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The Bivalent Side of the Nucleus Accumbens

Liat Levita^{1,*}, Todd A. Hare², Henning U. Voss³, Gary Glover⁴, Douglas J. Ballon³, and B.J. Casey¹

¹Sackler Institute for Developmental Psychobiology, Institute of Psychiatry, Weill Cornell Medical College, New York, NY

²California Institute of Technology, Division of the Humanities and Social Sciences Pasadena, CA

³Citigroup Biomedical Imaging Center, Weill Cornell Medical College, NY, NY

⁴Lucas Magnetic Resonance Image Center, Stanford University, Palo Alto, CA

Abstract

An increasing body of evidence suggests that the nucleus accumbens (NAcc) is engaged in both incentive reward processes and in adaptive responses to conditioned and unconditioned aversive stimuli. Yet, it has been argued that NAcc activation to aversive stimuli may be a consequence of the rewarding effects of their termination, i.e., relief. To address this question we used fMRI to delineate brain response to the onset and offset of unpleasant and pleasant auditory stimuli in the absence of learning or motor response. Increased NAcc activity was seen for the onset of both pleasant and unpleasant stimuli. Our results support the expanded bivalent view of NAcc function and call for expansion of current models of NAcc function that are solely focused on reward.

The role of the nucleus accumbens (NAcc) in behavior has tended to focus largely on responses toward rewarding and appetitive stimuli and events. However, based on evidence from both human neuroimaging studies and animal-based research a broader role for the NAcc function has been proposed in behavior modulated by aversive events. This imaging study has been designed to test the proposed bivalent function of the NAcc in behavior by addressing two arguments that have been raised against this idea: 1) that activation of the NAcc to aversive stimuli is secondary to some kind of relief as a result of the termination of that event; and 2) that it is a consequence of preparation and regulation of instrumental motor action.

The NAcc has been viewed as the key site for transference of motivational and other emotional signals received from the prefrontal cortex, amygdala and hippocampus to adaptive behavioral responses, and dopamine has been strongly implicated in facilitating this process (Laviolette, 2007; Meredith, 1999). However, this role has largely focused on rewarding appetitive processes (Day and Carelli, 2007), which has tended to overshadow work that has also demonstrated the involvement of the NAcc and the dopaminergic system in aversive emotional processes (Blackburn et al., 1992). For example, dopaminergic midbrain neurons increase firing in response to conditioned and unconditioned aversive stimuli (Guarraci and Kapp, 1999; Horvitz, 2000; but see, Ungless et al., 2004 and Schultz, 1997), as well as novel or unpredicted stimuli (Miller et al., 1981; Rasmussen et al., 1986). Moreover, while the abuse

*Corresponding author Liat Levita, The Sackler Institute for Developmental Psychobiology, Weill Cornell Medical College, 1300 York Ave, Box 140, New York, NY 10021, **Tel:** 212-746-5836, **Fax:** 212-746-5755, **Email:** lil2017@med.cornell.edu.

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potential of drugs such as amphetamine and cocaine is largely attributed to their rewarding actions, the effects of these drugs are not always hedonic, and there is evidence that their anxiogenic and psychomimetic effects (Mathias et al., 2008; Raven et al., 2000) might also be mediated by the NAcc and its dopaminergic innervation (Broderick et al., 2003; Hunt et al., 2005; Koob et al., 1989; Miczek et al., 1999).

Indeed, the function of the NAcc in gating and modulating goal-directed action (Cardinal et al., 2002) requires the detection of both safety and danger cues in the environment. Thus, an expanded, bivalent view of NAcc function has been advocated, whereby the NAcc is engaged in processing of both rewarding and aversive stimuli (Becerra et al., 2001; Jensen et al., 2003; Reynolds and Berridge, 2002). Consistent with the idea that the NAcc role in behavior is bivalent is that it is richly innervated, not only by the amygdala, which signals the salience of both positive and negative stimuli (Breiter et al., 1996; Demos et al., 2008; Hamann and Mao, 2002; Hamann et al., 2002; Paton et al., 2006), but also by other regions that process both aversive and reward information, such as the orbitofrontal cortex, insula, cingulate cortex, and the midline- and intra-laminar thalamic nuclei (Bourgeois et al., 2001; Haber et al., 2006; Hsu et al., 2000; Vogt, 2005). In turn the NAcc can affect expression of emotion via two routes: 1) It can influence motor action in response to emotive stimuli via its projections to the substantia nigra and ventromedial globus pallidus, which belong to the basal ganglia system involved in motor programming (Zahm and Heimer, 1993); and 2) It can induce significant changes in autonomic and physiological processes to these same stimuli, since it also projects to the lateral hypothalamus, which participates in the autonomic and endocrine expression of emotion (Kirouac and Ganguly, 1995).

The functional significance of the anatomical connectivity of the NAcc is reflected at the physiological level where different neurons in the NAcc can respond to either aversive or appetitive stimuli (Roitman et al., 2005; Setlow et al., 2003; Wheeler et al., 2008; Wilson and Bowman, 2005; Yanagimoto and Maeda, 2003). In addition, behavioral studies in both rodents and non-human primates have shown that the NAcc plays a critical role in aversive conditioning and active avoidance behavior (Ammassari-Teule et al., 2000; Hoebel et al., 2007; Iordanova et al., 2006; Levita et al., 2002; Schwienbacher et al., 2004), and an increasing number of human imaging studies have shown enhanced activity in this region in response to both conditioned and unconditioned aversive stimuli (Becerra et al., 2001; Gottfried et al., 2002; Jensen et al., 2003). Finally, in a manner similar to that previously demonstrated for the amygdala (Etkin et al., 2004; Stein et al., 2007; Straube et al., 2007), studies in non-human primates and humans have demonstrated that the positive association between anxiety levels and responses to aversive and anxiety-evoking stimuli is also modulated by the degree of NAcc activation (Kalin et al., 2005; Sturm et al., 2003).

A role for the NAcc in negative contexts is further supported by studies implicating the NAcc in contextual Pavlovian aversive conditioning (Haralambous and Westbrook, 1999; Levita et al., 2002; Westbrook et al., 1997), as well as conditioned inhibition of ongoing instrumental action (Parkinson et al., 1999). The latter is consistent with evidence implicating the NAcc in mediating the interaction between Pavlovian and instrumental contingencies (Hall et al., 2001). However, in contrast to the detrimental effect of lesions of the amygdala on fear learning (for review see, Maren, 2001; Phelps and LeDoux, 2005), some studies have failed to find an effect of lesions or pharmacological manipulations of the NAcc on discrete cue Pavlovian aversive conditioning (e.g., Levita et al., 2002; Westbrook et al., 1997). This finding is also mirrored in a number of human imaging studies failing to show NAcc activation in response to conditioned aversive stimuli (Chandrasekhar et al., 2008; Hamann and Mao, 2002; Phelps et al., 2004). Moreover, it could be argued that NAcc activity observed in anticipation of, or response to, negative events, is due to the rewarding effects of termination of an aversive event rather than a result of a response to the noxious stimuli (Ikemoto and Panksepp, 1999).

Additionally, since the NAcc influences instrumental behavior by allowing Pavlovian conditioned stimuli (CSs) to affect the level of instrumental responding (Cardinal et al., 2002), the engagement of the NAcc in some studies may reflect its role in modulating instrumental motor actions dissociable from emotion.

To address these two possibilities, we designed an fMRI study in which we could dissociate brain activation to the initiation and termination of unpleasant and pleasant auditory stimuli in the absence of learning or a motor response. Consequently, in this study subjects were required to passively listen to pleasant and unpleasant auditory stimuli that were randomly presented in a long-event related design while skin conductance response (SCR) was recorded. To dissociate activation related to onset versus offset, the duration of positively and negatively valenced auditory stimuli were jittered and regressors were created for onset and offset of the negative and positive stimuli. We predicted that NAcc activation would be observed for the initiation, but not termination of the unpleasant sounds, results that would be consistent with the idea of bivalent NAcc function.

Materials and Methods

Subjects

Twenty right-handed adults (10 male, 10 female; age: range 20-31, mean 25.7 ± 0.6 ; IQ = 116 ± 2.7) took part in the study. Subjects were free of any medical or neurological problems, and had no current or previous diagnosis of psychiatric or neurological disorder. All subjects gave informed consent in accordance with Weill Medical College of Cornell University IRB committee, and were paid for their participation.

Stimuli and Apparatus

Auditory stimuli consisting of two unpleasant (negative tones: n1, n2) and two pleasant (positive tones: p1, p2) tones were used in this study. These tones were presented for 2, 4, and 6 seconds. The auditory stimuli used were modified and generated using the digital audio editors: Audacity 1.2.6 (<http://audacity.sourceforge.net>) and PRAAT Version 4.5.08 (www.praat.org). The auditory stimuli generated were of 2 seconds duration and were looped to generate 4 and 6 second segments. Stimuli: n1, a combined 1000 Hz tone and white noise, which was intensity tiered for smooth onset and offset; n2, four bursts of a 1000 Hz square wave tone, duration 0.4s, and silence 0.1s; p1, a wind chime recording that was modified for a smooth rise and fall; p2, a second chime recording amplified and modified, like p1, for a smooth rise and fall. All stimuli were modified so they would have the same intensity (95 dB in scanner; Headphones; fMRI Devices Corporation, Waukesha, WI). These stimuli were chosen after a pilot study was run to select the most pleasant and unpleasant sounds from a selection of eight. In the pilot study a randomly mixed sequence of the eight sounds was presented three times to 11 subjects (age 26-36) who rated them individually on a 20-point unpleasantness–pleasantness scale. There was a significant difference in rating the pleasant and unpleasant sounds ($p < 0.001$). Average rating for the aversive sounds was 3.7 ± 0.33 and 17.1 ± 0.31 for pleasant sounds. From these eight sound stimuli the two sounds that were rated as most unpleasant and the two sounds that were rated as most pleasant were selected for the imaging study.

Skin Conductance Response

A skin conductance response (SCR) MRI compatible system (SCR100C Biopac, Goleta, CA) together with the AcqKnowledge (Biopac) software was used to monitor the SCR as it varied with the eccrine sweat gland activity. The computer running AcqKnowledge and the computer running E-prime (Psychology Software Tools, Inc, Pittsburgh, PA) were interfaced allowing generation of digital TTL timestamps for each stimulus on the Biopac channel recording, so

that stimuli presentations during scan were co-registered with SCR record. The SCR was sampled at 200 Hz using disposable electrodermal gel electrodes (Biopac model EL507) attached to the distal phalanx of the pointer and middle fingers of the left hand. The electrodes were connected to an MRI compatible cable set (MECMRI-TRANS) that interfaced with the SCR100C amplifier and the control panel. The SCR100C used a constant voltage (0.5 V) to measure skin conductance. The SCR was digitized at the electrodes and 1 Hz filter applied (Gain 2 $\mu\text{mho/V}$). Subjects were asked to wash their hands with water and dry them gently before the electrodes were attached. SCRs were analyzed by subtracting the peak skin conductance response occurring in a time window of 1-5 sec after stimulus onset from a baseline measure just prior to the stimulus onset. The small number of subjects which we successfully recorded SCR from ($n = 7$) precluded the inclusion of the SCR measures in our fMRI analysis.

Experimental Task

Subjects completed a passive listening task in which they heard pleasant and unpleasant sounds. Stimuli duration varied between 2, 4, and 6 seconds in order to deconvolve stimulus onset and offset BOLD responses. The interstimulus interval was 12 seconds (Figure 1A). The entire experiment consisted of 5 runs, each lasting 212 seconds. A total of 60 stimuli were presented, 30 negative and 30 positive sounds. The stimuli were presented in a pseudorandom order, with never more than two sounds of the same valence type following each other. Before the start of the experiment participants were told that they would hear sounds that were pleasant and unpleasant in nature and that no action was required on their part except to continue to pay attention to tones that would be presented. In addition, participants were instructed to close their eyes throughout the experiment, and were reminded of this at the beginning of each run. At the end of the experiment, while still in the scanner, subjects heard the auditory stimuli presented throughout the experiment, and rated their subjective experience of each tone on a 5-point scale (1 as most unpleasant and 5 as the most pleasant). The scanner was on during subjective rating so that the sounds would be experienced under the same conditions as during the experimental task. Subjects made their responses on a five button response glove. Stimuli and response collection (valence ratings of stimuli) were presented with the integrated functional imaging system (IFIS; PST, Pittsburgh) using an LCD video display in the bore of the MR scanner and a fiber optic response collection device. Self report ratings of state and trait anxiety were measured using the Spielberger's State-Trait Anxiety Inventory (Spielberger, 1983) administered following the scanning session.

Image Acquisition

Subjects were scanned with a General Electric Signa Excite 3.0 Tesla fMRI scanner (General Electric Medical Systems, Milwaukee, WI) with a quadrature head coil. Foam padding placed around the head was used to reduce motion. A whole brain, high resolution, T1 weighted anatomical scan (a 3D SPGR; 256×256 in-plane resolution, 240 mm field of view [FOV]; 124 1.5-mm axial slices) was acquired for each subject for transformation and localization of functional data into Talairach space (Talairach and Tournoux, 1988). A spiral in and out sequence (Glover and Thomason, 2004) was used to collect functional data (TR = 2000, TE = 30, FOV = 200 mm, Flip angle = 90 and 64×64 matrix). We obtained 29, 5 mm thick coronal slices with an in-plane resolution of 3.125×3.125 mm that covered the entire brain except for the posterior portion of occipital lobe.

Imaging data analysis

Functional imaging data were preprocessed and analyzed using the AFNI software package (Cox, 1996). The first 4 volumes (8 s) from each run were discarded to allow the scanner to reach magnetization equilibrium. Following slice time correction, images were registered to

the first image volume following the high-resolution anatomical dataset using rigid body transformations and smoothed using an isotropic 6 mm Gaussian kernel. Head motion was examined to confirm that all subjects had less than 2 mm of translation or 2° of rotational movement. Time series were normalized to percent signal change to allow comparisons across runs and individuals by dividing signal intensity at each time point by the mean intensity for that voxel and multiplying the result by 100. Four regressors were created for onset and offset of negative and positive sounds by convolving the stimulus timing files with a gamma-variant hemodynamic response function. Linear regression modeling was performed to fit the percent signal change time courses to each regressor. Linear and quadratic trends were modeled in each voxel time course to control for correlated drift. Motion parameters were included in the GLM as covariates of no interest. The resulting regression coefficients represent an estimate of percent signal change from the mean.

Group level analyses were conducted on the regression coefficients from the individual analysis after transformation into the standard coordinate space of Talairach and Tournoux (1988), using parameters obtained from the transformation of each subjects' high-resolution anatomical scan. Talairached transformed images had a re-sampled resolution of 3 cubic mm. Normalization to Talairach space was done using automatic talairach transformation in AFNI, where the anatomical volume was warped using 12-parameter affine transform to a template volume (TT_N27) in talairch space. An omnibus 2 (Valence; negative/positive) × 2 (Time; onset/offset) ANOVA that included subject as a random factor was conducted to determine the main effects of Valence, Time, and Valence × Time interaction. Correction for multiple comparisons was applied at the cluster level following Monte Carlo simulations conducted in the AlphaSim program within AFNI. Clusterwise false-positive rates of $p < 0.05$ corrected for multiple comparisons were determined for whole brain analyses. Additional simulations were restricted to the NAcc and amygdala based on the size of these regions unilaterally derived from the Talairach atlas included in the AFNI distribution (Volume: amygdala ~890 mm³; Nucleus accumbens ~1000 mm³). Whole brain simulations were conducted at individual voxel α probabilities set at 0.01, 0.001 and 0.0001 to allow identification of both broad and focal activations. Individual voxel α probabilities were set at 0.025 for simulations within the amygdala and NAcc. Only clusters with more than three voxels were considered for analysis.

For functional region of interest (ROI) analyses, anatomical masks of the NAcc and amygdala ROIs were defined from the Talairach atlas included in the AFNI software distribution. Voxels within these masks that showed activation above the threshold of $p < 0.025$ for the valence × time interaction at the group level were included in the functional ROI analysis. To address the concern that our group level functional ROI (defined by the average group level of activation) represented the same anatomical region in different participants we talairach transformed individual anatomical ROIs for the NAcc and amygdala generated with FreeSurfer to compare the individual FreeSurfer ROIs with the talairached group functional ROIs. Parcellation of the subcortical anatomy into regions of interest was performed using the FreeSurfer software suite (Fischl et al., 2004). These tools delineate anatomical divisions via automatic parcellation methods in which the statistical knowledge base derives from a training set incorporating the anatomical landmarks and conventions described by Duvernoy (1991). The resulting segmentation maps were viewed and the FreeSurfer derived-segmentation of regions of interest were evaluated and manually edited when found to be incorrect. We found that all subjects showed overlap within each of the two regions of interest, demonstrating that our group fROIs fit the individual subject anatomical data.

To examine the time course of activation, mean BOLD responses were plotted for selected clusters. To that end we applied functional masks of these clusters (based on the group level analysis) to extract individual time series averaged across voxels for each subject's fMRI time series. From these, percent signal change for each event was calculated relative to the mean of

the two TRs prior to stimulus onset in a time window of 0 to 12 seconds. We also analyzed our data using the mean as the baseline which did not appear to change any of the results. In addition, BOLD signal attenuation or enhancement with repeated presentations of the negative and positive stimuli was examined in individual subjects in two regions of interest, the NAcc and amygdala. In this analysis the peak hemodynamic response for each stimulus in each run was measured in the significant clusters observed after group analysis in the right NAcc and right amygdala. Mean of peak BOLD signal in early runs (1 & 2) and late runs (3 & 4) in these regions was calculated and a slope of best fit for early versus late trial peak response was generated. The gradient of the slope was taken as a measure of either habituation or sensitization to repeated presentation of stimuli across the experiment. Positive slopes are indicative of an increase in activation (sensitization) with time, whereas negative slopes are indicative of a decrease in activation (habituation) to the repeatedly presented stimuli.

Moreover, the interaction of the activity in the NAcc and amygdala in response to the onset of the negative and positive stimuli with the activity of other brain regions was characterized by performing a functional connectivity analysis. This analysis was computed in AFNI and performed by using the right amygdala functional ROI (derived from group analysis results valence \times time). Individual time series were extracted from the amygdala cluster for each subject's fMRI time series and averaged across voxels. Linear trend removal was first conducted on the entire time-series. Deconvolution was then run on the seed time series, and an interaction regressor created [deconvolved seed time series \times events of interest – the onset of the negative (ni) and positive (pi) stimuli]. Single subject GLM was then run exactly as for a regular analysis, but here, adding the two additional regressors of interest. In addition, all of the original regressors of interest (events) and no interest (motion parameters) were included to account for all sources of variability in the dataset. The resulting correlation coefficients for the interaction regressors were transformed to a normal distribution using Fisher's z transformation before group analyses on these values.

All Statistical analysis of the data was conducted in SPSS 15.0 (SPSS Inc. Chicago, IL).

Results

Validation of Stimulus Valence

At the end of the experimental session, while still in the scanner, subjects rated the auditory stimuli presented with respect to their positive and negative stimulus valence. There was a significant difference in valence ratings between the positive and negative stimuli presented (Figure 1B), where positive sounds were rated as pleasant and negative sounds were rated as unpleasant ($Z = -3.9$, $p \leq 0.001$). No gender differences were found in valence rating, nor did valence ratings differ with respect to the duration of the stimuli presentation (2, 4, or 6 sec).

Skin Conductance Response

Dissociation between the positive and negative sound stimuli was also found at the physiological level. Significantly greater skin conductance response (SCR) was observed to the negative versus the positive auditory stimuli (Figure 1C; $Z = -2.67$, $p = 0.008$). Measurement noise caused by the scanner environment prohibited reliable SCR in the majority of subjects tested, consequently the small number of subjects with robust SCR ($n = 7$) precluded the inclusion of the SCR measures in our fMRI analysis. No evidence was found of a SCR to the tone offset.

Imaging Results

Whole brain analysis—The fMRI data were analyzed using a generalized linear model (GLM) that evaluated BOLD responses to the initiation and termination of the auditory stimuli.

While this study was focused on NAcc activation, we first performed a whole-brain analysis to determine regions activated by negative versus positive stimuli at onset and offset using a 2 (Valence; negative versus positive) \times 2 (Time; onset versus offset) ANOVA. The complete list of brain regions showing main effects and interactions is given in Table 1 and in Supplementary Tables 1 and 2. Whole-brain contrast analysis probing the main effect of valence revealed greater activation to negative rather than positive auditory stimuli in the greater part of the striatal complex, as well as in the right amygdala (Table 2, and supplemental Figure 1). Moreover, whole brain contrast analysis for the main effect of time (stimulus offset minus onset) for the negative as well as the positive stimuli did not reveal any brain regions showing activation on termination of either of these stimuli (data not shown).

Valence \times time interaction (Figure 2) revealed greater activation for the negative auditory stimuli in the inferior frontal gyrus, cingulate cortex (Figure 2B), anterior and posterior insula (Figure 2C), globus pallidus, and cerebellum, all associated with greater relative deactivation on the offset of the negative stimulus. In contrast, the peak magnitude of onset BOLD activation was equivalent for both valence types in clusters observed in areas of the thalamus (Figure 2D) and the superior temporal gyrus (STG). However, these thalamic and STG activations were associated with greater relative deactivation on the offset of the negative stimulus.

Functional region of interest analysis—We conducted a functional region of interest (ROI) analysis on the NAcc to test our prior hypothesis regarding activation in response to positively and negatively valenced stimuli. Voxels within the NAcc that showed activation at the group level for the valence \times time interaction were included in this functional ROI. For this analysis, an anatomical mask of the NAcc was defined from the Talairach atlas included in the AFNI software distribution. Voxels within the NAcc were corrected for multiple comparisons at the $p < 0.05$ level using cluster thresholds determined by AlphaSim. The AlphaSim Monte Carlo simulations were run using an individual voxel threshold of $p < 0.025$ within an anatomical mask of the NAcc taken from the AFNI Talairach atlas.

Within the NAcc there was a main effect of time, whereby bilateral activation of the NAcc was observed in response to the onset of the positive and negative stimuli. No main effect of valence was observed. However, there was a significant interaction between valence and stimulus onset and offset in the right NAcc (Figure 3 A & B, Table 1), with significantly greater response to unpleasant than pleasant sound stimuli ($t = -3.61$ $p = 0.002$) at onset. Moreover, no evidence for activation of NAcc activity on offset of the positive or negative stimuli was found (Figure 3B). Further analysis of activation of the NAcc revealed a positive correlation between the mean BOLD activation to the onset of negative and positive stimuli, such that subjects who showed a high response magnitude to the positive stimuli, also showed a heightened response to the negative stimuli (Figure 3D; Pearson's $r = 0.881$; $p < 0.001$; $n = 20$). We also found that NAcc activation to the negative and positive stimuli remained largely constant throughout the experiment, as measured by comparing mean response magnitude during early, middle and late trials (Valence, $F_{1,38} = 2.71$; Time $F_{1,38} = 0.43$, Valence \times Time, $F_{1,38} = 0.001$; Supplemental Figure 2).

To examine regional specificity in response to aversive and pleasant stimuli, we investigated the response of the amygdala, a region implicated in affective processes and which sends significant projections to the NAcc. This analysis was performed in the same manner in which we conducted the functional ROI analysis for the NAcc. The amygdala functional ROI was defined by voxels showing a significant valence \times time interaction using small volume correction within an anatomical mask of the amygdala at $p < 0.05$. Within the amygdala there was a main effect of time as well as valence \times time interaction, but no main effect of valence. Post hoc t -tests on the main effect of time (onset vs. offset) revealed bilateral activation of the amygdala to both the negative and positive stimuli, as did valence \times time interaction (Figure

4, Table 1, and Supplementary Table 2). We also found sensitization of the amygdala, as indicated by increased amygdala activity to repeated presentations of the negative stimuli, but not positive stimuli. This increase was associated with self-ratings of anxiety, such that an increased amygdala activity with repeated presentations of the negative stimuli predicted greater state and trait anxiety in individual subjects (State; Pearson's $r = 0.674$; $p = 0.001$; Trait; Pearson's $r = 0.642$, $p = 0.003$, Figure 4C). No such association was found for the NAcc (State; Pearson's $r = 0.051$; Trait; Pearson's $r = -0.094$).

Based on anatomical data demonstrating NAcc-amygdala connectivity, we also conducted functional connectivity analysis with the right amygdala cluster set as the seed region (Figure 4A). We found a positive correlation between activity in the right amygdala and the right NAcc during the presentation of the negative, but not positive stimuli (Figure 4 D & E; and Supplemental Table 3).

Notably in this study auditory stimuli of different durations were presented to allow us to deconvolve stimulus onset versus offset. Plots of the hemodynamic response to the positive and negative stimuli of different durations revealed that only regions such as the superior temporal gyrus, which is involved in auditory perception, were sensitive to stimulus duration. In contrast, this was not the case in our regions of interest, the NAcc or amygdala, where the BOLD hemodynamic response was independent of stimulus duration (Figure 5, and Supplemental Figure 3).

Discussion

We found that the NAcc responds to the onset of both positive and negative stimuli. Onset and offset analysis of activation of the NAcc to pleasant and unpleasant sounds in a passive listening paradigm confirmed a direct activation of this region by aversive stimuli, rather than an effect secondary to some kind of relief, or a result of preparation and regulation of instrumental motor action. These results support the expanded view of NAcc function, whereby the NAcc plays a key role in modulating behavior to aversive and painful stimuli, and not just to stimuli that are rewarding in nature. Our findings are consistent with several studies that have reported striatal activity, including the NAcc, for primary and conditioned aversive stimuli (Blazquez et al., 2002; Ravel et al., 1999; Williams et al., 1993), as well as enhanced dopamine release in this region in response to similar events (Horvitz, 2002; Salamone et al., 2005). Moreover, consistent with the idea that the BOLD activations observed in this study did not reflect a simple sensory percept but rather valence, we found that neither the amygdala nor NAcc were sensitive to stimulus duration, unlike the superior temporal gyrus, a region involved in auditory perceptual processes (Pandya, 1995). Bivalent activation of the NAcc in this study is further supported by the clear dissociation in subjects' subjective valence rating of the positive and negative stimuli as being pleasant and unpleasant, respectively, which was also reflected in dissociable physiological response (SCR) to these stimuli. Nevertheless, it is possible that the NAcc was responding to the arousing or attention-grabbing quality of the stimuli presented rather than their valence, which would be consistent with studies that have suggested that the NAcc maybe responding to stimulus salience (Zink et al., 2006), or with other studies which find that both valence and salience are critical for NAcc activation (Cooper and Knutson, 2008). However, in this study, the responses we observe in the NAcc are not solely a reflection of stimuli's salience, since in auditory sensory areas as well as the thalamus we see equivalent activation to the positive and negative tones presented to the participants, suggesting matching of stimuli in terms of salience.

In this study we did not find activation of the NAcc on the offset of an aversive event. However, our negative result on offset of an aversive event needs to be interpreted with caution. It is

possible that subjects would never have felt relief on the offset of the aversive sound, since the scanner environment may in itself have been an unpleasant setting.

Notably, in this study we did not observe a dissociable temporal activation profile for NAcc activation in response to positive and negative primary auditory stimuli, as previously reported for conditioned aversive stimuli (Gottfried et al., 2002). Gottfried et al (2002) found that the NAcc showed significant activation to the aversively conditioned stimulus (CS+) early but not late in learning, the reverse being the case for the appetitive CS+. These temporal differences may relate to cue learning rather than unconditioned stimuli (US) responses as investigated in this study. They may also explain the failure to observe NAcc activation in some human imaging aversive conditioning studies (Chandrasekhar et al., 2008; Delgado et al., 2006; Hamann and Mao, 2002), since activation of the region in response to aversive CS+ may be masked if time is not a factor in the analysis.

Previous studies have suggested that the NAcc may play a role in the expression of anxiety (Kalin et al., 2005; Sturm et al., 2003). However, here we did not find an association between the rate of habituation of the NAcc to the negative auditory stimuli and subjects' self-rating of anxiety. Yet, consistent with previous studies (Etkin et al., 2004; Hare et al., 2008) the change in amygdala activity to the negative stimuli over time was associated with subjects' anxiety levels. The amygdala, specifically the basolateral nucleus, sends significant projections to the NAcc (Nauta, 1982) and hence if high anxiety levels enhance output from the amygdala, it might be that target sites like the NAcc would also show the same phenotype. Indeed, functional connectivity analysis revealed a positive coupling between the amygdala and the NAcc when subjects were exposed to the aversive, but not positive stimuli. However, while amygdala input can affect NAcc function (Cardinal et al., 2004; Setlow et al., 2002), other pathways to the NAcc can act to offset or change the degree by which the amygdala can drive this region (Everitt et al., 1999; Grace, 2000; Jackson and Moghaddam, 2001; Setlow et al., 2002). Moreover, a lack of correlation between levels of anxiety and NAcc activation may be a result of the passive nature of our task. Thus, while our task design enabled us to examine the response of the NAcc to emotive stimuli in the absence of possible confounds stemming from motor responses, it did not allow us to examine fully the functional significance of these activations in modulation of behavior. Tasks that involve instrumental approach-avoidance behavior may be more likely to demonstrate correlations between individual anxiety levels and both task performance and the degree of brain activation in the NAcc.

In this study, the amygdala, like the NAcc, showed a bivalent pattern of activation, consistent with a large body of evidence demonstrating that while the amygdala responds most reliably to negative stimuli (Hariri et al., 2000; Phelps et al., 2001; Whalen et al., 1998), it also performs operations such as signaling the salience of positive stimuli (Breiter et al., 1996; Demos et al., 2008; Hamann and Mao, 2002; Hamann et al., 2002). However, while the NAcc and amygdala respond to similar types of bivalent information, and are intimately connected, they belong to functionally dissociable neural circuits: 1) An amygdala-centered circuit that acts as a rapid response module that can engage affective response units even prior to conscious stimulus identification (Morris et al., 2001); and 2) A NAcc-centered circuit that can only fully engage down-stream sites for action-selection once stimulus identity has been established and its significance evaluated. Thus, while NAcc neurons respond to emotion-eliciting stimuli, they do so in a manner that is largely dependent on individual stimulus identity, i.e., object-specific, rather than responding to a single common physical or psychological property of these stimuli (Roitman et al., 2005; Setlow et al., 2003; Wilson and Bowman, 2005; Yanagimoto and Maeda, 2003). This is in sharp contrast to neurons in the amygdala which tend to respond to a single common psychological property (Belova et al., 2007; Maeda et al., 1993; Paton et al., 2006; Salzman et al., 2007).

The stimulus-identity dependency of NAcc neurons is consistent with the NAcc being a part of an approach-avoidance behavior network. Such a system must first be able to process information about the identity and value of unconditioned stimuli that can act either as rewards or punishers, and that once these events occur, motor systems must redirect behavior to gain maximal utility from rewarding events (Day and Carelli, 2007), or be engaged in a way that will allow the organism to avoid threat and aversive outcomes (Faure et al., 2008; Reynolds and Berridge, 2001). This idea is consistent with the role of the NAcc in both negative and positive reinforcement processes, for example in humans anticipating monetary gain and loss (Cooper and Knutson, 2008), and the damaging effect of NAcc lesions and pharmacological manipulations in tasks that require behavioral inhibition and modulation of instrumental action to optimize reward gain and avoid risk (Cardinal et al., 2004; Christakou et al., 2004; Martinez et al., 2002; Salamone et al., 1997; Wadenberg et al., 1990).

Concluding Remarks

In this study we were able to show that just as the amygdala is not solely responsive to negative events, the NAcc is not only responsive to anticipated positive rewards, but also aversive events. These results support models of NAcc function that are not solely focused on reward. This broader bivalent role for the NAcc is consistent with the anatomical connectivity of the NAcc that allows it to integrate a substantial amount of information from regions that process both positive and negative valence. Future work needs to investigate the precise role of this integration in emotional regulation via outputs of the NAcc to motor, cognitive and autonomic centers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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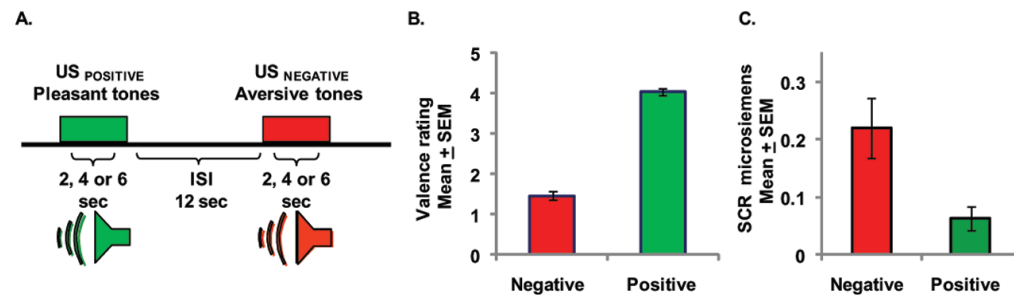


Figure 1.

Behavioral task and validation of stimulus valence. A. The paradigm consisted of presentations of negative and positive sound stimuli of variable duration (2, 4 or 6 s) presented with a fixed inter-stimulus interval (ISI) of 12s. In total 30 negative and 30 positive auditory stimuli were presented in a pseudorandom order. B. While in the scanner subjects rated the sounds as either pleasant or unpleasant on a 5 point rating scale, 1 being most unpleasant, and 5 being most pleasant. C. Skin conductance response (SCR) to the negative and positive auditory stimuli.

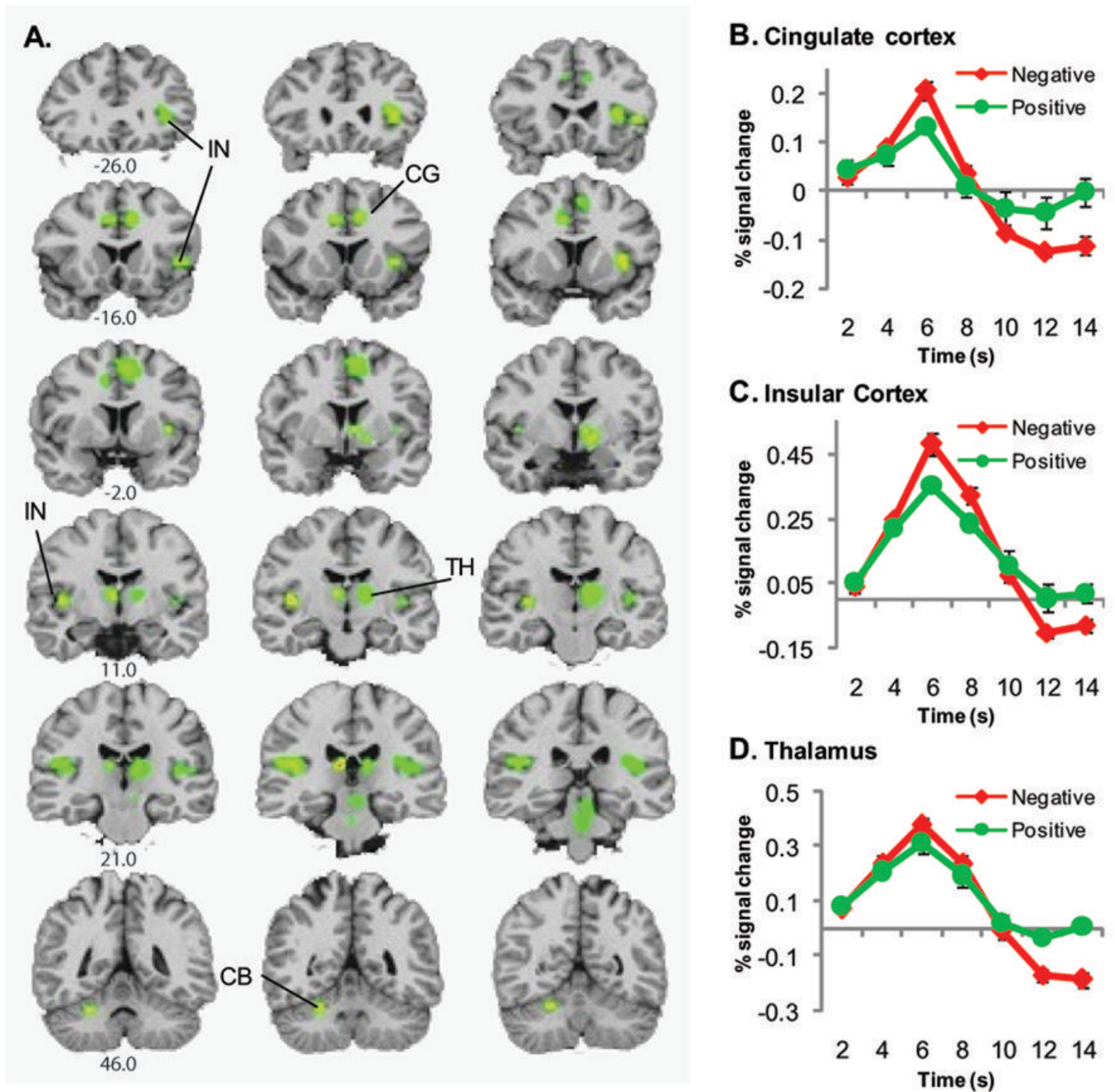


Figure 2.

Activation of nociceptive/emotive brain regions. A. Coronal sections illustrating regions that showed significant activation in group analysis of valence \times time ($p < 0.0001$, corrected to $p < 0.05$). Activations are displayed over a Talairach-normalized coronal templates in radiological convention (right = left). Greater activation was observed for the negative aversive stimuli in the (B) cingulate cortex (CIG), (C) insular cortex (IN), and cerebellum (CB). D. No difference in activation to negative and positive sounds was observed in thalamus (TH). Line plots represent mean \pm standard error of the mean (SEM) across participants. Anterior/posterior y-coordinates are specified below the coronal sections.

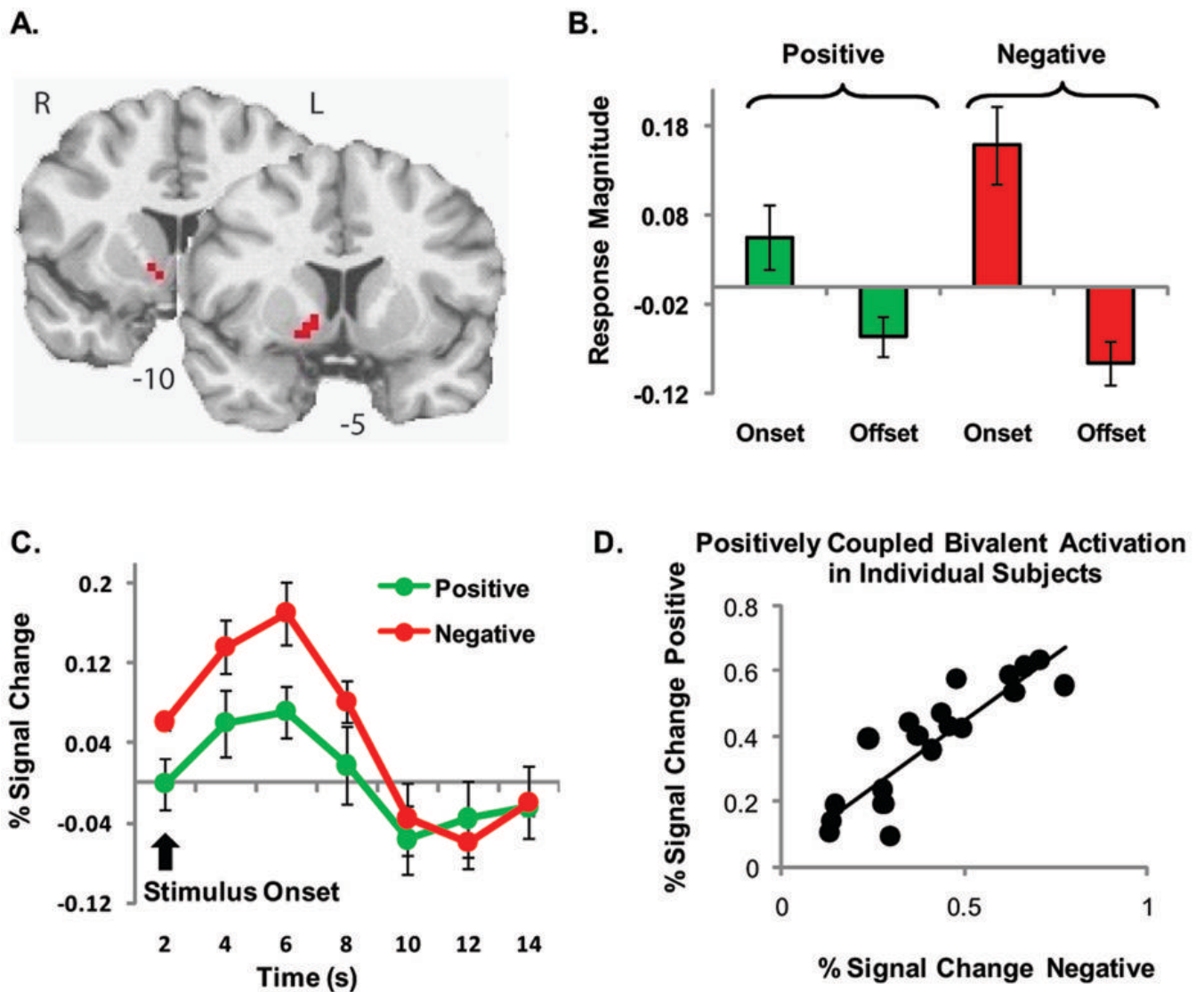


Figure 3. Bivalent activation of the nucleus accumbens. A. NAcc activation map in the coronal plane ($p < 0.05$, corrected). B. Activation in right NAcc on onset but not offset of both negative and positive events. C. Time course for the hemodynamic response in the right NAcc cluster. Image is in radiological format (right = left). D. Scatter plot of the positive correlation of individual subject's mean peak activation of the NAcc to the positive and negative stimuli throughout the experiment. Peak activation was defined for each individual by plotting the bold response for each event, and taking the peak value observed in resulting hemodynamic response function. Bar & line plots represent mean \pm standard error of the mean (SEM).

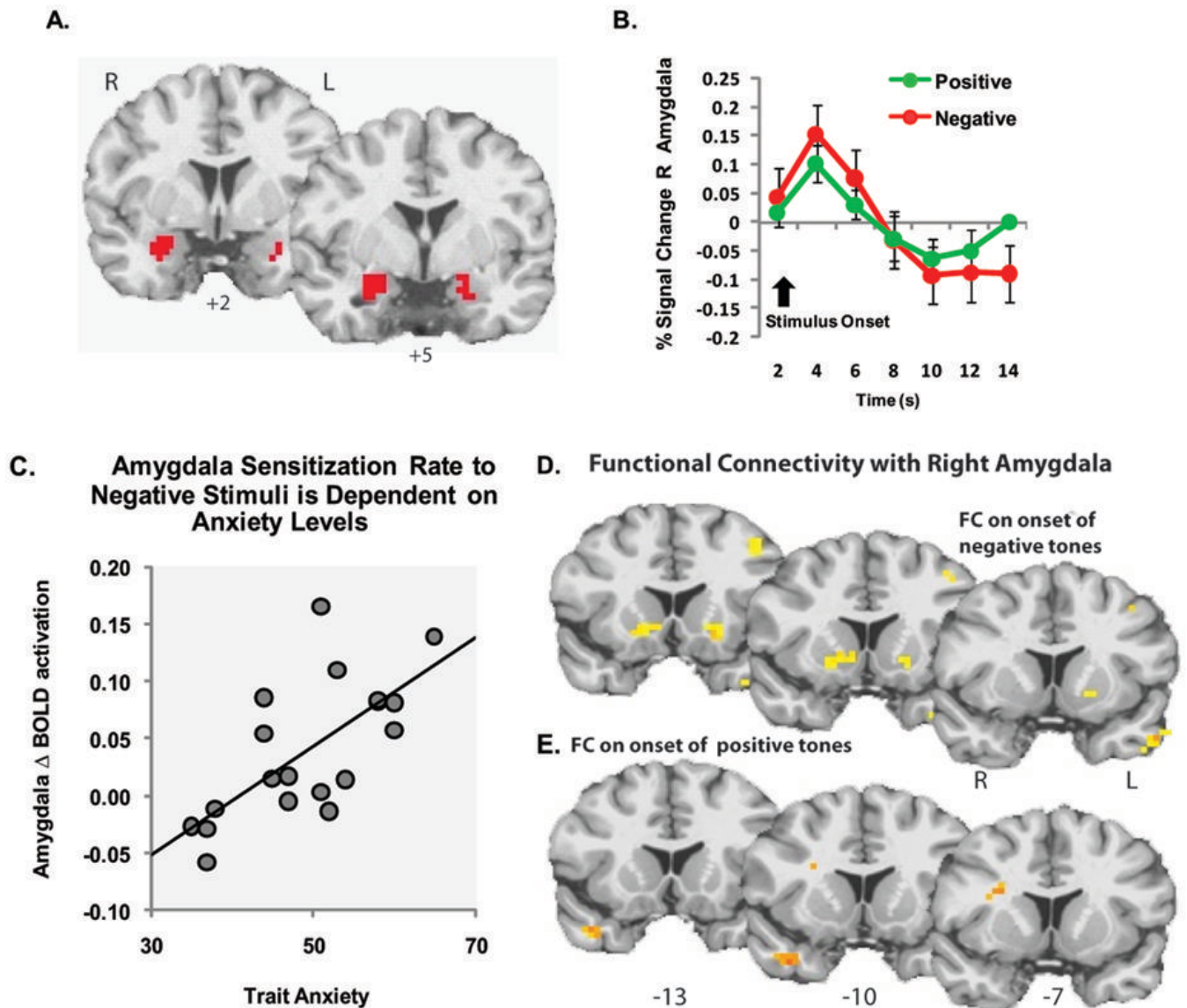


Figure 4. Bivalent amygdala activation. A. Coronal slice showing right and left amygdala clusters. B. Time course for the hemodynamic response in the right amygdala cluster. Images are in radiological format (right = left). Line plot represent mean \pm standard error of the mean (SEM) across participants. C. Scatter plot of the correlation between trait anxiety and change in right amygdala activation through time. Trait anxiety scores were positively correlated with increased amygdala activity on repeated presentations of the negative auditory stimuli. The y-axis represents rate of change in amygdala activation, positive values indicate sensitization, and negative scores indicate habituation. The x-axis represents trait anxiety score. D & E. Functional connectivity analysis with the right amygdala fROI cluster set as the seed region; Significant functional connectivity was observed between the right NAcc and right amygdala during presentation of the negative (D) but not positive tones (E). FC, functional connectivity; R, right; L, left.

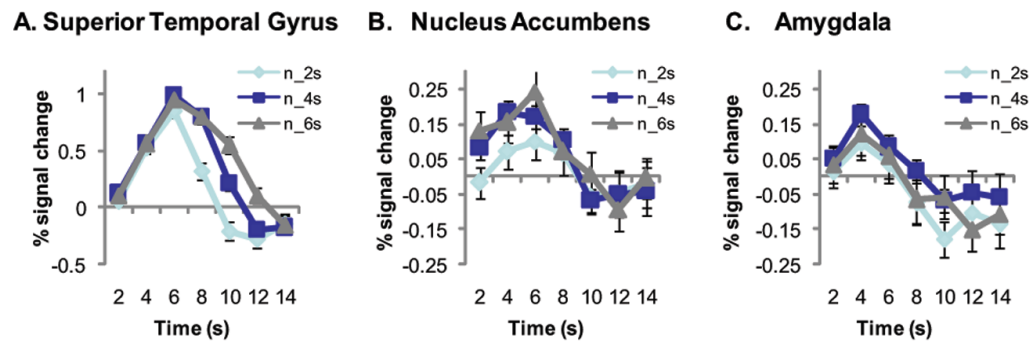


Figure 5.

Nucleus accumbens and amygdala activation are not sensitive to stimulus duration. Time course of the hemodynamic response on presentation of the different duration negative (n) stimuli (2, 4, and 6 s) in the right superior temporal gyrus (A), right nucleus accumbens (B) and right amygdala (C) clusters from group analysis valence \times time interaction. Line plots represent mean \pm standard error of the mean (SEM).

Table 1
Interaction of Valence (positive vs. negative) × Time (onset vs. offset)

Brain region	Side	Talairach coordinates (CM)						F Stats	Onset	Offset
		RL	AP	IS	Size (mm ³)					
Medial Frontal Gyrus	L	6.2	-1.7	19.1	1026		29.9	n > p	n > p	
Inferior Frontal Gyrus	L	43.8	-17.1	5.5	108		32.1	n > p	n = p	
Temporal Gyrus & Insula	L	39.3	24.5	13.2	1350		27.6	n > p	n > p	
Insula	R	-43.5	24.9	15.5	810		27.0	n > p	n = p	
Insula	L	31.6	-22.6	9.2	513		27.6	n > p	n > p	
Cingulate Gyrus	L	8.8	-13.5	36.4	270		28.4	n > p	n > p	
Cingulate Gyrus	R	-8.7	-10.3	-10.3	270		27.8	n > p	n > p	
Insula	L	33.5	-7.1	5.8	243		30.5	n > p	n > p	
Insula	R	-38.3	12.3	6.1	243		28.1	n > p	n > p	
Globus Pallidus	L	9.8	3.5	1.9	243		26.6	n > p	n > p	
Thalamus	L	11.7	17.3	11.3	1350		29.4	n = p	n > p	
Thalamus	R	-5.9	11.2	10.8	108		25.1	n = p	n > p	
Cerebellum	R	-22.9	48.5	-23.3	189		29.0	n > p	n > p	
Brain stem		3.1	27.0	15.7	1431		29.7	n > p	n > p	
Nucleus Accumbens	R	-12.9	-4.5	-1.1	189*		8.65	n > p	n = p	
Amygdala	R	-25.7	3.3	-14.3	702*		11.75	n = p	n > p	
Amygdala	L	19.1	4.5	-14.2	270*		8.04	n > p	n > p	

Group analysis ANOVA. CM, center of mass; L, Left; R, Right. Whole brain $p = 0.0001$, corrected to $p < 0.05$.

* Region of interest correction $p = 0.025$, corrected to $p < 0.05$.

Table 2
Whole-brain contrast analysis probing the main effect of valence

Brain region Negative > Positive*	Talairach coordinates (CM)						T stats
	Side	RL	AP	IS	Size (mm ³)		
Striatum, insula, globus pallidus, thalamus & brainstem	R&L	-3.8	14.7	4.3	41094	4.67	
Amygdala	R	-28.9	-0.2	-17.2	189	4.35	
Cingulate Gyrus	R&L	-0.9	-9.3	39.6	5265	4.47	
Posterior cingulate gyrus	R&L	-0.5	-23.0	40.8	324	44.5	
Insula & Inferior frontal gyrus	L	37.5	-11.8	9.8	375	4.33	
Cerebellum	R&L	35.2	49.5	-25.9	594	4.31	
Cerebellum	R	-23.6	47.2	-25.6	459	4.21	

Contrast Analysis: positive minus negative. CM, center of mass; L, Left; R, Right. Whole brain $p > 0.001$, corrected to $p > 0.05$.

* No brain regions with greater activation to the positive tones were found.