Unconscious processing of emotional content in hybrid faces

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Abstract

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Title: Unconscious processing of emotional content in hybrid faces

Seventeen participants were shown hybrid faces in an event-related design while undergoing functional magnetic resonance imaging (fMRI), we investigated the hypothesis that unconscious processing of emotion can take place, and that this process is driven by information the low spatial frequency spectrum. Furthermore we investigated the amygdala's role in a hypothesized subcortical pathway for emotional processing. The hybrid images either contained implicit emotional information in the low spatial frequency range (1-7 cycles/image) and a neutral expression in the rest of the bandwidth, or hybrids containing an implicit neutral expression in low spatial frequency range and an explicit emotional expression in the rest of the bandwidth. We found that manipulating spatial frequency information did not lead to significant increase in amygdala activity for single filtered or hybrid images with emotional content in the low spatial frequency range. Possible issues with non-independent ROI-analysis are discussed and how it may lead to inflated spurious results in previous studies. The behavioral data do however show that hybrid images with implicit emotional content were rated as significantly more unfriendly/friendly when compared to neutral broadband images. The behavioral data does support the idea that the low frequency information can influence a rather complex social judgment, but are not in line with the fMRI data. Too many conclusions could not be drawn due to the substantial inter-subject variability in the fMRI data.

During the last decade there has been a large interest on how salient, emotional and socially-charged visual stimuli are processed in the brain. This is an important topic to study because it can shed light how an animal assign biological value to target stimuli in the environment: which stimuli are good and which are bad; which should one approach and which to avoid? This raises the question of how and where salient, emotional and socially-charged visual stimuli are processed in the brain, and whether affective stimuli are processed in a specialized, or even dedicated neural substrates. One example of affective stimuli is the human face. With just a very brief glance we may form a "first impression" of a person's personality that does not differ significantly from the impression made by a longer exposure (Bar, Neta, & Linz, 2006). One possible explanation for the above phenomena is that some coarse structural features of the face can carry enough information about the emotional state and mood of a person that can rapidly be processed by a modular system that can be operated automatically (without attention) and mostly independently from conscious awareness (Tamietto & de Gelder, 2010). It has been argued for a long time for the existence of such a modular system which would have evolved for processing visual stimuli with emotional significance or biological value (LeDoux, 1996; LeDoux, 2000; Öhman, 2005). Research in neuroimaging has shown that visual stimuli with emotional importance are processed by a distinct neural network (Vuilleumier, Armony, Driver, & Dolan, 2001a, 2003; Öhman, Carlsson, Lundqvist, & Ingvar, 2007), and that such a visual stimulus can be processed automatically without attention (Whalen et al., 1998). Furthermore, it has been suggested that information from the visual stimulus is carried by the low spatial frequencies contained within the visual input (Loftus & Harley, 2005).

Initial evidence supporting the hypothesis of a rapid neural network for processing stimuli with emotional significance without conscious awareness derives largely from psychological research utilizing pattern backward masking technique (Esteves & Öhman, 1993). In backward masking one present one visual stimulus (a mask) immediately after another target stimulus. The duration of the target stimulus may be as short as ≤ 50 ms. This procedure in effect leads to a failure of conscious perception of the target stimulus. In a typical experiment on emotions utilizing backward masking, first one presents an emotional stimuli (e.g. spider, snake) followed by a neutral stimulus (mushroom, flowers, etc.). The participant may have no conscious experience of the masked stimulus but will exhibit physiological or emotional responses that reflect the information presented in the task. An example of research using backward masking technique found that participants that were

phobic to spiders and snakes had elevated skin conductance responses to snake and spider images as compared with neutral images under masking conditions (Öhman & Soares, 1994). Another study utilized backward masking by showing participants emotional face (angry or happy) for 16 ms, followed by a neutral face for 400 ms. Participants reported that they had not observed any emotional expressions, but their subsequent ratings of flavored drinks were positively affected by the subliminal presentation of a happy face or negatively affected by an angry face (Berridge & Winkielman, 2003). There is however some discrepancies about how long the duration of the masked stimulus has to be in order to be processed (Bar, et al., 2006).

Evidence for the existence of a dedicated neural substrate for processing emotional visual stimuli has also come from neuropsychological studies, and specifically cases of residual face-processing abilities in patients with hemispatial neglect, blindsight (Tamietto et al., 2009) and prosopagnosia. This research has shown that neglect patients normally show extinction to visual stimuli in the neglect field, but faces or stimuli arranged to give impression of a schematic face are able to capture their attention and overcome extinction (Vuilleumier, 2000; Vuilleumier & Sagiv, 2001). Patients with prosopagnosia experience difficulties with recognizing a face, but still retain the ability to differentiate between some facial expressions and detect the presence of a face. These patients have lesions or some other developmental deficit in the occipitotemporal areas (de Gelder, Frissen, Barton, & Hadjikhani, 2003). These findings suggest that faces are processed in the occipitotemporal areas, and that face detection and the ability to detect facial expression might also be supported by a subcortical route. In a case study of a patient with an extensive lesion of the left striate cortex, the patient was able to discriminate emotional expression presented in his blind (right) hemifield. The authors proposed that this residual ability to discriminate emotional expression depends on a subcortical visual pathway that circumvents the early visual cortices (Morris, DeGelder, Weiskrantz, & Dolan, 2001).

An influential neuroscience account of the proposed subcortical visual pathway was given by LeDoux (1996, 2000). He posited that there exist two distinct neural networks for processing emotional content and stimuli with biological value; the “low road” of the superior colliculus, the visual pulvinar of the thalamus and the amygdala (Tamietto & de Gelder, 2010). The superior colliculus receives direct projections from retinal ganglion cells with large receptive fields and with rapidly conducting axons that form the magnocellular pathways. This visual “low road” was originally developed from neurophysiological research on the rat auditory system by fear conditioning. Indirect evidence for this hypothetical

subcortical “low road” comes from neuroimaging studies that have revealed a connectivity pattern between amygdala, pulvinar, and superior colliculus when faces expressing fear were processed unconsciously (Liddell et al., 2005; Morris, Öhman, & Dolan, 1999; Vuilleumier, et al., 2003). Furthermore a physiological study found evidence that a neural pathway connecting the pulvinar thalamus to the amygdala does exist in other primates (Jones & Burton, 1976). The “low road” is hypothesized to support coarse processing of emotional information (Krolak-Salmon, Henaff, Vighetto, Bertrand, & Mauguiere, 2004) via the magnocellular pathway, which is characterized by low spatial resolution and rapid transmission of nerve impulses(Amaral, Behniea, & Kelly, 2003) and is thought to be phylogenetically old (Lamme, 2006). Thus, its output may remain implicit or unconscious (Carlsson et al., 2004; Vuilleumier, Richardson, Armony, Driver, & Dolan, 2004). This “low-road” is more importantly hypothesized to include a nucleus located in the medial temporal lobe called the amygdala. An influential view of the amygdala which stems from early studies of its function was that it acts as a generative locus of social cognition and behavior, required to link the perception of any stimuli in the environment to information about their emotional or biological value to the organism (Weiskrantz, 1956). Apparently, the amygdala requires minimal attentional resources to be engaged by stimuli with emotional value (Habel et al., 2007; Vuilleumier, Armony, Driver, & Dolan, 2001b; Williams, Morris, McGlone, Abbott, & Mattingley, 2004). Furthermore studies have found strong support for a modulatory role of the amygdala in visual processing, even at very early stages. Physiological studies on the amgydaloid projections in macaques found evidence for amygdala projections to the ventral visual stream, from rostral temporal cortical area TE to primary visual cortex (V1) (Amaral, et al., 2003). Indicating that the amygdala may have substantial modulatory control over sensory processing at all stages of the ventral-stream (Amaral, et al., 2003).

In contrast to the “low road”, the parallel “high road” extends from the thalamus into the higher visual areas in the occipital and temporal lobe. The higher visual areas receive input predominantly from the parvocellular pathway which is characterized by high spatial resolution and slow transmission of nerve impulses. Furthermore this neural pathway would have several processing stages from the primary visual cortex before reaching the inferotemporal cortex that provides a direct connection to the amygdala. The amygdala would receive a more detailed and accurate representation of a stimulus, but with each stage there would be increasingly complex processing of incoming information, and consequently adding processing time. Furthermore this complex processing requires extensive attentional resources

(Jiang & He, 2006; Vuilleumier, et al., 2001a). The apparent advantage of a "quick and dirty" thalamic-amygdala route is clear: It's better to mistake a stick for a snake, then a snake for a stick.

As pointed out above the subcortical "low road" supports coarse visual information through the magnocellular pathway which carries spatial frequencies in the low spectrum. The spatial frequency theory is based on a atomistic assumption meaning that even very complex images is an assemblage of many primitive spatial "atoms" (Palmer, 1999). The primitives in spatial frequency theory are two dimensional patterns whose luminance vary according to a sine wave over one spatial dimension and are constant over the perpendicular dimension (Palmer, 1999. p. 159) and are called sinusoidal gratings. Spatial frequencies refer to the width of the light and dark bars that the grating consists of. Low-frequency gratings have thick bars, and high-frequency gratings have thin bars. Spatial frequencies is in literature normally specified in term of the number of light/dark cycles per degree of visual angle, this quantity varies inversely with stripe width as in figure 1 (Palmer 1999. p.160).

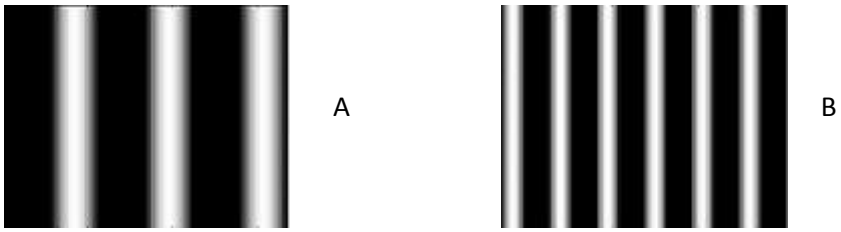

Figure 1. Two sinusoidal gratings A and B. B have a higher (6 cycles/image) spatial frequency than A (3 cycles/image).

The Fourier theorem states that any two-dimensional image can be analyzed into the sum of a set of sinusoidal gratings that differ in spatial frequency, orientation, amplitude, and phase (Palmer 1999. p.160). Fourier analysis may also be applied to highly complex images of people, objects and entire scenes. This provides us with the opportunity to decompose images in the same way as the visual system might do. A complex image consists of too many sinusoidal gratings to actually present here. But it is fully possible to show what kind of information is carried by different ranges of spatial frequencies. Figure 2. A is an image of a face containing spatial information in all spatial frequencies. Figure 2. B is an image of the same face as in A, but contains spatial information in the low spatial frequency spectrum and therefore only shows the coarse spatial structure of the image. Figure 2. C in contrast to B, only contains spatial information in the high spatial frequency spectrum and therefore only

shows the detailed spatial structure such as edges and smaller details. By using visual stimuli which has been decomposed according to the Fourier theorem it is possible to study the effect of isolating certain features of a visual scene and how it impacts the processing of spatial frequencies in the early stages of visual processing.

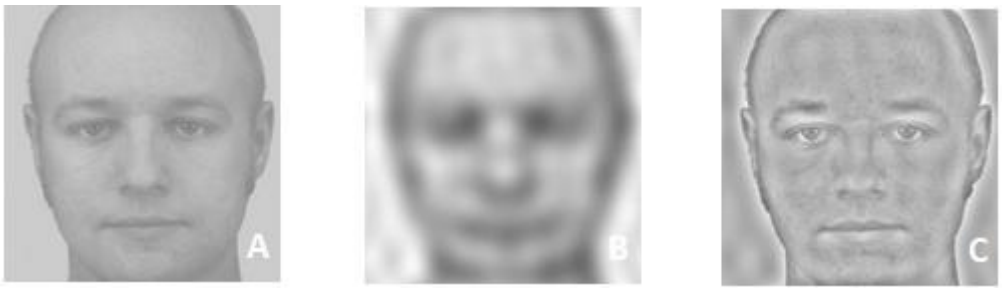

Figure 2. A broadband image (left image) containing all spatial frequencies have been Fourier transformed into its low spatial frequency information (middle image), and its high spatial frequency information. The low spatial frequency image contain the carries the global pattern of the light and dark areas (shading) of the face, whereas the high spatial frequency image carries the local contrast information (edges or contours) of the face.

Spatial frequency theory proposes that early visual system processing consists of a large number of psychophysical channels that are selectively tuned to a limited range of values within spatial frequencies or orientation of sinusoidal gratings (Palmer, 1999). Research has demonstrated that there exist specific spatial frequency channels that process high and low spatial frequencies in different areas in striate and extrastriate visual cortices (Iidaka, Yamashita, Kashikura, & Yonekura, 2004; Rotshtein, Vuilleumier, Winston, Driver, & Dolan, 2007).

A highly convincing study by Vuilleumier and colleagues' (2003) utilized emotional facial expressions in either low or high spatial frequencies found significantly greater activation in the amygdala for intact and low spatial frequency emotional faces then for high spatial frequency faces. They found that the amygdala essentially was "blind" to most of the visible spatial frequency range (<6 cycles/image) with no increase in amygdala activity from baseline. In contrast, the fusiform cortex in the temporal lobe showed significantly greater activity when participants were presented with either intact or high spatial frequency (≥24 cycles/image) images, regardless of emotional expression (Vuilleumier, et al., 2003). Another study showed enhanced fusiform cortex responses to hybrid faces containing fearful emotional expressions when such cues are present the low spatial frequency range. This effect was found in the fusiform face area (FFA) and might be a result of amygdala's modulatory control (Winston, Vuilleumier, & Dolan, 2003). Furthermore fMRI studies have shown that

processing of emotional expressions carried by low spatial frequency information is rapid, automatic and demand no attentional resources (Dumas et al., 2010; Vuilleumier, et al., 2003; Whalen, et al., 1998; Winston, et al., 2003). Indirect evidence for the importance of low spatial frequency components in face comes from statistical image analysis. The image analyses show that the decomposition of spatial frequency, which occurs in the human visual system, is able to classify fearful expression on the basis of low spatial frequency information alone. And at the same time indicating that high spatial frequency information might impair this classification. Consequently, the visual information provided to amygdala by the magnocelluar layers would seem to be efficient at least on a statistical level (Mermillod, Vuilleumier, Peyrin, Alleysson, & Marendaz, 2009). This is consistent with studies showing that wide-open eyes are one of the most important perceptual cues for identifying fear (Adolphs et al., 2005). These studies provides evidence which indicates that processing of emotional expressions takes place in a neural "low road" that supports rapid processing of low spatial frequency information and demands no attentional resources. One thing that is still unclear is where the low and high spatial frequency information streams converge to produce our conscious perception of faces. One area that several studies has been pointed out as a candidate for performing this task is the FFA (Eger, Schyns, & Kleinschmidt, 2004; Vuilleumier, et al., 2003; Winston, et al., 2003).

This study takes advantage of a perceptual technique, originally pioneered by Schyns and Oliva (Oliva, Torralba, & Schyns, 2006; Schyns & Oliva, 1994, 1999). By using a two-dimensional Fourier transformation on an image it is possible extract different range of spatial frequencies. To produce a "hybrid" image you superimpose a facial image at a coarse spatial scale upon a different facial image at a fine spatial scale. The low spatial frequencies carry the global information about the coarse spatial structure (such as wide-open eyes) and high spatial frequencies carry information about the edges and smaller details of the face. The result is a hybrid image that carries different information in different spatial frequency ranges. Hypothetically this gives hybrid stimuli an advantage over backward masking technique since hybrid stimuli avoid the interruption of visual processing of the emotional information (Laeng et al., 2010). This means that an image with emotional content in the low spatial frequency spectrum, and emotionally neutral content in the high spatial frequency spectrum, will result in processing of the emotional content even though participants can not report having a conscious experience of the emotional content. The fact is that the unconsciously perceived low spatial frequency information is a constituent part of the stimulus that remains available

to all visual areas at all times, and in principle, it could be attended together with the remaining spatial frequency information (Laeng, et al., 2010).

Several studies using this perceptual technique have shown that using hybrid images is an ideal way of studying the neural basis for processing of spatial frequencies (Iidaka, et al., 2004), and testing dissociable processing of high and low spatial frequency information in faces (Rotshtein, et al., 2007; Winston, et al., 2003). The only study known to the author to have utilized hybrid faces to investigate the possibility of unconscious processing of emotional facial expression is by Laeng and colleagues' (2010). This study is also the only study which used congruent faces (same sex and identity). In their study Laeng and colleagues' (2010) demonstrated that participants rated portrayed persons as "friendly" when the lowest spatial frequencies contained a positive facial expression and "unfriendly when the lowest spatial frequencies contained a negative facial expression. They also showed that one patient who had the left anterior temporal lobe surgically resected (including amygdala), did not show the same effect of unconscious processing as the healthy participants.

There is however one potential weakness in studies which uses stimuli with a limited spatial frequency range (either high or low spatial frequency images) as pointed out by Rotshtein and colleagues' (2007). The observed activation may be the result of the fact that these stimuli can differ remarkably in their visual appearance and in their energy, contrast and luminance. As explained above these activations may be the result of the brain choosing another processing strategy to interpret the visual stimuli (Morrison & Schyns, 2001; Schyns & Oliva, 1997). This same weakness may also be relevant in studies using hybrid images with an incomplete spatial frequency spectrum. Rotshtein and colleagues' (2007) used a high bandpass at >24 cycles/image and a low spatial bandpass at <8 cycles/image. As stated by spatial frequency theory any visual scene can be decomposed into spatial frequencies from the entire spectrum, thus removing some of the frequencies that gives invaluable information in perceptual categorization and visual recognition (Costen, Parker, & Craw, 1996; Palmer, 1999; Ruiz-Soler & Beltran, 2006). The observed activations might potentially reflect processing of ambiguous and/or unusual stimuli rather than processing of emotional content. Studies have shown that the mid-level spectrum of spatial frequencies is important in processing faces (see Ruiz-Soler & Beltran, 2006, for extensive review).

This paper consists of two experiments; the first experiment is an attempt to replicate the seminal study of Vuilleumier and colleagues' (2003) whereas the second experiment

utilizes the hybrid picture paradigm first developed by Schyns and Oliva (1994) and later modified by Laeng and colleagues' (2010) to study the processing of core emotions. The stimulus material in the Vuilleumier replication (Experiment 1) consists of six different conditions. The conditions are as follows: 1) fully neutral broadband images of males and females containing all the original spatial information (NeuBB); 2) fully fearful broadband images of males and females containing all the original spatial information (FearBB); 3) neutral images with a low-pass cut-off of <7 cycles/image of males and females extracted from the original spatial information (NeuLF); 4) fearful images with a low-pass cut-off of <7 cycles/image of males and females extracted from the original spatial information (FearLF); 5) neutral images with a high-pass cut-off of <8 cycles/image of males and females extracted from the original spatial information (neutral NeuHF); 6) fearful images with a high-pass cut-off of <8 cycles/image of males and females extracted from the original spatial information (FearHF)(see Figure 4). The stimulus material in the hybrid experiment (Experiment 2) consisted of ten different conditions.

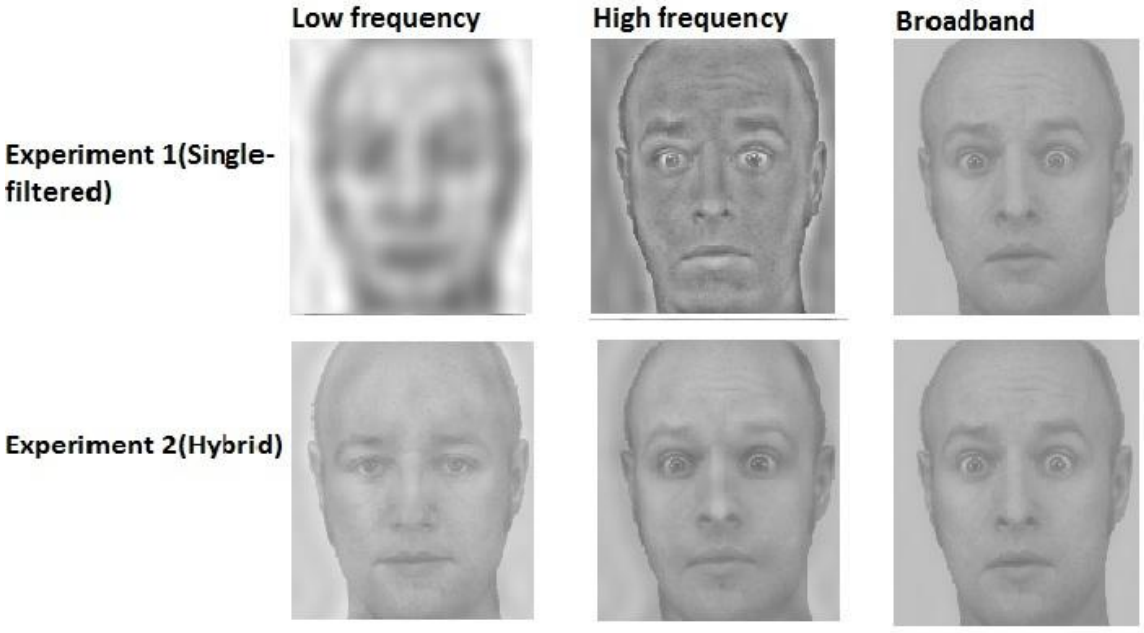

Figure 3. First row show how images have been filtered in Experiment 1. Second row demonstrates of FearHybLF, FearHybHF and a broadband images differ from each other.

The conditions were as follows: 1, 2, 3) fully neutral, fearful, angry and happy broadband images of males and females containing all the original spatial information (NeuBB, FearBB, AngryBB, HappyBB); 5, 6, 7) hybrid images composed of high frequency fearful, happy and angry expressions (8-128 cycles/image; FearHybHF, AngerHybHF, HappyHybHF) superimposed onto a neutral image in the low spatial frequencies (1-7 cycles/image); 8, 9, 10) hybrid images composed of low frequency fearful, happy and angry expressions (1-8

cycles/image; FearHybLF, AngerHybLF, HappyHybLF) superimposed onto a neutral image in the high spatial frequencies (8-128 cycles/image)(see Figure 3).

Previous research has shown that fearful expressions generate significantly stronger emotional activation in the amygdala than other emotions (Whalen et al., 2001), but we also took under consideration that the amygdala could rapidly habituate to repeated emotional faces, with 'fear' producing the largest decrement in amygdala activation (Fischer et al., 2003; Strauss et al., 2005; Wright et al., 2001). Therefore we decided to include happy and angry facial expressions in the hybrid experiment so as to hinder rapid habituation (Breiter et al., 1996; Whalen, et al., 1998).

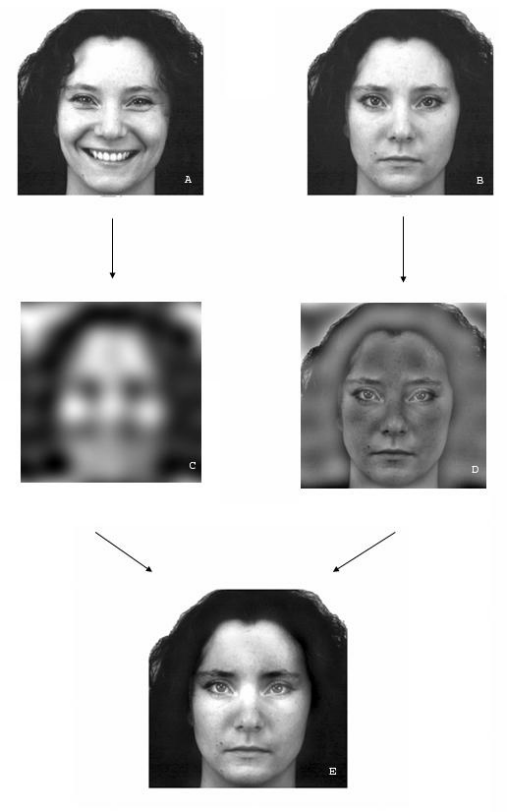

Figure 4. An example of the process of creating a hybrid face: original A and B are different images of the same model expressing two different emotions (happy and neutral). Image C is a low pass version (≤6 cycles/image) of Image A, whereas Image D is the high pass version (≥7 cycles/image) of Image B. Image E is the hybrid image or the combination of Image C and Image D. (Courtesy of Bruno Laeng; from (Laeng, et al., 2010).

The goal in the present study is therefore twofold: 1) to replicate the result from the seminal study by Vuilleumier and colleagues' (2003); 2) and to further investigate the neural substrate of unconscious processing of emotions by using the hybrid paradigm in an event-related fMRI design. Based upon results from previous research indicating the amygdala as an

important structure in emotional processing, the main focus of this paper will be on activations in amygdala.

In Experiment 1, we predict higher amygdala activity for broadband and low frequency fearful expressions compared with high frequency fearful expressions. Furthermore we predict higher amygdala activity in the condition FearLF in comparison to fully neutral broadband images and neutral low frequency images due to the low spatial emotional information in the FearLF images. In Experiment 2 we expect higher amygdala activity for FearHybLF (emotion is "unseen") in comparison to fully neutral broadband images. FearHybLF is also expected to produce more amygdala activity than FearHybHF because of the low frequency emotional information. Provided that these predictions are supported by the results then there would be support for the idea that processing of emotional information in amygdala is mainly carried by low spatial frequency information. Such evidence would also provide indirect evidence of the existence of a subcortical "low-road" with direct connections from thalamus to amygdala. Findings supporting the predictions in Experiment 2 would indirectly support the idea that emotional information could be processed by the brain without reaching conscious awareness.

Materials and Methods

Overview

This study consists of two experiments both using fMRI to estimate activity changes in amygdala when participants are simply viewing spatially filtered images of neutral and emotional images, and when participants are rating hybrid face images from unfriendly to friendly in the MR-scanner. One fMRI session consisted of a localizer phase for mapping of individual regions of interest, and three experimental phases (one phase for Experiment 1 and two phases for Experiment 2).

Subjects

Seventeen healthy participants (females = 9), all with Norwegian as native language, volunteered to participate in the study (mean age= 23.2; SD= 4.9; range = 19-31). All participants had normal or corrected-to-normal vision, no reported neurological or psychiatric history and no structural brain abnormality. All participants underwent a MRI-security check list and gave informed consent in line with the regional ethics regulations.

Experiment 1 (Single filtered image paradigm)

Stimuli and stimulus presentation

The original stimulus material consisted of 140 colour photos of fearful and neutral faces from the Karolinska Directed Emotional Faces (Calvo & Lundqvist, 2008; Lundqvist, Calvo, & Öhman, 1998). Thirty-five male and 35 female models were chosen from the Karolinska database. Each model displayed either a fearful or neutral expression. All photos were cropped; gray scaled, matched on contrast and luminance, and resized to 256 × 256 pixels using standard routines in Photoshop (version CS5; Adobe Systems Inc., USA).

Spatial frequency information was filtered from each processed image using a custom script written by the author in MatLab (version 2009b; The MathWorks Inc., USA). Low frequency emotional images (FearLF) were filtered with a low pass cut-off of ≤7 cycles/image (1-7 cycles/image). The same low pass cut-off was used to make low frequency neutral images (NeuLF). High frequency emotional images (FearLF), was filtered with a high pass cut-off of ≥8 cycles/image (8-128 cycles/image). The same high pass cut-off was used to make the high frequency neutral images (NeuHF). Emotional and neutral broadband images were created by conducting an inverse Fourier transform on the two spectra combined (the

coinciding facial expressions were combined with another image portraying the same person, resulting in the final images FearBB and NeuBB).

Stimuli were back-projected (resolution 1400 × 1050 pixels) onto a mirror mounted mirror on the MRI head coil (visual angle 7°, distance 67.5 cm). The visual angle was 7° and the distance 67.5 cm, ensuring that the cycle/image parameters corresponded with those of Winston and colleagues' (2003).

Procedure

Participants were shown faces from six different conditions in an event-related design. Each of the six conditions had 44 images each (22 females). Each face was only shown once in each condition. In total, there were 264 trials. All the conditions were shown in a randomized order. The start of the first trial was synchronized with a trigger from the MR-scanner using a SyncBox (NordicNeuroLab, Norway, Bergen). The duration of one trial was 1.5 TRs (3000 ms). A centrally presented fixation cross was present on screen during a trial and rest, except when a stimulus was presented. Each stimulus was presented for 250 ms, and stimulus onset-time was 1000 ms. All trials were jittered with a variable inter-trial interval (ITI) with an average of 6 seconds. The task consisted in simply passive viewing. Participants were informed before the experiment that they would be asked to see a series of facial images. Furthermore participants were instructed to not move their bodies while in the scanner, and to stay alert and focused during the experimental phases. To further prevent movement foam rubber cushions were used to restrain the head within the MRI head coil. All participants used double hearing protection, and were able to communicate with the experimental leader through an intercom system between each sequence. This experimental phase lasted about 16 minutes.

Experiment 2 (Hybrid paradigm)

Stimuli and stimulus presentation

The original stimulus material consisted of 264 colour photos from the Karolinska database. The selected models were 33 male and 33 female models. Each model displayed four different emotions in full frontal view (fear, anger, happiness and neutral).

The spatial frequency content from each image was filtered with the same custom MATLAB script (version 2009b; The MathWorks Inc., USA) used in the previous

experiment. To produce hybrid images with the emotional content in the low frequency range (FearHybLF, AngerHybLF, HappyHybLF), emotional images were filtered with a low pass cut-off of ≤7 cycles image to obtain the low frequency images (1-7 cycles/image); neutral images were filtered with a high pass cut-off of ≥8 cycles/image producing the high frequency range images (8-128 cycles/image). To obtain the images with emotional content in the high frequency range (FearHybHF, AngerHybHF, HappyHybHF) and a neutral expression in the low frequency range, a low pass cut-off of 7 cycles/image were used for the neutral images, and a high pass cut-off of 8 cycles/image for the emotional images. To create the final hybrid images an inverse Fourier transform were conducted on the two spectra combined (low frequency emotional from one image combined with high frequency neutral from another image, or vice versa). Broadband images (FearBB, AngerBB, HappyBB and NeuBB) were also obtained with an inverse Fourier transform (two corresponding low and high frequency expressions are combined).

Stimuli were presented with the same parameters and equipment as in the previous experiment.

Procedure

Participants were shown faces from seven different conditions in an event-related design. Each of the six conditions had 66 images each (33 females). Each face was only shown once in all conditions. In total, there were 440 trials divided between two experimental phases (220 trials in each phase). All conditions were presented in a randomized order. As in the previous experiment the first trial synchronized with a trigger from the scanner. The duration of one trial was 1.5 TRs (3000 ms). A centrally presented fixation cross was present on screen during a trial and rest, expect when a stimulus and response options were presented. Each stimulus was presented for 250 ms directly followed by response options that were presented for 1750 ms, and stimulus onset-time was 1000 ms. All trials were jittered with a variable intertrial interval (ITI) with an average of 6.2 seconds. The participants' task was to rate how unfriendly – friendly they perceived each image with a MRI compatible response pad (1 = unfriendly; 2 = slightly unfriendly; 3= slightly friendly; 4 = friendly). Participants were told that they were to rate and observe a series of facial images, and to answer as best as they could. Furthermore participants were instructed to not move their bodies or heads while in the scanner and to stay alert and focused during the experimental phases. To further prevent movement foam rubber cushions were used to restrain the head within the MRI head coil. All

participants used double hearing-protection, and were able to communicate with the experimental leader through an intercom system between each sequence. Each experimental phase lasted 16 minutes.

ROI-localizer

After the three experimental phases, participants went through a ROI-localizer phase. The localizer allow for region of interest definition independently from the main experiments. The localizer was not created by the author and the information that follows were provided by the creators of this localizer (Kristiansen & Viken, 2008). Participants were shown images of non-manipulated emotional faces, neutral faces and buildings in a block-design. A study on improved mapping of human amygdala (Morawetz et al., 2008) showed that a blocked design with passive viewing gave improved functional mapping of amygdala. The set of images showing buildings were chosen on the basis of research done by Henderson and colleagues' (Henderson, Larson, & Zhu, 2008) where they showed that full scenes (the entire building is observable) produce greater activation in parahippocampal area (PPA) than close-up scenes. The stimuli was presented foveally (in the central eye field) with a size of 500 × 500 pixels, and used the same equipment and fMRI parameters as the experimental phases. One block consisted of 16 stimuli from the same condition. Each trial started with the presentation of a fixation cross (200 ms), followed by a stimulus (300 ms). This order was followed until all 16 stimuli constituting a block had been presented. The duration for one block was 8000 ms. Each block was presented 10 times. Every face was presented on average 2.5 times; the same face was never presented more than 3 times and never less than 2 times. Every building was presented 4 times during the localizer phase. Before the first block, between each block, and after the last block there was a rest-period of lasting of 12000 ms. The order of the blocks was counterbalanced between subjects, and a condition could never be followed by the same condition. Furthermore all stimuli could never be presented twice within the same block. Total duration for the localizer phase was about 12 minutes.

Image Acquisition

Functional magnetic resonance imaging (fMRI) data were acquired on a Philips Achieva 3 Tesla whole body MR unit (Philips Medical Systems, Best, The Netherlands) at the The Interventional Centre at the Oslo University Hospital. The scanner is equipped with an 8-channel Phillips SENSE head coil. Functional images were obtained with blood oxygen level-dependent (BOLD) sensitive T2*-weighted echo-planar imaging (EPI) sequence. The same

functional imaging parameters were used in both experimental and localizer runs: 34 transversally oriented slices (no gap) were placed to include the participants' amygdala and fusiform cortex. Volumes were acquired with an interleaved slice acquisition with a repetition time (TR) of 2000 ms, an echo time (TE) of 35 ms, and a flip angle of 70°. Voxel size was 3×3×3 mm, and the field of view (FOV) measured 240×240×102 mm.

Anatomical T1-weighted images were obtained with a turbo field echo (TFE) pulse sequence with a TR of 8.48 ms, TE of 2.3 ms, and a flip angle of 8°. This whole-brain structural volume consisted of 170 sagittally-oriented slices with a voxel size of 1×1×1.2 mm. The FOV measured 256×256×204 mm. The slices of the structural volume were aligned with the AC-PC line.

A total of 500 scans were acquired in the first experimental run, 960 scans in total from the second experimental run (two phases), and 380 scans were acquired in the ROI-localizer run. The total time for a complete session in the scanner was 53 minutes.

Preprocessing

Preprocessing was conducted in SPM8 (Friston et al., 1995) The functional scans were realigned by estimating six parameters for rigid-body transformation and translation (quality 0.9; separation 4; registered to mean; 2^{th} degree B-spline interpolation). Scans were further co-registered against the individual whole-brain anatomical volume. Functional scans were spatially normalized to a standard template from the Montreal Institute of Neurology (MNI) (3×3×3 mm voxel size for the functional scans; trilinear interpolation). Reported coordinates in this paper are in MMI space.

fMRI data analysis

A statistical analysis was first conducted on a fixed effects single-subject level based on the General Linear Model in SPM8 (Friston, et al., 1995). Low-frequency drifts were removed using a standard temporal high-pass filter (cut-off 128 s). The design matrices were generated using event-related regressors convolved with the canonical hemodynamic response function. In the second-level group analysis a whole brain *t*-test analysis was modelled with a random-effects group model in SPM8. ROIs were calculated based on the localizer run, and percentage signal change within the ROI was calculated by the SPM8 region of interest

toolbox (Brett, Anton, Romain, & Poline, 2002) MarsBaR (version0.42; http://marsbar.sourceforge.net).

The ROI model was specified by using 3 regressors, each representing the onset-times of one of the three following conditions: EmoFaces, NeutralFaces and Places. The three regressors were modelled as epochs with duration of 8 s (corresponding with the duration of a block in the ROI localizer). Two contrasts were defined for the localizer data: EmoFaces and NeutralFaces over Places (Faces>Places); Emofaces over Places (Faces>Places). The first contrast was used to detect face responsive voxels in the fusiform cortex that did not respond to buildings. This contrast also produced a strong right amygdala activity, and was used to define the right amygdala ROIs. The second contrast produced a strong left amygdala activity, and was used to define the left amygdala ROIs. How these ROIs are generated is described later.

For Experiment 1, a model was specified using 6 regressors, each representing the onset times of the 6 conditions: EmoLF-faces; EmoHF-faces; Emo broadband faces; NeuLF-faces; NeuHF-faces; and Neutral broadband faces. All regressors were modelled as events with duration of 0 s. Four t-contrasts were specified for the data: FearLF>NeuBB; FearBB>NeuBB; FearLF>NeuLF; and FearHF>NeuHF.

For Experiment 2, a model was specified using 10 regressors, each representing the onset times of the 10 conditions; FearLF-faces; FearHF-faces; Fear broadband faces; AngerLF-faces; AngerHF-faces; Anger broadband faces; HappyLF-faces; HappyHF-faces; Happy broadband faces; and Neutral broadband faces. All regressors were modelled as events with duration of 0 s. Three t-contrasts of interest were specified for the data: FearHybLF>NeuBB; FearHybLF>FearHybHF; and FearHybHF>FearHybLF.

Whole-Brain Analysis

To investigate differences in activation patterns outside the region of interest, we performed a whole-brain analysis for the seven contrasts specified above for the two experiments. The contrasts from the single subject fixed effects model was used to specify a random effects group model in SPM8. One sample *t*-tests were run for all contrasts. Significance threshold was set to $p < 0.001$ (uncorrected; cluster defining threshold: 10 voxels). The number of slices

used in the EPI sequence did not cover the whole brain; therefore patterns of activation outside these areas could not be detected.

ROI Analysis

A combination of functional and anatomical approach was used to define bilateral amygdala as regions of interest. The anatomical ROI was made by following a well established protocol to measure the amygdaloid volume (Watson et al., 1992). Bilateral amygdala was manually drawn by using the volume of interest (VOI) tool in MRIcroN (version 1, 2010; http://www.cabiatl.com/mricro/).

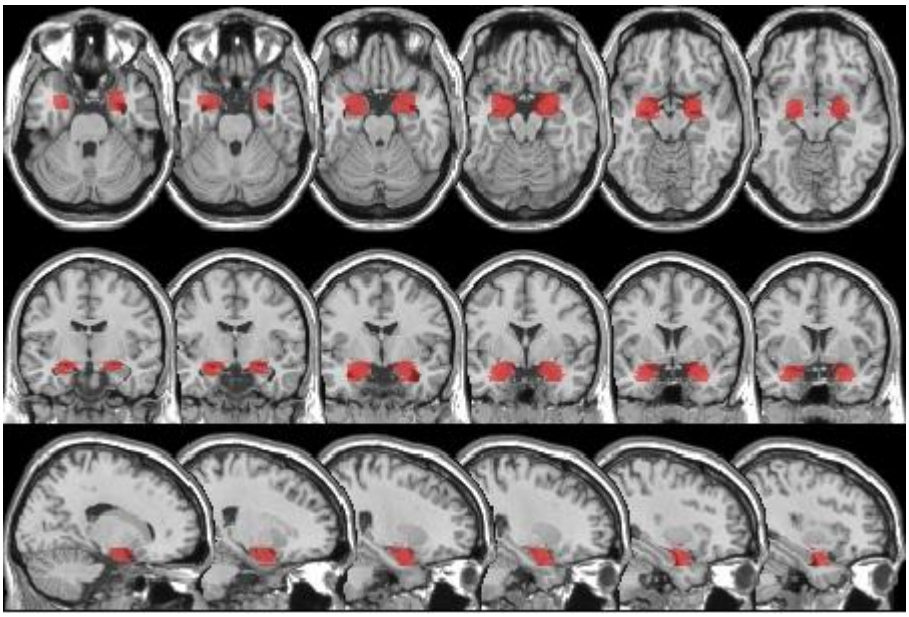

Figure 5. The picture shows six different axial, coronal and sagittal slices of an averaged anatomical T1-weighted image (SPM template). The volume of interest (red) was drawn in MRIcroN, using Watson's protocol for defining the amygdala.

The amygdalae's structures were drawn upon an averaged single subject anatomical T1-weighted image (see Figure 4). The VOI was checked up against each subject's normalized anatomical structural image to ensure that the VOI matched their amygdaloid structures. The MRIcroN-VOI was then converted into a SPM8 mask with the toolbox MarsBaR for statistical modelling. The contrast images from the single subject fixed effects localizer models (contrast: Faces>Places and EmoFace>Places) were used to extract the coordinated for bilateral amygdala cluster maximas within the anatomically defined ROIs in each subject. All statistical analyses of bilateral amygdala were conducted on data extracted from the functional ROIs (See Figure 5; see Appendix A, Table 1 for individual cluster sizes).

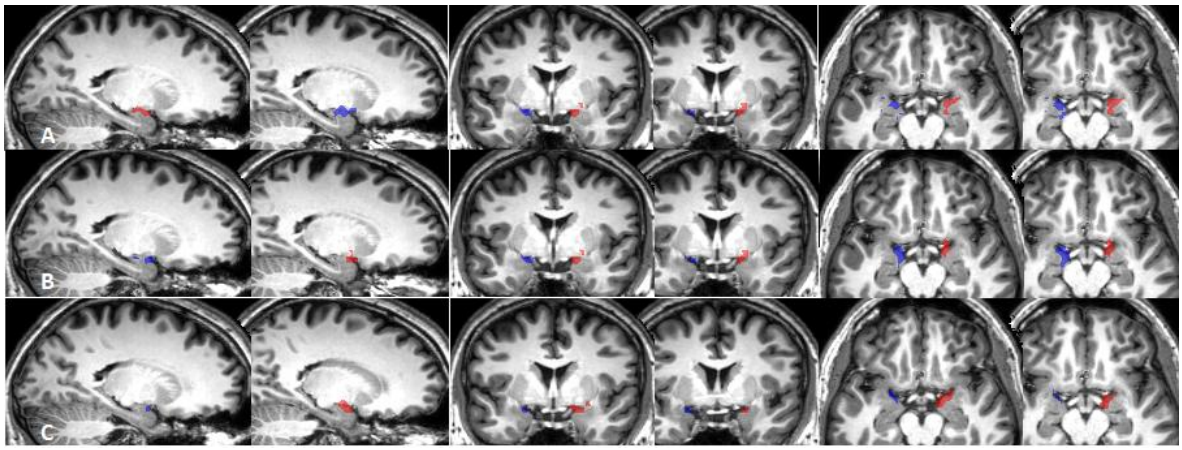

Figure 6. The image shows the functional ROIs for three participants (A, B and C) in sagittal, coronal and axial slices.

The statistical analysis for estimation of percentage signal change within bilateral amygdala ROIs was conducted in the following steps: The fixed effects model for each participant was imported into MarsBaR, and then the mean values for each condition within the individual functional ROIs were extracted. The percentage signal change values for each participant was exported to SPSS and a paired *t*-tests were run for the contrasts in interest for both experiments.

Rating Task Analysis

The analysis of the behavioural results from Experiment 2 was conducted on group level using SPSS. We wanted to validate whether the emotional content was truly hidden in the low spatial frequency range and also replicate Laeng and colleagues' (2010) findings. Paired *t*-tests was applied to all contrasts and corrected for multiple within-subject comparisons.

Results

Experiment 1 (Single filtered image paradigm)

Amygdala modulation

Our initial hypothesis aimed to replicate the findings by Vuilleumier and colleagues' (2003) but contrary to these prediction the estimation of percentage signal change in the two amygdala ROIs did not show any significant difference in the FearBB>NeuBB comparison, the FearHF>NeuHF, nor the FearLF>NeuLF comparison (Table 1). Possible explanations for these null results will be the presented in the discussion. A significant difference in signal change was found when we compared the condition EmoLF with the condition NeuBB in left amygdala ROI (Table 1). Furthermore there was a significant difference in signal change when we compared FearHF with FearLF in right amygdala (Table 1).

Table 1. Results: left and right amygdala. Negative numbers indicate that there is negative activity in contrast to baseline. *$P < 0.05$ (uncorrected)

	SD	S.E.M	*P*<	% signal change
Left Amygdala ROI				
FearLF>NeuBB	.110	.028	.025*	.071
FearBB>NeuBB	.127	.032	.15	.05
FearHF>NeuHF	.090	.023	.30	.025
FearLF>NeuLF	.137	.035	.24	.043
FearHF>FearLF	.101	.026	.91	.003
Right Amydala ROI				
FearLF>NeuBB	.15	.04	.56	-.023
FearBB>NeuBB	.122	.031	.42	.026
FearHF>NeuHF	.093	.024	.08	.045
FearLF>NeuLF	.161	.041	.46	-.031
FearHF>FearLF	.116	.03	.04*	.066

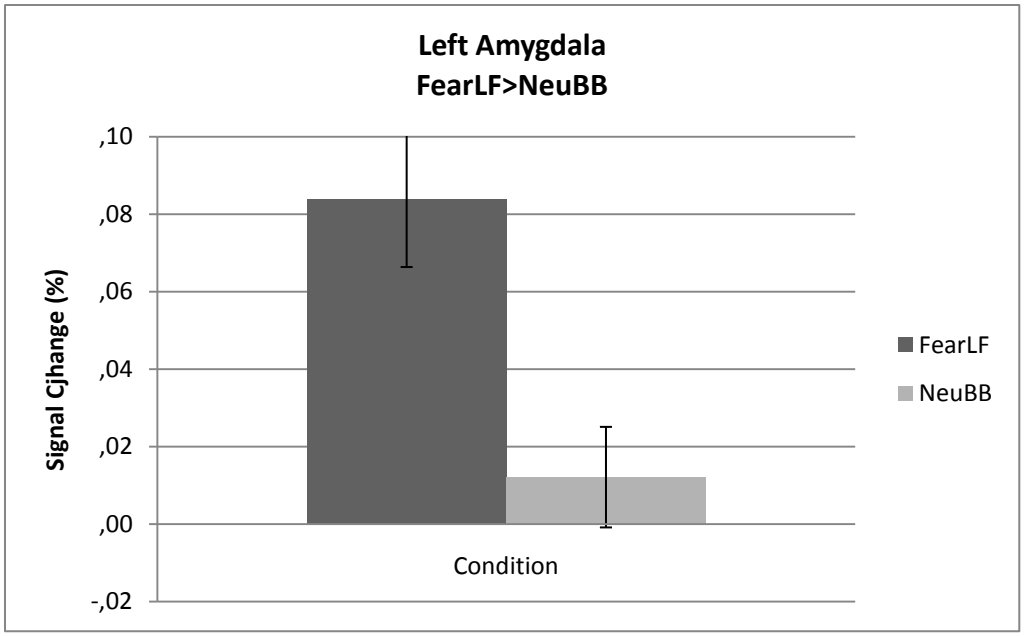

Figure 7. Estimated percent signal change (± s.e.m.) in left amygdala for the comparison FearLF>NeuBB ($p < 0.05$).

The difference in percentage signal change in left and right amygdala is shown in Figure 7 and Figure 8, respectively. Signal change in bilateral amygdala for the comparison FearHF>FearLF are shown in Figure 9 and Figure 10, respectively.

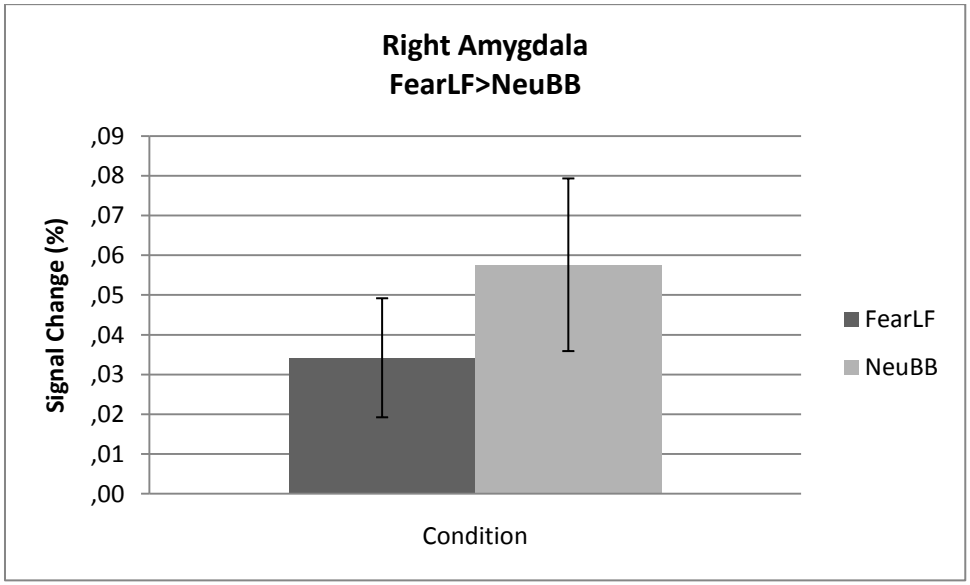

Figure 8. Estimated percent signal change (± s.e.m.) in right amygdala for the comparison FearLF>NeuBB ($p > 0.05$).

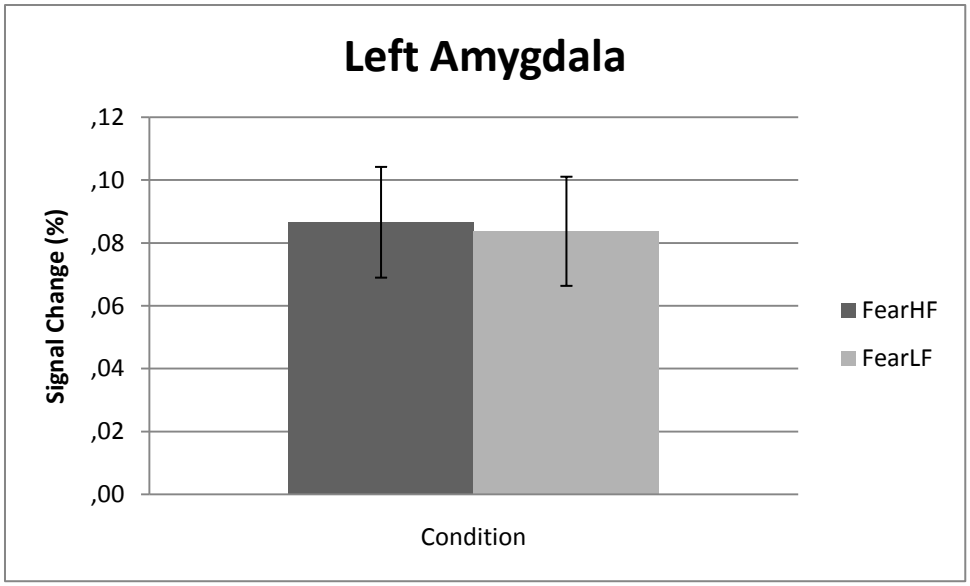

Figure 9. Estimated percent signal change (± s.e.m.) in left amygdala for the comparison FearHF>FearLF (p>0.05)

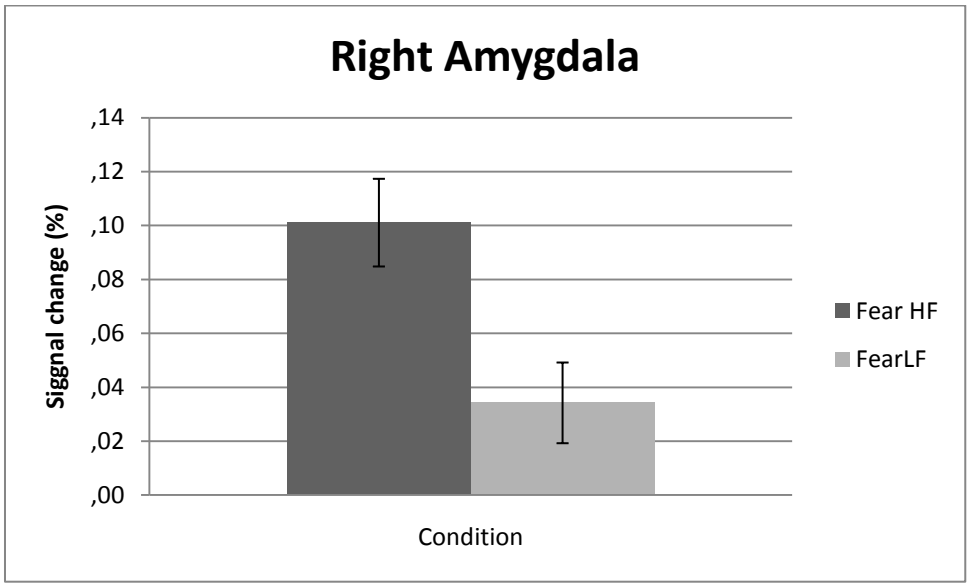

Figure 10. Estimated percent signal change (± s.e.m.) in right amygdala for the comparison FearHF>FearLF (p<0.05)

Whole-brain Analysis

The whole brain random effects group analysis did not show any clusters with increased activation surviving the threshold $p < 0.001$ for the comparisons in interest: FearBB>NeuBB; FearLF>NeuLF; and FearHF>NeuHF. Possible explanations will be investigated in the discussion.

Experiment 2 (Hybrid paradigm)

Behavioral data

Descriptive statistics were estimated to obtain the individual mean ratings so as to perform within-group pair wised comparisons. The confidence interval was set to 95% and was corrected for multiple comparisons when comparing individual emotions with NeuBB. HappyBB, HappyHybLF and HappyHybHF were significantly rated friendlier than NeuBB. The remaining nine conditions were significantly rated as less friendly as NeuBB.

Table 2. Paired *t*-tests comparing all emotions to NeuBB. A value of 0 is considered neutral, negative numbers indicate negative valence, while positive numbers indicate positive valence. $P < 0.05$ corrected within-group comparison.

Contrasts	SD	s.e.m.	p<	Mean difference
AngerBB>NeuBB	.35	.071	.000	-.956
AngerHybLF>NeuBB	.11	.022	.000	-.255
AngerHybHF>NeuBB	.32	.064	.000	-1.055
FearBB>NeuBB	.23	.058	.000	-.404
FearHybLF>NeuBB	.12	.024	.169	-.035
FearHybHF>NeuBB	.29	.058	.000	-.47
HappyBB>NeuBB	.32	.064	.000	1.22
HappyHybLF>NeuBB	.27	.054	.000	.311
HappyHybHF>NeuBB	.43	.086	.000	.973

Amygdala Modulation

The estimation of percentage signal change did not reveal any significant modulations in bilateral amygdala ROIs for the contrasts FearHybLF > NeuBB, FearHybLF>FearHybHF, and FearHybHF>FearHybLF ($p > 0.05$).

Whole-brain Analysis

The whole-brain analysis did not reveal any significant increase in activation for the FearHybLF condition compared with the other two conditions; FearHybHF and NeuBB. This was not in line with our initial hypotheses. Neither the comparison between FearHybHF and FearHybLF did reveal significant patterns of activation.

Discussion

The goal of Experiment 1 was to replicate the results of Vuilleumier and colleagues (2003) seminal study with single-filtered, low-passed, images of fearful and neutral expressions versus fearful and neutral expressions in the high frequency spectrum. Broadband images of neutral and fearful expressions were also included. In contrast to Vuilleumier and colleagues original paradigm, we included the entire spatial frequency spectrum (low frequency images 1-7 cycles/image; high frequency images 8-128 cycles/image without excluding a mid-range window (8-24 cycles/image). Therefore by including the middle spatial range we avoid results that could reflect changes in processing strategies, due to the loss of invaluable spatial information which might contain critical information for perceptual categorization and visual recognition of emotional expressions. In our initial predictions we expected significantly higher amygdala activation for single-filtered low-passed fear (FearLF) in comparison with low frequency neutral (NeuLF) and high frequency fearful faces (FacesHF). Furthermore we predicted that FearLF would result in increased amygdala activity in comparison with neutral broadband images (NeuBB).

Contrary to our main hypothesis for Experiment 1 we did not find a significant difference in amygdala activation for FearLF compared with FearHF. On the contrary we found significantly higher activation for FearHF compared with FearLF in right amygdala. This finding was limited to the right amygdala and there were no significant differences in left amygdala between FearHF and FearLF. Furthermore there was no significant increase in amygdala activation when comparing FearLF to NeuLF. We did however find a significant difference in amygdala activation when comparing low frequency fear with neutral broadband images in the left ROI. These findings might seem in contradiction with each other, but the explanation for this finding is that a single filtered low frequency expressions increases amygdala activation more than a neutral broadband image (Table 2).

Thus, the fMRI results from Experiment 1 did not support the results found by Vuilleumier and colleagues' (2003). One possible reason for the failure to replicate could be due to the fact that, compared to similar studies investigating the neural basis of emotional processing and spatial frequency content using fMRI methodology, we used the entire spatial frequency spectrum. Our low frequency images had a cut-off of $\leq$7 cycles/image and our high frequency images had a cut-off of $\geq$8 cycles/image while the study that we tried to replicate had a low pass cut-off of <6 cycles/image and a high pass cut-off of >24 cycles/image

(Vuilleumier, et al., 2003). One possible explanation for the low signal change for high frequency information in Vuilleumier and colleagues' (2003) is that the medium spatial frequency range contains critical spatial information for recognizing not just identity (Ruiz-Soler & Beltran, 2006) but also facial expressions. This could possibly explain why amygdala seems to be essentially "blind" for high spatial frequency information. In our experiment we included the 8-24 cycles/image range which several studies are missing. More importantly there has yet to be established a threshold for which spatial frequencies are leading to optimal processing in amygdala. If the medium spatial frequency range contains critical information for recognizing facial expressions it might explain why we did not find at significant difference between FearLF and FearHF in left amygdala,. Furthermore it could explain why there are significantly higher activations for FearHF in contrast to FearLF in right amygdala. Human amygdala receives projections from the anterior inferotemporal cortex that conveys highly processed object information (Pessoa & Adolphs, 2010). To further investigate the above described scenario one could conduct a study to establish which specific spatial frequency range or ranges lead to optimal processing in the amygdala. Such a study would be extensive and is beyond the scope of this study.

As stated above we did however find a significant difference in activation when we compared FearLF with neutral broad band images which is consistent with our initial hypothesis. However, to replicate Vuilleumiers and colleagues' (2003) findings the following comparisons should also have reached a significant level: FearBB>NeuBB; FearHF>NeuHF; and FearLF>NeuLF. However, we do see a trend that the mean differences between the above comparisons are in the predicted direction although these differences do not reach significance. There might very well be a methodological problem with our design or fMRI parameters. One major issue is that we did not find significantly higher amygdala activations for fearful broadband images compared to neutral broadband images, even though research has shown reliably that amygdala activity increases when presented with a fearful facial expression in comparison to a neutral facial expression (Morawetz, et al., 2008; Vuilleumier, et al., 2003; Whalen, et al., 2001; P. J. Whalen, et al., 1998; Williams, et al., 2004). Several pilot studies were performed to best determine how to design the experiment, but there might very well have been a habituation effect to emotional stimuli. Studies which have investigated rapid habituation to emotional stimuli have repeatedly shown the same emotion to participants (Fischer, et al., 2003; Wright, et al., 2001), but there might as well be a habituation effect in our design even though the emotional stimuli is jittered with variable inter-trial intervals.

Since we decided for a randomized experimental design, two stimuli from the same condition could follow each other. This could be corrected with a pseudo-randomized design which would ensure that stimulus from the same condition could not directly follow the other. In this study we utilized a rather standard EPI sequence since we also wanted reasonable volume coverage for whole-brain analysis.

There is another difference between this study and the study by Vuilleumier and colleagues (2003). They did not utilize a localizer task in their study to define their functional ROI's which was used in a percent signal change analysis. First they ran a whole-brain analysis contrasting fearful faces versus neutral faces (collapsing over all spatial frequency ranges used in their study). These fear responsive voxel clusters were then defined as functional amygdala ROI's which then were used in a signal change analysis (Vuilleumier, et al., 2003). In other words they conducted a non-independent ROI analysis (Poldrack & Mumford, 2009; Vul, Harris, Winkielman, & Pashler, 2009). This could potentially bias the results (Vul, et al., 2009). A recent article (Kriegeskorte, Simmons, Bellgowan, & Baker, 2009) have also pointed out that using the same data for selection of a ROI and selective analysis will result in invalid statistical inference. Kriegeskorte and colleagues' (2009) simulated ROI analysis based upon generated non-independent fMRI data and independent fMRI data. When they used the same data which was used in the initial mapping of the ROI for later analysis, they discovered that the noise at fringes of the ROI could lead to improved statistics and inflated effect sizes (Kriegeskorte, et al., 2009). However when they used independent data to map the ROI they did not observe the same distortions by noise as in the former example. The benefit of defining a ROI based upon a localizer task is that when you test that region with this independent dataset it is unlikely that these fringe voxels will be significant due to noise. In conclusion analyses by Vuilleumier and colleagues (2003) could have led to spurious significant results and could therefore explain why we could not replicate the results. This could especially hold true when investigating percent signal change where effects are small.

A recent study was also not able to find any evidence supporting the hypothesis of preferential activation to certain spatial frequency ranges in amygdala (Morawetz, Baudewig, Treue, & Dechent, 2011). There were no significant differences in signal between low spatial frequency information and high spatial frequency information. Neither did they find any differential response between low spatial frequencies and broadband images (Morawetz, et

al., 2011). However there was one main difference between the above mentioned study and the study by Vuilleumier and colleagues was that the former did not include neutral faces as control stimuli. The argument for not including neutral faces was that the previous study had shown that neutral faces did not modulate amygdala activity to any significant degree and that the effect of different spatial frequency ranges only were expected in the fearful context (Morawetz, et al., 2011). One could argue that since they only included fearful faces (low frequency, high frequency and broadband), the study investigates face processing in general. However to validate the stimulus material regarding valence and emotional recognition they included a behavioral experiment outside the scanner. They found that fearful faces were rated as more negative than neutral faces regardless of spatial frequency filtering. Furthermore broadband fearful faces were rated as more negative than low and high spatial frequency fearful faces. The validation ratings were in agreement with the fMRI data which showed that broadband images were associated with the highest signal changes in the amygdala. They concluded that the differential signal changes in the amygdala in response to fearful faces were not due to the result of greater intensity of consciously perceived emotion in the low spatial frequency range compared to the high spatial frequency range (Morawetz, et al., 2011). However we did find a significant differentiation between high and low spatial frequency fearful faces in right amygdala. This finding could be explained by the substantial inter-subject variability in our data. Another explanation might be that fearful faces are not recognized as fearful when only low frequency information is present. Indirect evidence comes from a study which showed that categorizing an emotional expression (happy, sad, fear and anger) is impaired when only low spatial frequency information is present (Goren & Wilson, 2006). Furthermore they discovered that fear was the hardest emotion to recognize and was often confused with a sad facial expression and also fear was often mistaken with surprise (Dailey, Cottrell, Padgett, & Adolphs, 2002). In the debriefing several participants reported that they had observed surprised faces in the experiment. These same results were also found by the validation rating in the study by Morawetz and colleagues (2011). The fMRI findings in our study and Morawetz and colleagues (2011) do not support the hypothesis that low spatial frequency information has a special role in a subcortical "low road".

In Experiment 2 we utilized hybrid images which consisted of spatial frequencies from the entire spectrum, FearHybLF (AngerHybLF and HappyHybLF) with emotional information in the low spatial frequency spectrum, and FearHybHF (AngerHybHF and

HappyHybHF) with emotional information in high range of the spectrum. The goal was to further investigate that processing of emotional information in amygdala is mainly carried by low spatial frequency information. Furthermore we investigated whether emotional information could be processed in a subcortical "low-road" without reaching conscious awareness by using stimulus where the actual emotional information is implicit or "unseen". Another reason for using hybrid images was that the material had been tested before. Laeng and colleagues' (2010) tested whether the hybrid images differed significantly from each other in respect to contrast. This procedure was done to ensure that there was no underlying differentiation in contrast between the different emotions utilized in the experiment. No significant differences between the various emotions were found. This is important in respect to whether modulations could be due to differences in contrast. Our initial hypotheses predicted that hybrids with implicit fearful expressions (FearHybLF) would result in significantly higher signal change in the amygdala in comparison to broadband neutral images and hybrids with explicit fearful expressions (FearHybHF). Furthermore we expected to replicate Laeng and colleagues' (2010) behavioral results where hybrids with explicit emotional information were rated as significantly more unfriendly or friendly depending on facial expression compared to broadband neutral images and hybrids with implicit emotional information. In fact, in the present experiment, hybrids with implicit emotional content were rated close to neutral but they were still judged as significantly more friendly or unfriendly than the broadband neutral images. These findings seem to be in accordance with Laeng et al. (2010).

In line with our initial predictions negative broadband emotions were rated as significantly more unfriendly than broadband neutral and hybrids with implicit emotional content. Positive emotions were rated as significantly friendlier than broadband neutral and hybrids with implicit emotional content. However it is interesting that hybrids with implicit emotional content but has an explicit neutral expression is rated as significantly friendlier or more unfriendly than broadband neutral. However, FearHybLF was not rated significantly more unfriendly than neutral. A possible explanation for this might be that fear is not a directly 'threatening' expression and it might not always be rated as an "unfriendly" expression. Therefore, the results from the rating task are consistent with those of Laeng and colleagues' (2010). However, contrary to our main hypothesis, there was no significant differentiation in signal change in amygdala when comparing FearHybLF with either NeuBB or FearHybHF. As in the previous experiment, FearBB did not even result in increased

amygdala activity when compared to NeuBB. None of the above described comparisons resulted in significant activation outside our ROIs when we conducted a whole-brain analysis.

In contrast to Experiment 1 our second experiment included a behavioral task that the participants had to do in the scanner. We choose to include an active task in the fMRI experiment where the participants had to rate the stimulus material. Our reasoning behind this decision was that participants seemingly lost interest for the stimuli in our pilot. However what we did not consider at the time was how increased cognitive load could affect amygdala modulation due to low frequency information. A large meta-analysis of 385 fMRI and PET studies which investigated amygdala activation during emotional processing found that an active task decreased the odds ratio of amygdala activation relative to baseline level. (Costafreda, Brammer, David & Fu, 2008). The ACC and other prefrontal cortices are recruited when the cognitive load increases. Studies have shown that ACC activity increases with task difficulty (Fu et al., 2002; Paus, Koski, Caramanos, & Westbury, 1998) and is negatively correlated with amygdala activation (Blair et al., 2007; Pezawas et al., 2005). Furthermore the ACC have strong reciprocal connections with amygdala (Pessoa & Adolphs, 2010). Right after a stimulus was presented, participants had to rate the facial expression on a four point scale (unfriendly-friendly). With the studies presented above in mind, one possible explanation for the lack of significant modulation in the amygdala could be that when participants have to make an explicit judgment about the friendliness of a face, ACC and other prefrontal cortices are recruited and amygdala receives inhibitory signals from connections with these higher order cortices. One possible interpretation during a demanding task amygdala activity is inhibited to ensure that performance is optimal when potentially disrupting emotional stimuli is present (Costafreda, et al., 2008). Another scenario that could explain lack of amygdala activity may be a shortage of attentional resources in presence of a competing task In the above mentioned scenario the lack of amygdala activity would not be caused by inhibitory signals but rather that amygdala is ‘passive’ under high cognitive load (Costafreda, et al., 2008). This is in contrast with existing data that suggest that amygdala can process emotional stimuli with minimal attentional resources (Habel, et al., 2007; Vuilleumier, et al., 2001b; Williams, et al., 2004) and even without attention (Öhman, 2002), yet there is conflicting data that show that the amygdala requires some degree of attention suggesting that processing of emotional stimuli such as facial expressions are under top-down control (Pessoa, Kastner, & Ungerleider, 2002). Interestingly the data showed that when cognitive load was high, amygdala activity was equivalent and not significantly different from

zero regardless of stimulus valence. Furthermore when participants were simply told to attend the emotional stimuli the data showed significant amygdala modulation. Thus providing evidence that amygdala needs some attentional resources. The above findings by Pessoa and colleagues' (2002) could possibly explain the present findings. The rating task would in this scenario increase the cognitive load to such a degree that there simply are not enough attentional resources available for amygdala.

If we do assume that there amygdala is part of some early warning system which allocates resources for further processing (LeDoux, 2003) then why do we not find differential responses to the stimuli? One study in our fMRI group (Kristiansen & Viken, 2008) pointed out that a conflict could occur since high and low spatial frequencies carried different emotional information about the stimulus. Furthermore if different spatial frequency ranges are processed by two distinct neural pathways the conflict between the competing stimuli has to be resolved. Their whole-brain analysis showed a significant increase in activity in the precentral gyrus an area implicated in processing of conflicting incongruent information. This activation was interpreted as the brain trying to resolve the conflict between incongruent spatial frequency information (Kristiansen & Viken, 2008). However the present data does not support their findings. Another possible explanation is that initial and automatic (Morris, et al., 1999; Whalen, et al., 1998) fear responses in the amygdala are inhibited by other cortices. Pessoa and Adolphs (2010) suggested that initial processing of visual information may indeed proceed simultaneously along several parallel neural pathways. This would in turn result in what they call "multiple waves" of activation across the visual cortex and beyond (Pessoa & Adolphs, 2010). Thus emotional stimuli which have biological value could engage multiple brain regions such as amygdala, orbitofrontal cortex (OFC), ACC and anterior insula. The article by Pessoa and Adolphs (2010) points out that there exist reciprocal connections through amygdala via pulvinar to cortical areas included the OFC and ACC. Furthermore it is suggested that the amygdala is part of a larger distributed system involved in processing emotionally significant stimulus where activity serving different purposes is spread out in time and space. The amygdala's role in this system could be to facilitate initial fear-responses until more elaborate conscious processing is possible. Another role that the amygdala might serve in this affective system is to allocate processing resources to different stimuli by modulating the anatomical components that are required to prioritize particular features of information processing in a given situation (Pessoa & Adolphs, 2010). This scenario is also pointed out as plausible by Kristiansen and Viken (2008). There is evidence

that indicates that amygdala is a part of a bigger affective system. A PET study (Carlsson, et al., 2004) presented fear-relevant or neutral visual stimuli to participants with variable presentation times. They discovered that when the presentation time of stimulus was long enough for a participant to consciously perceive fear-relevant stimuli there was no significant increase amygdala activity in contrast to neutral stimuli. This deactivation in amygdala was negatively correlated with increased activity with cortical areas that was mentioned above. However when short presentation times did not allow stimuli to reach consciousness they found significant increase in amygdala activity (Carlsson, et al., 2004). This would suggest that initial responses towards emotional stimuli in both Experiment 1 and 2 could have been inhibited by prefrontal cortices which have projections back to amygdala.

For Experiment 2 we predicted an increase in amygdala when participants were presented with hybrid images containing implicit fear (FearHybLF). The increased cognitive load caused by the rating task could have resulted in prefrontal areas inhibiting amygdala activity to ensure possible disrupting emotional stimuli does not interfere with the competing behavioral task. Another explanation is that initial fear responses are inhibited by higher order cortices. Evidence indicates that there is a negative correlation between increased ACC activity and decreased amygdala activity (Carlsson, et al., 2004) when participants have enough time to consciously process emotional visual stimuli much. These inhibitory signals might be down regulations of initial fear responses which are sent after the biological value of a certain stimulus is determined. This would necessarily have some effect on reaction times in the rating task. It would be interesting to compare the reaction times between ratings of emotional and neutral faces. It has been suggested that emotional stimuli are able to bias the competition for processing resources (see Pessoa et al, 2002) and interfere with an on-going task. To investigate whether amygdala does indeed have an initial fear response then current fMRI methodology would not suffice because of low temporal resolution. However one could use focused fMRI imaging which have a sampling rate at 100 ms (Sabatinelli, Lang, Bradley, Costa, & Keil, 2009). The drawback with this focused imaging technique is that the volume coverage is small. One could also possibly use MEG which has high temporal resolution and has previously been used in amygdala research (Dumas, et al., 2010).

From the results of the behavioral data from Experiment 2 it is clear that participants did not find the fearful expressions particularly threatening or unfriendly. When the participants were forced to consciously make a judgment about the friendliness, the initial

neural activity might have been modulated by the affective system which is involved in making conscious judgments about stimuli. Yet the low frequency information in our hybrids seems to evoke some emotions towards the portrayed model in the hybrids and influence participants' social judgment. Participants rated the portrayed persons when compared to neutral broadband images as significantly more "friendly" when the there was a positive expression in the low frequency spectrum and "unfriendly" when the lowest frequencies showed negative emotions. Laeng and colleagues' (2010) concluded in their study that these hybrids could evoke "core" emotions without conscious awareness of a specific emotion but that these emotions can convey a clear "impression" of a person's character. There is however a possibility that there is something in the hybrid images that give away what emotion is present in the low spatial frequency information. It is a possibility that there is some 'shadowing' around the mouth that could influence participants' social judgment. As pointed out by Bar (2006) there is a possibility that a person's repeated expression could affect muscular structure or even skeletal properties. Thus even a neutral expression could be conceived as threatening because of subtle cues in a person's the facial structure. Low spatial frequency information in hybrid faces could possibly give these same or comparable subtle cues. The results from the behavioral data are not in agreement with the fMRI data. Research does show that there are structures besides the amygdala which may be capable of supporting unconscious processing of fear, this is clear from studying a patient with bilateral amygdala lesions that still could perform implicit rapid visual search of fearful faces (Tsuchiya, Moradi, Felsen, Yamazaki, & Adolphs, 2009). Furthermore the proposal that the amygdala is specialized for rapid detection of fear is also challenged by the fact that this patient is still able to perform normal rapid detection of fearful faces (Tsuchiya, et al., 2009). With this in mind one could explain the discrepancy between the behavioral data and the fMRI data. The low frequency information in the hybrids has influence on participants' judgment but activates some brain region which also is capable of supporting unconscious processing of fear which has been outside our region of interest.

General Discussion

We found that manipulating spatial frequency information did not lead to significant increase in amygdala activity for single filtered or hybrid images with emotional content in the low spatial frequency range. Yet, hybrid images with implicit emotional content were rated as significantly more unfriendly/friendly when compared to neutral broadband images. Thus, we found supportive evidence to the idea that the low frequency information can influence a rather complex social judgment (Laeng, et al., 2010).

There was a substantial intersubject variability in signal change in both experiments which might very well had impact on our findings. Substantial inter-subjects variability has consequences for studies which rely on averaged group responses (Davis, Kwan, Crawley, & Mikulis, 1998). Furthermore fMRI investigations have found that although participants rate their experiences in a similar manner there is substantial inter-subjects variability in neural activity. This could be attributed to participants having different sensory-cognitive experience of the stimulus (Davis, et al., 1998). The variation in our data might be a function of anatomical and functional differences in our population. The findings from the study mentioned above might give a possible explanation for the discrepancies between the behavioral data from Experiment 2 and the fMRI data from both Experiment 1 and 2. Possible effects of habituation could also have an impact on inter-subjects variability. Several participants showed negative activations to stimuli regardless of expression when compared to baseline. This might reflect habituation over time in amygdala to a given emotion. As described above in the method section our functional ROIs were extracted by utilizing data from the ROI localizer. Only voxels that were responsive to faces were extracted for the ROI. One drawback with this approach is the fact that localizer tasks normally take place during the last phase of an fMRI session. Given that in one fMRI session participants view a total of 704 faces before the localizer task, even though we placed several structural sequences between the experimental phases, there is a chance that the amygdala habituated to faces over time. There was also considerable inter-subjects variability in the number of voxels that were activated by faces. Unfortunately there is no practical solution to this, in other words you cannot simply run the localizer first in an fMRI session. We see two possible solutions to this issue. In total there are four experimental phases in one fMRI session this could be resolved with two sessions. The second solution would be to determine a threshold value, participants a low number of voxels would be excluded from the experiment based on probable habituation

effects. However the drawback would be that this threshold value would be an arbitrary number since there is no answer to how many activated voxels is an acceptable amount.

Furthermore there are several challenges in amygdala research that need to be pointed out which are also highlighted by our results. Studying the amygdala with neuroimaging methodology has proven to be problematic in the past (Fredrikson, Wik, Annas, Ericson, & Stoneelander, 1995). Furthermore fMRI research has shown that the amygdalae and especially the right amygdala rapidly habituate to repeatedly presented emotional stimuli (Fischer, et al., 2003; Wright, et al., 2001). Moreover, fMRI research targeting the human amygdala has also suffered from susceptibility-induced magnetic field in homogeneities caused by the neighboring air-filled bony cavities at the base of the skull (Merboldt, Fransson, Bruhn, & Frahm, 2001). Furthermore, meta-studies have reported substantial individual variations in amygdala volumes in the range of 1050 – 3880 mm^3, plus interhemispheric asymmetry, gender differences and age differences (Brierley, Shaw, & David, 2002; Pedraza, Bowers, & Gilmore, 2004). However, not all meta-studies are in agreement with the former findings (Pruessner et al., 2000). Analyses of fMRI parameters indicates that differences in positional correction, MRI magnetic field strength and slice thickness might contribute to volumetric asymmetry (Brierley, et al., 2002; Pedraza, et al., 2004). There is also a possibility that the specific method employed to anatomically assess the amygdala's boundaries may have an effect on the final reported volume. Future research focusing on the amygdala as a main area of interest might follow some directions pointed out by Morawetz and colleagues' (2008) for some easy alternations to the fMRI parameters. Their results gave a clear indication of what parameters would result in improved functional mapping of the amygdala. They found that a TE of 27 ms with a voxel size of $2\times2\times2$ mm^3 resulted in the least susceptibility artifacts in the anteromedial aspect of the temporal lobe. Their emotional stimulation paradigm resulted in robust bilateral amygdala activation for the approaches with 2 mm sections only and not with 4 mm section thickness. Furthermore they found larger activation volumes for a TE of 36 ms when compared with a TE of 27 ms. Analysis showed that smoothing with a 4 mm spatial filter represented a good compromise between increased sensitivity and preserved specificity (Morawetz, et al., 2008). The increase of spatial resolution which seems to paramount for reliable amygdala activations come at the cost of volume coverage. As pointed out above inhomogeneities caused by air-filled bony cavities at the base of the skull could be resolved by using different unwarping techniques. Furthermore it is advised to use probability mapping

based on cytoarchitecture mapping of the amygdala due to that the subnuclei of the amygdala differ in function architecture and connectivity (Amunts et al., 2005). Future research could possibly split a study into a two-part experiment where one focuses on the amygdaloid structure in one session, with optimized fMRI parameters for that region on the cost of spatial resolution, whereas the second experimental session focusing on volume coverage with more standard fMRI parameters. As pointed out several times amygdala may be part of an affective system which might be an extensive distributed network encompassing both cortical and subcortical structure (Pessoa & Adolphs, 2010). Further research should also focus on areas involved in this system (e.g ACC, OFC). Furthermore a potential weakness in this study is the relatively low number of subjects recruited, resulting in decreased statistical power of the data analysis. There have been consistent findings in the literature that emotional images (especially images of fearful expressions) reliably yield greater activation in the amygdala compared to neutral ones. We are confident that with an increased number of subjects, and some changes in the fMRI parameters, this paradigm would produce similar results.

A substantial inter-subject variability makes it hard to draw any definite conclusions, but we hope that this study has been able to shed some light on the complex nature of emotional processing and to provide some directions in respect to both experimental and other methodological problems for future research.

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Appendix A

Pilot – Experimental design and Scanner Parameters

Amygdala is prone to rapid habituation to emotional faces we tested several different designs to minimize the chance of habituation. Our initial design for the experiment utilizing hybrids was initially split into three phases each 12 minutes long. The original stimuli consisted of fearful and neutral expressions from two different angles, with a total of 420 images. Analysis of the fMRI data from the pilot study revealed that there was a habituation effect; four participants were included in this piloting phase. The experiment was redesigned to its current form.

Furthermore we wanted to find the optimal TE-time for finding amygdala activation; another four participants were scanned. Based on literature we tested two TE-times: 25ms and 35ms. TR-time was 2 seconds. Our findings indicated that a TE of 35ms gave the strongest signal in amygdala. We further followed several advices in the literature for improved mapping (Morawetz, et al., 2008). The localizer had proven activate amygdala, FFA and PPA effectively in a previous study.

Localizer – Individual functional ROIs

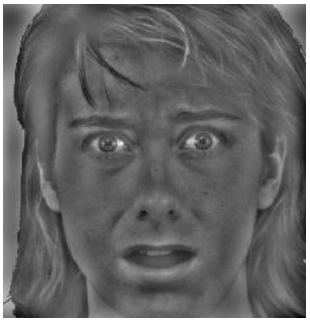
Table 1. The cluster size (number of voxels) and MMI coordinates (center of mass) for the functional ROIs extracted from the Localizer session.

FP	*x*	y	z	Cluster size
Left Amygdala				
218	-21	-4	-17	15
219	-19.5	1	-17.7	18
220	-24	-3	-11	18
221	-20	-4	-20	17
222	-18.5	-7	-16	32
223	-22.5	-5.5	-18	10
224	-27.5	-5.5	-15	34
225	-22.5	-6.5	-17.5	8
226	-23	-5.5	-11	9
228	-16	-2	-15	19
229	-18.5	-4	-15	30
230	-18.4	-4	-15	14
233	-17	-2	-16.5	13
235	-18.8	-8	-15	15
238	-19.5	-9.5	-13	11
239	-26	-4	-11.5	8
240	-21.5	-1.5	-12	23
Right Amygdala				
218	20	-2.5	-14.5	8
219	20	-1	-16	34
220	20	1	-14.7	13
221	24	-5	-27.5	8
222	21	0	-13.5	15
223	21	-3	-10.5	8
224	21	-2.5	-14.7	37
225	25	3	-19	19
226	20	1	-13.7	10
228	20	1.5	-16.5	45
229	19.5	1	-14.5	29
230	18.8	-1	-14	12
233	21.5	1	-14	9
235	24.5	-5	-14	16
238	19.5	-2	-15	15
239	18.8	-1.5	-12	8
240	25	2	-21.5	20

Stimuli Examples

Stimuli from Experiment 1: Single filtered low frequency images (One fearful, one neutral)


Stimuli from Experiment 1: Single filtered high frequency images (One fearful, one neutral)




Stimuli from Experiment 2: Hybrid faces with low frequency content (One fearful, one angry, one happy)



Stimuli from Experiment 2: Hybrid faces with high frequency content (One fearful, one angry, one happy)

Stimuli from Experiment 2: Broadband faces with high frequency content (One fearful, one angry, one happy, one neutral)

Stimuli from Localizer experiment. Places, Fearful faces and Neutral faces