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## The face of negative affect: Trial-by-trial corrugator responses to negative pictures are positively associated with amygdala and negatively associated with ventromedial prefrontal cortex activity

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## Abstract

The ability to simultaneously acquire objective physiological measures of emotion concurrent with fMRI holds the promise to enhance our understanding of the biological bases of affect, and thus improve our knowledge of the neural circuitry underlying psychiatric disorders. However, the vast majority of neuroimaging studies to date examining emotion have not anchored the examination of emotion-responding circuitry to objective measures of emotional processing. To that end, we acquired electromyographic (EMG) activity of a valence-sensitive facial muscle involved in the frowning response (corrugator muscle) concurrent with fMRI while twenty-six human participants viewed negative and neutral images. Trial-by-trial increases in corrugator EMG activity to negative pictures were associated with greater amygdala activity, and a concurrent decrease in ventromedial prefrontal cortex activity. Thus, this study highlights the reciprocal relation between amygdalar and ventromedial prefrontal cortex in the encoding of emotional valence as reflected by facial expression.

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## Introduction

Darwin was among the first noted biologists to highlight the importance of facial expression as a read-out of emotion and underscore the continuity across species in its form and function (Darwin, 1872). This nonverbal form of communication plays an essential role in social and emotional functioning. While much has been published on the neural correlates of human emotion since the advent of neuroimaging, the relationship between activity in brain regions involved in emotion processing and objective measures of emotion, such as the production of spontaneous facial expressions, has not been examined. In humans, it is unknown whether the amygdala is solely involved in the perception of affect, or whether it also mediates emotional experience as revealed by facial displays of emotion. Thus, studies integrating neuroimaging with methods that simultaneously make inferences about facial expression are vital to map and understand how the brain coordinates (and incorporates) the varied programs accompanying emotion.

To investigate whether activity in emotion networks underlie the facial movements accompanying affect, it is necessary to measure the production of emotion simultaneous with the recording of neural activity following emotion provocation. Objective physiological measurements of emotion-responding systems can validate that emotion was induced, provide an on-line index of the magnitude of emotional responding, and enable the characterization of the neural systems with which it is associated. It is well agreed among emotion researchers that a bipolar dimension of affective experience ranging from pleasure to displeasure (Lang, Greenwald, Bradley, & Hamm, 1993) is observable in organisms with rudimentary nervous systems where behavior is present – typically called valence (Davidson, 1992). Additionally, emotion researchers consider the dimension of arousal, reflecting the intensity of the experienced emotion (Lang et al., 1993).

Of the various psychophysiological methods putatively providing objective measures of emotion (which include eye-blink startle, electrodermal activity, pupil dilation), measurement of facial muscle activity via electromyography (EMG) is the only current measure which is simultaneously valence specific, objective, continuous, unobtrusive, and has high temporal resolution (~ 30Hz) (for comparison, electrodermal activity is continuous, objective, and unobtrusive, but it is not valence specific, and has poorer temporal resolution). Specifically, the corrugator muscle on the face – that which spans above the eye-brows – is involved in frowning and has been shown to linearly covary with subjects' experience affect, increasing with negative affect, and attenuating with positive affect (Cacioppo, Petty, Losch, & Kim, 1986; Cacioppo & Tassinary, 1990; Larsen, Norris, & Cacioppo, 2003). Furthermore, an elegant series of studies by Peter Lang's group suggest that when viewing visual stimuli, facial EMG (most specifically corrugator EMG) corresponds uniquely to the *valence* of the stimulus, and not to the more general property of *arousal*. Arousal, on the other hand, is accounted for most strongly by self-reported ratings of arousal as well as skin conductance (Lang, 1995). Given the utility of facial EMG for emotion research generally, and for research on individual differences in valence-specific affective processing more specifically, we developed a method to simultaneously acquire fMRI BOLD and facial EMG (Heller, Greischar, Honor, Anderle, & Davidson, 2011). Understanding the neural circuitry involved in the encoding and processing of valence is

critical as pervasive mood disorders, such as depression, are characterized primarily by deficits in the valence, rather than in the arousal dimension of affective processing (Heller et al., 2009; Watson et al., 1995).

Therefore, to determine the neural correlates of a valence-sensitive facial expressive signal during emotional processing we examined whether trial-by-trial magnitude of corrugator EMG predicted activity in brain networks thought to be involved in affect. Given the large literature suggesting a role for the amygdala in emotion, we were particularly interested in whether activity in the amygdala was associated with corrugator EMG magnitude, as would be predicted given its previously described association with valence ratings of emotional stimuli (e.g., Anders, Lotze, Erb, Grodd, & Birbaumer, 2004).

## Methods

Twenty-eight subjects (15 female, mean age: 21.8), recruited from the Madison, WI area participated in the study. This sample included the subset of 16 participants included in our original study (Heller et al., 2011). Two participants' fMRI data were excluded due to excessive motion (defined as several large spikes (> 2 mm) or severe drift (> 2.5 mm)), leaving 26 participants for fMRI analyses. All subjects were recruited via the use of flyers posted in public places around the Madison, WI area. Subjects reported no current Axis I disorder and were not currently taking any psychotropic medication. This research was approved by the University of Wisconsin-Madison Health Sciences Institutional Review Board, and all participants provided informed consent. Subjects passively viewed a set of 200 standardized pictures randomly presented during scanning (Lang PJ, 2005). Half of the pictures were “negative” and have been reliably shown to induce negative affect (Larsen et al., 2003); the other 100 pictures were “neutral” and reliably induce little or no affect. Images were chosen based on the normative ratings, where negative images had significantly lower valence ratings (mean=2.39, sd=0.72), than neutral pictures (mean=5.46, sd=0.35;  $t(198)=38.55$ ,  $p<.001$ ). Pictures were presented for 4 seconds, followed by an 8 second fixed inter-trial interval. Upon stimulus onset, subjects made a two-button forced choice response indicating whether the image was negative or neutral.

During scanning, facial EMG was recorded from the corrugator supercillii muscle using 4 mm electrodes. Corrugator EMG data were recorded using a Biopac MP150 recording system and EMG100C electromyogram amplifier with MECMRI cable and filter components for MRI installation. EL254RT Ag-AgCl radio translucent electrodes were applied to the corrugator muscle separated by ~1 cm using adhesive collars and electrolyte gel. To minimize wire movement due to scanner noise and motion, leads were affixed to a foam tube exiting the bore. Grounding was provided by EDA sensors located on the index and middle fingers. EMG amplifier gain was 1000 with 1 Hz highpass and 500 Hz lowpass filtering. Sampling rate was 1000 Hz with a TTL pulse from the scanner recorded on one channel for precise timing of the start of each TR. Biopac EMG data were read into a Matlab program for hand scoring of data between TRs (TR intervals were automatically scored as bad using the TTL pulse channel from the scanner). Each run was divided into 1 sec intervals and power spectral density PSD for each interval computed using Welch's method on 0.1 sec windows with 50% overlap. A threshold of  $15\text{microV}^2/\text{Hz}$  was used to eliminate

any 1 sec intervals exceeding this value. Corrugator EMG was estimated as the mean power in the 45–200 Hz band excluding 60 Hz (i.e. 45–48; 62–200 Hz). Linear interpolation was used to estimate picture epoch (–1 to +12sec) 1 sec values from the 1 sec recording intervals with a maximum of 0.5 sec for good recorded intervals. Corrugator EMG values were log<sub>10</sub> transformed for normalization and are expressed in log<sub>10</sub> (uV<sup>2</sup>/Hz) units (For more detail, see: Heller et al., 2011). As stated above, corrugator EMG data were not analyzed for the 4 consecutive seconds of picture presentation. This was because we found that as the scanner acquired EPI data (1.5 out of every 2.5 s), the EMG signal was overwhelmed by the electromagnetic noise induced by the collection of EPI images. Thus, 1.5 out of every 2.5 s was automatically scored as bad (during collection of EPIs) and removed from further analysis. However, we were able to reconstruct an average time course of EMG signal during negative and neutral trials because trial onset was jittered with respect to EPI onset. For example, on some trials, the scanner acquired EPI data for the initial 1.5 s, and then from 2.5 to 4 s, leaving corrugator EMG to be analyzed at 1.5 to 2.5 s. On other trials, EPI data would have been acquired from –0.5 to 1 s and 2 to 3.5 s and corrugator EMG would be analyzed from 1 to 2 s and 3.5 to 4 s. On other trials, EPI data would be acquired from seconds 1–2.5 and from 3.5 to the end of the trial, allowing corrugator EMG data to be analyzed from 0 to 1 s and 2.5 to 3.5 s. The average number of seconds corrugator EMG was analyzed per trial was 1.67 s, with a range of 1–2 s. Again, this was possible because trial presentation was jittered randomly with respect to TR onset. By averaging corrugator EMG magnitude across trials (but within condition), the jittering of EPI data acquisition with respect to trial onset allowed us to reconstruct the time course of corrugator EMG activity for negative and neutral trials.

One drawback with this approach (for a trial-by-trial regression with EMG data) is that each trial contains EMG data corresponding to distinct portions of the trial. However this approach is necessary to reconstruct a full timecourse. Because it was paramount to us to demonstrate a significant main effect of condition on corrugator EMG we designed the experiment to have this approach. It should be emphasized, however, that each trial used in the amplitude modulation analysis contained valid EMG data from some portion of that trial.

To assess whether there was a significant main effect of valence on corrugator EMG activity, the mean EMG magnitude across the 4s image presentation was calculated in response to both negative and neutral stimuli and a paired t-test was computed. As stated above, the EMG signal used for trial-by-trial analyses encompasses the epoch within the 4s stimulus presentation during which the scanner acquisition was off.

FMRI data were collected using a “bunched slice acquisition sequence” in which each EPI volume was collected in 1.5 sec, while the effective TR was 2.5 sec (with 1 second of silent time between volumes). The 1 sec silent time between volumes was used as the time window to capture the EMG signal for analysis (see below). 30 slices were collected, with a native resolution of [3.75 × 3.75 × 5 mm]; TE=25, flip angle=60. Given the sluggishness of the BOLD response, it was hypothesized that the 1.0 sec of quiet time would not disrupt our ability to detect regional brain activity. Data were slice-time corrected and motion corrected in AFNI (Cox, 1996). A study specific template with a resolution of 1mm<sup>3</sup> was created using ANTS (Avants & Gee, 2004) and EPI data were normalized to that template using

FSL's linear normalization algorithm FLIRT followed by ANTS' nonlinear normalization routine (Avants & Gee, 2004) and smoothed (5mm FWHM). Individual subject GLMs were performed in FSL using the canonical double gamma HRF in FSL (Smith et al., 2004; Woolrich et al., 2009). Due to several previous reports demonstrating that the amygdala and other regions robustly distinguish between negative and neutral stimuli, we separated negative and neutral trials in individual subject's GLMs. Thus any associations between trial-by-trial EMG magnitude with fMRI BOLD would not be simply due to a main effect of valence. Using FSL's 3-level approach, a separate GLM was performed for each run of EPI data. A fixed effects GLM was then performed on all 8 runs for each subject, which was used in group analyses. Random effects GLMs were used to examine which brain areas showed a significant association with trial-by-trial corrugator EMG magnitude in response to each negative and neutral stimuli.

To examine whether trials with greater EMG activity predicted greater amygdala activity, we applied an a priori, anatomically defined bilateral amygdala region of interest (ROI) based on the Harvard-Oxford Atlas in which there was a minimum of a 50% probability that the voxel was indeed within the amygdala (Desikan et al., 2006). Correction for multiple comparisons in this restricted search as well an exploratory search across the whole brain to assess whether activity in additional brain regions was related to changes in corrugator EMG following emotional processing was performed by using Gaussian random field theory at the cluster level,  $Z > 2.3$ ,  $p < .05$ .

## Results

### EMG

To confirm our previous findings that we were able to acquire EMG in the scanner with sufficient quality to capture its well-described modulation by valence (Lang et al., 1993), we examined whether corrugator EMG was greater during negative as compared with neutral trials. As predicted, there was a significant main effect of valence across the 4s image presentation such that there was greater corrugator EMG in response to negative stimuli during the image as compared with neutral stimuli ( $t(27) = 2.21$ ,  $p = 0.04$ ; Figure 1).

### fMRI: Main Effect of Negative vs. Neutral Condition

A direct comparison of negative vs. neutral revealed a very robust network of regions including the bilateral amygdala, visual cortex, anterior Insula/inferior frontal gyrus (bilateral), thalamus and brainstem. Notable areas showing suprathreshold activity in the neutral vs. negative contrast include: inferior parietal lobule (bilateral) and orbitofrontal cortex (bilateral). Because this analysis revealed such a robust network, the threshold was set at  $t > 4.58$ ,  $p < .0001$  for the figure (Figure 2) and table (Table 1). Below, we have included a few selected images to illustrate this network. We have included this figure in the manuscript as well as a table describing the suprathreshold regions.

**EMG & fMRI: Amygdala ROI**—In the negative picture trials, greater corrugator EMG activity on a trial by trial basis predicted greater left amygdala activity (small volume corrected – see Methods;  $[x,y,z] = -17, -3, -23$ ; mean across significant, suprathreshold left

amygdala cluster:  $t(25)= 2.62, p=0.01$ ; Figure 3, Table 2). Across this same suprathreshold amygdala cluster, changes in corrugator activity following the presentation of neutral pictures were not a significant predictor of amygdala activity ( $t(25)=0.80, z=0.78, p=0.43$ ). Suggesting that the positive relationship between amygdala activity and corrugator EMG may be specific to the context of negative stimuli, the difference in correlations between amygdala activity and corrugator magnitude during negative trials compared with neutral trials was marginally significant ( $z=1.65, p=0.049$  [one-tailed]).

**EMG & fMRI: Whole-brain analysis**—Across the whole brain (i.e., corrected for multiple comparison conducted at the whole-brain level), trials with greater corrugator EMG activity in response to negative stimuli predicted lower levels of vmPFC (max  $[x,y,z]=14, 52, -19$ ; mean across cluster:  $t(25)= -3.05, p<.001$ ) and greater levels visual cortex activity (max  $[x,y,z]=43, -76, 4$ ; mean across cluster:  $t(25)= 2.77, p<.001$ ; Figure 4, Table 2). During the presentation of neutral stimuli, corrugator activity did not significantly predict activity in the vmPFC cluster on a trial-by-trial basis ( $z\text{-stat}=-0.46, p=0.64$ ) and the relationship between corrugator activity and vmPFC activity was more significant on negative trials than on neutral trials ( $z=2.32, p=.02$ ). In the visual cortex cluster, corrugator activity during neutral stimuli marginally predicted activity on a trial-by-trial basis (mean  $z\text{-stat}=1.81, p=.07$ ).

## Discussion

In this study, twenty-six individuals watched negative and neutral pictures while we simultaneously acquired functional neuroimaging data and EMG recordings of the corrugator supercilii, a valence-sensitive facial muscle group. By examining the neural correlates of trial-by-trial fluctuations in corrugator activity for each participant, we found that negative-picture-induced increases in corrugator activity were associated with concurrent increases in amygdalar activation. Conversely, *decreases* in corrugator EMG activity were instead associated with greater engagement of the vmPFC during negative-picture viewing. Thus, this study provides compelling evidence that the experience of negative emotion as reflected by potentiation of the corrugator muscle is reliably associated with activation of the amygdala and deactivation of the vmPFC.

It is well established that the activity of the corrugator supercilii muscle, used for frowning, covaries linearly and inversely with the valence of self-reported emotion (Lang et al., 1993). Specifically, EMG activity of the corrugator muscle is potentiated by negative emotion provoked by pictures (Lang et al., 1993) and sounds (Larsen et al., 2003), and attenuated by positive stimuli (Larsen et al., 2003; Neta, Norris, & Whalen, 2009; Rymarczyk, Biele, Grabowska, & Majczynski, 2011). Importantly, when used to index changes in emotional responses following the instruction to regulate negative-picture induced emotion, corrugator EMG activity shows high test-retest reliability (Lee, Shackman, Jackson, & Davidson, 2009), which compares favorably to another valence-sensitive measure previously used to index affective changes in a functional neuroimaging study, the acoustic startle probe (Anders et al., 2004). Thus, corrugator EMG recordings constitute an adequate valence-sensitive measure for emotion researchers who seek to unobtrusively validate that affective changes are successfully induced in emotion-elicitation paradigms conducted in the

laboratory. Despite its utility, only recently has this measure been successfully incorporated during functional neuroimaging recordings in emotion processing (Heller et al., 2011) and facial mimicry paradigms (Likowski et al., 2012). By taking advantage of a bunched slice acquisition sequence developed in our laboratory, we were able to examine how moment-to-moment increases and decreases in negative affect (as manifested by modulation of the corrugator supercilii muscle) were associated with corresponding changes in neural activity.

While this study demonstrates a correlation between amygdala activity and facial movements, it does not provide causal evidence for the amygdala subsequently effecting facial movements. However, a prior study examining the relationship between activity in emotion related networks and facial expression production utilized intracranial stimulation of the amygdala in individuals with partial epilepsy and found increased corrugator EMG activity (on approximately 50% of the trials), skin conductance as well as ratings of fear and anxiety (Lanteaume et al., 2007) following amygdalar stimulation. However, in that previous study, the use of a patient population and lack of an external emotional stimulus (i.e., solely used intracranial stimulation) limited the generalizability of that initial finding. Our results corroborate and extend the intracranial work by demonstrating similar findings in a non-patient sample and using external stimuli. Taken together, this suggests that the amygdala plays an important role in facial expressions of negative affect.

The finding that increases in negative affect (as reflected by corrugator activity potentiation) predicted increased amygdalar activation to negative pictures adds to an extensive literature previously documenting a role for the amygdala in the appraisal of emotionally significant stimuli. Indeed, increases in amygdala activation have been consistently found during the processing of negative facial expressions, negative pictures, and aversively conditioned stimuli [for a meta-analysis, see (Costafreda, Brammer, David, & Fu, 2008)]. As would be predicted by the well-described descending projections from the amygdala to modulators of the sympathetic nervous system, [reviewed in (LeDoux, 2012)], the amygdala modulates emotional responding, which is reflected in trial-by-trial covariation of amygdalar activity and sympathetic nervous system measures such as pupil dilation (Dubé et al., 2009; Phelps et al., 2001; Williams et al., 2007; Wood, Ver Hoef, & Knight, 2012). However, the role of the amygdala in specifically mediating affectively *valenced* processes has been less studied [for an exception, see (Anders et al., 2004)], in part due to the challenge that it is to ascertain that a valenced process took place given the scarcity of reliable, valence-sensitive measures. Here, by recording a reliable and well-validated valence-sensitive output channel, corrugator EMG, we showed that increases in the activity of this muscle group reliably correlated with engagement of the amygdala, thus providing critical evidence for an amygdalar role in decoding and producing appropriate responses to an aversive stimulus. Future work, however, should ideally also include a positively valenced condition. As the amygdala has been shown to respond to both negative and positive stimuli (e.g., Small et al., 2003), work integrating objective facial markers of affective responding with fMRI will be helpful in disambiguating the role of the amygdala in affective processing.

The vmPFC has figured prominently in studies of automatic and voluntary emotional regulation, as this region is a key source of descending inhibitory projections to the amygdala [for a review, see (M. J. Kim et al., 2011)]. Accordingly, activity of the vmPFC

typically increases when individuals learn that a cue previously associated with a shock signals *safety* (i.e., extinction) (Delgado, Nearing, Ledoux, & Phelps, 2008), and when individuals retrieve such an extinction memory (Milad et al., 2007). Accordingly, greater vmPFC thickness is associated with greater retrieval of the extinction memory (Milad et al., 2005). Moreover, the vmPFC role in potentially producing amygdalar inhibition and positively valenced interpretations of a cue is not limited to contexts such as fear conditioning: When confronted with emotionally ambiguous stimuli such as surprised faces, individuals who interpret them more positively show increased vmPFC activation to them, with concomitant decreases in amygdalar activation (H. Kim, Somerville, Johnstone, Alexander, & Whalen, 2003). Extending this amygdalar-inhibitory and appraisal-modifying role of vmPFC to situations when individuals *deliberately* attenuate their emotional responses, vmPFC activation increases following cognitive reappraisal to reinterpret a negative visual stimulus as less threatening (Delgado et al., 2008), which in turn predicts decreased amygdalar activation (Urry et al., 2006). Collectively, these studies suggest that the engagement of the amygdala and vmPFC may result in opposing appraisals of emotionally provocative stimuli, with vmPFC function biasing towards a positively valenced evaluation [presumably via amygdalar downregulation; (M. J. Kim et al., 2011)]. Of note, a recent account for the role of vmPFC is that of processing conceptual information of affective stimuli and generating affective meaning. (Roy, Shohamy, & Wager, 2012). The authors suggest that this is partially determined by information stored in long-term memory and predictions about the self in that particular context. Therefore, one possible mechanistic explanation for why there was a significant amygdala EMG association in response to negative but not neutral stimuli is that the vmPFC may be assessing the potential impact of a stimulus (e.g., affective and potentially threatening or not) at a conceptual level, and subsequently serving to bias downstream limbic activity and guiding facial responses. One potential example of such a phenomenon may be in that of affect labeling whereby “putting feelings into words” diminishes amygdala and increases ventral PFC activity (Lieberman et al., 2007).

In our study, increases in visual cortical activity correlated with increased corrugator EMG to negative pictures. Modulation of visual cortical regions following negative visual stimulus processing have been consistently reported in neuroimaging and electrophysiological studies (Bradley et al., 2003; Pizzagalli et al., 2002; Sabatinelli, Bradley, Fitzsimmons, & Lang, 2005). Mechanistically, recent findings in humans are consistent with feedback projections from the amygdala to visual cortex (Furl, Henson, Friston, & Calder, 2013; Sabatinelli, Lang, Bradley, Costa, & Keil, 2009; Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004), projections which are well described in tracing studies in monkeys (Amaral, Behniea, & Kelly, 2003). Thus, our findings are consistent with these data.

A long debated question in the field of facial expressions concerns what facial movements actually represent – whether they are an external reflection of the internal emotional experience (Ekman, 1993) or their function is merely in the service of social communication of affect (Fridlund, 1994). Although there are empirical data supporting both views, facial EMG studies in general provide support for the former (Larsen et al., 2003) theory and high

intra-subjects correlations between facial displays and subjective experience are well documented (Mauss, Levenson, McCarter, Wilhelm, & Gross, 2005). Moreover, the fact that facial EMG responses can be detected in response to non-conscious, masked emotional stimuli (Dimberg, Thunberg, & Elmehed, 2000) undermines the view that facial expressions primarily serve a voluntary explicit communicative function. In our study, participants were lying in the bore of the scanner and simply viewing pictures, a context far removed from one of social communication. The fact that facial expressive signals were associated with neural circuits implicated in emotion and emotion regulation suggests that the expressive signals convey information about internal emotional experience.

Simultaneous acquisition of peripheral-physiological data and functional neuroimaging is a critical endeavor to reveal the neurobiology underlying emotional experience. Although the import of self-reported affect in emotion paradigms should not be minimized, repeatedly probing subjects for how they feel during emotion-induction experiments engages additional reflective processes that impact the ongoing emotional experience (Kassam & Mendes, 2013). As mentioned in the introduction, facial EMG recordings are a particularly well-suited measure to probe the neurobiology of affective processes as they can be valence specific (such as the corrugator and zygomaticus muscles), and can be collected unobtrusively and continuously. Therefore, future studies on the neural correlates of affective processing, including its temporal dynamics and regulation, will greatly benefit from concurrent acquisition of fMRI and facial EMG data.

In conclusion, intra-individual increases in negative affect as indexed by a valence-sensitive facial muscle group (corrugator supercillii) are positively associated with amygdalar activation and inversely associated with vmPFC activation.

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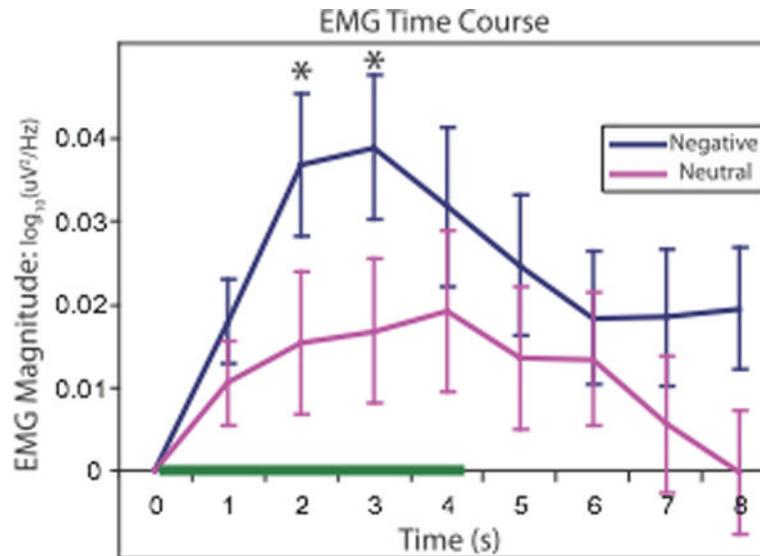
## References

- Amaral DG, Behniea H, Kelly JL. Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey. *Neuroscience*. 2003; 118(4):1099–1120. [PubMed: 12732254]
- Anders S, Lotze M, Erb M, Grodd W, Birbaumer N. Brain activity underlying emotional valence and arousal: a response-related fMRI study. *Hum Brain Mapp*. 2004; 23(4):200–9. doi:10.1002/hbm.20048. [PubMed: 15449355]
- Avants B, Gee JC. Geodesic estimation for large deformation anatomical shape averaging and interpolation. *NeuroImage*. 2004; 23(Suppl 1):S139–150. doi:10.1016/j.neuroimage.2004.07.010. [PubMed: 15501083]

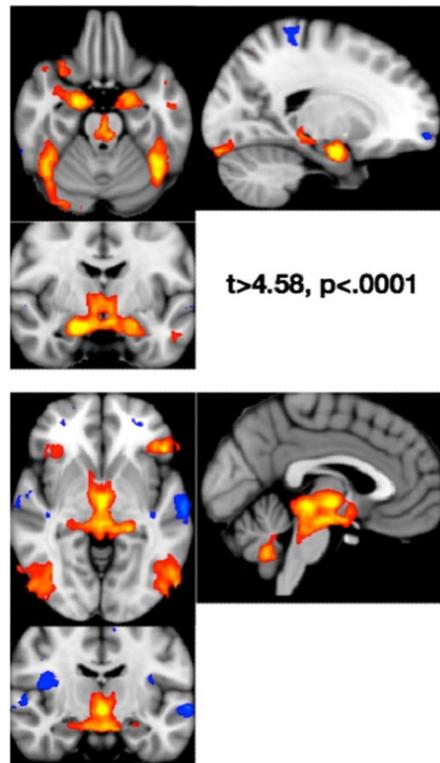
- Bradley MM, Sabatinelli D, Lang PJ, Fitzsimmons JR, King W, Desai P. Activation of the visual cortex in motivated attention. *Behavioral Neuroscience*. 2003; 117(2):369–380. [PubMed: 12708533]
- Cacioppo JT, Petty RE, Losch ME, Kim HS. Electromyographic activity over facial muscle regions can differentiate the valence and intensity of affective reactions. *J Pers Soc Psychol*. 1986; 50(2): 260–8. [PubMed: 3701577]
- Cacioppo JT, Tassinary LG. Inferring psychological significance from physiological signals. *Am Psychol*. 1990; 45(1):16–28. [PubMed: 2297166]
- Costafreda SG, Brammer MJ, David AS, Fu CHY. Predictors of amygdala activation during the processing of emotional stimuli: a meta-analysis of 385 PET and fMRI studies. *Brain Research Reviews*. 2008; 58(1):57–70. doi:10.1016/j.brainresrev.2007.10.012. [PubMed: 18076995]
- Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research, an International Journal*. 1996; 29(3):162–173.
- Darwin, C. *The Expression of the Emotions in Man and Animals*. Oxford University Press; Oxford, UK: 1872.
- Davidson RJ. Anterior cerebral asymmetry and the nature of emotion. *Brain and Cognition*. 1992; 20(1):125–151. [PubMed: 1389117]
- Delgado MR, Nearing KI, Ledoux JE, Phelps EA. Neural circuitry underlying the regulation of conditioned fear and its relation to extinction. *Neuron*. 2008; 59(5):829–38. doi:S0896-6273(08)00579-5 [pii] 10.1016/j.neuron.2008.06.029. [PubMed: 18786365]
- Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Killiany RJ. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage*. 2006; 31(3):968–980. doi:10.1016/j.neuroimage.2006.01.021. [PubMed: 16530430]
- Dimberg U, Thunberg M, Elmehed K. Unconscious facial reactions to emotional facial expressions. *Psychol Sci*. 2000; 11(1):86–9. [PubMed: 11228851]
- Dubé A-A, Duquette M, Roy M, Lepore F, Duncan G, Rainville P. Brain activity associated with the electrodermal reactivity to acute heat pain. *NeuroImage*. 2009; 45(1):169–180. doi:10.1016/j.neuroimage.2008.10.024. [PubMed: 19027077]
- Ekman P. Facial expression and emotion. *American Psychologist*. 1993; 48(4):384. [PubMed: 8512154]
- Fridlund, AJ. *Human facial expression: An evolutionary view*. Academic; San Diego, CA: 1994.
- Furl N, Henson RN, Friston KJ, Calder AJ. Top-down control of visual responses to fear by the amygdala. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2013; 33(44):17435–17443. doi:10.1523/JNEUROSCI.2992-13.2013. [PubMed: 24174677]
- Heller AS, Greischar LL, Honor A, Anderle MJ, Davidson RJ. Simultaneous acquisition of corrugator electromyography and functional magnetic resonance imaging: a new method for objectively measuring affect and neural activity concurrently. *NeuroImage*. 2011; 58(3):930–934. doi: 10.1016/j.neuroimage.2011.06.057. [PubMed: 21742043]
- Heller AS, Johnstone T, Shackman AJ, Light SN, Peterson MJ, Kolden GG, Davidson RJ. Reduced capacity to sustain positive emotion in major depression reflects diminished maintenance of fronto-striatal brain activation. *Proc Natl Acad Sci U S A*. 2009; 106(52):22445–50. doi: 0910651106 [pii] 10.1073/pnas.0910651106. [PubMed: 20080793]
- Kassam KS, Mendes WB. The Effects of Measuring Emotion: Physiological Reactions to Emotional Situations Depend on whether Someone Is Asking. *PloS One*. 2013; 8(7):e64959. doi:10.1371/journal.pone.0064959. [PubMed: 23785407]
- Kim H, Somerville LH, Johnstone T, Alexander AL, Whalen PJ. Inverse amygdala and medial prefrontal cortex responses to surprised faces. *Neuroreport*. 2003; 14(18):2317–2322. doi: 10.1097/01.wnr.0000101520.44335.20. [PubMed: 14663183]
- Kim MJ, Loucks RA, Palmer AL, Brown AC, Solomon KM, Marchante AN, Whalen PJ. The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. *Behavioural Brain Research*. 2011; 223(2):403–410. doi:10.1016/j.bbr.2011.04.025. [PubMed: 21536077]

- Lang PJ. The emotion probe. *Studies of motivation and attention. The American Psychologist.* 1995; 50(5):372–385. [PubMed: 7762889]
- Lang PJ, Greenwald MK, Bradley MM, Hamm AO. Looking at pictures: affective, facial, visceral, and behavioral reactions. *Psychophysiology.* 1993; 30(3):261–273. [PubMed: 8497555]
- Lang PJ, BM. International affective picture system (IAPS): affective ratings of pictures and instruction manual. Technical Report A-6. University of Florida; Gainesville, FL: 2005.
- Lanteaume L, Khalifa S, Régis J, Marquis P, Chauvel P, Bartolomei F. Emotion induction after direct intracerebral stimulations of human amygdala. *Cerebral Cortex (New York, N.Y.):* 1991). 2007; 17(6):1307–1313. doi:10.1093/cercor/bhl041.
- Larsen JT, Norris CJ, Cacioppo JT. Effects of positive and negative affect on electromyographic activity over zygomaticus major and corrugator supercilii. *Psychophysiology.* 2003; 40(5):776–85. [PubMed: 14696731]
- LeDoux J. Rethinking the emotional brain. *Neuron.* 2012; 73(4):653–676. doi:10.1016/j.neuron.2012.02.004. [PubMed: 22365542]
- Lee H, Shackman AJ, Jackson DC, Davidson RJ. Test-retest reliability of voluntary emotion regulation. *Psychophysiology.* 2009; 46(4):874–879. doi:10.1111/j.1469-8986.2009.00830.x. [PubMed: 19473303]
- Lieberman MD, Eisenberger NI, Crockett MJ, Tom SM, Pfeifer JH, Way BM. Putting feelings into words: affect labeling disrupts amygdala activity in response to affective stimuli. *Psychological Science.* 2007; 18(5):421–428. doi:10.1111/j.1467-9280.2007.01916.x. [PubMed: 17576282]
- Likowski KU, Mühlberger A, Gerdes ABM, Wieser MJ, Pauli P, Weyers P. Facial mimicry and the mirror neuron system: simultaneous acquisition of facial electromyography and functional magnetic resonance imaging. *Frontiers in Human Neuroscience.* 2012; 6:214. doi:10.3389/fnhum.2012.00214. [PubMed: 22855675]
- Mauss IB, Levenson RW, McCarter L, Wilhelm FH, Gross JJ. The tie that binds? Coherence among emotion experience, behavior, and physiology. *Emotion (Washington, D.C.).* 2005; 5(2):175–190. doi:10.1037/1528-3542.5.2.175.
- Milad MR, Quinn BT, Pitman RK, Orr SP, Fischl B, Rauch SL. Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory. *Proceedings of the National Academy of Sciences of the United States of America.* 2005; 102(30):10706–10711. doi:10.1073/pnas.0502441102. [PubMed: 16024728]
- Milad MR, Wright CI, Orr SP, Pitman RK, Quirk GJ, Rauch SL. Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biological Psychiatry.* 2007; 62(5):446–454. doi:10.1016/j.biopsych.2006.10.011. [PubMed: 17217927]
- Neta M, Norris CJ, Whalen PJ. Corrugator muscle responses are associated with individual differences in positivity-negativity bias. *Emotion (Washington, D.C.).* 2009; 9(5):640–648. doi:10.1037/a0016819.
- Phelps EA, O'Connor KJ, Gatenby JC, Gore JC, Grillon C, Davis M. Activation of the left amygdala to a cognitive representation of fear. *Nature Neuroscience.* 2001; 4(4):437–441. doi:10.1038/86110.
- Pizzagalli DA, Lehmann D, Hendrick AM, REGARD M, Pascual-Marqui RD, Davidson RJ. Affective judgments of faces modulate early activity (approximately 160 ms) within the fusiform gyri. *NeuroImage.* 2002; 16(3 Pt 1):663–677. [PubMed: 12169251]
- Roy M, Shohamy D, Wager TD. Ventromedial prefrontal-subcortical systems and the generation of affective meaning. *Trends in Cognitive Sciences.* 2012; 16(3):147–156. doi:10.1016/j.tics.2012.01.005. [PubMed: 22310704]
- Rymarczyk K, Biele C, Grabowska A, Majczynski H. EMG activity in response to static and dynamic facial expressions. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology.* 2011; 79(2):330–333. doi:10.1016/j.ijpsycho.2010.11.001. [PubMed: 21074582]
- Sabatelli D, Bradley MM, Fitzsimmons JR, Lang PJ. Parallel amygdala and inferotemporal activation reflect emotional intensity and fear relevance. *NeuroImage.* 2005; 24(4):1265–1270. doi:10.1016/j.neuroimage.2004.12.015. [PubMed: 15670706]
- Sabatelli D, Lang PJ, Bradley MM, Costa VD, Keil A. The timing of emotional discrimination in human amygdala and ventral visual cortex. *The Journal of Neuroscience: The Official Journal of*

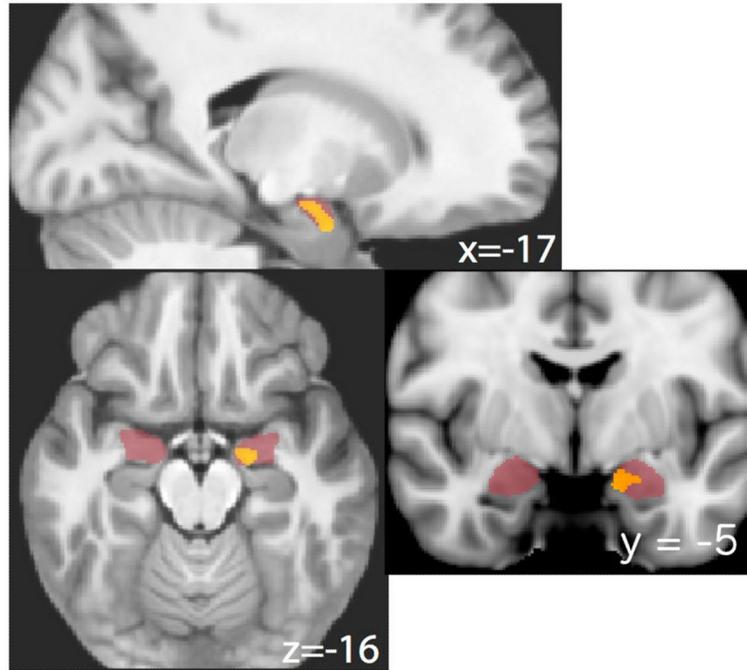
- the Society for Neuroscience. 2009; 29(47):14864–14868. doi:10.1523/JNEUROSCI.3278-09.2009. [PubMed: 19940182]
- Small DM, Gregory MD, Mak YE, Gitelman D, Mesulam MM, Parrish T. Dissociation of neural representation of intensity and affective valuation in human gustation. *Neuron*. 2003; 39(4):701–711. [PubMed: 12925283]
- Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*. 2004; 23:208–219.
- Urry HL, van Reekum CM, Johnstone T, Kalin NH, Thurow ME, Schaefer HS, Davidson RJ. Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*. 2006; 26(16):4415–4425. doi:10.1523/JNEUROSCI.3215-05.2006. [PubMed: 16624961]
- Vuilleumier P, Richardson MP, Armony JL, Driver J, Dolan RJ. Distant influences of amygdala lesion on visual cortical activation during emotional face processing. *Nature Neuroscience*. 2004; 7(11):1271–1278. doi:10.1038/nn1341.
- Watson D, Weber K, Assenheimer JS, Clark LA, Strauss ME, McCormick RA. Testing a tripartite model: I. Evaluating the convergent and discriminant validity of anxiety and depression symptom scales. *Journal of Abnormal Psychology*. 1995; 104(1):3–14. [PubMed: 7897050]
- Williams LM, Das P, Liddell BJ, Olivieri G, Peduto AS, David AS, Harris AWF. Fronto-limbic and autonomic disjunctions to negative emotion distinguish schizophrenia subtypes. *Psychiatry Research*. 2007; 155(1):29–44. doi:10.1016/j.psychres.2006.12.018. [PubMed: 17398080]
- Wood KH, Ver Hoef LW, Knight DC. Neural mechanisms underlying the conditioned diminution of the unconditioned fear response. *NeuroImage*. 2012; 60(1):787–799. doi:10.1016/j.neuroimage.2011.12.048. [PubMed: 22227141]
- Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, Smith SM. Bayesian analysis of neuroimaging data in FSL. *NeuroImage*. 2009; 45(1 Suppl):S173–186. doi:10.1016/j.neuroimage.2008.10.055. [PubMed: 19059349]



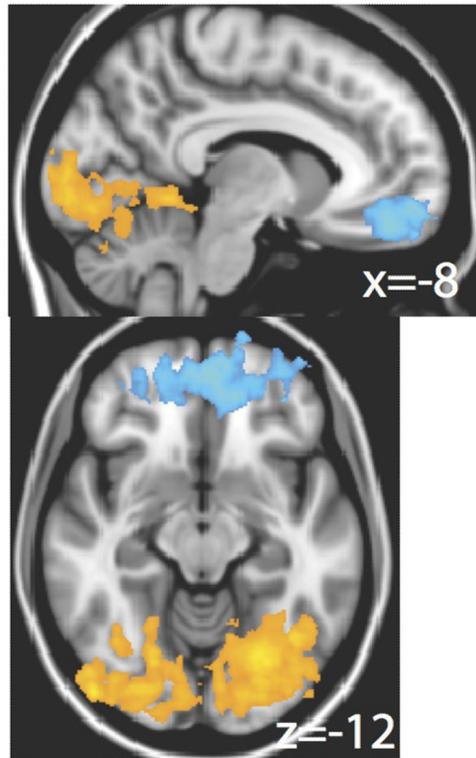
**Figure 1.** Corrugator EMG time course for scan session. There was a significant main effect of valence across the 4s image presentation such that there was greater corrugator EMG in response to negative stimuli during the image as compared with neutral stimuli ( $t(27) = 2.21$ ,  $p=0.04$ ). Further inspection of the timecourse (and only for descriptive purposes) revealed that this significant main effect was driven by differences at seconds 2 ( $t(27) = 2.58$ ,  $p=0.02$ ) and 3 ( $t(27) = 2.64$ ,  $p=0.01$ ). Asterisks indicate a significant main effect of valence at those timepoints. The green bar indicates duration of image presentation.



**Figure 2.**  
Main effect of Negative vs. Neutral contrast. Threshold is  $t > 4.58, p < .0001$ .



**Figure 3.** Significant activity within Amygdala ROI demonstrating a correlation between amplitude of amygdala activity and corrugator EMG magnitude on a trial-by-trial basis ( $p < .05$  small volume corrected). A priori ROI based on Amygdala structure presented in pink. The significant cluster within this small volume is presented in orange.



**Figure 4.** Significant activity across the whole brain demonstrating an inverse correlation between amplitude of vmPFC activity, and a positive correlation between amplitude of visual cortex activity, and corrugator EMG magnitude on a trial-by-trial basis ( $p < .05$  corrected).

**Table 1**

Results from main effect of the Negative vs. Neutral contrast. These results correspond to a threshold of  $t > 4.58$ ,  $p < .0001$ .

#Voxels	Peak-x	Peak-y	Peak-z	max-t	Location
34543	16	-4	-19	9.78	R Amygdala
23931	57	-61	6	9.2	R Middle Temporal Gyrus
20142	-41	-61	-24	9.62	L Cerebellum/Fusiform Gyrus
2940	-44	8	18	7.28	L Inferior Frontal Gyrus/Insula
2874	15	-30	65	-7.01	R Precentral Gyrus
2845	-48	28	-5	8.59	L Inferior Frontal Gyrus
2399	61	-28	10	-6.58	R Superior Temporal Gyrus
1976	-43	-65	48	-5.55	L Inferior Parietal Lobule
1893	-2	-58	-42	7.34	Cerebellum
1859	-63	-18	-6	-6.96	L Middle Temporal Gyrus
1771	45	-13	12	-5.91	R Insula
1660	-43	11	-36	6.02	L Middle Temporal Gyrus
1411	-8	-21	70	-5.89	L Precentral Gyrus
1249	68	-46	-17	-6.01	R Inferior Frontal Gyrus
736	-25	-92	-13	5.52	L Inferior Occipital Gyrus
705	46	6	27	6.61	R Inferior Frontal Gyrus
386	-22	-53	47	6.21	L Intraparietal Sulcus
219	29	-48	16	-5.13	R Optic Radiation
212	-40	-23	-3	-5.49	L Posterior Insula/Superior Temporal Sulcus
205	-10	-56	46	6.17	Precuneus
193	21	62	-8	-6.05	R Superior Frontal Gyrus
182	14	-38	11	-5.65	R Posterior Cingulate
180	12	-53	-52	4.92	Cerebellum
163	-35	-16	21	-5.1	L Insula

**Table 2**

Whole brain analysis & analysis with Amygdala ROI. Due to the large spatial extent of the clusters from the whole brain analysis, included several local maxima from the entire suprathreshold region.

<i>Region (BA)</i>	<i>x, y, z (mm)</i>	<i>Cluster size</i>	<i>Max z-value</i>
Visual cortex (19)	43, -76 -4	104,565	3.55
Local Maxima 1	43, -76 -4		3.55
Local Maxima 2	49, -79, -1		3.52
Local Maxima 3	49, -83, -4		3.51
Local Maxima 4	-39, -82, -2		3.5
Local Maxima 5	-18, -89, 17		3.46
Local Maxima 6	23, -67, -21		3.4
vmPFC (10/11)	14, 52, -19	23,419	-4.02
Local Maxima 1	14, 52, -19		-4.02
Local Maxima 2	-6, 56, -9		-4.01
Local Maxima 3	0, 53, -14		-3.95
Local Maxima 4	-13, 42, -16		-3.94
Local Maxima 5	-27, 52, -4		-3.92
Local Maxima 6	-32, 54, -8		-3.79
<i>Region</i>	<i>x, y, z (mm)</i>	<i>Cluster size</i>	<i>Max z-value</i>
L Amygdala	-17, -3, -23	341	2.69