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Functionally distinct amygdala subregions identified using DTI and high-resolution fMRI

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Abstract

Although the amygdala is often directly linked with fear and emotion, amygdala neurons are activated by a wide variety of emotional and non-emotional stimuli. Different subregions within the amygdala may be engaged preferentially by different aspects of emotional and non-emotional tasks. To test this hypothesis, we measured and compared the effects of novelty and fear on amygdala activity. We used high-resolution blood oxygenation level-dependent (BOLD) imaging and streamline tractography to subdivide the amygdala into three distinct functional subunits. We identified a laterobasal subregion connected with the visual cortex that responds generally to visual stimuli, a non-projecting region that responds to salient visual stimuli, and a centromedial subregion connected with the diencephalon that responds only when a visual stimulus predicts an aversive outcome. We provide anatomical and functional support for a model of amygdala function where information enters through the laterobasal subregion, is processed by intrinsic circuits in the interspersed tissue, and is then passed to the centromedial subregion, where activation leads to behavioral output.

Key words: fMRI; streamline tractography; amygdala; novelty; fear conditioning

The amygdala is at the core of the brain's emotion processing network (Phelps, 2006). Although often treated as a unitary structure in functional neuroimaging studies, the amygdala is comprised of a set of distinct subnuclei (de Olmos, 1972; Amaral *et al.*, 1992; Sah *et al.*, 2003; Amunts *et al.*, 2005). The amygdala receives extensive sensory input, and the basolateral nucleus receives highly processed visual information from higher order visual regions along the ventral visual path pathway (Aggleton *et al.*, 1980; Sah *et al.*, 2003). The central nucleus acts as the main output of the amygdala and projects to regions of the brainstem, basal forebrain and dienchephalon. By influencing these regions, the central nucleus plays a key role in generating fear, characterized by species-specific behavioral responses, release of stress hormones and changes in autonomic nervous system activity (Ledoux, 2000; Cheng *et al.*, 2006a; Kim and Jung, 2006). This fear state is thought to prepare the subject to react appropriately when a threat is encountered in the environment (Öhman and Mineka, 2001).

Pavlovian fear conditioning can be used to study emotional processing in the laboratory (Kim and Jung, 2006). During fear conditioning an initially neutral conditioned stimulus (CS) is presented so that it predicts an aversive outcome (UCS; Pavlov, 1927). Once the subject learns that the CS predicts the occurrence of the UCS, they begin to show conditioned emotional responses (CR) in the presence of the CS. These conditioned emotional responses are dependent upon associative learning that takes place in amygdala circuits (McKernan and Shinnick-Gallagher, 1997; Blair *et al.*, 2001; Schroeder and Shinnick-Gallagher, 2005; Sah *et al.*, 2008; Johansen *et al.*, 2010). Sensory information about the CS and UCS converges on the amygdala, allowing associative connections to form in amygdala synapses, and subsequent reexposure to the CS activates output cells within the central nucleus (Ledoux, 2000; Kim and Jung, 2006). Although fear learning appears to be one of the primary functions of the amygdala, there is also ample evidence to suggest the amygdala is engaged in other types of psychological phenomena.

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In several recent articles, our lab and others have found that the amygdala responds to simple stimulus novelty (Wright *et al.*, 2003; Blackford *et al.*, 2010; Weierich *et al.*, 2010; Balderston *et al.*, 2011). For example, we compared novel and repeated images of faces and scenes. We found that novel faces, but not scenes, evoked larger responses than repeated stimuli of the same type. In addition, we found that this novelty effect was due to a response evoked by the initial presentation of a unique face; this response was not evoked on subsequent presentations (Balderston *et al.*, 2011). This was clearly the case even for emotionally neutral faces, which is notable given the large literature suggesting that angry and fearful faces preferentially activate the amygdala (Gamer and Büchel, 2009; Whalen *et al.*, 1998, 2001, 2004).

One possible explanation for this novelty effect evoked by neutral faces is that the amygdala's response to novelty represents the processing necessary to initially identify threats in the environment, while fear responses represent the actual response to an identified threat. According to this hypothesis, novelty and fear may engage distinct, separable processes in the amygdala, which has been suggested by others (Weierich *et al.*, 2010). Alternatively, it could be that novel stimuli are more salient than repeated stimuli, and the amygdala is simply tracking this difference in salience (Sander *et al.*, 2003). According to this idea, novelty and fear are examples of a single superordinate principle, and thus engage the same process in the amygdala. The purpose of this experiment was to test these hypotheses by examining the effects of both novelty and fear on neural activity within individual subregions of the amygdala. If these psychological properties are representations of different processes occurring in the amygdala, it is possible that they activate distinct subregions of the amygdala. If they are representations of a superordinate process occurring in the amygdala, they should activate similar subregions of the amygdala.

Methods

Participants

Twenty-three (13 female) neurologically healthy University of Wisconsin-Milwaukee students (Age: $M = 24.81$, s.d. $= 6.18$) participated for extra credit in their psychology courses. Participants also received 20 dollars and a picture of their brain for participation. All participants gave informed consent, and the protocol was approved by the Institutional Review Boards for human subject research at the University of Wisconsin-Milwaukee and the Medical College of Wisconsin. Four subjects were excluded from the analysis. Two were excluded for movement, one due to equipment failure, and one because the functional slab was not properly placed to cover the amygdala.

Stimuli

Seven neutral images were selected from the international affective picture system (IAPS) database (Lang *et al.*, 2008). Images were of single individuals, displaying neutral facial expressions (Image indices: 2190, 2200, 2210, 2305, 2493, 2506, 2516). We presented the stimuli centrally against a black background, using the software package Presentation (Neurobehavioral Systems, Inc., Albany, CA). Participants viewed the stimuli using a back projection video system with prism glasses mounted to the head coil.

Electrical stimulation

Participants received presentations of an electrical stimulation. The stimulation was administered via an AC (60 Hz) source (Contact Precision Instruments, Model SHK1, Boston, MA) through two surface cup electrodes (silver/silver chloride, 8 mm diameter, Biopac model EL258-RT, Goleta, CA) filled with electrolyte gel (Signa Gel, Parker laboratories Fairfield, NJ). Stimulation electrodes were placed on the skin over the subject's right tibial nerve over the right medial malleolus. The shock was presented for 500 ms, at a level that the subject rated as painful but tolerable.

UCS expectancy

Participants continuously rated their expectancy of receiving the electrical stimulation. They controlled a cursor placed on a visual analog scale anchored with 0 and 100. They were instructed to place the cursor near 0 if they were sure they would not receive an electrical stimulation, near 100 if they were sure that they would receive an electrical stimulation, and near 50 if they were unsure. Responses were recorded throughout the experiment and sampled at 40 Hz. We then averaged the values across the last four seconds of the stimulus period for each trial. These averages were then used in subsequent group level analysis.

Skin conductance responses

We recorded skin conductance level (SCL) via two surface cup electrodes (silver/silver chloride, 8 mm diameter, Biopac model EL258-RT, Goleta, CA) filled with electrolyte gel (Signa Gel, Parker laboratories Fairfield, NJ) attached to the bottom of the participants' left foot approximately 2 cm apart. SCL was sampled at 200 Hz throughout the experiment. We identified the peak SCL value during the 8-s trial and expressed it as a percent change from the average of the preceding 2-s baseline (Balderston and Helmstetter, 2010; Balderston *et al.*, 2011). These values were used in subsequent group level analyses.

Magnetic resonance imaging

We conducted whole brain imaging using a 3 T GE MRI 750 system, with a 32-channel head coil. To identify the amygdala, we collected high resolution T1-weighted images (TR $= 8.2$ s; TE $= 3.9$ ms; field of view $= 24$ cm; flip angle $= 12\circ$; voxel size $= 0.9375 \times 0.9375 \times 1.0$ mm). We then segmented these images using the Freesurfer software package, which is freely available online and has been described previously (Fischl *et al.*, 2002, 2004). Freesurfer generated volumes were then realigned to native space using The Analysis of Functional NeuroImages software package (AFNI). These realigned volumes were then manually inspected to ensure that they conformed to previously described standards (Morey *et al.*, 2009).

Streamline tractography

We collected diffusion-weighted images (DWI) images, which were used to determine the anatomical connectivity of the amygdala. Thirty-eight whole brain images containing 70 contiguous 2 mm axial slices were acquired using an echoplanar pulse sequence (TR $= 10$ s; TE $= 81$ ms; field of view $= 240$ mm; matrix $= 128 \times 128$; b value $= 800$ s/mm^2; diffusion directions $= 35$, number of b value $= 0$ s/mm^2 volumes $= 3$). We calculated diffusion tensors from the DWI images using the AFNI command 3dDWItoDT. We then computed the tensor coefficients using the DTI-query program dtiprecompute (pathway tracing algorithm $=$ STT, step size $= 2$mm, FA termination threshold $= 0.15$, and angular threshold $= 90$), which creates a

database of fiber tracts that can then be queried using the DTI-query user interface (Sherbondy *et al.*, 2005).

High-resolution fMRI

We collected high-resolution functional magnetic resonance images (fMRI) to record amygdala blood oxygenation level-dependent (BOLD) during the experimental run. Functional images were acquired from a slab of eight contiguous 2 mm axial slices with an in plane resolution of 1×1 mm, using a T2* weighted gradient echo, echoplanar pulse sequence (TR = 2 s; TE = 30 ms; field of view = 256 mm; matrix = 256×256; flip angle = 77°). Slices were manually centered on the amygdala, as identified on the T1-weighted images.

We used AFNI to reconstruct and process the fMRI data (Cox, 1996). EPI images were preprocessed using a standard processing stream that included motion correction, image registration, and z-score normalization. Runs were manually inspected for large head movements, and for proper T1-EPI registration. Images that contained discrete head movements were censored, and participants showing excessive movement (greater than 2 mm displacement or more than five instances of discrete head movements; Balderston *et al.*, 2011) were excluded from further analyses. Head motion and dial movement regressors were included in the analysis as regressors of no interest. Timeseries data were deconvolved with stimulus canonicals using AFNI's 3dDeconvolve command, to yield average impulse response functions (IRFs). The peak of the IRF was identified and used for subsequent group level analyses.

Procedure

During the experiment, we presented a series of novel (NOV), repeated but not shocked (CS−), and repeated but shocked (CS+) faces (Figure 1). Pictures were presented for 8 s, with a 20-s variable intertrial interval. The 500 ms shock UCS coterminated with the CS+, and was presented on every CS+ trial. The analysis included five trials of each stimulus type, and we only counted repeated presentations in the CS+ and CS− categories. Two repeated images (CS+ and CS−) were each presented six times, five novel images were each presented once. The initial presentation of the CS− was included in the NOV category because it was novel at the time of the presentation. Although the initial presentation of the CS+ was also novel, we did not include it in the NOV category because it was paired with the shock. Additionally, to remain consistent with the treatment of the CS−, the initial presentation of the CS+ was not included in the CS+ category, and was therefore not included in the analysis.

Prior to the experiment, we situated the participant comfortably in the scanner, secured their head with cushions, and attached the physiological monitoring equipment. Next, we instructed the subject on the proper use of the dial, and set the level of the electrical stimulation using previously described methods (Balderston *et al.*, 2011; Schultz *et al.*, 2012). We began by collecting T1-weighted images, followed by four minutes of resting state data (not shown here). Prior to the functional scan, we manually identified the amygdala and placed the slices for the high-resolution functional scan. Next we began the experimental run, and recorded the high-resolution functional data. Afterward we collected an additional four minutes of resting, and concluded by collecting the diffusion weighted images. At the end of the experiment, the subject completed a brief post experimental questionnaire.

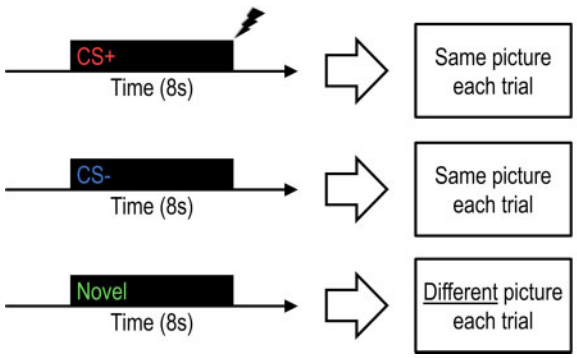

Fig. 1. We presented face images in an event-related fMRI design. One image was repeatedly presented and paired with a shock (CS+). One image was repeatedly presented and not paired with a shock (CS−). Novel images were presented and not repeated. Images were presented for 8 s. The initial (novel) presentation of the CS+ and CS− were not used included in their respective categories. Instead the initial presentation of the CS− was considered novel, and the initial presentation of the CS+ was excluded from the analysis.

Identification of amygdala subregions

We identified subregions of the amygdala based on anatomical connectivity using the T1 and DTI data (Figure 2). We began by identifying the amygdala for each subject using the Freesurfer segmented T1-weighted images. Next we identified the white matter intersecting with the amygdala mask, using the precomputed fiber database. Across subjects we noticed two prominent pathways: one that connected the amygdala with the ventral visual pathway, and one that connected the amygdala with the diencephalon. The visual pathway observed in the tractography data may reflect afferent connections from the visual cortex,

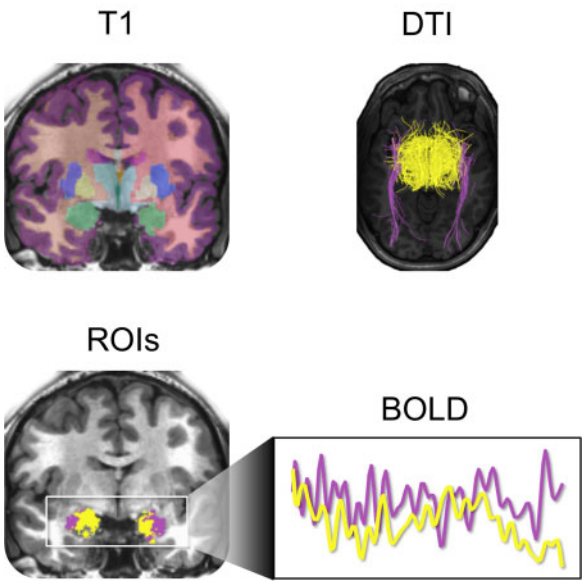

Fig. 2. We identified subregions of the amygdala using anatomical connectivity. First we defined the amygdala for each individual using the Freesurfer-segmented T1. Next we identified white matter pathways from the diffusion tensor images (DTI) using probablistic tractography. Purple pathways connect the amygdala with the visual cortex. Yellow pathways connect the amygdala with the diencephalon. Subsequently we identified the regions of interest (ROIs) within the amygdala containing these white matter pathways. Finally we sampled the high-resolution BOLD activity using these ROIs.

while the diencephalic pathway may reflect efferent connections to the hypothalamus (Krettek and Price, 1977; Amaral *et al.*, 1992; Price, 2003). Next we selected the fibers that intersected with both the amygdala, and the destination ROI (visual cortex, diencephalon), and created anatomical masks from these two pathways. Finally, we exported these masks as NIFTI volumes, and subdivided the amygdala by overlaying the white matter volumes on the amygdala volumes.

Our analysis identified four distinct amygdala subregions: one region connected with the visual cortex (laterobasal), one region connected with the diencephalon (centromedial), one region representing the overlap between these two regions, and the interspersed tissue showing no anatomical connectivity (interspersed). In order to determine which subregion the overlap area predominantly belonged to, we compared the pattern of activity in the overlap region to the pattern of activity of the two other connected regions for each subject. Then, for each subject we assigned the overlap region to the subregion in such a way that it minimized the sum of the squared deviations across stimulus types. Next, we sampled the BOLD activity from the functional run using these three subregions.

Results

UCS expectancy

In order to determine whether the participants were able to explicitly learn the picture shock contingencies, we recorded their UCS expectancy on each trial. We performed a 3 (CS+ *vs* CS− *vs* Novel) × 5 (Trial) repeated measures ANOVA, and found a significant main effect for CS ($F(2,36) = 82.81$; $P < 0.01$) and a significant CS × Trial interaction ($F(8,144) = 3.27$; $P < 0.01$). The main effect for CS type suggests that subjects expected the shock on the CS+ presentations, expected no shock on the CS− presentations, and were unsure whether or not to expect the shock on the novel stimulus presentations (Figure 3A). We performed the corresponding pairwise t-tests to support this conclusion (CS+ > CS−: $t(18) = 10.90$; $P < 0.01$; CS+ > Nov: $t(18) = 8.07$; $P < 0.01$; Nov > CS−: $t(18) = 6.18$; $P < 0.01$).

Skin conductance responses

In order to determine whether the participants were able to implicitly learn the picture shock contingencies, we recorded their SCRs on each trial. We performed a 3 (CS+ *vs* CS− *vs* Novel) × 5 (Trial) repeated measures ANOVA, and found a significant main effect for CS ($F(2,36) = 6.49$; $P < 0.01$) and a significant main effect for Trial ($F(8,72) = 12.46$; $P < 0.01$). The main effect for CS type suggests an effect for conditioning (Figure 3B). This is supported by a significant CS+ > CS− pairwise t-test ($t(18) = 3.46$; $P < 0.03$). Consistent with previous results (Balderston *et al.*, 2011), we found that novelty evokes an intermediate level SCR (CS+ > Nov: $t(18) = 1.61$; $P = 0.12$; Nov > CS−: $t(18) = 2.23$; $P = 0.04$).

Distinct response profiles in amygdala subregions

Next, we wanted to determine whether novelty and fear activate similar subregions within the amygdala. To do so, we performed a 3 (CS+ *vs* CS− *vs* Novel) × 3 (Centromedial *vs* Interspersed *vs* Laterobasal) repeated measures ANOVA, and found a significant main effect for subregion ($F(2,36) = 3.87$; $P = 0.03$) and a significant CS × subregion interaction ($F(4,72) = 2.85$; $P = 0.03$). The results from this analysis suggest that the three amygdala subregions have distinct response profiles, which we verified using pairwise statistics (Figure 4). The laterobasal region seemed to be responding to all CS types (post hoc *ps* > 0.05). The interspersed tissue seemed to be responding to only the salient stimulus types (one-way repeated measures ANOVA: $F(2,36) = 3.31$; $P = 0.05$; CS+ > CS−: $t(35) = 2.46$; $P = 0.02$; NOV > CS−: $t(35) = 2.29$; $P = 0.03$). The centromedial region seemed to be responding only to the CS+ (Planned comparison, CS+ > NOV and CS−: $F(1,54) = 3.96$; $P = 0.05$).

Discussion

In this experiment, we measured the effect of novelty and fear on behavior and amygdala BOLD responses. We subdivided the amygdala into three distinct subregions based on anatomical connectivity, which we identified on a subject by subject basis. Importantly, the pathways used to subdivide the amygdala are consistent with the known anatomical connectivity of the amygdala (Krettek and Price, 1977; Amaral *et al.*, 1992; Price, 2003). The laterobasal subregion shared white matter pathways with the visual cortex and responded to all stimulus categories. The centromedial subregion shared white matter pathways with the diencephalon and responded only to stimuli that predicted an aversive outcome. The interspersed tissue was connected with neither the visual cortex nor the diencephalon. This region responded both to novel stimuli, and stimuli that predicted an aversive outcome. Interestingly, these results suggest that these three subregions within the amygdala represent different nodes within an information processing circuit, and that the activation of these different subregions may represent the flow of information through the amygdala. According to this model, information enters the amygdala through the

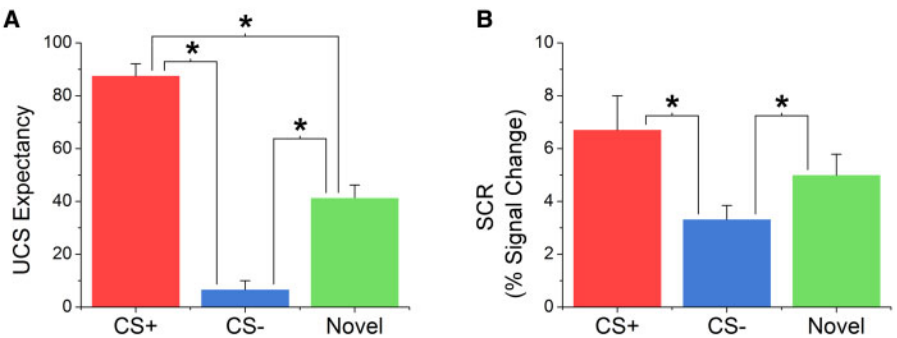

Fig. 3. Subjects showed evidence of implicit and explicit conditional learning. (A) Subjects expected the shock on CS+ trials, expected no shock on CS− trials, and were unsure whether to expect the shock on Novel trials. (B) Subjects showed larger skin conductance responses (SCRs) to the CS+ and Novel stimuli than to the CS−. (bars = M ± SEM, *$P \leq 0.05$)

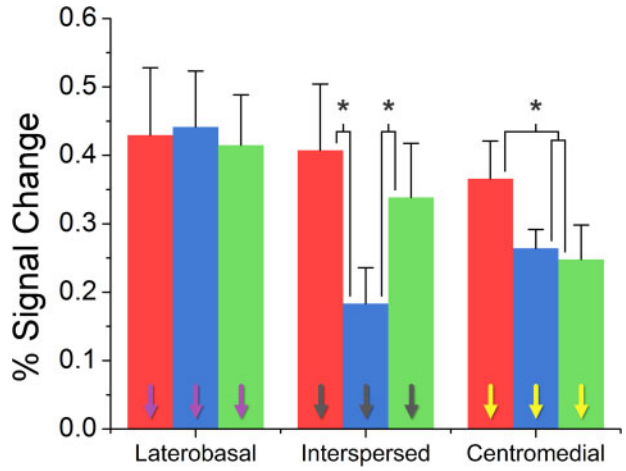

Fig. 4. Subregions of the amygdala show distinct patterns of activity. The laterobasal subregion (purple arrows) responds to all stimulus types. The interspersed tissue (grey arrows) responds to salient stimulus types (CS + and Novel). The centromedial subregion (yellow arrows) responds only to stimulus types that predict an aversive outcome (CS+). Colored arrows indicate region of anatomical connectivity (purple = visual cortex; grey = no connectivity; yellow = diencephalon). (bars = M ± SEM, *P ≤ 0.05)

laterobasal subregion, is processed in intrinsic circuits within the interspersed tissue, and is passed to the centromedial subregion if and only if the source of visual information signals a potential threat in the environment.

The wide variety of experimental paradigms and the often conflicting results have led to the formulation several theories of amygdala function (Ledoux, 2000; Öhman and Mineka, 2001; Sander *et al.*, 2003; Whalen, 2007; Pessoa, 2010). Some suggest that the amygdala is primarily involved in the expression of fear (Ledoux, 2000), while others suggest that it is primarily involved in the detection of motivationally significant environmental and social signals (Davis and Whalen, 2001; Tamietto and de Gelder, 2010). At a fundamental level, these theories can be broadly categorized into two different categories that differ in whether they define the amygdala primarily in terms of input or output. Some of these issues can be resolved by considering that these two processes are both mediated by the amygdala, but by different subregions. Additionally, our results suggest that a third process—evaluation—must also be considered. That is, the amygdala is not simply a region that detects or responds to motivationally significant events; rather it is a region that actively monitors the environment, evaluates environmental signals for evidence of motivationally significant events, and generates responses if and only if such an event is detected.

The laterobasal subregion: feature detection

There are many theories of amygdala function that suggest that the amygdala is specialized to detect specific visual features, such as forms that may indicate the presence of a dangerous snake or a threatening face (Öhman and Mineka, 2001; Adolphs, 2002; Isbell, 2006). For instance, it is well known that both angry and fearful faces activate the amygdala more than neutral faces (Whalen *et al.*, 1998, 2001; Reinders *et al.*, 2006), and that this effect can occur without awareness (Whalen *et al.*, 2004; Carlson *et al.*, 2009; Feng *et al.*, 2009). In fact, some have shown that specifically the eye region of the face can evoke similar effects (Whalen *et al.*, 2004; Gamer and Büchel, 2009). Accordingly, individuals with bilateral amygdala lesions have difficulty spontaneously using the eye region of the face to identify fearful facial expressions (Kennedy and Adolphs, 2010). In addition to faces, other types of stimuli have been shown to receive preferential processing by the amygdala. For instance, snakes and spiders have been shown to evoke amygdala response with and without awareness (Britton *et al.*, 2006; Larson *et al.*, 2006; Ahs *et al.*, 2009; Larson *et al.*, 2009; Nili *et al.*, 2010). Snakes and spiders also tend to capture attention (Kindt and Brosschot, 1997; Miltner *et al.*, 2004; Van Strien *et al.*, 2009), and pop out in complex visual displays (Larson *et al.*, 2007). They can support fear learning in the absence of awareness (Öhman and Soares, 1993; Flykt *et al.*, 2007), and learning with these types of stimuli typically leads to a stronger fear memory that is more difficult to distinguish (Fredrikson *et al.*, 1976; Öhman *et al.*, 1976; Hugdahl and Öhman, 1980). The amygdala shares reciprocal connections with many levels of the ventral visual pathway, supporting the notion that it is involved in visual processing (Sah *et al.*, 2003). Our results suggest that this feature level processing occurs in the laterobasal subregion.

The interspersed tissue: evaluation

In contrast to those theories that suggest the amygdala is specialized for visual processing, others have suggested that the amygdala plays a major role in the identification of behaviorally relevant stimuli or events, independent of specific visual features (Sander *et al.*, 2003). Support for these theories comes from studies showing that the amygdala responds to psychological features independent of perceptual features (Schwartz *et al.*, 2003; Herry *et al.*, 2007; Whalen, 2007; Ousdal *et al.*, 2008; Weierich *et al.*, 2010; Blackford *et al.*, 2010; Balderston *et al.*, 2011). In one study in mice and humans, Herry *et al.* (2007) showed that a series of unpredictable tones lead to immediate early gene expression and increases in BOLD activity, compared to a series of predictable tones. In several recent studies, our lab and others have shown that the amygdala responds to novel stimuli, independent of emotional content (Schwartz *et al.*, 2003; Blackford *et al.*, 2010; Weierich *et al.*, 2010; Balderston *et al.*, 2011). Although it is clear that the amygdala plays a key role in the expression of emotion, the novelty evoked amygdala responses that we have observed are not necessarily accompanied by increases in arousal, suggesting that fear expression is not a sufficient explanation of amygdala function.

These results suggest that defining amygdala function in terms solely of either detection or response is not sufficient. Rather, amygdala activity should be considered within an emotional decision-making framework. Our results suggest that salient visual stimuli activate intrinsic circuits within the amygdala, and that this activation represents the evaluation of this visual information for further evidence of a motivationally significant event. Importantly, this activation is not necessarily predictive of behavior, suggesting that it potentially precedes behavioral output.

The centromedial subregion: response expression

According the traditional view, the primary function of the amygdala is to form associative memories involving biologically significant events (Ledoux, 2000; Kim and Jung, 2006). Information about stimuli and motivationally significant outcomes converges on the basolateral nuclei and these associations lead to the activation of the central nucleus, which initiates a fear response (Ledoux, 2000; Kim and Jung, 2006). Work from Pavlovian fear conditioning studies shows that the

amygdala is necessary for the acquisition, and consolidation of learned fear associations (Bailey *et al.*, 1999; Parsons *et al.*, 2006; Kwapis *et al.*, 2011). In addition, amygdala activity in fMRI experiments has been shown to correlate with conditioned fear responses (Cheng *et al.*, 2003; Knight *et al.*, 2005; Cheng *et al.*, 2006b; Cheng *et al.*, 2007). Work from studies employing emotional visual stimuli has shown that emotional responses evoked by such images are dependent upon the normal functioning of the amygdala (Bechara *et al.*, 1995; Gläscher and Adolphs, 2003), and correlated with amygdala activation (Williams *et al.*, 2001).

Our results suggest indeed that response expression is one function of the amygdala. Further, our results suggest that the activity needed to produce a conditioned response occurs within the centromedial subregion of the amygdala, which is anatomically connected with the diencephalon. Although we do not have the anatomical specificity to equate the centromedial subregion defined here with the location of the central nucleus, the central nucleus likely makes up at least a portion of the centromedial region in the majority of the subjects (Sah *et al.*, 2003; Amunts *et al.*, 2005).

Limitations

Although the current results suggest that there are distinct subregions of the amygdala that mediate different aspects of amygdala function, these results should be considered within the context of the limitations of the study. First, by increasing the resolution of our functional images, we necessarily decreased the signal to noise ratio. Future studies at high-field should be conducted to expand upon these findings. In this study, we created single-subject masks based on the anatomical connectivity of the amygdala. One of the limitations of this approach is that these masks do not encompass the entire amygdala, and the remainder of the tissue is distributed heterogeneously across subjects, making it difficult to summarize at the group level. Although it is unclear whether this absence of connectivity reflects a limitation of our imaging procedure or a feature of the underlying anatomy, our results suggest that the amygdalar tissue not accounted for by our connectivity masks and the amygdalar tissue in our masks are playing fundamentally different roles in the psychological processes commonly identified as 'amygdala-dependent'. Another limitation is that we did not use a strictly seed-based approach to identify white matter tracts. Instead, we pre-computed the white matter pathways and interactively identified those that passed through the amygdala, making it difficult to identify the origin of the fibers. However, it should be noted that the white matter pathways were similar across subjects, and our results are consistent with the known anatomical connectivity of the amygdala (Aggleton *et al.*, 1980; Sah *et al.*, 2003). One final limitation of this study is that we do not address the intra-amygdala connectivity of the subregions. Although intra-amygdala connectivity is an interesting question, identifying short-range connections within the grey matter of the amygdala is beyond the scope of the current work. Future studies using high-resolution DTI may be able to fill this gap in the literature.

Summary

On the basis of our results, we propose a three-stage information processing model to describe amygdala function (Figure 5). (1) The amygdala receives information about the environment via inputs to the laterobasal subregion. (2) Intrinsic processing circuits within the amygdala process this information, often giving weight to specific types of input. (3) The amygdala modifies the behavior of the organism according to the motivational significance of the information presented via projections from the centromedial subregion.

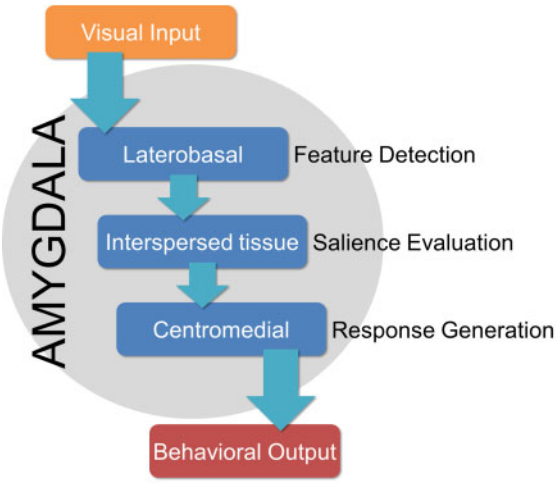

Fig. 5. Theoretical model depicting information flow through the amygdala. According to our model visual input enters the amygdala through the laterobasal subregion, which processes visual features. Salient visual features are then evaluated in intrinsic processing nodes in the interspersed tissue. Finally, behavioral output is generated by the centromedial region if and only if the salient visual features predict a motivationally significant event in the environment.

Our results provide an empirical framework for an opinion put forth by Pessoa (2010). According to this view, amygdala function can be understood from within a multilevel decision making framework. He argues that the amygdala is specialized to answer the questions "What is it?" and "What is to be done?" about environmental input. Specifically, he argues that via reciprocal connections with the ventral visual pathway, the amygdala receives and modifies visual information, allowing the organism to assess the motivational significance of specific objects in the environment. Interestingly, our results suggest that this evaluation may not necessarily occur within regions of the amygdala that receive projections from visual regions, but that the visual processing and evaluation may occur in different amygdala subregions. Additionally, he argues that the amygdala modifies the attention and behavior of the organism via projections from the central nucleus to the basal forebrain. Although it is not possible to make such a specific anatomical confirmation based on our data, our data are at least consistent with this conclusion. In contrast to Pessoa, we argue that the 'What is it?' and 'What is to be done?' questions may be part of a single hierarchical decision making process, with the ultimate product being output from the centromedial subregion. Although not directly tested here, future studies should be designed to distinguish between these two possibilities. No matter what the outcome, it is clear that it will no longer be sufficient to report on what the amygdala is doing, rather it will be necessary to identify the contributions of individual amygdala subregions to the psychological principles under investigation.

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