensity rating over time following intramuscular (gray) or subcutaneous (black) hypertonic saline injections. The vertical dashed line indicates the onset of each hypertonic saline injection. The scans during which the fMRI analysis was performed is also indicated (i.e. -120 to 120 seconds relative to the injection).

and decreases during muscle pain and during cutaneous pain ($P < 0.01$ random effects, corrected for multiple comparisons, minimum cluster size 5 voxels). Binary images of these group statistical maps were made and regions in which signal intensity increased or decreased during both cutaneous and muscle pain were assessed. In addition, a two-sample *t*-test was performed to determine differences between brainstem activations during cutaneous and muscle pain ($P < 0.01$ uncorrected, minimum cluster size 5 voxels). Finally, a volume-of-interest (VOI) analysis was performed to examine whether muscle and/or cutaneous pain stimuli evoked signal intensity changes in the region of the ipsilateral spinal trigeminal sub-nucleus caudalis (SpVc). In each subject the SpVc was selected, using an anatomical atlas as a guide [De Armond et al., 1976], and the percent signal-intensity changes calculated.

For the lip brushing scans, significant signal intensity changes were assessed using a repeated box-car model convolved with a hemodynamic delay. Since we were interested in defining the location of Vp, we restricted our analysis to the ipsilateral pons (random effects, corrected $P < 0.01$, minimum cluster size 5 voxels). The resulting statistical map was then binarized and regions in which both lip brushing and cutaneous pain and both lip brushing and muscle pain overlapped were determined. Mean ($\pm$ SEM) percentage changes in signal intensity plot over time were calculated for each of these areas. Finally, in the 3 subjects who received both hypertonic and isotonic saline intramuscular injections, signal intensity changes within Vp were calculated for these two challenges. All statistical maps were overlaid onto a T1-anatomical image set.

Since an uncorrected threshold was used for the comparison between brainstem activation during cutaneous and muscle pain, the significance of these signal intensity

Figure 2.

Regions of the pons and medulla in which signal intensity increased significantly during muscle (left) and cutaneous (middle) noxious stimuli. To the right are corresponding myelin-stained sections indicating the locations of the trigeminal sensory complex and medullary raphe nuclei. The slice locations in MNI space are indicated at the bottom left of each image. Vp: principal trigeminal sensory nucleus; SpVo: spinal trigeminal oralis; SpVi: spinal trigeminal interpolaris.

changes was confirmed using a repeated measures ANOVA procedure. For every significant cluster, the mean (i.e. mean of all voxels in each cluster) percentage change in signal intensity was calculated for each time point in each subject. For each challenge (cutaneous and muscle pain), the individual subject mean signal intensity changes were averaged across all subjects to create a mean ($\pm$ SEM) percentage signal intensity change plot over time for each significant cluster. A repeated measures ANOVA with Bonferroni-Dunn analysis ($P < 0.05$) was then conducted to determine whether the signal intensity changes were significantly different from each other during cutaneous and muscle pain as well as being different from the baseline period. Finally, to assess if signal intensity in each significant cluster remained above baseline following the cessation of the pain period, we extended the signal intensity change to include the entire 90 volume challenge period.

RESULTS

Pain Perception and Spread

Injection of hypertonic saline into the masseter muscle, or into the overlying skin, evoked a perception of pain within 10 sec that reached a peak within 90 sec and then subsided within approximately 7 min (see Fig. 1). The average maximum pain scores following subcutaneous and intramuscular hypertonic saline injections were 4.73 ± 0.51 and 4.35 ± 0.56 , respectively. These scores were not significantly different (t -test; $P > 0.05$). Most subjects described the cutaneous pain as being restricted to a small region surrounding the needle tip and commonly described it as "sharp" and "hot". In contrast, most subjects described the muscle pain as being diffuse, larger in spread as well as "aching" and "cramping" in nature. In contrast, no subject reported pain following the isotonic saline injection, nor was lip-brushing considered painful.

TABLE I. MNI co-ordinates of clusters within the lower brainstem, which displayed increases in signal intensity during muscle and cutaneous orofacial pain

	MNI co-ordinates		
	X	Y	Z
Muscle pain			
Ipsilateral Vp	-12	-28	-36
Ipsilateral SpVo	-4	-38	-52
Medullary Raphe	2	-36	-50
Cutaneous pain			
Ipsilateral SpVo	-2	-38	-48
Ipsilateral SpVi	-4	-38	-56
Medullary Raphe	0	-36	-52

fMRI Signal Intensity Changes

Group and region-of-interest analyses revealed that both subcutaneous and intramuscular injections evoked significant increases in signal intensity within discrete regions of the ipsilateral lateral medulla and within the medial medulla. Both muscle and cutaneous injections evoked significant increases in signal intensity in the region of the ipsilateral and contralateral oralis (SpVo) division of SpV, and in the medullary raphe nuclei (Fig. 2; Table I). Volume-of-interest analysis also revealed that during both noxious cutaneous and muscle stimuli signal intensity increased significantly within the caudalis subdivision of SpV (see Fig. 3). Within the region of SpVo both muscle and cutaneous hypertonic saline injections evoked signal increases that began immediately following the hypertonic

saline injection and persisted beyond the pain period, remaining at significantly increased levels for the entire 90 volume challenge period (Figs. 4 and 7).

In addition to SpVc and SpVo activation, cutaneous hypertonic saline injections evoked significant increases in signal intensity within the ipsilateral interpolaris (SpVi) subdivision of SpV, that began at the onset of the injection and remained above baseline for the entire challenge period (Figs. 4 and 7; Table II). In contrast, muscle hypertonic saline injections did not alter SpVi signal intensity. We also found that noxious muscle stimulation activated a discrete cluster within the ipsilateral pons, in the region activated by innocuous brushing of the lip (i.e., the trigeminal principal sensory nucleus (Vp; Fig. 5; Tables I and II). The Vp signal intensity began immediately following the hypertonic saline injection and gradually increased during the entire challenge period (see Fig. 7). In contrast, cutaneous pain was not associated with a significant change in Vp signal intensity.

Finally, in contrast to Vp activation by intramuscular injection of hypertonic saline, intramuscular injection of the same volume of isotonic saline had no effect on the signal intensity within the same Vp region (see Fig. 6).

DISCUSSION

The results of this study demonstrate that noxious orofacial cutaneous and muscle stimulation are differentially represented within the brainstem. It should be noted here that pain is a multi-faceted perception consisting of sensory discriminative, emotional, and behavioural qualities and thus the pain experience requires cortical processing. While our subjects perceived the hypertonic saline

Figure 3.

Volume-of-interest analysis showing the signal intensity changes in the caudalis division of the spinal trigeminal nucleus (SpVc) during muscle and cutaneous noxious stimuli. To the left are raw functional MRI and myelin-stained images showing the approximate location of the SpVc volume of interest. To the right is a graph of the mean ($\pm$ SEM) percent changes in signal intensity over time for the SpVc following muscle (gray) and cutane-

ous (black) hypertonic saline injections. The vertical dashed line indicates the onset of each hypertonic saline injection. The black * indicates time points during cutaneous noxious stimulation where signal intensity is significantly greater than baseline. The gray * indicates time points during muscle noxious stimulation where signal intensity is significantly greater than baseline.

Figure 4.

Brainstem regions in which signal intensity increased during both muscle and cutaneous noxious stimulation (blue shading) and where signal increased following cutaneous but not muscle hypertonic saline injections (hot colour scale). The mean ($\pm$ SEM) percentage changes in signal intensity during cutaneous (green) and muscle (gray) nociception are plotted against time for the spinal trigeminal oralis (SpVo) and spinal trigeminal interpolaris (SpVi) nuclei. Slice locations in MNI space are indicated

at the bottom left of each image. The onset of the hypertonic injection is represented by vertical dashed lines. The green * indicates time points during cutaneous pain where signal intensity is significantly greater than baseline. The gray * indicates time points during muscle pain where signal intensity is significantly greater than baseline. The red # indicates time points where signal intensity is significantly different during cutaneous compared to muscle pain.

injections as painful, our aim was to investigate nociception by determining the signal intensity changes at the level of the primary synapse. Although both cutaneous and muscle nociceptive input activated the caudalis and oralis subdivisions of SpV, only cutaneous nociception evoked a large and sustained signal intensity increase in the interpolaris division of SpV, and only muscle nociception evoked a significant signal intensity increase within Vp. It is well established that cutaneous and muscle noxious stimuli evoke different perceptual, behavioural and cardiovascular changes [Burton et al., 2009; Lewis, 1942], and we speculate that the differential activation evoked by cutaneous and muscle noxious stimuli within the trigeminal sensory complex may contribute to the neural basis for these differences.

The processing of nociceptive information from the face has classically been seen as the role of the SpVc [Sessle, 2000a]. Lesions encompassing SpVc can alter orofacial pain perception and provide relief from chronic pain; microinjection of morphine into SpVc can induce ipsilateral facial analgesia and reduce pain behaviours [Duale et al., 1996; Luccarini et al., 1998]. In humans, trigeminal tractotomy has been used for the treatment of atypical facial pain [Kanpolat et al., 2005] and trigeminal neuralgia [Gybels, 1989], although this approach is often ineffective.

To the best of our knowledge only two studies have used brain imaging techniques to define orofacial pain processing and these investigations report signal increases within SpVc during noxious cutaneous stimulation [DaSilva et al., 2002; Mainero et al., 2007]. To date, no study has examined brain activation patterns during orofacial pain which originates in deeper structures such as muscle. This is

TABLE II. MNI co-ordinates of clusters within the trigeminal brainstem sensory complex, which displayed significantly different activations during muscle and cutaneous pain

	MNI co-ordinates		
	X	Y	Z
Cutaneous > muscle pain			
Ipsilateral SpVi	-4	-38	-56
Brushing			
Ipsilateral Vp	-10	-32	-34

The location of significant signal intensity increases during innocuous brushing of the lip is shown in the bottom row.

Figure 5.

Region of pons in which signal intensity increased during innocuous brushing of the lip (top; hot colour scale) and where both lip brushing and muscle noxious stimulation evoked signal increases (bottom, blue shading). To the right is the mean ($\pm$ SEM) percentage change in signal intensity changes for the region of the principal trigeminal sensory nucleus (Vp) during muscle (grey) and

cutaneous (green) nociception and lip brushing (blue). Slice locations in MNI space are indicated at the bottom left of each image. The onset of the hypertonic injection is represented by vertical dashed lines. The gray * indicates time points during muscle nociception where signal intensity is significantly greater than baseline. Vertical grey bars indicate periods of brushing.

Intramuscular Injections

Figure 6.

Signal intensity changes in the trigeminal principal sensory nucleus following intramuscular injections of hypertonic saline (grey) and isotonic saline (black) in three individuals. Slice location in MNI space is indicated in the bottom left of the image. The percentage change in signal intensity during hypertonic and isotonic saline is plotted against time. Time of injection is represented by vertical dashed lines.

surprising, given that chronic orofacial pain rarely derives from skin but most often involves facial muscles and/or the temporomandibular joint.

Consistent with these previous human brain imaging studies, we found that noxious cutaneous injections evoked signal increases in the region of SpVc. Further, we found that masseter muscle injection also activates SpVc, which is consistent with previous animal investigations which report increases in *c-fos* expression in SpVc during orofacial cutaneous [Strassman and Vos, 1993; Strassman et al., 1993], temporomandibular joint [Hathaway et al., 1995], and masseter muscle noxious stimuli [Imbe et al., 1999, 2001; Ro and Capra, 1999]. These results are also supported by anatomical tract tracing studies which show that small-diameter primary afferents from deep and superficial facial structures synapse in laminae I, II and V of SpVc [Hayashi, 1985] and electrophysiological investigations that report SpVc excitation during deep and superficial orofacial noxious stimulation [Amano et al., 1986; Bolton et al., 2005; Imbe et al., 2001]. It appears that the SpVc plays a multidimensional role in the processing of orofacial pain: SpVc projects directly to the ventroposterior medial thalamic nucleus (VPM), which projects to the primary somatosensory cortex [De Chazeron et al., 2004; Guy et al., 2005], and is thought to process the sensory discriminative aspects of pain. The SpVc also projects to the lateral column of the midbrain periaqueductal gray matter, a region critical for the expression of flight/fight reactions to noxious stimuli [Bandler et al., 2000].

Although the majority of studies have focused on the role of SpVc, our data and evidence from animal work reveal that the rostral SpVo is also involved in the processing of orofacial pain. Recently, Dessem et al. [2007] revealed in rodents that SpVo receives nociceptive primary afferent input from high threshold mechanosensitive neurons and Hu and colleagues have reported increases in the excitability of SpVo neurons during mustard oil injections into the masseter muscle [Hu et al., 1992]. Further, SpVo lesions result in perioral analgesia and decreased pain-evoked behaviours [Luccarini et al., 1998; Pickoff-Matuk et al., 1986]. Unlike SpVc, which receives only direct primary afferent input, it has been reported that SpVo receives both direct primary, and second-order nociceptive inputs from neurons originating in SpVc [Dallel et al., 1998; Greenwood and Sessle, 1976; Hu et al., 1992; Voisin et al., 2002]. Like SpVc, SpVo projects directly to VPM in the thalamus and therefore is likely to play a role in the sensory discriminative aspects of orofacial pain [De Chazeron et al., 2004; Guy et al., 2005].

In contrast to the strict ipsilateral input to SpVo shown in rodents, we found that both muscle and cutaneous nociception also evoked increases in signal intensity in the contralateral SpVo. Studies in rodents have revealed that SpVo receives strictly unilateral primary and secondary afferent inputs that code information about both nociceptive and non-nociceptive stimuli [Dallel et al., 1990]. In the rat, SpVo contains both nociceptive-specific and wide

dynamic range neurons that respond to oral and perioral noxious stimulation as well as non-noxious inputs from multiple whisker receptive fields [Dallel et al., 1990; Desilets-Roy et al., 2002; Pierret et al., 2000]. It is thought that these multiple inputs allow for the integration of complex sensory information regarding the animals surrounding environment. In contrast to the rodent, our data suggests that in humans, the SpVo receives bilateral noxious inputs which originate in both skin and muscle. Although we cannot say with certainty that SpVo activation results from direct primary afferent drive, the SpVo signal intensity changes begin immediately following the hypertonic saline injection and are similar to those signal changes that occur in other regions of the SpV complex. Given that humans do not use facial stimulation as a major sensory tool, it may be the case that the human SpVo has altered connectivity compared with that of rodents. The differences we see between our research and the data presented from animal studies may represent a difference in the way in which pain and sensation from the face are processed in the two species.

While there is considerable evidence that SpVc and SpVo are involved in orofacial pain processing there is much less evidence in regards to SpVi function. It is known from animal studies that a discrete transitional zone between SpVc and SpVi is consistently activated by noxious stimulation, particularly that originating in muscle [Dubner and Ren, 2004; Hathaway et al., 1995; Imbe et al., 1999; Ro and Capra, 1999; Strassman et al., 1993; Wang et al., 2006]. However, the limited spatial resolution of fMRI does not allow us to determine whether this interopolaris/caudalis transition zone is also activated by nociceptive input from muscle in humans. Our data suggest that noxious cutaneous but not muscle stimulation activates SpVi throughout its entire rostro-caudal extent.

Although the precise role played by SpVi in the processing of orofacial painful stimuli remains unknown, the preferential activation of SpVi by noxious stimulation of the skin suggests that this subdivision plays a unique role in cutaneous pain processing. It is well established that cutaneous and deep pain evoke differential sensory perceptions and behavioural responses [Lewis, 1942]. Whereas cutaneous pain is easily localized, feels sharp and evokes active emotional coping behaviours (i.e., flight or fight), deep pain is often dull, diffuse and evokes passive coping behaviours (i.e. conservation/withdrawal response). We have previously shown in humans that cutaneous and muscle pain in the forearm and leg evoke differential patterns of fMRI signal changes in the cerebral cortex [Henderson et al., 2006, 2007] and animal studies have revealed differential patterns of *c-fos* expression in the brainstem [Bandler et al., 2000]. It may be the case that the activation of SpVi by noxious cutaneous and not muscle stimulation underlies at least part of this differential response to cutaneous and muscle pain.

In contrast to SpVc which projects heavily to the PAG, SpVi does not project to the PAG but instead projects

Figure 7.

Mean signal intensity changes in regions in which hypertonic saline injections evoked sustained increases in signal intensity. Signal intensity changes within the principal trigeminal sensory nucleus (Vp) are indicated by the light green, the oralis subdivision of the spinal trigeminal nucleus (SpVo) by light blue and the interpolaris division of the spinal trigeminal nucleus (SpVi) by dark blue. The mean pain intensity profiles during muscle (grey) and cutaneous (black) pain are also shown. Time of injection is represented by vertical dashed lines.

heavily to the superior colliculus [Wiberg et al., 1986]. An important behavioural reaction in animal models of pain is for the animal to direct its attention to the site of pain and to bite or lick the source of discomfort. It has been shown in rodents that these oral behaviours are suppressed following inhibition of the superior colliculus [Wang and Redgrave, 1997] and it is possible that a projection from SpVi to the superior colliculus in humans is responsible for directing movements and attention towards the painful area. Alternatively, it has been suggested that the SpVi is involved in mediating more generalized changes in behaviour. The zona incerta, a region which

integrates diverse sensory inputs and regulates arousal, attention and locomotor responses [Mitrofanis, 2005], receives robust, direct input from SpVi and Vp, whereas both SpVc and SpVo contribute very little to the trigemino-incerta pathway [Simpson et al., 2008]. It is possible that the activation of SpVi by cutaneous noxious stimuli underlies the increased hypervigilance which characterizes pain of superficial origin.

Surprisingly, some of the signal intensity increases within the SpV complex during both noxious muscle and cutaneous stimuli remained well above baseline levels even after the perceived pain intensity had subsided (see Fig. 7). In addition to the potential lingering effects of a noxious stimulus on an animal's vigilance and other behaviours, other mechanisms that may be responsible for the prolonged nature of the SpV complex BOLD signal is the endogenous analgesic circuitry. It has been shown that the SpV receives input from sites involved in pain modulation. For example, it has been reported that the locus coeruleus (a noradrenergic region involved in endogenous pain modulation) projects to—and can modulate—the activity of neurons in the rostral SpV complex, particularly SpVo and Vp [Couto et al., 2006; Sasa et al., 1974]. Furthermore, brainstem regions that form part of the serotonergic endogenous analgesic system, namely the nucleus raphe magnus, send projections to the caudal regions of SpV, specifically SpVc and SpVi, and these connections have been shown to modulate the response of SpV neurons during acute noxious stimuli [Basbaum and Fields, 1984]. If it is assumed that the suggestion that the BOLD signal reflects total synaptic activity is true [Logothetis et al., 2001], then during a prolonged noxious stimulus both primary afferent activity and inputs from the endogenous pain modulatory circuitry would result in an increase in SpV synaptic activity that may not follow the pattern of perceived pain and extend well after the perceived pain has subsided.

In direct contrast to the SpVi, we found that only orofacial muscle nociception activates the principal sensory nucleus (Vp). This result was surprising given that it has been a long held view that Vp transmits only non-noxious somatosensory information from the face [Kirkpatrick and Kruger, 1975; Mantle-St John and Tracey, 1987; Smith, 1973]. We are certain that the region activated by muscle noxious stimuli was located in Vp as we functionally defined this region by innocuous brushing of the lower lip. In addition to this, we can also conclude that the activation seen in Vp is related to muscle pain *per se* and not to the activation of muscle stretch receptors (produced by the injection-induced distension within the muscle), as isotonic saline injection failed to cause an increase in signal intensity. Consistent with our findings, Dessem et al. [2007] have shown in rodents that nociceptive muscle afferents in the masseter nerve project to a discrete region of Vp. It is known that Vp projects to both the VPM as well as to the zona incerta [Simpson et al., 2008]. While the projection to VPM may conduct information regarding

the spatial and temporal quality of the stimulus, the zona incerta pathway may regulate arousal and motor responses. Furthermore, Simpson and colleagues [2008] report that within the ventral aspect of the zona incerta, inputs from SpVi are distributed lateral to those inputs from Vp. Given that, unlike cutaneous pain, muscle pain is characterized by the conservation-withdrawal reaction, this topographically organized zona incerta projection raises the possibility that a Vp–zona incerta pathway underlies the dampened arousal and motor activity evoked by muscle pain and a SpVi–zona incerta pathway underlies the increased arousal and hypervigilance evoked by cutaneous pain.

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