Central Circuitry Responsible for the Divergent Sympathetic Responses to Tonic Muscle Pain in Humans

Sophie Kobuch,¹ Azharuddin Fazalbhoy,^{2,3} Rachael Brown,^{1,2}
Luke A Henderson,⁴ and Vaughan G Macefield^{1,2*}

¹*School of Medicine, Western Sydney University, Sydney, Australia*

²*Neuroscience Research Australia, Sydney, Australia*

³*School of Health Sciences, RMIT University, Melbourne, VIC, Australia*

⁴*Discipline of Anatomy and Histology, University of Sydney, Sydney, Australia*

Abstract: Experimentally induced tonic muscle pain evokes divergent muscle vasoconstrictor responses, with some individuals exhibiting a sustained increase in muscle sympathetic nerve activity (MSNA), and others a sustained decrease. These patterns cannot be predicted from an individual's baseline physiological or psychological measures. The aim of this study was to investigate whether the different muscle sympathetic responses to tonic muscle pain were associated with differential changes in regional brain activity. Functional magnetic resonance imaging (fMRI) of the brain was performed concurrently with microelectrode recording of MSNA from the peroneal nerve during a 40-min infusion of hypertonic saline into the ipsilateral tibialis anterior muscle. MSNA increased in 26 and decreased in 11 of 37 subjects during tonic muscle pain. Within the prefrontal and cingulate cortices, precuneus, nucleus accumbens, caudate nucleus, and dorsomedial hypothalamus, blood oxygen level dependent (BOLD) signal intensity increased in the increasing-MSNA group and remained at baseline or decreased in the decreasing-MSNA group. Similar responses occurred in the dorsolateral pons and in the region of the rostral ventrolateral medulla. By contrast, within the region of the dorsolateral periaqueductal gray (dIPAG) signal intensity initially increased in both groups but returned to baseline levels only in the increasing-MSNA group. These results suggest that the divergent sympathetic responses to muscle pain result from activation of a neural pathway that includes the dIPAG, an area thought to be responsible for the behavioral and cardiovascular responses to psychological rather than physical stressors. *Hum Brain Mapp* 38:869–881, 2017. © 2016 Wiley Periodicals, Inc.

Key words: muscle pain; muscle sympathetic nerve activity; functional magnetic resonance imaging

INTRODUCTION

Research in experimental animals has revealed that noxious stimulation of different body tissues evokes different

behavioral and cardiovascular responses. That is, cutaneous pain elicits fight/flight behaviors, coupled with increases in heart rate, blood pressure and sympathetic vasoconstrictor drive, whereas muscle and visceral pain

Contract grant sponsor: National Health and Medical Research Council of Australia; Contract grant number: GNT 1029782 and GNT1100040

*Correspondence to: Vaughan Macefield, School of Medicine, Western Sydney University, Locked Bag 1797, Penrith NSW 2751, Australia. E-mail: v.macefield@westernsydney.edu.au

Received for publication 12 August 2016; Revised 22 September 2016; Accepted 27 September 2016.

DOI: 10.1002/hbm.23424

Published online 3 October 2016 in Wiley Online Library (wileyonlinelibrary.com)

bring about quiescence and decreases in heart rate, blood pressure and vasoconstrictor drive [Keay and Bandler, 2001]. Although some clinical observations in humans suggested similar divergent responses [Lewis, 1942; Feinstein et al., 1954], we found that acute cutaneous or muscle pain, induced by bolus subcutaneous or intramuscular injection of hypertonic saline, both evoked transient (~30 s) increases in skin sympathetic nerve activity (SSNA) and heart rate, with small sustained increases in blood pressure and muscle sympathetic nerve activity (MSNA) that matched the duration of pain [Burton et al., 2009a,b; Hall et al., 2012; for review see Burton et al., 2016]. These data demonstrate that, in humans, differential sympathetic responses to acute cutaneous and muscle pain do not occur. Moreover, we had shown that these sympathetic responses are psychogenic rather than of spinal reflex origin, as they do not occur when noxious stimuli are delivered to the legs in individuals with complete spinal cord injury [Brown and Macefield, 2008].

We recently found that prolonged muscle pain, induced by intramuscular infusion of hypertonic saline, is accompanied by two patterns of change in MSNA, with subjects showing either a sustained increase or a sustained decrease in MSNA [Fazalbhoy et al., 2012, 2014; Kobuch et al., 2015, 2016; for review see Burton et al., 2016]. These divergent patterns of MSNA are reproducible over time [Fazalbhoy et al., 2014], and not based on sex, or differences in resting MSNA, blood pressure, or heart rate [Kobuch et al., 2015]. Moreover, anxiety and attitudes to pain do not account for these divergent sympathetic responses to muscle pain [Kobuch et al., 2016]. Interestingly, chronic pain is associated with a significantly greater risk of hypertension, particularly in females, and it has been proposed that this comorbidity reflects fundamental alterations in the relationship between the cardiovascular and pain regulatory systems [Bruehl et al., 2005]. Although the underlying brain circuitry responsible for hypertension in individuals with chronic pain is not known, it is possible that the divergent responses we previously found to occur in healthy individuals during tonic muscle pain also

reflect differences in interactions between pain and cardiovascular circuits even in healthy subjects.

As noted above, given that noxious stimuli below the lesion in patients with complete spinal cord injury do not evoke any changes in blood pressure, heart rate, skin blood flow or sweat release, the circuitry responsible for pain-related changes in MSNA must lie within supraspinal sites [Burton et al., 2008]. Studies in the cat suggest that the autonomic responses to pain form part of a behavioral response mediated by neurons in the midbrain periaqueductal gray matter (PAG) [Bandler et al., 1991, 2000]. Of course, numerous human brain imaging studies have also revealed a “central autonomic network” consisting of higher brain regions such as the insular, cingulate, and prefrontal cortices, as well as precuneus and amygdala [Benarroch, 1993; Macefield and Henderson, 2016]. Furthermore, recent concurrent functional magnetic resonance imaging (fMRI) and microneurography studies from our laboratory have confirmed the function of the well-described autonomic-related regions of the brainstem, such as the rostral and caudal ventrolateral medulla, nucleus tractus solitarius and dorsolateral pons [Lundblad et al., 2014; Macefield and Henderson, 2010, 2016]. The aim of the current study was to use concurrent fMRI and microelectrode recordings of MSNA to determine if these autonomic regions respond differentially in individuals who displayed increases versus those who displayed decreases in MSNA during tonic muscle pain. Given that the dorsomedial hypothalamus (DMH) is part of the classical hypothalamic defense area, evoking increases in blood pressure and heart rate in the cat [Coote et al., 1979], we tested the hypothesis that a sustained increase in MSNA during long-lasting muscle pain is associated with a sustained increase in BOLD signal intensity of the DMH, while a sustained decrease in MSNA is associated with a sustained decrease in DMH activity. And given that the DMH has connections with the PAG and RVLM, we tested the hypothesis that a sustained increase or decrease in MSNA during tonic muscle pain is associated with parallel increases or decreases in signal intensity of the PAG and RVLM.

METHODS

Participants

Experiments were performed on 37 healthy university subjects (11 females; mean $\pm$ SEM age: 21.9 ± 0.5 years), each of whom had contributed psychometric data to our recent study in which we examined differences in anxiety and attitudes to pain in 66 subjects [Kobuch et al., 2016]. Subjects responded to an advertisement that outlined the procedures to be undertaken, including the nerve recording, muscle pain and fMRI; all procedures were approved by the Human Research Ethics Committees of Western Sydney University and the University of New South Wales. Written consent was obtained from all subjects in accordance with the Declaration of Helsinki.

Abbreviations

BOLD	Blood oxygen level dependent
dlPAG	Dorsolateral PAG
DMH	Dorsomedial hypothalamus
fMRI	Functional magnetic resonance imaging
FWHM	Full-width at half-maximum
MNI	Montreal Neurological Institute
MSNA	Muscle sympathetic nerve activity
PAG	Periaqueductal gray
RMS	Root-mean-square
SSNA	Skin sympathetic nerve activity
SUIT	Spatial Unbiased Infratentorial Template
TE	Echo time
TR	Repetition time

Microneurography

In the laboratory, subjects lay supine on an MRI bed in a comfortable position with the legs supported by a foam cushion. The course of the right common peroneal nerve was identified via external stimulation (2–10 mA) using a 1 mm surface probe that delivered 0.2 ms pulses at 1 Hz from an isolated constant-current stimulator (Stimulus Isolator; ADInstruments, Sydney, Australia). Once twitches were evoked, the skin was marked over the optimal site for generating a twitch. This allowed the localization of the common peroneal nerve and the site for percutaneous insertion of tungsten microelectrodes (FHC, Bowdoin, ME, USA). In order to locate a specific nerve fascicle, intraneural stimulation through the microelectrode, with a maximal current of 1 mA at 1 Hz, was used to generate muscle twitches, while adjusting the depth and angle of the microelectrode to optimize the responses. As the microelectrode approached the fascicle the current was progressively reduced; twitches at currents of 0.02 mA or lower indicated that the microelectrode tip had entered a muscle fascicle. Once the fascicle was identified, its nerve signal was amplified 100 times via an MR-compatible head-stage located close to the recording site and further amplified to a total gain of 20,000 times and a band pass of 300 Hz to 5 kHz (NeuroAmp EX; ADInstruments, Sydney, Australia). Neural activity was stored on a computer using a computer-based data acquisition and analysis system (PowerLab16SP hardware and LabChart 7 software; ADInstruments, Sydney, Australia). The microelectrode was then manually adjusted to search for spontaneous bursts of MSNA. Bursts were identified as MSNA by their tight cardiac rhythmicity and generation of a sustained increase in MSNA during an inspiratory-capacity apnea. To facilitate the identification of the bursts, a root-mean-square (RMS) processed version of the neural signal was computed using a moving average of 200ms. MSNA was recorded continuously for 5 minutes of rest, prior to the subjects being wheeled from the laboratory to the scanner with the microelectrode remaining *in situ*.

Cardiovascular Monitoring

Before the scanning period, 5 minutes of continuous blood pressure were recorded using radial arterial tonometry (Colin 7000 NIBP; Colin Corp., Aichi, Japan). Respiration was measured using a strain-gauge transducer (Pneumotrace, UFI, Morro Bay, CA, USA), and heart rate was monitored with a piezoelectric pulse transducer placed on the big toe. The blood pressure monitor was removed before entering the scanner.

MRI

Using a 3 Tesla MRI scanner (Philips Achieva, 32-channel SENSE head coil), two scans encompassing the whole brain were collected: a high-resolution 3D

Figure 1.

A: Plots of perceived pain intensity and MSNA amplitude changes during continuous hypertonic saline solution infusion into the right tibialis anterior muscle of 37 subjects. The MSNA plot indicates mean ($\pm$ SEM) percentage change in MSNA amplitude during pain relative to the baseline period in the increasing (black, $n = 26$) and decreasing groups (grey, $n = 11$). The vertical dashed line indicates the start of the hypertonic saline infusion. The grey shading indicates the volumes during which all subjects were in moderate pain. **B:** Plots of mean ($\pm$ SEM) pain intensity, pain area, and infusion volume in subjects that displayed increases and decreases in MSNA. **C:** Plots of the frequency of words most often chosen by subjects from the McGill pain questionnaire to describe the ongoing muscle pain. Note there is no difference in any of the pain parameters between the increasing and decreasing MSNA groups.

T1-weighted anatomical image [200 axial slices, echo time (TE)=2.5 ms, repetition time (TR)=5600 ms, raw voxel size = 0.87 mm³], followed by a series of 250 gradient echo echo-planar sensitive to blood oxygen level dependent contrast (BOLD) fMRI images [46 axial slices, (TE)=40 ms, (TR)=8 s, raw voxel size = 1.5 × 1.5 × 3.25 mm].

During the fMRI scan, all axial slices were collected during the first 4 seconds of the 8 s TR. Two 10 ml syringes filled with hypertonic saline were placed in an infusion pump (Harvard Instruments, USA), and connected to a three-way tap via a 30 cm length of extension tubing primed with hypertonic saline. A 23 G butterfly cannula was then attached to the three-way tap, primed, and inserted 1.5 cm deep into the belly of the right tibialis anterior muscle, approximately 5 cm lateral and 10 cm inferior to the tibial tuberosity. An infusion of hypertonic saline solution (7%) was commenced during the 50th scan (volume 50) for all subjects and was sustained throughout the duration of the entire scanning period. Each subject was unaware of when the hypertonic saline infusion was to begin. The initial rate of infusion was set at 0.25 ml/min and was constantly adjusted to maintain a pain level of 5 out of 10 on a Numerical Rating Scale (NRS) (0 = no pain, 10 = most intense pain imaginable). The subjects' pain ratings were monitored continuously and adjusted according to each subject pressing four color-coded buttons, each color associated with either "pain onset," "pain at 5/10," "pain below 5/10," or "pain above 5/10." At the completion of the scanning session, each subject was asked to complete a McGill Pain Questionnaire [Melzack, 1975] and draw the area of perceived pain on a standard diagram of the leg.

Analysis

Pain psychophysics

Using the points at which each subject indicated the onset of pain as well as when the pain reached 5/10, we plotted an estimate of the average pain intensity over time for all subjects. We found that, on average, pain began approximately 18 volumes after the start of the infusion, i.e., at volume 68, and first reached a 5/10 rating at approximately volume 72, after which it was sustained for the remaining scanning period (Fig. 1A). These pain intensity changes were similar in all subjects, whether they were subsequently categorized as displaying an increase or a decrease in MSNA.

MSNA

Individual bursts of MSNA were displayed as a mean-voltage neurogram, computed as the root-mean-square (RMS) processed signal with a moving time average window of 200 ms. The 4 s inter-scan (OFF) period (see below) allowed the measurement of MSNA bursts. Bursts were manually counted and the amplitude was measured from the RMS-processed nerve signal. Scans 16 to 75 were

selected as the baseline period and scans 76 to 250 as the tonic pain period. In each subject, the absolute values for MSNA amplitude were averaged for the pain period and reported as percentage change relative to the baseline period.

Functional MRI

Using Statistical Parametric Mapping 12 (Wellcome Trust Center for Neuroimaging, University College London, UK) and custom software, all fMRI images were realigned, spatially normalized to the Montreal Neurological Institute (MNI) template, intensity normalized to eliminate any slow drift in signal intensity, and bias corrected. Scans were then smoothed using a 6mm full-width at half-maximum (FWHM) Gaussian filter. In addition, we performed a brainstem-only analysis for the subjects for whom the acquired images extended to the bottom of the caudal medulla ($n = 30$). The brainstem was isolated and spatially normalized to the Spatial Unbiased Infratentorial Template (SUIT) using the SUIT toolbox [Diedrichsen et al., 2011], intensity normalized, bias corrected, and smoothed using a 4mm FWHM Gaussian filter.

Signal intensity changes were measured during the subsequent 4 s (ON period), taking into account the (+) 5 s neurovascular coupling delay between a neuronal event and the peak BOLD signal [Logothetis et al., 2001] and the (−) 1 s required for the burst of MSNA to travel from the brain to the peripheral recording site [Fagius and Wallin, 1980]. The first 15 scans were excluded to allow for global signal equilibration. We searched for signal intensity changes using a box-car design, with a baseline period of 60 volumes (scans 16–75) and a pain period of 175 volumes (scans 76–250). A hemodynamic delay function was not included, given that we accounted for this delay in our analysis design. A one-sample random effects analysis was performed with all subjects in a single group to determine signal intensity changes associated with the pain period ($P < 0.05$, family wise error corrected). To determine regions in which signal intensity changes were different in the increasing versus decreasing MSNA group, a two-sample random effects analysis was performed using both the whole-brain and brainstem only images ($P < 0.001$, uncorrected). Significant clusters were overlaid onto a standard whole-brain and brainstem template in MNI space. For each significant cluster, the percentage changes in signal intensity were extracted by comparing the signal intensity of the pain period to baseline. In addition, we performed correlation analyses in each subject to determine whether there were significant linear relationships between the peak or trough in MSNA amplitude against the corresponding BOLD signal intensity changes (Pearson's correlation, $P < 0.05$); for each subject the average change in MSNA or BOLD signal intensity was calculated over 10 volumes. All results were expressed as mean ± SEM.

Figure 2.

Signal intensity increases (hot color scale) during continuous hypertonic saline solution infusion into the right tibialis anterior muscle in all 37 subjects. Significant clusters were overlaid onto a mean T1-weighted anatomical image set created from all 37 subjects. Slice locations in MNI space are indicated in the upper

left of each image. The left side of the image is the side contralateral to the noxious stimulus. M1: primary motor cortex; PCC: posterior cingulate cortex; S1: primary somatosensory cortex; S2: secondary somatosensory cortex.

RESULTS

Subjective Experience during Tonic Muscle Pain

Intramuscular infusion of hypertonic saline solution induced moderately intense pain that began approximately

18 volumes (144 s) following the start of the infusion and quickly reached a pain rating of ~5/10 within approximately 4 volumes (32 s). The pain intensity remained at approximately this level for the remainder of the MRI session. A two-sample *t*-test revealed that there was no

TABLE I. Locations in Montreal Neurological Institute space, *t* values, and cluster sizes of regions in which signal intensity increased in all subjects during tonic muscle pain

Signal intensity increases	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value	Cluster size
Orbitofrontal cortex					
Left (contralateral)	-10	10	-20	6.85	9
Insular cortex					
Left	-30	18	-16	8.10	74
	-38	10	-14	7.12	
	-36	18	-10	6.87	
Primary somatosensory cortex					
Left	-10	-36	46	5.95	6
Secondary somatosensory cortex					
Left	-38	-30	18	7.08	55
	-44	-22	18	5.26	
Primary motor cortex					
Right (ipsilateral)	4	-14	60	6.39	28
	4	-20	58	6.28	
	4	-14	54	6.17	
Posterior cingulate cortex					
	2	-40	26	7.02	78
	6	-42	24	6.98	
	-4	-42	26	6.62

Figure 3.

A: Brain regions above the brainstem in which signal intensity increases were greater (hot color scale) or lower (cool color scale) in the increasing MSNA than in the decreasing MSNA group during tonic muscle pain. Significant clusters were overlaid onto a mean T1-weighted anatomical image set created from all 37 subjects. Slice locations in MNI space are indicated in the upper right of each image. The left side of the image is the side

contralateral to the noxious stimulus. **B:** Plots of mean ($\pm$ SEM) percentage signal intensity changes during pain relative to the baseline period for significant clusters in the increasing MSNA (orange) and decreasing MSNA (blue) groups. The vertical dashed line indicates the start of the hypertonic saline infusion and the grey shading the pain period. ACC: anterior cingulate cortex; DMH: dorsomedial hypothalamus.

TABLE II. Locations in Montreal Neurological Institute space, *t* values and cluster sizes of regions in which signal intensity was significantly different in subjects that displayed increased muscle sympathetic nerve activity (MSNA) compared with those that showed decreased MSNA during tonic muscle pain. Only regions above the brainstem are included

Increases>decreases	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value	Cluster size
Orbitofrontal cortex					
Left (contralateral)	-20	24	-22	3.76	24
Subgenual anterior cingulate cortex					
Right (ipsilateral)	6	18	-16	3.25	6
Dorsomedial hypothalamus					
Left	-2	-10	-8	3.51	7
Nucleus accumbens					
Right	10	10	0	3.67	18
Left	-4	10	-6	3.18	5
Dorsolateral prefrontal cortex					
Right	32	48	6	3.78	14
Caudate nucleus					
Left	-14	8	14	3.69	22
Precuneus					
Left	-6	-52	66	4.40	22

significant difference in the mean pain intensity ratings in the increasing MSNA compared with the decreasing MSNA group (increasing: 5.7 ± 0.2 ; decreasing: 6.0 ± 0.2 ; $P = 0.28$). In addition, there was no significant group

difference between the total volume of hypertonic saline infused in either group (increasing: 18.8 ± 2.6 ml; decreasing: 18.1 ± 0.9 ml; $P = 0.82$) or the area of perceived pain spread (increasing: 1054 ± 102 pixels; decreasing:

Figure 4.

A: Brainstem regions in which signal intensity increases were greater (hot color scale) or lower (cool color scale) in the increasing MSNA ($n = 20$) than in the decreasing MSNA ($n = 10$) group during tonic muscle pain. Significant clusters were overlaid onto a mean SUI T1-weighted anatomical image set created from 30 subjects. Slice locations in MNI space are indicated in the upper right of each image. The left side of the image is the

side contralateral to the noxious stimulus. **B:** Plots of mean ($\pm$ SEM) percentage signal intensity changes during pain relative to the baseline period for significant clusters in the increasing MSNA (orange) and decreasing MSNA groups (blue). The vertical dashed line indicates the start of the hypertonic saline infusion and the grey shading the pain period. RVLM: rostroventrolateral medulla; dlPons: dorsolateral pons; PAG: midbrain periaqueductal gray.

TABLE III. Locations in Montreal Neurological Institute space, *t* values and cluster sizes of regions in which signal intensity was significantly different in subjects that displayed increased muscle sympathetic nerve activity (MSNA) compared with those that showed decreased MSNA during tonic muscle pain. Only brainstem regions are included

Signal intensity increases	<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i> value	Cluster size
Rostroventrolateral medulla					
Right (<i>ipsilateral</i>)	8	-40	-49	4.30	12
Dorsolateral pons					
Right	8	-30	-29	3.28	3
Left (<i>contralateral</i>)	-6	-32	-35	3.60	13
Midbrain periaqueductal gray					
Left	-4	-34	-5	3.20	2

1009 ± 131 pixels; $P = 0.80$) (Fig. 1B). There was also no difference in the words chosen from the McGill pain questionnaire to describe the ongoing pain; both groups chose “dull,” “aching,” and “throbbing” to describe the pain (Fig. 1C).

MSNA During Tonic Muscle Pain

Consistent with our previous findings, tonic muscle pain induced two distinct sympathetic responses: 26 subjects exhibited a significant increase in MSNA burst amplitude ($71.8 \pm 26.7\%$), while 11 subjects showed a significant decrease ($-32.5 \pm 18.6\%$) throughout the pain period ($P < 0.0001$, *t*-test) (see Fig. 1A). The changes in MSNA began at approximately the same time as the subjects first perceived pain and were sustained for the duration of the infusion.

Signal Intensity Changes

Pain was associated with significant increases in BOLD signal intensity in a number of brain regions that have been previously shown to be activated during acute muscle pain. In all 37 subjects BOLD signal intensity increased in the left (contralateral to the noxious stimulus) primary somatosensory cortex and right primary motor cortex in the region representing the leg and foot, contralateral secondary somatosensory cortex, contralateral anterior and dorsal posterior insular cortex, as well as bilaterally in the posterior cingulate cortex and the thalamus (Fig. 2 and Table I).

In none of these regions (Fig. 2, Table I) were there any significant differences in signal intensity between subjects in whom MSNA increased compared with those in whom MSNA decreased. However, a two-sample analysis revealed significant differences in other regions (Fig. 3, Table II). Signal intensity increased in the increasing MSNA group and remained unchanged in the decreasing MSNA group in the right (ipsilateral) dorsolateral prefrontal cortex [dlPFC: % change over entire pain period: increasing 1.13 ± 0.08 vs. decreasing -0.29 ± 0.07], left nucleus accumbens [NAc: increasing 1.46 ± 0.10 vs.

decreasing -0.27 ± 0.09], right subgenual anterior cingulate cortex (sgACC: increasing 2.71 ± 0.18 vs. decreasing -0.46 ± 0.19) and left dorsomedial hypothalamus (DMH: increasing 1.52 ± 0.09 vs. decreasing 0.27 ± 0.09). By contrast, signal intensity increased during the pain period in the increasing MSNA group and decreased in the decreasing MSNA group in the left orbitofrontal cortex (OFC: increasing 1.76 ± 0.11 vs. decreasing -0.26 ± 0.09), left caudate nucleus (increasing 1.08 ± 0.09 vs. decreasing -0.99 ± 0.08) and left precuneus (increasing 0.61 ± 0.10 vs. decreasing -1.01 ± 0.10). In no region was signal intensity lower in the MSNA increasing group relative to the MSNA decreasing group.

In the brainstem-specific analysis ($n = 30$), we found significantly different signal intensity changes in the increasing compared with the decreasing MSNA group (Fig. 4, Table III). Signal intensity increased in the increasing MSNA group and remained at baseline levels in the MSNA decreasing group in the right lateral medulla, within the region of the rostral ventrolateral medulla (RVLM: increasing 1.08 ± 0.10 vs. decreasing 0.32 ± 0.09), right dorsolateral pons (dlPons: increasing 1.53 ± 0.10 vs. decreasing 0.27 ± 0.08) and the left dlPons (increasing 1.60 ± 0.10 vs. decreasing 0.39 ± 0.07). In direct contrast, BOLD signal intensity was significantly greater in the MSNA decreasing group compared with the increasing group in the region of the midbrain encompassing the left periaqueductal gray matter (PAG: increasing 0.61 ± 0.17 vs. decreasing 1.45 ± 0.17). This was the only region in the entire brain where signal intensity was greater in the MSNA decreasing group.

Correlation With MSNA

In addition to differences in cortical and subcortical signal intensity changes between the increasing and decreasing MSNA groups, we found that changes in signal intensity in most of these regions were correlated with the peak/trough (maximum change) in MSNA amplitude (Fig. 5). Positive correlations occurred in the left OFC (Pearson's, $r = 0.41$, $P = 0.02$), right dlPFC ($r = 0.43$, $P = 0.01$), left precuneus ($r = 0.50$, $P = 0.002$), left dlPons

Figure 5.

Brain regions in which peak or trough MSNA amplitude during pain were significantly correlated with signal intensity changes. PAG: midbrain periaqueductal gray.

($r = 0.45$, $P = 0.01$), and right dlPons ($r = 0.51$, $P = 0.004$). In contrast, a negative correlation between increasing MSNA and signal intensity increases occurred in the left dorsolateral PAG (dlPAG) ($r = -0.43$, $P = 0.02$). We found no significant linear relationships in the right RVLM ($r = 0.33$, $P = 0.09$), left NAc ($r = 0.32$, $P = 0.07$), left caudate nucleus ($r = 0.23$, $P = 0.21$), right sgACC ($r = 0.27$, $P = 0.14$), or left DMH ($r = 0.25$, $P = 0.15$).

DISCUSSION

In this investigation, we revealed brain responses associated with divergent changes in MSNA during tonic muscle pain. Although all subjects reported similar pain intensity ratings, the long-lasting pain evoked robust increases in MSNA in some individuals and decreases in others. These divergent MSNA responses were associated with different signal intensity changes in a number of brain regions, namely the prefrontal and cingulate cortices, precuneus and DMH, and brainstem regions such as the PAG and RVLM. These data suggest that, during tonic muscle pain, descending modulation of the brainstem circuits, which are thought to control the cardiovascular responses to pain, can evoke different MSNA responses in different individuals.

Consistent with our previous investigations, we found that tonic muscle pain resulted in divergent muscle sympathetic responses [Fazalbhoy et al., 2012, 2014; Kobuch et al., 2015, 2016]. Since these opposite MSNA responses were associated with different signal intensity changes in cortical regions involved in higher-order emotional and cognitive function, such as the prefrontal and cingulate cortices, one might expect that psychological differences underlie the different MSNA responses. However, we previously reported that pain catastrophizing, pain anxiety, and pain vigilance, as well as state and trait anxiety levels, did not differ between groups [Kobuch et al., 2016]. In addition, here we report no group difference in pain parameters such as the intensity, spread, or quality of the pain. Although there is a well-documented relationship between psychological variables such as pain catastrophizing and the development of chronic pain [Chaves and Brown, 1987; Keefe et al., 1989; McCracken and Dhingra, 2002; Pavlin et al., 2005; Picavet et al., 2002; Spanos et al., 1979], our findings suggest that these variables do not predict the MSNA response to pain and likely do not play a role in the development of hypertension associated with chronic pain.

In almost all regions showing differences in signal intensity changes, signals increased in the increasing MSNA

group and remained unchanged in the decreasing group. Moreover, most of these regions were positively correlated with maximum MSNA amplitude. One clear exception was the region of the dIPAG, in which the signal intensity initially increased in both groups but then returned to baseline levels in the increasing MSNA group, and continued to increase in the decreasing MSNA group. In addition, there was a negative correlation between signal intensity in the PAG and peak MSNA. It is well established in the cat that the PAG is activated by both superficial and deep pain and is critical for the expression of defensive behaviors [Bandler et al., 1991; Keay and Bandler, 2001]. Keay and Bandler's [2001] review discusses studies in which cutaneous pain activates the lateral PAG, and direct stimulation of this PAG region evokes fight/flight behaviors that are associated with behaviorally-relevant increases in blood pressure, heart rate, and sympathetic activity [Bandler et al., 2000; Carrive et al., 1987; Carrive et al., 1988; Hilton and Redfern, 1986; Meller and Dennis, 1991; Van Bockstaele et al., 1991; Yardley and Hilton, 1986]. In direct contrast, deep pain, such as that arising from muscle, activates the ventrolateral PAG, and stimulation of this PAG region evokes quiescence with decreases in blood pressure, heart rate, and sympathetic activity [Carrive and Bandler, 1991; Lovick, 1992]. Given these data, one would expect that tonic muscle pain in humans would evoke decreases in MSNA in all individuals, and that this would be associated with signal changes in the ventrolateral part of the PAG, which did not occur in the current study.

An alternate proposal of PAG organization is one based on whether the pain is interpreted as being escapable or inescapable. That is, when the situation is escapable, active coping strategies such as fight/flight and sympathoexcitation are evoked, whereas an inescapable stressor is met with passive coping mechanisms, such as a conservation-withdrawal response and sympathoinhibition [Keay and Bandler, 2001]. In our experiment, the noxious stimulus is inescapable in the sense that the MRI scanner is a confined environment and requires the subject to remain stationary. Alternatively, the stimulus could be deemed escapable - given that each individual is informed that they can stop both the pain and the scanning at any point. It might be that those individuals that deemed the stimulus inescapable displayed decreases in MSNA, whereas those that judged the stimulus as escapable displayed MSNA increases. If this were the case, one would then predict that decreased MSNA would be associated with increases in signal intensity in the ventrolateral PAG, and increased MSNA with lateral PAG signal increases, which again did not occur.

It is curious that the divergent MSNA responses seen in our study during long-lasting muscle pain are associated with different signal intensity changes in the region of the dIPAG, and not the lateral or ventrolateral PAG. Although stimulation of the dIPAG evokes active coping

behaviors, unlike the lateral and ventrolateral PAG, it does not receive noxious inputs from the spinal cord or brainstem but, instead, receives inputs from primarily prefrontal and cingulate cortices [Floyd et al., 2000]. The predominance of cortical inputs into the dIPAG has led to the suggestion that this region produces active coping strategies in response to psychological stressors [Keay and Henderson, 2010]. This idea is supported by studies in the rat, which show active coping behaviours and dIPAG activation during threat signals such as the odour or sight of a cat [Canteras and Goto, 1999; Dielenberg et al., 2004]. Furthermore, it has been shown in the rat that the dIPAG projects to, and receives input from the hypothalamus - which in turn receives input from the prefrontal and cingulate cortices [Beckstead, 1979; Domesick, 1969; Floyd et al., 2001; Gabbott et al., 2005]. Microinjection of excitatory amino acids into the dIPAG in the rat evokes a significant increase in sympathetic nerve activity and respiratory activity [Iigaya et al., 2010; Huang et al., 2000]. Imaging studies in humans have demonstrated the role of the dIPAG in the physiological responses to psychological stressors, such as induced panic attack [Reiman et al., 1989], or the approach of a virtual predator [Mobbs et al., 2007]. Moreover, stimulation of the dIPAG in humans can evoke increases in blood pressure [Basnayake et al., 2011, 2012] as well as increases in MSNA burst amplitude [Sverrisdóttir et al., 2014]. Further investigations in rats have proposed that these increases are mediated via a pathway to the DMH [Horiuchi et al., 2009], and since there is only a small direct projection from the dIPAG to the DMH [Thompson and Swanson, 1998], it has been suggested that the dIPAG produces sympathoexcitation through indirect projections to the DMH via the cuneiform and/or parabrachial nuclei [Fulwiler and Saper, 1984; Lam and Verberne, 1997; Dampney et al., 2013]. The DMH then projects directly to the RVLM and/or raphe pallidus, where it can excite sympathetic premotor neurons and increase sympathetic output, as shown in the rat [Fontes et al., 2001].

Consistent with this proposed dIPAG output pathway and descending inputs, we found that in subjects in whom MSNA increased, signal intensity also increased in the dIPFC, sgACC, DMH, dorsolateral pons, and RVLM. The increased signal intensity in DMH and RVLM in the increasing MSNA group supports our hypothesis. Interestingly, within the dIPAG, signal intensity increased during the initial period of pain but then decreased towards baseline levels for the remainder of the scanning period whilst MSNA remained elevated. It has been suggested the dIPAG control of sympathetic activity may involve a facilitatory summation of inputs from the dIPAG and DMH [de Menezes et al., 2009; Dampney et al., 2013]. This raises the possibility that, following the initial period of muscle pain, facilitatory effects of the dIPAG on the DMH, in combination with descending inputs from the prefrontal and cingulate cortices - which

also project to the DMH [Beckstead, 1979; Domesick, 1969; Floyd et al., 2001; DiMicco et al., 2002; Gabbott et al., 2005] - are adequate to maintain increased sympathetic drive without increased dIPAG activity.

Intriguingly, dIPAG signal increased and remained elevated during the entire pain period in individuals who displayed a fall in MSNA. In these subjects, signal intensity did not change in the prefrontal or cingulate cortices, DMH, dorsolateral pons or RVLm. Indeed, the only regions besides the dIPAG that showed signal changes were the precuneus and caudate nucleus, which displayed robust decreases in signal intensity. It is possible that in these subjects inputs onto the dIPAG from the precuneus and caudate over-ride the pathway described above and, instead, elicit decreased dIPAG output and reduced muscle sympathetic activity. In fact, the dIPAG contains a high concentration of receptors for the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) [Barbaresi, 2005, 2010], and the blockade of these receptors elicits strong increases in sympathetic and respiratory activity [Iigaya et al., 2012]. Given this, increased dIPAG signal intensity in the decreasing MSNA group could reflect increased excitation of tonic local GABAergic activity, resulting in a decrease in MSNA. Additionally, the dIPAG contains high concentrations of NADPH diaphorase and nitric oxide synthase (NOS) [Bandler and Shipley, 1994; Herbert and Saper, 1992; Onstott et al., 1993]. Blocking NOS in the dIPAG evokes an increase in mean arterial pressure, and injection of nitric oxide donating compounds can evoke hypotensive responses [Hall and Behbehani, 1997, 1998]. Why differences in the effects of higher centers, such as the prefrontal and cingulate cortex, or precuneus and caudate nucleus, on the dIPAG occur in some individuals and not others remains unknown.

Finally, it is important to emphasize that, although we used a physical stressor (tonic muscle pain), we did not find differential signal changes in the region of the lateral or ventrolateral PAG, but instead found such changes in the dIPAG. Although this difference may reflect interspecies variability, we suggest instead that the natural behavioral-coupled cardiovascular responses to the physical stressor were not expressed due to the need for the individual to remain still. We speculate that the MSNA changes and associated changes in brain activity reflect differences in individuals' psychological stress responses to the unpleasant painful event. If this is true, it significantly alters the interpretation of brain imaging studies that explore PAG activity during physical stressors, since natural behavioral responses and their associated changes in cardiovascular control and even analgesic responses may not be properly expressed. Nevertheless, this experimental method of inducing pain provides insight as to how pain may modulate MSNA and its underlying central circuitry. However, in order to interpret these changes in chronic pain patients, one would need to know their baseline levels prior to the development of chronic pain.

Limitations

As with our previous studies on the sympathetic responses to muscle (or cutaneous) pain, it may be that there was a form of selection bias in our subjects. While we did not knowingly introduce a bias, one must recognize that those subjects who volunteered for the experiment did so knowing that they were going to have a microelectrode inserted into a nerve, another needle inserted under the skin, and a cannula inserted into a muscle. Moreover, they knew they were going to experience strong muscle pain during the intramuscular infusion of hypertonic saline for up to an hour. As such, subjects with a needle phobia, or a morbid fear of pain, would not volunteer for this type of experiment. None of the subjects we did study reported having a fear of needles, and none fainted during the course of the experiment (although they were supine). Of course, it may well be that had we actively selected those with a needle phobia, or those with a tendency to faint, we may have uncovered further differences, but it is fair to say that all our subjects were essentially normal. Another potential limitation is that we always recorded MSNA from the right common peroneal nerve, ipsilateral to the intramuscular infusion of hypertonic saline. Accordingly, we must be circumspect in any consideration of the lateralization of the changes in BOLD signal intensity we uncovered, but it is fair to say that changes in MSNA are usually distributed symmetrically to muscles on either side of the body. The most significant limitation of this study is the spatial resolution of imaging brainstem structures such as the PAG. Whilst we argue that the differential signal intensity changes within the midbrain were located in the region of the dIPAG, given the relatively poor spatial resolution of human brain imaging at 3 T, it is possible that these changes were indeed located in other PAG columns. Recent developments in MRI at higher field strengths, such as 7 T, offer to provide superior spatial acuity and will provide greater confidence in the spatial accuracy of future imaging studies.

REFERENCES

- Bandler R, Carrive P, Zhang SP (1991): Integration of somatic and autonomic reactions within the midbrain periaqueductal gray: Viscerotopic, somatotopic and functional organization. *Prog Brain Res* 87:269–305.
- Bandler R, Keay KA, Floyd N, Price J (2000): Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. *Brain Res Bull* 53:95–104.
- Bandler R, Shipley MT (1994): Columnar organization in the midbrain periaqueductal gray: Modules for emotional expression? *Trends Neurosci* 17:379–389.
- Barbaresi P (2005): GABA-immunoreactive neurons and terminals in the cat periaqueductal gray matter: A light and electron microscopic study. *J Neurocytol* 34:471–487.
- Barbaresi P (2010): Postnatal development of GABA-immunoreactive neurons and terminals in rat periaqueductal

- gray matter: A light and electron microscopic study. *J Comp Neur* 518:2240–2260.
- Basnayake SD, Hyam JA, Pereira EA, Schweder PM, Brittain J-S, Aziz TZ, ... Paterson DJ (2011): Identifying cardiovascular neurocircuitry involved in the exercise pressor reflex in humans using functional neurosurgery. *J Appl Physiol* 110: 881–891.
- Basnayake SD, Green AL, Paterson DJ (2012): Mapping the central neurocircuitry that integrates the cardiovascular response to exercise in humans. *Exp Physiol* 97:29–38.
- Beckstead RM (1979): An autoradiographic examination of cortico-cortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. *J. Comp. Neuro* 184: 43–62.
- Benarroch EE (1993): The central autonomic network: Functional organization, dysfunction, and perspective. *Mayo Clinic Proceedings* 68:988–1001.
- Brown R, Macefield VG (2008): Assessing the capacity of the sympathetic nervous system to respond to a cardiovascular challenge in human spinal cord injury. *Spinal Cord* 46: 666–672.
- Bruehl S, Chung OY, Jirjis JN, Biridepalli S (2005): Prevalence of clinical hypertension in patients with chronic pain compared to nonpain general medical patients. *Clin J Pain* 21:147–153.
- Burton AR, Brown R, Macefield VG (2008): Selective activation of muscle and skin nociceptors does not trigger exaggerated sympathetic responses in spinal-injured subjects. *Spinal Cord* 46: 660–665.
- Burton AR, Birznies I, Bolton PS, Henderson LA, Macefield VG (2009a): The effects of deep and superficial experimentally-induced acute pain on muscle sympathetic nerve activity in human subjects. *J Physiol* 587:183–193.
- Burton AR, Birznies I, Spaak J, Henderson LA, Macefield VG (2009b): The effects of deep and superficial experimentally-induced acute pain on skin sympathetic nerve activity in human subjects. *Exp Brain Res* 195:317–324.
- Burton AR, Fazalbhoy A, Macefield VG (2016): Sympathetic responses to noxious stimulation of muscle and skin. *Front. Neurol* 109.
- Canteras NS, Goto M (1999): Fos-like immunoreactivity in the periaqueductal gray of rats exposed to a natural predator. *Neuroreport* 10:413–418.
- Carrive P, Bandler R (1991): Viscerotopic organization of neurons subserving hypotensive reactions within the midbrain periaqueductal grey: A correlative functional and anatomical study. *Brain Res* 541:206–215.
- Carrive P, Dampney RA, Bandler R (1987): Excitation of neurones in a restricted portion of the midbrain periaqueductal grey elicits both behavioural and cardiovascular components of the defence reaction in the unanaesthetized decerebrate cat. *Neuroscience Letters* 81:273–278.
- Carrive P, Dampney RA, Bandler R (1988): Anatomical evidence that hypertension associated with the defence reaction in the cat is mediated by a direct projection from a restricted portion of the midbrain periaqueductal grey to the subretrofacial nucleus of the medulla. *Brain Res* 460:339–345.
- Chaves JF, Brown JM (1987): Spontaneous cognitive strategies for the control of clinical pain and stress. *J. Behav. Med* 10: 263–276.
- Coote JH, Hilton SM, Perez-Gonzalez JF (1979): Inhibition of the baroreceptor reflex on stimulation in the brain stem defence centre. *J Physiol* 288:549–560.
- Dampney RAL, Furlong TM, Horiuchi J, Iigaya K (2013): Role of dorsolateral periaqueductal grey in the coordinated regulation of cardiovascular and respiratory function. *Auton. Neurosci* 175:17–25.
- de Menezes RCA, Zaretsky DV, Fontes MAP, DiMicco JA (2009): Cardiovascular and thermal responses evoked from the periaqueductal grey require neuronal activity in the hypothalamus. *J Physiol* 587:1201–1215.
- Diedrichsen J, Maderwald S, Kuper M, Thurling M, Rabe K, Gizewski ER, et al. (2011): Imaging the deep cerebellar nuclei: A probabilistic atlas and normalization procedure. *Neuroimage* 54:1786–1794.
- Dielenberg RA, Leman S, Carrive P (2004): Effects of dorsal periaqueductal gray lesions on cardiovascular and behavioral responses to car odor exposure in rats. *Behav. Brain Res* 153: 487–496.
- DiMicco JA, Samuels BC, Zaretskaia MV, Zaretsky DV (2002): The dorsomedial hypothalamus and the response to stress: Part renaissance, part revolution. *Pharmacol Biochem Behav* 71: 469–480.
- Domesick VB (1969): Projections from the cingulate cortex in the rat. *Brain Res* 12:296–320.
- Fagius J, Wallin BG (1980): Sympathetic reflex latencies and conduction velocities in normal man. *J Neurol Sci* 47:433–448.
- Fazalbhoy A, Birznies I, Macefield VG (2012): Individual differences in the cardiovascular responses to tonic muscle pain: Parallel increases or decreases in muscle sympathetic nerve activity, blood pressure and heart rate. *Exp Physiol* 97: 1084–1092.
- Fazalbhoy A, Birznies I, Macefield VG (2014): Consistent interindividual increases or decreases in muscle sympathetic nerve activity during experimental muscle pain. *Exp Brain Res* 232: 1309–13015.
- Feinstein B, Langton JNK, Jameson RM, Schiller F (1954): Experiments on pain referred from deep somatic tissues. *J Bone Joint Surg* 36:981–997.
- Floyd NS, Price JL, Ferry AT, Keay KA, Bandler R (2000): Orbitomedial prefrontal cortical projections to distinct longitudinal columns of the periaqueductal gray in the rat. *J Comp Neurol* 422:556–578.
- Floyd NS, Price JL, Ferry AT, Keay KA, Bandler R (2001): Orbitomedial prefrontal cortical projections to hypothalamus in the rat. *J Comp Neurol* 432:307–328.
- Fontes MA, Tagawa T, Polson JW, Cavanagh SJ, Dampney RA (2001): Descending pathways mediating cardiovascular response from dorsomedial hypothalamic nucleus. *Am J Physiol Heart Circ Physiol* 280:H2891–H2901.
- Fulwiler CE, Saper CB (1984): Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. *Brain Res* 319:229–259.
- Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ (2005): Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. *J Comp Neurol* 492:145–177.
- Hall CW, Behbehani MM (1997): The medial preoptic nucleus of the hypothalamus modulates activity of nitric oxide sensitive neurons in the midbrain periaqueductal gray. *Brain Res* 765: 208–217.
- Hall CW, Behbehani MM (1998): Synaptic effects of nitric oxide on enkephalinergic, GABAergic, and glutamatergic networks of the rat periaqueductal gray. *Brain Res* 805:69–87.
- Hall SC, Fazalbhoy A, Birznies I, Macefield VG (2012): Biphasic effects of tonic stimulation of muscle nociceptors on skin

- sympathetic nerve activity in human subjects. *Exp Brain Res* 221:107–114.
- Herbert H, Saper CB (1992): Organization of medullary adrenergic and noradrenergic projections to the periaqueductal gray matter in the rat. *J Comp Neurol* 315:34–52.
- Hilton SM, Redfern WS (1986): A search for brain stem cell groups integrating the defence reaction in the rat. *J Phys* 378: 213–228.
- Horiuchi J, McDowall LM, Dampney RAL (2009): Vasomotor and respiratory responses evoked from the dorsolateral periaqueductal grey are mediated by the dorsomedial hypothalamus. *J Phys* 587:5149–5162.
- Huang ZG, Subramanian SH, Balnave RJ, Turman AB, Moi Chow C (2000): Roles of periaqueductal gray and nucleus tractus solitarius in cardiorespiratory function in the rat brainstem. *Respir Physiol* 120:185–195.
- Iigaya K, Horiuchi J, McDowall LM, Dampney RAL (2010): Topographical specificity of regulation of respiratory and renal sympathetic activity by the midbrain dorsolateral periaqueductal gray. *Am J Physiol* 299:R853–R861.
- Iigaya K, Müller-Ribeiro FC-F, Horiuchi J, McDowall LM, Nalivaiko E, Fontes MAP, Dampney RAL (2012): Synchronized activation of sympathetic vasomotor, cardiac, and respiratory outputs by neurons in the midbrain colliculi. *Am J Physiol* 303:R599–R610.
- Keay KA, Bandler R (2001): Parallel circuits mediating distinct emotional coping reactions to different types of stress. *Neurosci Biobehav Rev* 25:669–678.
- Keay KA, Henderson LA (2010): Physical and emotional pain. In: George K, Michel LM, Richard T, editors. *Encyclopedia of Behavioral Neuroscience: Three-Volume Set*. United States: Elsevier. pp. 54–63.
- Keefe FJ, Brown GK, Wallston KA, Caldwell DS (1989): Coping with rheumatoid arthritis pain: Catastrophizing as a maladaptive strategy. *Pain* 37:51–56.
- Kobuch S, Fazalbhoy A, Brown R, Macefield VG (2015): Inter-individual responses to experimental muscle pain: Baseline physiological parameters do not determine whether muscle sympathetic nerve activity increases or decreases during pain. *Front Neurosci* 9:471. Available at: <http://doi.org/10.3389/fnins.2015.00471>
- Kobuch S, Fazalbhoy A, Brown R, Macefield VG (2016): Inter-individual responses to experimental muscle pain: Baseline anxiety ratings and attitudes to pain do not determine the direction of the sympathetic response to tonic muscle pain in humans. *Int J Psychophysiol* 104:17–23.
- Lam W, Verberne AJ (1997): Cuneiform nucleus stimulation-induced sympathoexcitation: Role of adrenoceptors, excitatory amino acid and serotonin receptors in rat spinal cord. *Brain Res* 757:191–201.
- Lewis T (1942): *Pain*. London: McMillan.
- Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A (2001): Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412:150–157.
- Lovick TA (1992): Inhibitory modulation of the cardiovascular defence response by the ventrolateral periaqueductal grey matter in rats. *Exp Brain Res* 89:133–139.
- Lundblad LC, Fatouleh RH, Hammam E, McKenzie DK, Macefield VG, Henderson LA (2014): Brainstem changes associated with increased muscle sympathetic drive in obstructive sleep apnoea. *NeuroImage* 103:258–266.
- Macefield VG, Henderson LA (2010): Real-time imaging of the medullary circuitry involved in the generation of spontaneous muscle sympathetic nerve activity in awake subjects. *Hum Brain Mapp* 31:539–549.
- Macefield VG, Henderson LA: 'Real-time' imaging of cortical and subcortical sites of cardiovascular control: Concurrent recordings of sympathetic nerve activity and fMRI in awake subjects. *J Neurophysiol* 116:1199–1207.
- McCracken LM, Dhirga L (2002): A short version of the pain anxiety symptoms scale (pass-20): Preliminary development and validity. *Pain Res Manag: J Can Pain Soc* 7:45–50.
- Meller ST, Dennis BJ (1991): Efferent projections of the periaqueductal gray in the rabbit. *Neurosci* 40:191–216.
- Melzack R (1975): The McGill pain questionnaire: Major properties and scoring methods. *Pain* 1:277–299.
- Mobbs D, Petrovic P, Marchant JL, Hassabis D, Weiskopf N, Seymour B, ... Frith CD (2007): When fear is near: Threat imminence elicits prefrontal-periaqueductal gray shifts in humans. *Science* 317:1079–1083.
- Onstott D, Mayer B, Beitz AJ (1993): Nitric oxide synthase immunoreactive neurons anatomically define a longitudinal dorsolateral column within the midbrain periaqueductal gray of the rat: Analysis using laser confocal microscopy. *Brain Res* 610: 317–324.
- Pavlin DJ, Sullivan MJL, Freund PR, Roesen K (2005): Catastrophizing: A risk factor for postsurgical pain. *Clin J Pain* 21:83–90.
- Picavet HSJ, Vlaeyen JWS, Schouten JSAG (2002): Pain catastrophizing and kinesiophobia: Predictors of chronic low back pain. *Am J Epidemiol* 156:1028–1034.
- Reiman EM, Raichle ME, Robins E, Mintun MA, Fusselman MJ, Fox PT, Hackman KA (1989): Neuroanatomical correlates of a lactate-induced anxiety attack. *Arch Gen Psychiatry* 46: 493–500.
- Spanos NP, Radtke-Bodorik HL, Ferguson JD, Jones B (1979): The effects of hypnotic susceptibility, suggestions for analgesia, and the utilization of cognitive strategies on the reduction of pain. *J Abnorm Psychol* 88:282–292.
- Sverrisdóttir YB, Green AL, Aziz TZ, Bahuri NFA, Hyam J, Basnayake SD, Paterson DJ (2014): Differentiated baroreflex modulation of sympathetic nerve activity during deep brain stimulation in humans. *Hypertension* 63:1000–1010.
- Thompson RH, Swanson LW (1998): Organization of inputs to the dorsomedial nucleus of the hypothalamus: A reexamination with Fluorogold and PHAL in the rat. *Brain Res Rev* 27: 89–118.
- Van Bockstaele EJ, Aston-Jones G, Pieribone VA, Ennis M, Shipley MT (1991): Subregions of the periaqueductal gray topographically innervate the rostral ventral medulla in the rat. *J Comp Neurol* 309:305–327.
- Yardley CP, Hilton SM (1986): The hypothalamic and brainstem areas from which the cardiovascular and behavioural components of the defence reaction are elicited in the rat. *J Auton Nerv Syst* 15:227–244.