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# Real-time imaging of cortical areas involved in the generation of increases in skin sympathetic nerve activity when viewing emotionally charged images

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ABSTRACT

The sympathetic innervation of the skin not only primarily subserves thermoregulation, but has also been commandeered as a means of emotional expression. While the majority of brain imaging studies of emotion have utilised the galvanic skin response as a means of inferring changes in skin sympathetic nerve activity (SSNA), spontaneous fluctuations in the galvanic skin response bear little relation to spontaneous fluctuations in SSNA. To improve our understanding of the central neural processes involved in the generation of autonomic emotional markers, we recorded SSNA concurrently with brain functional magnetic resonance imaging in 13 subjects. Emotional changes were evoked by presentation of positively-charged (erotica) or negatively-charged (mutilation) images from the International Affective Picture System. Positive and negative emotionally-charged images evoked significant increases in total SSNA and signal intensity in the orbital, dorsolateral and ventromedial prefrontal cortices, amygdala, nucleus accumbens and anterior insula. Increases in signal intensity during increases in SSNA occurred in a number of brain regions, including the central and lateral amygdala, dorsolateral pons, thalamus, nucleus accumbens, and cerebellar cortex. Signal intensity decreases during SSNA increases occurred in the left orbitofrontal, frontal and right precuneus cortices. These data reveal for the first time, cortical and subcortical sites involved in generating SSNA changes during emotions.



## Introduction

Emotions are complex products of cognitive, behavioral and neurobiological processes, which result from an interaction between a person and their environment (Ellsworth, 1991; Frijda, 1986). Although theories of emotion are continually being developed and refined, it is clear that emotional state changes involve alterations in the activity of organs controlled by the autonomic nervous system (Lacey and Lacey, 1970). For example, a person in fear will display sympathetically-mediated responses, such as increased sweating, cutaneous vasoconstriction and piloerection. Furthermore, although the cognitive appraisal of emotions can vary greatly from individual to individual, it has been shown that most emotions evoke fairly standardized autonomically-mediated changes in heart rate and arterial blood pressure (Carter et al., 2008; Hare et al., 1970). However, whether particular patterns of autonomic activity are characteristic of particular emotions, or are common to a range of emotional states, remains hotly debated. Assessment of the patterns of cardiorespiratory activity in distinct emotional states suggests that there are specific "autonomic signatures" that identify each emotion (Bradley et al., 2008; Kreibig, 2010; Low et al., 2008; Rainville et al., 2006; Stephens et al., 2010).

Although the changes in autonomic activity that occur during emotions are largely considered to be pathologically inconsequential during short emotional episodes, it has been suggested that intense emotional arousal plays a significant role in the onset of acute coronary events, such as sudden cardiac death and myocardial infarction (Gabbay et al., 1996; Mittleman et al., 1995). In addition, emotionally-evoked autonomic changes are known to be disadvantageous to various organ systems if experienced over prolonged periods of time (Kubzansky and Kawachi, 2000). For example, it has been hypothesized that repeated cardiovascular responses during emotional episodes provide the basis for the increased incidence of cardiovascular disease in individuals with negative psychological traits such as anger and anxiety (Manuck, 1994; Rozanski et al., 1999).

As reviewed by Critchley et al. (2011), there is an increasing interest in the use of brain imaging techniques, in particular functional magnetic resonance imaging (fMRI), to identify areas of the human

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brain involved in autonomic control, both in healthy individuals and in those with specific neurological deficits. However, relatively few investigators have explored regions responsible for the autonomic changes evoked by emotions. Various approaches have been used to assess the physiological responses to emotional processes, with the majority of studies monitoring changes in heart rate or changes in sweat release, or occasionally pupil diameter (Harrison et al., 2009); electrical activity of the stomach has also been used to quantify subjective reports of disgust (Harrison et al., 2010). An important consideration in measuring the physiological responses to emotional stimuli is that they provide an *indirect* marker of changes in autonomic outflow, one that may be difficult to interpret. Changes in heart rate, diameter of the pupil and gastric activity are controlled by both sympathetic and parasympathetic nerves, whereas skin blood flow and sweat release are controlled exclusively by sympathetic nerves. Of course, this is an advantage of recording sweat release, but it needs to be recognized that effector-organs respond in a sluggish manner. Changes in heart rate are fairly rapid, but inferring changes in sympathetic and parasympathetic outflow from assessment of heart-rate variability (see Gray et al., 2009) is problematic (Goldstein et al., 2011).

Recordings from single sudomotor neurons from the common peroneal nerve have shown that the decrease in skin resistance associated with sweat release lags the sudomotor discharge by $1.12 \pm 0.05$ s (Macefield and Wallin, 1996). As stated by Bini et al., (1980), "*no simple quantitative relationship could be seen between the size of individual sudomotor bursts and [the] accompanying electrodermal responses.*" Moreover, there is a very poor relationship between changes in electrical resistance (or conductance) of the skin and sudomotor activity, as produced by controlled intraneural electrical stimulation (Kirno et al., 1991; Kunimoto et al., 1991, 1992). Indeed, because of the non-linear relationship between sudomotor activity and skin resistance, it has been argued that changes in skin resistance cannot be used to quantify changes in skin sympathetic nerve activity (Kirno et al., 1991). Conversely, direct microelectrode recordings of skin sympathetic nerve activity (SSNA), which is directed to the cutaneous blood vessels (and hairs in non-glabrous skin) as well as to the sweat glands, should provide a better measure of sympathetic outflow from the brain than a measurement of sweat release alone, given that measuring individual bursts of SSNA provides a better temporal resolution than indirect measurement of effector-organ function (changes in electrical resistance and/or changes in skin blood flow).

Accordingly, the aim of this study is to define brain regions responsible for emotionally evoked increases in SSNA. Specifically, we used neutral and emotionally-charged images from the International Affective Picture System (Lang et al., 1997) to induce increases in SSNA. We adopted the technique we had recently developed that allows us to perform direct recordings of sympathetic nerve activity coupled with concurrent functional magnetic resonance imaging (fMRI) of the brain (Macefield and Henderson, 2010). Since it has been suggested that brain regions such as the amygdala, insula and cingulate cortex are critically involved in emotional expression, we hypothesize that these regions will display signal intensity increases during emotional state changes. Furthermore, we hypothesize that activity in brainstem regions known to be involved in generating changes in sympathetic drive, such as the nucleus of the solitary tract, will be significantly correlated to emotion-related changes in skin sympathetic nerve activity. The fundamental knowledge gained from this investigation will allow us to better understand the physiological changes associated with states of disturbed emotional processing.

## Methods

### Subjects

Thirteen subjects (10 males, 3 females) aged 18–49 years participated in this study. All procedures were carried out with understanding and written informed consent of each subject. All procedures were approved by the local institutional Human Research Ethics Committees (University of New South Wales, University of Western Sydney) and were conducted in accordance with the conditions established by the Declaration of Helsinki. All experiments were conducted at the Neuroscience Research Australia Imaging Centre (Achieva 3 T, Philips Medical Systems, Netherlands).

### Nerve recording and MR imaging

Subjects lay supine on an MRI bed in the laboratory. A knee was supported on a foam block and the common peroneal nerve located at the fibular head by electrical stimulation through a surface probe (3–10 mA, 0.2 ms, 1 Hz; Stimulus Isolator, ADInstruments, Sydney, Australia). An insulated tungsten microelectrode (FHC, Maine, USA) was inserted percutaneously into the nerve and manually guided into a cutaneous fascicle of the nerve while delivering weak electrical stimuli to evoke "pins and needles" sensations (0.01–1 mA, 0.2 ms, 1 Hz). A nearby subdermal microelectrode, with 1 mm insulation removed, served as the reference electrode and a surface AgAgCl electrode on the leg as the ground electrode. Once a cutaneous fascicle had been entered, as defined by radiating paresthesia and the absence of muscle twitches during intraneural stimulation, neural activity was amplified (gain $10^4$, bandpass 0.3–5.0 kHz) using a low-noise, electrically isolated, headstage (NeuroAmpEX, ADInstruments, Sydney, Australia). The innervation territory on the hairy skin of the dorsum of the foot or lateral aspect of the leg was mapped out by stroking the skin to activate low-threshold cutaneous mechanoreceptors. Adjustments of the microelectrode were made until spontaneous bursts of skin sympathetic nerve activity (SSNA) were encountered. A brisk sniff, a loud sound or an unexpected tap on the head evoked an arousal burst and thereby confirmed that we were recording SSNA (Delius et al., 1972). Neural activity was amplified (gain 20,000, bandpass 300 Hz–5 kHz) and acquired (10 kHz sampling) on a computer-based data acquisition and analysis system (LabChart 7, PowerLab 30SP; ADInstruments, Sydney, Australia).

A piezoelectric transducer was applied to the pad of a toe to measure the pulsatile fluctuations in skin blood volume; from this signal heart rate and pulse amplitude were calculated using the Cyclic Measurements feature in the LabChart 7 software. Decreases in pulse amplitude were used to indicate decreases in skin blood volume and hence skin blood flow. Skin potential (0.1–10 Hz; BioAmp, ADInstruments, Sydney, Australia) was measured across the sole and dorsum of the foot; this signal represented sweat release. Respiration was monitored using piezoelectric transducers strapped around the chest and abdomen and both respiratory rate and amplitude measured (Cyclic Measurements, LabChart 7, ADInstruments, Sydney, Australia).

The subject's head was enclosed in an 8 channel SENSE head coil and stabilized with foam pads to minimise head movement. Headphones were provided to minimise noise and to allow communication with the subject. A continuous series of 200 gradient echo echo-planar images, sensitive to Blood Oxygen Level Dependent (BOLD) contrast and encompassing the entire brain were collected over a 27 minute period (46 axial slices, TR = 8 s, TE = 40 ms, flip angle = 90°, raw voxel size = $1.5 \times 1.5 \times 2.75$ mm thick). All 46 axial slices were collected during the first 4 s of the 8 second TR.

### Emotional stimuli

Emotional state changes were produced by viewing standard images from the International Affective Picture System (IAPS (Lang et al., 1997). Four of the subjects had participated in fMRI experiments previously, but none of the subjects had been exposed to the IASP images. Each picture used in the system has been extensively tested and rated for valency (its subjective impact ranging from extremely negative to extremely positive) and arousal. In our investigation, positive

emotions were evoked by viewing images of erotica with high positive valence ratings; negative emotions were evoked by viewing images of mutilation with high negative valence ratings. Following a 4-minute (30 volume) baseline, during which 30 neutral images were viewed (1 image per volume), a 2 minute (15 volume) period of emotional engagement occurred, during which 15 erotica or 15 mutilation images (1 image per volume) were viewed. This was followed by a 2-minute period of 15 neutral images. This 30 volume period was then repeated a further 5 times so that in total each subject viewed 3 periods of erotica and 3 periods of mutilation and 6 intervening neutral periods. The periods of erotica or mutilation were randomized.

*SSNA and physiology analysis*

Bursts of sympathetic neural activity were analysed by measuring the amplitude of each burst, following processing of the nerve signal: a high-pass digital filter at 300 Hz was applied to the recorded signal to remove artifacts picked up by the cable from the headstage to the amplifier, and mean-voltage signal generated by constructing a root-mean square processed signal with a time constant of 200 ms. Individual bursts of SSNA were measured in each 4 s the inter-scan period and the cumulative sum computed. Mean values over each 2 min block of stimuli were calculated. Mean values of heart rate, skin blood flow, skin potential and respiratory rate and amplitude were also measured over each 2-min block, including the scanning periods. Repeated Measures Analysis of variance of each physiological parameter across the three stimulus conditions, coupled with a Newman–Keuls test for multiple comparisons, were used for statistical analysis of the data (Prism 5 for Mac, GraphPad Software Inc, USA). Data in the Erotica and Mutilation conditions were also normalized to values obtained in the Neutral condition and the same analyses performed.

*MRI analysis*

Using SPM8 (Friston et al., 1995), functional images were motion corrected, global signal drifts removed using the detrending method described by Macey et al. (2004), spatially normalized to the Montreal Neurological Institute (MNI) template, and smoothed using a 5 mm full-width-at-half-maximum Gaussian filter. Changes in fMRI signal intensity were assessed during the subsequent 4 s period to take into account the ~5 s neurovascular coupling delay and the ~1 s required for conduction of the sympathetic bursts from the brain to the peripheral recording site, as described previously (Macefield and Henderson, 2010).

Significant changes in BOLD signal intensity were assessed in each individual using four separate models. For the first two analyses, signal intensity changes during exclusively erotica or exclusively mutilation were assessed using repeated box-car models. For the third analysis, erotica and mutilation were assessed using a repeated box-car model, that is, brain regions activated during both erotica and mutilation periods. For the final analysis, regional BOLD signal intensity changes associated with SSNA were determined using a SSNA model which was created for each individual subject. Since we were assessing signal intensity changes associated with SSNA increases during emotional state changes, i.e. erotica and/or mutilation periods, and not during baseline, i.e. neutral periods, a SSNA model for each subject was created by placing a value of 0 for all neutral image periods, and 1 for each time point during erotica or mutilation periods in which SSNA was at least 1 SD greater than the SSNA during the entire previous neutral period. The contrast maps generated from each of the four analyses were then placed into random effects analyses for group comparisons (uncorrected, $p<0.005$).

We and others have previously used frequency analyses to explore fine spatial activation maps (Coghill et al., 2003; Henderson et al., 2007). In using a frequency analysis we are exploring primarily the number of individuals which show a significant activation. To perform a frequency analysis, each individual SSNA contrast maps (corrected, $p<0.05$) was binarized and summed to create a frequency map, i.e. the value of each voxel represents the number of subjects in which signal intensity increased significantly. Finally, for each subject, the percentage change in signal intensity during increased SSNA, relative to signal intensity during neutral periods and emotional periods during which there were no changes in SSNA, was determined for each significantly activated brain region. The individual results were then averaged across subjects to give an overall mean ($\pm$SEM) percentage change in signal-intensity and significance assessed using raw intensity values and paired t-tests ($p<0.05$). Furthermore, for the lateral amygdala, the left cluster was reflected to the right side and signal intensity changes calculated for each subject. Significant differences between baseline and SSNA periods and between the left and right sides of the lateral amygdala were assessed using paired t-tests ($p<0.05$).

## Results

*Emotion evoked changes in SSNA*

Fig. 1 shows an example of the raw and calculated data obtained during scanning in one subject. It can be seen that spontaneous bursts of SSNA occurred while viewing neutral images, presumably related to the emotional salience of the image: one of our subjects, a champion barista, displayed bursts of SSNA whenever an (neutral) image of a coffee cup was shown. It can also be seen that fluctuations in heart rate and skin blood flow occurred, but no changes in skin potential occurred.

Mean changes in the physiological parameters during viewing of the erotica and mutilation images are shown in Fig. 2. Mean values were calculated over each 2 min set of images and normalized to the values obtained while viewing the neutral images. There were no significant changes in heart rate, respiratory rate or respiratory depth, but skin blood flow increased slightly when viewing images of mutilation. Skin potential (sweat release) increased when viewing both types of emotionally-charged images, but because of the high variability this failed to reach statistical significance. Conversely, significant increases in SSNA occurred during exposure to both positively and negatively-charged emotional images.

Fig. 3 shows representative examples in three subjects during the entire 200 volume period. Emotionally-charged images are represented by the gray bars. On average, both the number and amplitude of bursts of SSNA increased significantly during erotica and mutilation, relative to their levels when viewing neutral images, but the differences between the erotica and mutilation periods were not significantly different (Fig. 4). There was a significant positive correlation between the number and amplitude of SSNA bursts during neutral periods and the increase in SSNA bursts during the erotica and mutilation periods: the greater the SSNA associated with viewing neutral images, the greater the increase in SSNA evoked by positively or negatively-charged emotional images.

*fMRI signal intensity changes*

*Erotica only*

Significant signal intensity changes during periods of erotica occurred in a number of brain regions. Increases in signal intensity occurred in the left and right cerebellar, posterior cingulate, medial prefrontal and visual cortices and in the left and right medial thalamus. Signal increases also occurred in the left amygdala and right precuneus. A significant decrease in signal intensity occurred in the left and right premotor cortex, as seen in Fig. 5.

*Mutilation only*

As with the images of erotica, images of mutilation evoked signal intensity increases in the left and right cerebellar and visual cortices. In addition, mutilation evoked signal increases in the right amygdala

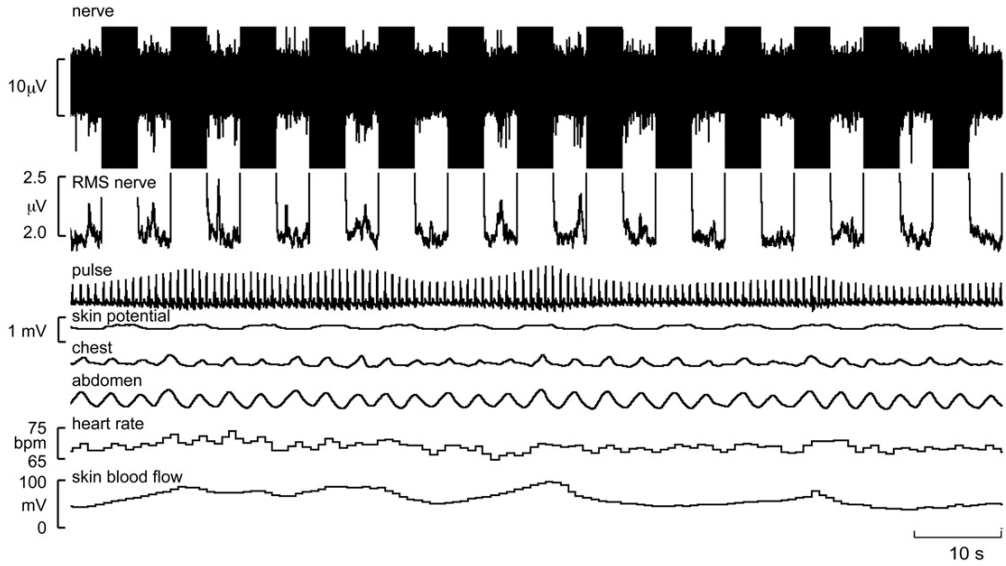



**Fig. 1.** Raw and calculated data obtained from one subject during exposure to neutral images. Scanning artifacts are shown as the black areas in the raw nerve signal. Spontaneous bursts of skin sympathetic nerve activity can be observed during each of the illustrated 4 s inter-scan periods. Heart rate and skin blood flow were calculated from the pulse signal.

as well as the deep cerebellar nuclei. Mutilation also evoked signal decreases in the premotor cortex, changes we had seen also with images of erotica. However, unlike erotica, mutilation evoked signal decreases in the left orbitofrontal cortex and in the left and right supplementary motor cortex (Fig. 5).

*Erotica and mutilation*

In addition to signal intensity changes associated specifically with positively or negatively-charged emotional images, there was significant regional brain activation that occurred during presentations of both erotica and mutilation. Signal increases occurred in the deep cerebellar nuclei and the left amygdala, as well as the cerebellar and visual cortices. In addition, signal intensity increased in the region of the ventral tegmental area, the medial left and right thalamus and the right precuneus. Signal intensity decreases occurred in the premotor cortex and the supplementary motor cortex (Fig. 5).

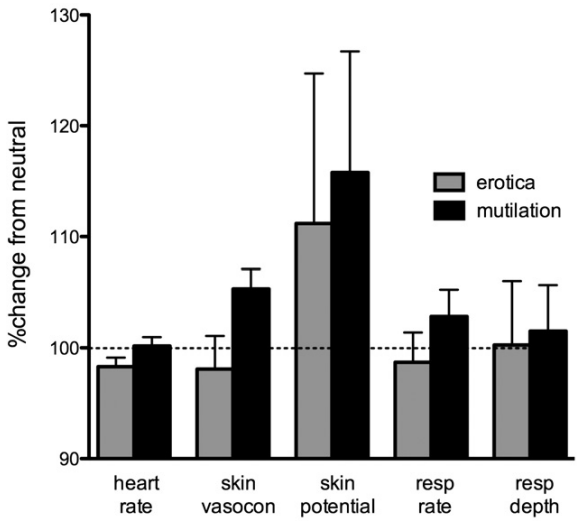



**Fig. 2.** Relative changes (mean + SEM) in heart rate, skin blood volume (skin vasoconstriction), sweat release (skin potential), respiratory rate and depth during viewing of 2 minute blocks of images of erotica or mutilation, normalized to the values recorded during 2 minute blocks of viewing neutral images. Physiological parameters were recorded continuously during the scan and inter-scan trials.

*SSNA*

Signal intensity changes correlated to individual subject's SSNA changes also occurred in a number of brain regions. Increases in signal intensity occurred bilaterally in the dorsolateral pons, nucleus accumbens and cerebellar cortex, the right amygdala and thalamus. Signal intensity decreases occurred in the left orbitofrontal cortex, frontal cortex and right precuneus (Fig. 6, Table 1). Signal intensity changes in each individual subject and the overall mean (SEM) signal intensity changes in areas in which there were significant increases in signal intensity during exposure to erotica and mutilation images are shown in Fig. 7.

Close inspection of both the random effects and frequency analyses revealed differential regional activation of the amygdala that were related to the increases in SSNA. Both random-effects and frequency analyses revealed that changes in SSNA were associated with activation of the lateral nucleus of the left amygdala. In addition, frequency analysis revealed bilateral activation of the central nucleus of the amygdala (Fig. 8). Finally, comparison of the right and left lateral amygdala revealed that although the right lateral amygdala displayed a significant signal intensity increase, the left lateral amygdala did not. Furthermore, there was a significant difference between the signal intensity changes in the left compared with the right lateral amygdala.

## Discussion

We have used, for the first time, concurrent microneurography and fMRI to correlate direct measures of skin sympathetic nerve activity with BOLD signal intensity while subjects view neutral and emotionally charged images. It is well known that emotional state changes are associated with changes in sympathetic drive. In this investigation we found that the presentation of emotionally-charged images resulted in a significant increase in burst frequency and amplitude of skin sympathetic nerve activity. Furthermore, both positive and negative emotional stimuli evoked similar increases in SSNA, suggesting that – regardless of valence – emotional state changes provoke the body to generate an increase in sympathetic outflow to the skin, as well as to other targets. These emotion-evoked increases in

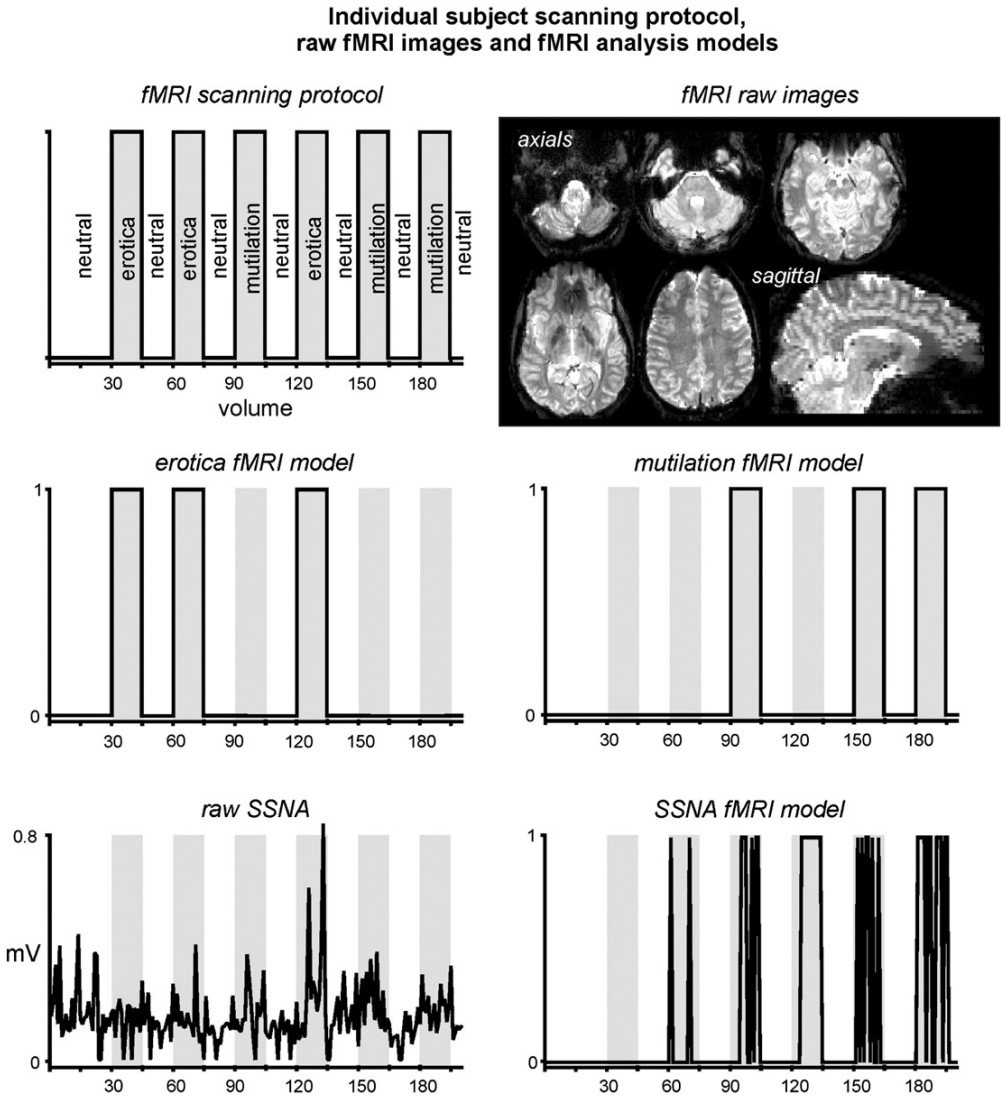



**Fig. 3.** Raw skin sympathetic nerve activity (SSNA) recordings during the entire 200 volume MRI scan in three subjects. To the right are the individual subject fMRI models used to search for signal intensity changes associated with SSNA increases evoked by positive and negative emotions. Note that SSNA increases during periods in which erotica or mutilation images were shown (gray vertical bars). SSNA increases during erotica and mutilation that were above one standard deviation of the mean SSNA during the immediately previous period of neutral images were treated as "on" periods for the fMRI analysis. mV: millivolt; au: arbitrary units.

SSNA were associated with regional fMRI signal intensity changes, including increases in the lateral and central amygdala nuclei, nucleus accumbens, medial thalamus and dorsolateral pons, and signal decreases in the frontal and orbitofrontal cortices.

Although the cognitive appraisal of emotions can vary between individuals, some investigators have suggested that most emotions evoke fairly standardized autonomically-mediated changes in heart rate and arterial blood pressure (Carter et al., 2008; Hare et al., 1970). Indeed, we show that both positive and negative emotions generated by the viewing of images of erotica and mutilation evoke similar increases in SSNA. This lack of difference argues against the suggestions that the patterns of autonomic changes are emotion-specific (Ekman and Davidson, 1994) or that the autonomic nervous system is more reactive to stimuli which present a threat to survival (Low et al., 2008). However, a wider range of autonomic variables and greater variations in emotional states would need to be investigated to confirm this suggestion.

The sympathetic innervation of the skin primarily subserves thermoregulation, through its actions on cutaneous blood vessels, sweat glands and hairs. However, the system has been commandeered as a means of emotional expression, with anxiety or an increase in arousal causing coactivation of sudomotor and cutaneous vasoconstrictor neurons (Delius et al., 1972). It is known that, in the absence of arousal, sudomotor outflow to the glabrous skin of the hand is negligible at rest, and only appears at ambient temperatures exceeding 45 °C (Bini et al., 1980). Stress-related sudomotor activity to the glabrous skin appears in patients with idiopathic hyperhidrosis (Macefield et al., 2008). Moreover, we also know that while sudomotor drive to the hairy skin primarily subserves thermoregulatory demands it is also activated by arousal stimuli; in thermoneutral conditions any sweat release is related to emotional state (Bini et al., 1980; Delius et al., 1973). We should also point out that the sympathetic outflow was directed to the hairy skin on the dorsum of the foot or the lateral aspect of the leg; it may well be that regional differences occur, as discussed by (Bini et al., 1980). Somewhat surprisingly, the effects of viewing emotionally-charged images on skin sympathetic nerve activity have never been reported. Indeed, only one study has used the IAPS images to examine human sympathetic nerve activity: Carter et al. (2008)

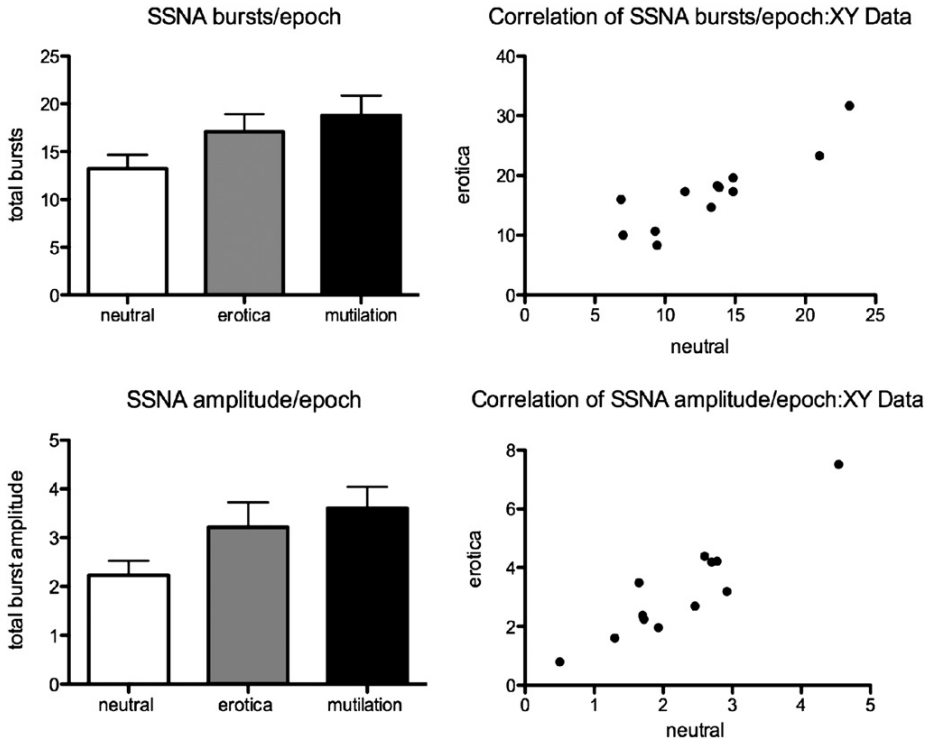


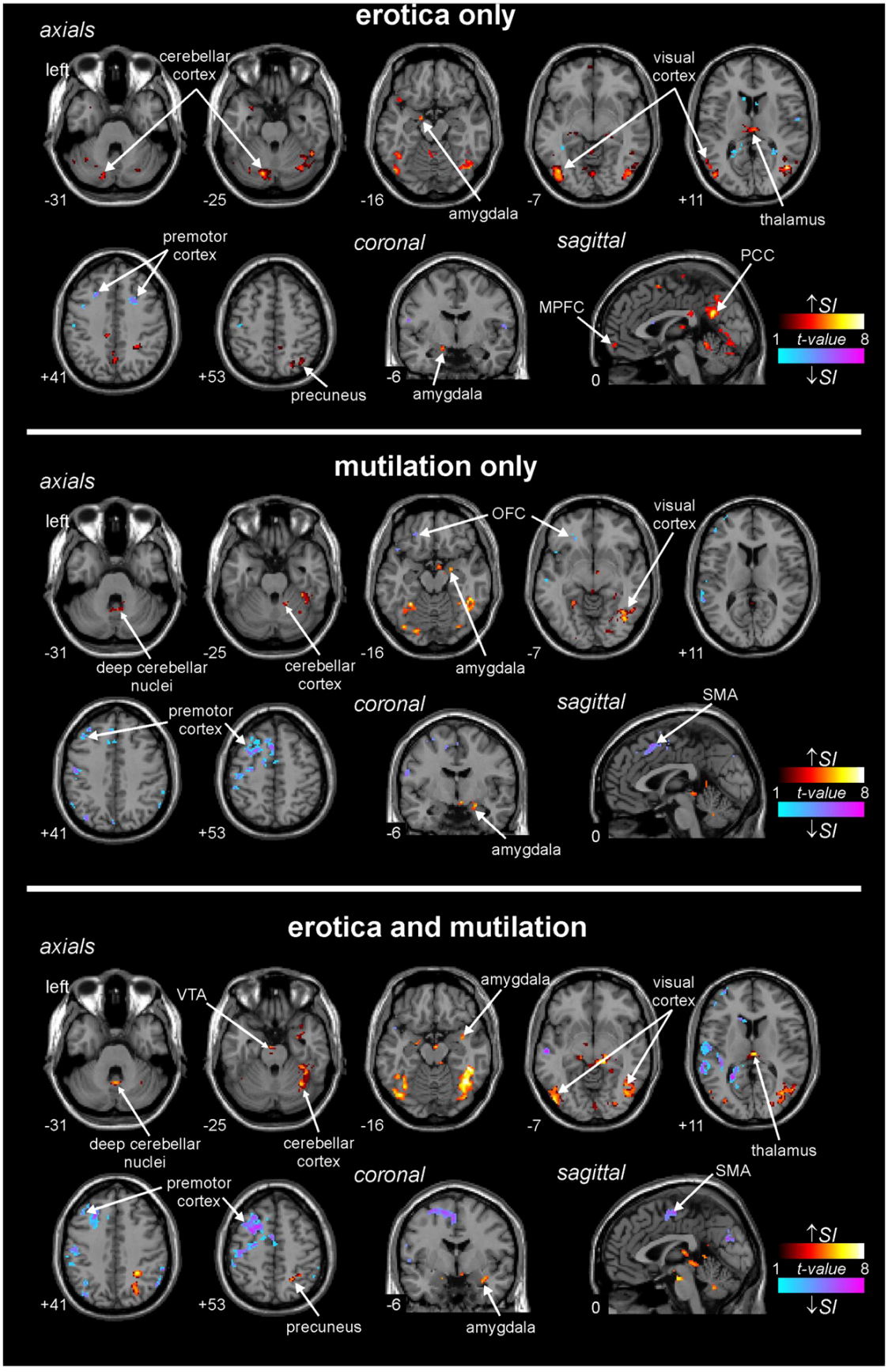

**Fig. 4.** Changes in skin sympathetic nerve activity (SSNA), measured as the number of bursts per 2-minute epoch, or as the total burst amplitude, during viewing of neutral or emotionally-charged images (erotica or mutilation). The right panels indicate that there was an essentially linear relationship between the levels of SSNA recorded when viewing neutral images and when viewing images of erotica.

showed that viewing images of mutilation had no effect on muscle sympathetic nerve activity. While we could observe changes in the co-recorded physiological parameters in individual subjects, on average there were – somewhat surprisingly – no significant differences in heart rate, skin blood flow, sweat release or respiratory depth or rate across the three stimulus conditions. Despite this, there were significant increases in SSNA during exposure to positively-charged (erotica) and negatively-charged (mutilation) images. This emphasizes that recording the skin sympathetic nerve activity directly affords us a much better measure of the autonomic responses to emotional stimuli than measuring the physiological parameters alone. Indeed, it may well be that the sluggish nature of the physiological changes we recorded did not allow us to differentiate across the different emotional conditions.

Although many investigations have explored brain regions responsible for the cognitive appraisal of emotions, few studies have explored brain regions responsible for emotion-evoked autonomic changes. Those studies have used indirect markers of sympathetic outflow, typically using sweat release and heart rate. As noted above, changes in electrical skin resistance correlate very poorly with skin sympathetic nerve activity (Kunimoto et al., 1992). Ours is the first investigation to correlate changes in brain activity with changes in directly recorded SSNA during emotional state changes. We found that changes in SSNA during positive and negative emotions are associated with activation of the left lateral amygdala and the left and right central nucleus. Not surprisingly, we found that SSNA changes were associated with significant activation of the amygdala and other brain regions which connect directly to the amygdala such as the OFC, nucleus accumbens and dorsolateral pons.

It is well known that the amygdala is involved in processing many forms of emotional state changes. Our data confirms this, with amygdala activation during both positive and negative emotions. However, this appeared to be lateralized: left amygdala during positive and right amygdala during negative emotions. The left amygdala has been implicated in the processing of negative stimuli such as fearful faces (Breiter et al., 1996; Morris et al., 1998) and the induction of sad mood, as well as positive stimuli such as including happy mood induction (Schneider et al., 1997). In contrast, while activation of the right amygdala has been loosely linked to positive stimuli such as positive words (Yoshimura et al., 2009), most studies show activation of the right amygdala during negative stimuli such as negative words (Hamann and Mao, 2002; Yoshimura et al., 2009) and hearing crying voices (Sander et al., 2003).

In addition, we found that signal intensity within the right (but not the left) lateral amygdala was associated with changes in SSNA. Although this finding may reflect lateralized amygdala function in generating SSNA, it may also result from the more robust SSNA response to negative emotions and thus a greater influence of this on the overall amygdala activation pattern. Much of our knowledge of regional amygdala function arises from experimental animal investigations into the role of the amygdala in the conditioned fear response. These studies have revealed that the lateral amygdala receives direct sensory input and may be the site at which plastic synaptic events that contribute to fear learning occur (Blair et al., 2001; LeDoux, 2000; Malkani and Rosen, 2000). The lateral amygdala then projects to the central amygdaloid nucleus which in turn projects to hypothalamic and brainstem sites to produce autonomic, endocrine and behavioral changes (Bellgowan and Helmstetter, 1996; Davis and Shi, 2000; LeDoux et al., 1988). Our data suggests that a similar mechanism may also occur in humans during the viewing of positively and negatively-charged emotions. We found that the SSNA increases that occurred during positive and negative emotions were correlated to signal intensity changes in the left and right central amygdaloid region. Critchley et al. (2005) recently reported a similar result, revealing that HR changes evoked by happy, sad, angry or disgusted facial expressions were all associated with activation of the amygdala in the region of the central nucleus.


erotica only
axials
left
cerebellar cortex
visual cortex
-31
-25
-16
amygdala
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+11
thalamus
premotor cortex
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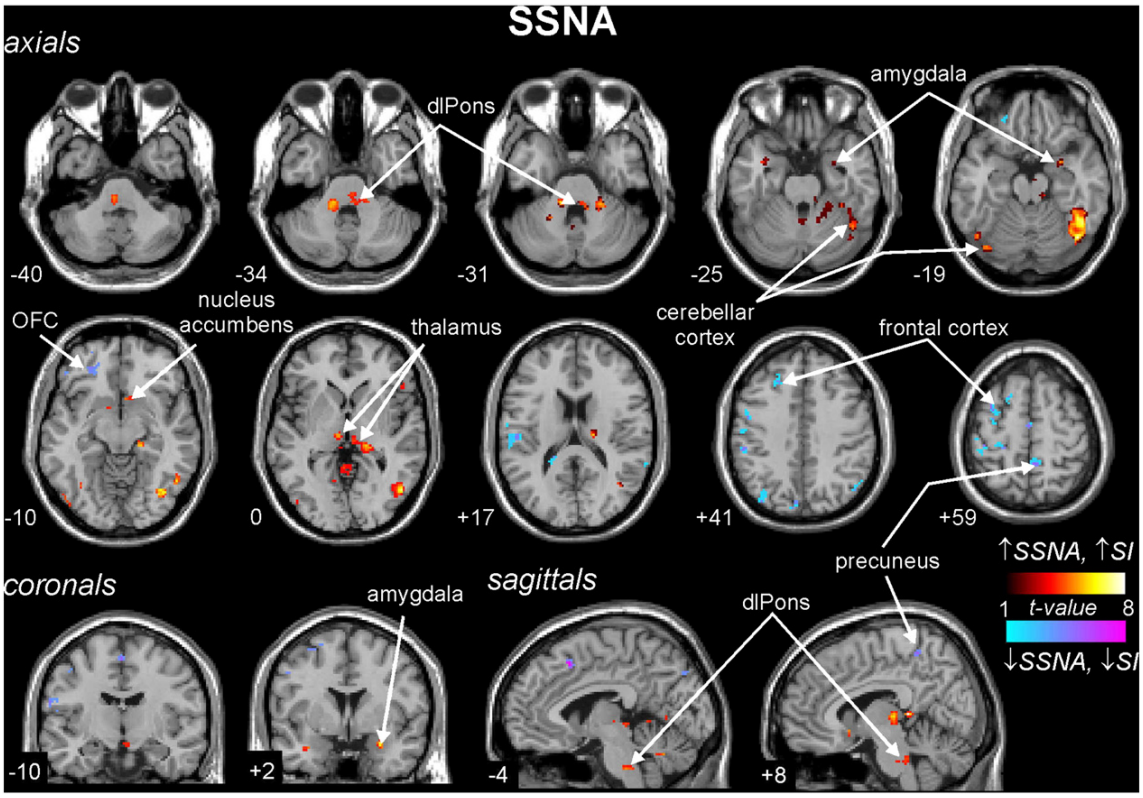


**Fig. 6.** Significant signal intensity (SI) increases (hot color scale) and decreases (cool color scale) correlated to changes in skin sympathetic nerve activity (SSNA) changes during periods of erotica and mutilation image presentation. Slice locations in Montreal Neurological Institute space are indicated at the lower left of each image slice. dlPons: dorsolateral pons; OFC: orbitofrontal cortex.

**Table 1**
Montreal Neurological Institute coordinates of significant signal intensity changes correlated to skin sympathetic nerve activity evoked by emotional state changes.

| | Brain region | MNI coordinate | | | Cluster size | t-Score |
|---|---|---|---|---|---|---|
| | | x | y | z | | |
| *Signal intensity increases* | Dorsolateral pons: | | | | | |
| | Right | 6 | −34 | −34 | 11 | 4.26 |
| | Left | −12 | −34 | −30 | 37 | 5.23 |
| | Right cerebellar cortex | 24 | −36 | −30 | 45 | 5.16 |
| | Right nucleus accumbens | 8 | 14 | −8 | 18 | 4.43 |
| | Thalamus: | | | | | |
| | Right | 6 | −24 | 4 | 45 | 7.36 |
| | Left | −8 | −18 | 0 | 10 | 4.90 |
| *Signal intensity decreases* | Left orbitofrontal cortex | −20 | 42 | −14 | 61 | 5.11 |
| | Left frontal cortex | −20 | 10 | 54 | 12 | 4.41 |
| | Right precuneus | 6 | −46 | 56 | 28 | 4.17 |

Tract tracing studies in animals have shown that the central amygdala can be divided into two major regions based on projection patterns. The medial part has extensive brainstem projections, including projections to the periaqueductal gray, pontine reticular formation, dorsal motor nucleus of the vagus and the nucleus of the solitary tract (Hopkins and Holstege, 1978; Rosen et al., 1991; Schwaber et al., 1982; Veening et al., 1984). In striking contrast, the brainstem projections of the lateral part of the central amygdaloid nucleus are limited to the parabrachial region (Petrovich and Swanson, 1997). Although we did not have the spatial resolution to determine precisely where in the central amygdaloid nucleus our signal increases occurred, we did find that, within the brainstem, SSNA-correlated signal increases were restricted to the dorsolateral pons, i.e. encompassing the left and right parabrachial nuclei. This raises the possibility that SSNA increases evoked by positive and negative emotionally charged images are mediated by a projection from the lateral part of the central amygdaloid to the parabrachial nucleus.

In contrast to the viewing of erotica and mutilation images, emotional states such as conditioned fear involve a significant behavioral response. This requires the coordinated action of both the motor and cardiovascular systems, and is likely mediated through brainstem regions and/or cortical regions known to evoke motor and cardiovascular responses upon stimulation. For example, it is well established from studies in experimental animals that stimulation of the midbrain periaqueductal gray matter produces the motor and cardiovascular changes associated with fight/flight and freezing responses (Bandler et al., 2000; Walker and Carrive, 2003). It is likely that, in these situations, central amygdaloid activation results in activity changes in the dorsolateral pons, as well as in the midbrain periaqueductal gray matter and lower brainstem regions such as the nucleus of the solitary tract. A future high-resolution human brainstem investigation will aid in determining the precise brainstem activations patterns evoked by different emotional state changes.

Surprisingly, we found that erotica alone, mutilation alone, erotica and mutilation or SSNA responses (both group and frequency analyses)

**Fig. 5.** Significant signal intensity (SI) increases (hot color scale) and decreases (cool color scale) during erotica image presentation alone (top panel), mutilation image presentation alone (middle panel) and during erotica and mutilation image presentations (lower panel). Slice locations in Montreal Neurological Institute space are indicated at the lower left of each image slice. MPFC: medial prefrontal cortex; OFC: orbitofrontal cortex; PCC: posterior cingulate cortex; SMA: supplementary motor area; VTA: ventral tegmental area.

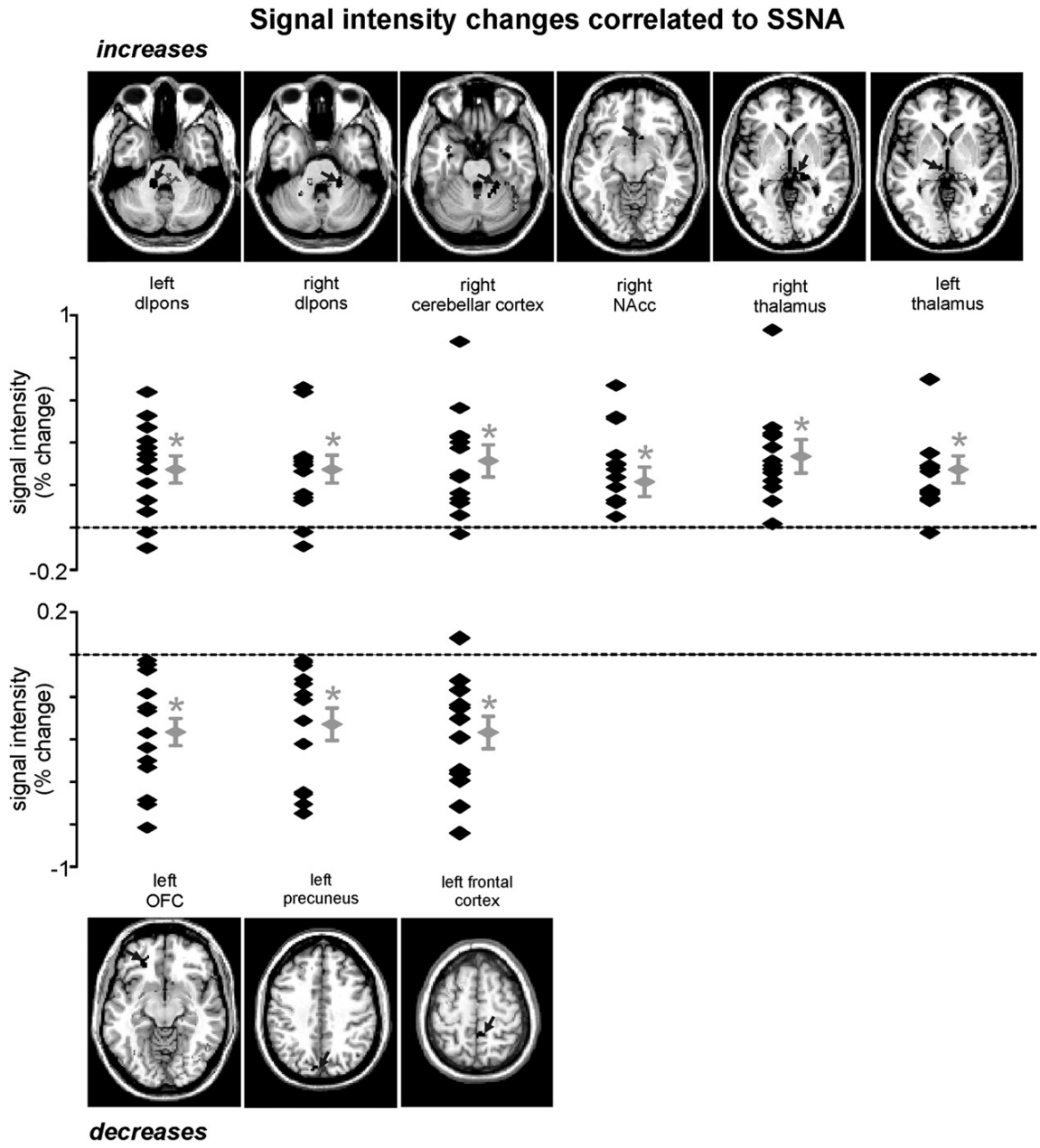


**Fig. 7.** Percentage change in signal intensity during erotica and mutilation image presentations compared with neutral image periods in brain regions that were significantly correlated to skin sympathetic nerve activity. Black triangles indicate the percentage change for each individual subject. The gray triangle represents the mean (±SEM) of all subjects. The black arrow in each image slice points to the regions from which the signal intensity changes were derived. *$p<0.05$.

did not evoke signal intensity increases within the insular or cingulate cortices. This is interesting given that it has been suggested that coactivation of the insular and cingulate cortices occurs during virtually all emotions and it has been hypothesized that the insular and cingulate cortices form the neural basis for the subjective awareness of emotions (Craig, 2005). Furthermore, it has been reported that electrical stimulation of the right insular cortex evokes increases in arterial pressure and heart rate, while stimulation of the left evokes decreases (Oppenheimer et al., 1992). Although these reports raise the possibility that the insula may encode both the subjective experience as well as the associated cardiovascular changes, our data suggests that this is not the case for all emotional state changes. Indeed, as mentioned above, since the insula also projects extensively to the motor-related regions such as the basal ganglia (Chikama et al., 1997), it may be the case that those emotional state changes which require rapid behavioral changes evoke activity changes in cortical and brainstem regions which have the ability to evoke cardiovascular change to support motor activity. In contrast, our emotional stimuli did not activate the cingulate and insular cortices but instead activated the orbitofrontal cortex (mutilation and SSNA) and the medial prefrontal cortices (erotica). These regions may be responsible for the subjective experience, whereas amygdala projections to the dorsolateral pons may mediate the associated changes in SSNA.

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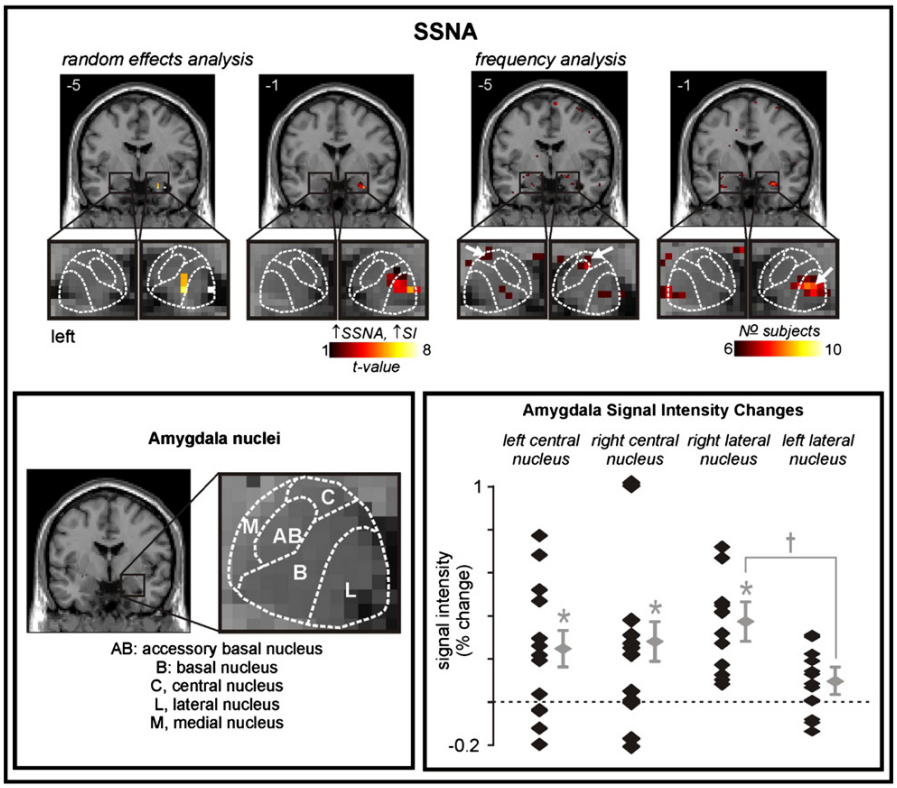


**Fig. 8.** Signal changes in the amygdala correlated to changes in skin sympathetic nerve activity (SSNA). Significant signal increases (hot color scale) assessed using a random effects analysis and a frequency analysis reveal signal increases in the right lateral amygdala and the left and right central amygdala nucleus. Slice locations in Montreal Neurological Institute space are indicated at the lower left of each image slice. The lower left panel shows the approximate nuclear subdivision of the amygdala. In the lower right panel, black triangles indicate the percentage change for each individual subject. The gray triangle represents the mean (±SEM) of all subjects. *$p<0.05$.

## References


Bandler, R., Keay, K.A., Floyd, N., Price, J., 2000. Central circuits mediating patterned autonomic activity during active vs. passive emotional coping. Brain Res. Bull. 53, 95–104.

Bellgowan, P.S., Helmstetter, F.J., 1996. Neural systems for the expression of hypoalgesia during nonassociative fear. Behav. Neurosci. 110, 727–736.

Bini, G., Hagbarth, K.E., Hynninen, P., Wallin, B.G., 1980. Thermoregulatory and rhythm-generating mechanisms governing the sudomotor and vasoconstrictor outflow in human cutaneous nerves. J. Physiol. 306, 537–552.

Blair, H.T., Schafe, G.E., Bauer, E.P., Rodrigues, S.M., LeDoux, J.E., 2001. Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. Learn. Mem. 8, 229–242.

Bradley, M.M., Miccoli, L., Escrig, M.A., Lang, P.J., 2008. The pupil as a measure of emotional arousal and autonomic activation. Psychophysiology 45, 602–607.

Breiter, H.C., Etcoff, N.L., Whalen, P.J., Kennedy, W.A., Rauch, S.L., Buckner, R.L., Strauss, M.M., Hyman, S.E., Rosen, B.R., 1996. Response and habituation of the human amygdala during visual processing of facial expression. Neuron 17, 875–887.

Carter, J.R., Durocher, J.J., Kern, R.P., 2008. Neural and cardiovascular responses to emotional stress in humans. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, R1898–R1903.

Chikama, M., McFarland, N.R., Amaral, D.G., Haber, S.N., 1997. Insular cortical projections to functional regions of the striatum correlate with cortical cytoarchitectonic organization in the primate. J. Neurosci. 17, 9686–9705.

Coghill, R.C., McHaffie, J.G., Yen, Y.F., 2003. Neural correlates of interindividual differences in the subjective experience of pain. Proc. Natl. Acad. Sci. U. S. A. 100, 8538–8542.

Craig, A.D., 2005. Forebrain emotional asymmetry: a neuroanatomical basis? Trends Cogn. Sci. 9, 566–571.

Critchley, H.D., Rotshtein, P., Nagai, Y., O'Doherty, J., Mathias, C.J., Dolan, R.J., 2005. Activity in the human brain predicting differential heart rate responses to emotional facial expressions. Neuroimage 24, 751–762.

Critchley, H.D., Nagai, Y., Gray, M.A., Mathias, C.J., 2011. Dissecting axes of autonomic control in humans: Insights from neuroimaging. Auton. Neurosci. 161, 34–42.

Davis, M., Shi, C., 2000. The amygdala. Curr. Biol. 10, R131.

Delius, W., Hagbarth, K.E., Hongell, A., Wallin, B.G., 1972. Manoeuvres affecting sympathetic outflow in human skin nerves. Acta Physiol. Scand. 84, 177–186.

Delius, W., Wallin, G., Hagbarth, K.E., 1973. Role of sympathetic nerve impulses in regulation of peripheral circulation. Scand. J. Clin. Lab. Invest. Suppl. 128, 47–50.

Ekman, P., Davidson, R., 1994. The Nature of Emotion. Oxford University Press, New York.

Ellsworth, P., 1991. Some implications of cognitive appraisal theories of emotion. In: Strongman, K.T. (Ed.), International Review of Studies on Emotion. Wiley, NewYork, pp. 143–161.

Frijda, N. (Ed.), 1986. The Emotions. Studies in Emotion and Social Interaction. Cambridge University Press, New York.

Friston, K.J., Holmes, A.P., Worsley, K.P., Proline, J.B., Frith, C.D., Frackowiak, R.S.J., 1995. Statistical parametric maps in functional imaging: a general imaging approach. Hum. Brain Mapp. 2, 189–210.

Gabbay, F.H., Krantz, D.S., Kop, W.J., Hedges, S.M., Klein, J., Gottdiener, J.S., Rozanski, A., 1996. Triggers of myocardial ischemia during daily life in patients with coronary artery disease: physical and mental activities, anger and smoking. J. Am. Coll. Cardiol. 27, 585–592.

Goldstein, D.S., Bentho, O., Park, M.Y., Sharabi, Y., 2011. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. Exp. Physiol. 96, 1255–1261.

Gray, M.A., Minati, L., Harrison, N.A., Gianaros, P.J., Napadow, V., Critchley, H.D., 2009. Physiological recordings: basic concepts and implementation during functional magnetic resonance imaging. Neuroimage 47, 1105–1115.

Hamann, S., Mao, H., 2002. Positive and negative emotional verbal stimuli elicit activity in the left amygdala. Neuroreport 13, 15–19.

Hare, R., Wood, K., Britain, S., Shadman, J., 1970. Autonomic responses to affective visual stimulation. Psychophysiology 7, 408–417.

Harrison, N.A., Gray, M.A., Critchley, H.D., 2009. Dynamic pupillary exchange engages brain regions encoding social salience. Soc. Neurosci. 4, 233–243.

Harrison, N.A., Gray, M.A., Gianaros, P.J., Critchley, H.D., 2010. The embodiment of emotional feelings in the brain. J. Neurosci. 30, 12878–12884.

Henderson, L.A., Gandevia, S.C., Macefield, V.G., 2007. Somatotopic organization of the processing of muscle and cutaneous pain in the left and right insula cortex: a single-trial fMRI study. Pain 128, 20–30.

Hopkins, D.A., Holstege, G., 1978. Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. Exp. Brain Res. 32, 529–547.

Kirno, K., Kunimoto, M., Lundin, S., Elam, M., Wallin, B.G., 1991. Can galvanic skin response be used as a quantitative estimate of sympathetic nerve activity in regional anesthesia? Anesth. Analg. 73, 138–142.

Kreibig, S.D., 2010. Autonomic nervous system activity in emotion: a review. Biol. Psychol. 84, 394–421.

Kubzansky, L.D., Kawachi, I., 2000. Going to the heart of the matter: do negative emotions cause coronary heart disease? J. Psychosom. Res. 48, 323–337.
Kunimoto, M., Kirno, K., Elam, M., Wallin, B.G., 1991. Neuroeffector characteristics of sweat glands in the human hand activated by regular neural stimuli. J. Physiol. 442, 391–411.
Kunimoto, M., Kirno, K., Elam, M., Karlsson, T., Wallin, B.G., 1992. Neuro-effector characteristics of sweat glands in the human hand activated by irregular stimuli. Acta Physiol. Scand. 146, 261–269.
Lacey, J.I., Lacey, B.C., 1970. Some autonomic-central nervous system interrelationships. In: Black, P. (Ed.), Physiological Correlates of Emotion. Academic Press, New York, pp. 205–227.
Lang, P., Bradley, M., Cuthbert, B., 1997. International Affective Picture System (IAPS): Technical Manual and Affective Ratings. NIMH Center for the Study of Emotion and Attention.
LeDoux, J.E., 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23, 155–184.
LeDoux, J.E., Iwata, J., Cicchetti, P., Reis, D.J., 1988. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J. Neurosci. 8, 2517–2529.
Low, A., Lang, P.J., Smith, J.C., Bradley, M.M., 2008. Both predator and prey: emotional arousal in threat and reward. Psychol. Sci. 19, 865–873.
Macefield, V.G., Henderson, L.A., 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, V.G., Wallin, B.G., 1996. The discharge behaviour of single sympathetic neurones supplying human sweat glands. J. Auton. Nerv. Syst. 61, 277–286.
Macefield, V.G., Sverrisdottir, Y.B., Elam, M., Harris, J., 2008. Firing properties of sudomotor neurones in hyperhidrosis and thermal sweating. Clin. Auton. Res. 18, 325–330.
Macey, P.M., Macey, K.E., Kumar, R., Harper, R.M., 2004. A method for removal of global effects from fMRI time series. Neuroimage 22, 360–366.
Malkani, S., Rosen, J.B., 2000. Specific induction of early growth response gene 1 in the lateral nucleus of the amygdala following contextual fear conditioning in rats. Neuroscience 97, 693–702.
Manuck, S.B., 1994. Cardiovascular reactivity in cardiovascular disease: “once more unto the breach”. Int. J. Behav. Med. 1, 4–31.
Mittleman, M.A., Maclure, M., Sherwood, J.B., Mulry, R.P., Tofler, G.H., Jacobs, S.C., Friedman, R., Benson, H., Muller, J.E., 1995. Triggering of acute myocardial infarction onset by episodes of anger. Determinants of Myocardial Infarction Onset Study Investigators. Circulation 92, 1720–1725.
Morris, J.S., Friston, K.J., Buchel, C., Frith, C.D., Young, A.W., Calder, A.J., Dolan, R.J., 1998. A neuromodulatory role for the human amygdala in processing emotional facial expressions. Brain 121 (Pt 1), 47–57.
Oppenheimer, S.M., Gelb, A., Girvin, J.P., Hachinski, V.C., 1992. Cardiovascular effects of human insular cortex stimulation. Neurology 42, 1727–1732.
Petrovich, G.D., Swanson, L.W., 1997. Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit. Brain Res. 763, 247–254.
Rainville, P., Bechara, A., Naqvi, N., Damasio, A.R., 2006. Basic emotions are associated with distinct patterns of cardiorespiratory activity. Int. J. Psychophysiol. 61, 5–18.
Rosen, J.B., Hitchcock, J.M., Sananes, C.B., Miserendino, M.J., Davis, M., 1991. A direct projection from the central nucleus of the amygdala to the acoustic startle pathway: anterograde and retrograde tracing studies. Behav. Neurosci. 105, 817–825.
Rozanski, A., Blumenthal, J.A., Kaplan, J., 1999. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 99, 2192–2217.
Sander, K., Brechmann, A., Scheich, H., 2003. Audition of laughing and crying leads to right amygdala activation in a low-noise fMRI setting. Brain Res. Brain Res. Protoc. 11, 81–91.
Schneider, F., Grodd, W., Weiss, U., Klose, U., Mayer, K.R., Nagele, T., Gur, R.C., 1997. Functional MRI reveals left amygdala activation during emotion. Psychiatry Res. 76, 75–82.
Schwaber, J.S., Kapp, B.S., Higgins, G.A., Rapp, P.R., 1982. Amygdaloid and basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. J. Neurosci. 2, 1424–1438.
Stephens, C.L., Christie, I.C., Friedman, B.H., 2010. Autonomic specificity of basic emotions: evidence from pattern classification and cluster analysis. Biol. Psychol. 84, 463–473.
Veening, J.G., Swanson, L.W., Sawchenko, P.E., 1984. The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: a combined retrograde transport-immunohistochemical study. Brain Res. 303, 337–357.
Walker, P., Carrive, P., 2003. Role of ventrolateral periaqueductal gray neurons in the behavioral and cardiovascular responses to contextual conditioned fear and poststress recovery. Neuroscience 116, 897–912.
Yoshimura, S., Ueda, K., Suzuki, S., Onoda, K., Okamoto, Y., Yamawaki, S., 2009. Self-referential processing of negative stimuli within the ventral anterior cingulate gyrus and right amygdala. Brain Cogn. 69, 218–225.