# Amygdala Activation in Response to 2D and 3D Emotion-Inducing Stimuli

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#### **ABSTRACT**

Studying changes in brain activation according to the valence of emotion-inducing stimuli is essential in the research on emotions. Due to the ecological potential of virtual reality, it is also important to examine whether brain activation in response to emotional stimuli can be modulated by the three-dimensional (3D) properties of the images. This study uses functional Magnetic Resonance Imaging to compare differences between 3D and standard (2D) visual stimuli in the activation of emotion-related brain areas. The stimuli were organized in three virtual-reality scenarios, each with a different emotional valence (pleasant, unpleasant and neutral). The scenarios were presented in a pseudo-randomized order in the two visualization modes to twelve healthy males. Data were analyzed through a GLM-based fixed effects procedure. Unpleasant and neutral stimuli activated the right amygdala more strongly when presented in 3D than in 2D. These results suggest that 3D stimuli, when used as "building blocks" for virtual environments, can induce increased emotional loading, as shown here through neuroimaging.

Keywords: virtual reality, 3D/2D visual stimuli, valence (pleasant, unpleasant, neutral), amygdala, functional Magnetic Resonance Imaging (fMRI).

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#### 1. Introduction

During the last decades many studies have been published concerning the neural basis of visually-driven emotional responses (e.g., LeDoux, 1996; Panksepp, 1998) and their influence on cognitive processes such as attention (Lang, Bradley and Cuthbert, 1997), memory (Ochsner and Schacter, 2000), or cognitive skills involving composite functions, such as decision making (Damásio, 1994). Many of these studies used emotion-inducing visual pictures in the context of laboratory experimentation (Lane, Chua and Dolan, 1999; Paradiso et al., 1999).

Functional Magnetic Resonance Imaging (fMRI) studies have been particularly relevant for the study of emotions (e.g., Morris et al., 1996; Keightley et al., 2003), since this technique has provided additional information about the neural substrates involved in processing stimuli with emotional content. The increasing availability of fMRI has greatly stimulated this field of research. Although functional neuroimaging has provided strong support for the critical role of the amygdala in the processing and memory of emotions (e.g., Costafreda et al., 2008; Fanselow and Gale, 2003; LeDoux, 2003; Phan et al., 2002), this way of exploring emotional phenomena has mainly used simple slide-like pictures or other simple stimuli that allow additional experimental control.

The use of two-dimensional (2D) images is the most commonly used method to obtain emotional responses to visual stimuli in experimental settings. This method was proven effective. In particular the stimuli in the International Affective Picture System (IAPS; Lang, Bradley and Cuthbert, 1999) have shown great consistency (e.g., Ito, Cacioppo and Lang, 1998; Lang, Bradley and Cuthbert, 2005; Libkuman, Otani, Kern, Viger and Novak, 2007). However, since virtual reality (VR) environments were shown to be immersive and realistic, providing a sense of presence (the "sense of being in the simulated scenarios") in the created three-dimensional (3D) environment (Baños et al., 2004; Carter, Bordnick, Traylor, Day and Paris, 2008; Coelho, Tichon, Hine, Wallis and Riva, 2006), it is important to explore the comparative value of 2D and 3D scenarios in the elicitation of the emotional responses. It has been suggested that 3D features may induce emotions more effectively than 2D features in experimental settings (Riva et al., 2007). VR is often used in clinical settings with proven value, particularly in the treatment of phobias and other anxiety disorders (e.g., Coelho et al. 2008; Coelho, Silva, Santos, Tichon and Wallis, 2008; Meyerbroker and Emmelkamp, 2010), post-

traumatic stress disorder (e.g., Reger et al. 2011), autism spectrum disorders (e.g., Wang and Reid, 2011), addictive behaviors (e.g., Bordnick et al., 2009), and cognitive impairments after brain injury (e.g., Cernich, Kurtz, Mordecai and Ryan, 2010).

A large meta-analysis from Sergerie, Caroline and Armony (2008) demonstrated that processing stimuli with emotional content consistently activates the amygdala. However, the 148 studies selected for the analysis did not use 3D stimuli. Despite the fact that realistic environments such as VR scenarios are avoided in favor of experimental control, some researchers such as Hoffman, Richards, Coda, Richards and Sharar (2003) demonstrated the possibility of combining VR technology with fMRI. Hoffman's participants reported a strong sense of immersion during data acquisition, despite constraints such as immobilization and noise. Shortly after, Lee, Lim, Wiederhold and Graham (2005) studied the effect of 2D and 3D stimuli as cues for inducing smoking craving, and concluded that the latter produce greater activation in brain areas involved in attention, visual balance and movement coordination. To the best of our knowledge no other studies have compared the effects of 2D and 3D emotion-inducing stimuli through measures of brain activation.

The main aim of the present research is to test the hypothesis that 3D stimuli produce greater amygdalae activation, compared to the conventional 2D display. Concerning emotional valence, we also hypothesize an increase in amygdalae activation for the pleasant and unpleasant conditions (compared with the neutral one), considering the role of this brain structure in processing pleasant and aversive stimuli, and in the recognition of pleasant and unpleasant emotions, such as fear (Baxter and Murray, 2002; Costafreda et al., 2008; Siebert, Markowitsch and Bartel, 2003). The investigation of emotions, of their neural substrates and of their effects on other mental functions lacks optional stimuli that are capable to optimize the induction of emotional responses in controlled laboratory settings. The use of VR technology contributes to overcome this limitation. It enables the development of stimuli which, while still passible of manipulation, control and replication to allow quantification of experimental results, have properties that bring them closer to reality.

Next, we present the methods used in this study (including participants, materials, procedures, data acquisition and data analysis) followed by the study's results, which are discussed in a final section that also includes the study's limitations and suggestions for future research.

#### 2. Method

# 2.1 Participants

This study is part of a broader project that uses male subjects to minimize confounding variables such as hormonal variation. The present study included 12 right-handed (self-reported), healthy male subjects without contraindications for MRI, or pathologies of the central nervous system (CNS), psychiatric disorders, trauma, and visual acuity deficits (assessed through a screening interview), which were exclusion criteria. The sample's mean age was 26.58 years old (SD = 5.16, range = 18-35 years). Participants signed a written informed consent form before taking part in this study.

#### 2.2 Materials

This study uses functional MRI to evaluate the brain activation of subjects watching virtual scenarios. Each scenario (Figure 1) included three sets of 15 stimuli-objects, one set per valence (3DAIS – 3D Affective Scenarios). Each of these stimuli (the most pleasant, unpleasant and neutral stimuli) was chosen from our database of 3D objects. The stimuli were based on those developed by Lang et al. (1999) in the IAPS, which had been previously validated with the Self-Assessment Manikin – SAM (Lang, 1980). Our database was validated for emotional arousal and emotional valence using rating procedures similar to those of Lang and colleagues (1999) in the development of the IAPS. The database comprises 131 3D objects, each rated by 214 individuals on the 9-point SAM scale for emotional arousal (1 - lower, 9 - higher) and emotional valence (1 - unpleasant, 9 – pleasant) (Monteiro, Barbosa and Silvério, 2011).

The stimuli were chosen according to the following criteria:

- 1. The stimuli receiving the highest scores in both valence and arousal (valence  $\geq$  6.0; arousal  $\geq$  4.0) were grouped in the pleasant condition (n=15);
- 2. The stimuli receiving the lowest scores in valence but scoring the highest in arousal (valence  $\leq$  4.0; intensity  $\geq$  4.0) were grouped in the unpleasant condition (n=15);
- 3. Finally, the stimuli receiving intermediate values in the valence scale and low scores in arousal (valence  $\geq 4.5$  and  $\leq 5.5$ , arousal  $\leq 3.0$ ) were grouped in the neutral emotional condition (n=15).

The selected objects are described in Table 1.

Objects	Pleasant			Neutral		Objects	Unpleasant	
	M (SD)		Objects	M (SD)			M (SD)	
	Arousal	Valence		Arousal	Valence		Arousal	Valence
Cocktail	5.84	7.99	Roll the	2.57	5.07	Droppings	6.89	1.44
	(2.37)	(1.36)	dough	(2.09)	(1.56)		(2.98)	(1.78)
Woman	5.50	6.27	Cookware	2.76	5.36	Old mobile	4.23	2.27
	(2.95)	(2.03)		(2.23)	(1.95)		(2.12)	(1.85)
Champagn	5.26	7.46	Valence	1.99	5.02	Knife	4.90	2.76
e	(2.66)	(2.06)		(1.98)	(1.37)		(2.40)	(1.97)
Bag of	5.52	6.98	Dryer	2.20	5.19	Syringe	4.82	2.18
money	(2.22)	(2.04)	Diyei	(1.78)	(1.59)		(2.63)	(2.04)
Flower 3	5.16	7.14	Iron ironing	2.10	4.83	Dirty toilet	5.97	1.80
	(2.67)	(1.71)		(2.08)	(1.66)		(2.61)	(1.23)
Cake	5.27	6.94	Cutlery	2.39	5.15	Mouse	5.11	2.22
	(2.71)	(2.26)		(2.01)	(1.70)		(2.42)	(2.15)
Cake 2	4.49	6.67	Glass 2	1.88	5.11	Corpse	4.35	2.12
	(2.82)	(1.78)		(1.38)	(1.63)		(2.62)	(1.92)
Butterfly 2	3.89	6.39	Glass	2.04	5.16	Dirty sink	4.66	2.46
	(2.31)	(2.01)		(1.46)	(1.72)		(2.16)	(1.92)
Champagn e	4.19	6.94	Books	2.21	5.24	Pistol	5.36	2.50
	(2.55)	(1.77)		(2.40)	(2.12)		(2.68)	(2.21)
Chocolates	5.29	7.63	Shoes	1.77	5.12	Old couch	4.97	2.77
	(2.61)	(1.76)		(2.18)	(1.26)		(2.63)	(2.46)
Lobster	5.89	7.33	Springs	1.98	4.97	Old bed	5.21	2.43
	(2.44)	(1.83)	clothing	(1.39)	(1.61)		(2.23)	(1.73)
Strawberrie	5.26	7.45	Craps	2.28	4.89	Cobweb	5.03	2.49
s	(2.61)	(1.62)		(2.07)	(1.69)		(2.12)	(1.80)
Ice cream	5.10	7.11	Clock	2.21	5.12	Snake	6.85	1.45
	(2.80)	(1.74)		(1.99)	(1.52)		(1.83)	(1.91)
Biscuits	4.65	7.01	Table	2.15	5.04	Fly	5.19	2.29
	(2.61)	(1.81)		(1.83)	(1.48)		(2.58)	(1.94)
Beach	6.61	8.19	Fan	1.00	5.12	Spider	6.25	1.97
	(1.63)	(1.31)		(1.72)	(1.53)		(2.45)	(2.40)

**Table 1**. Arousal and valence scores for selected objects in each scenario: Means (M) and standard deviations (SD).

A stimulation unit (a laptop computer running Windows Vista) connected to a rear projection system (a multimedia projector XGA with 1024x768 pixels and 2200 ANSI lumens) was used to present the scenarios (Figure 1) in a 150x100 cm screen.

The MR scanning was carried out using a 1.5 T scanner (MAGNETON, Sonata, Siemens) with a gradient of Max. Amplitude of 40 mT/m, Min. Rise Time of 200  $\mu$ s, and Max slew rate of 200 T/m/s. Participants watched the screen through a mirror while lying in the scanner. The 3D visualization effect was achieved through analyph image technique and the use of passive glasses (Zone, 2005).



**Figure 1**. Emotion-inducing scenarios of different (pleasant - left, neutral - middle, unpleasant - right) valences, all projected in 2D and 3D visualization modes.

#### 2.3 Procedure

We implemented a simple within-subjects, 3x2 experimental design, with emotional valence (pleasant, neutral, unpleasant scenarios) and visualization (2D, 3D) mode as factors.

A block-design paradigm with two fMRI runs was used: one for the 2D scenarios and the other for the 3D scenarios. The scenarios were counterbalanced. There was no interval between runs, except the time that was necessary for participants to put on or take off the anaglyph glasses, depending on the initial (2D or 3D) visualization condition.

Both runs consisted of three cycles of rest and activation, one for each (counterbalanced) emotional valence, with no interval between cycles. During resting periods, a fixation cross was displayed for 40 sec, immediately followed by one of the scenarios. Each run was preceded by four seconds of no stimulation (which were discarded) to allow for the MR signal to reach its equilibrium. Thus, the duration of a whole run was 4'4", as represented in Figure 2.

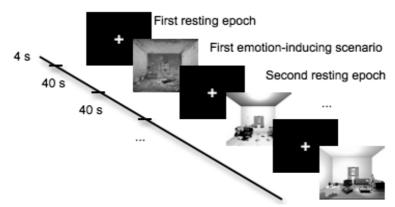


Figure 2. Schematic representation of one of the runs of the experimental paradigm.

During data acquisition, participants' heads were positioned in a standard radiofrequency head coil (equipped with a mirror) with tape and cushioning to minimize head motion. Participants were instructed to keep their eyes open during the entire session and pay attention to the image that was projected on the screen.

# 2.4 Data acquisition

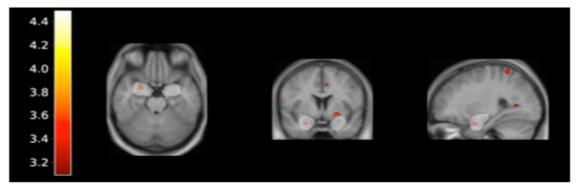
For functional imaging, a gradient—echo T2\*-weighted Echo-Planar Imaging (EPI) sequence [Repetition Time (TE) = 50ms, flip angle of 90°, Echo Time (TR) = 4s] was used to measure the BOLD (Blood Oxygenation Level Dependent) effect (Ogawa, Lee, Kay and Tank, 1990). The Field of View (FOV) was 240 x 240mm² with a 64 x 64 matrix resulting in an effective resolution of 3.75mm x 3.75mm x 3mm (Thk). Sixty volumes with 36 slices each were acquired in axial orientation, with slices parallel to the anterior—posterior commissure. The slice thickness was 3mm with a gap of 25%. For anatomic reference, a high-resolution MPRAGE T1-weighted scan was acquired for each participant with the following parameters: TR = 2s, TE = 3.69ms, 256 x 256 matrix, FOV = 240 x 240mm², and slice thickness of 1mm leading to an effective resolution of 0.9 x 0.9 x 1 mm.

## 2.5 Data analysis

Data preprocessing was performed using Statistical Parametric Mapping (SPM 8; (Wellcome Trust Centre for Neuroimaging, UCL, London, UK) running on MATLAB 2008A (The MathWorks Inc., Natick, MA, USA). The functional scans were realigned to correct for bulk motion of the head. After co-registration of functional and anatomical scans the data were normalized to the MNI standard brain (Montreal Neurological Institute, Montreal, Canada) using SPM's segment function. Finally, the functional data were smoothed with an 8x8mm isotropic Gaussian kernel. A GLM-based fixed effects analysis was run on the data using SPM8. Movement parameters were used as regressors of no interest in the analysis. A region of interest (ROI) analyses was carried out for both amygdalae. The ROI was created using the Harvard-Oxford cortical and subcortical structural atlas (Harvard Center for Morphometric Analysis, Boston, MA, USA). Results were corrected for multiple comparisons using FWE (Family-Wise Error) correction. A p-value of 0.05 was considered significant. In addition, whole brain results were calculated for all contrasts. An uncorrected p-value of 0.001 was considered significant in this case.

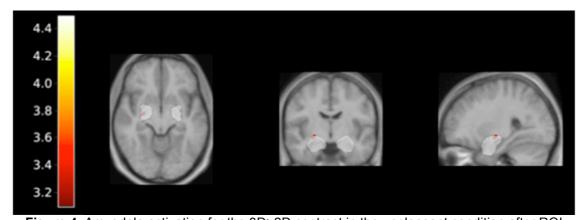
## 3. Results

ROI analysis showed a significant activation of the right amygdala for the neutral scenario in the contrast showing higher activation for the 3D mode than for the 2D visualization mode (3D>2D), p = .016, FWE-corrected, x=20.0, y=-2.0, z=-26.0 (Figure 3).



**Figure 3.** Amygdala activation for the 3D>2D contrast in the neutral condition after ROI analyses.

The unpleasant scenario (Figure 4) shows a similar tendency, with the right amygdala showing a marginally significant increased activation for the 3D visualization mode in comparison to the 2D mode (p = .062, FWE-corrected, x=26.0, y=-10.0, z=-10.0).



**Figure 4**. Amygdala activation for the 3D>2D contrast in the unpleasant condition after ROI analyses.

At the level of the whole brain we found significant activations (p = .012, FWE-corrected and p = .049, FWE-corrected) for the 3D>2D contrast within the neutral condition in the left rolandic operculum (x=-40.0, y=-6.0, z=12.0) and in the right postcentral gyrus (x=62.0, y=0.0, z=14.0), respectively. No significant activations were found for the opposite contrast (2D>3D) or for other emotional conditions.

#### 4. Discussion

The addition of a third dimension to certain virtual scenarios seems to produce augmented activation in some brain regions. This study found increased activation of the right amygdala, particularly in the neutral and unpleasant 3D scenarios. This suggests that the higher realism and presence of the 3D specific stimuli upregulates the amygdala response. Under the assumption that 3D stimuli are more arousing than 2D stimuli, this would be reflected in augmented amygdala activation. This is shown in early lesion studies reporting that bilateral amygdalae damage results in the inability to recognize arousal resulting from emotion-inducing stimuli (e.g., Adolphs, Russel and Tranel, 1999), and also in recent research indicating that the amygdalae are essential in the alteration of attention associated with emotional arousal (see Todd and Anderson, 2010).

This study corroborates our first hypothesis. The right amygdala activation in the unpleasant 3D scenario is in line with previous studies that show right hemisphere dominance processing negative emotions (Davidson, Ekman, Saron, Senulis and Friesen, 1990) and the right amygdala to be more closely related to affective pictorial information (Markowitsch, 1998; Phelps, O'Connor, Gatenby, Grillion and Davis, 2001).

Contrary to our second hypothesis, pleasant scenarios did not produce increased amygdala activation. These results were consistent with some studies that report the activation, or at least a higher probability of activation, of the amygdala in response to unpleasant stimuli but not to pleasant ones (Costafreda et al., 2008; Paradiso et al., 1999; Phan et al., 2002), although higher activation in response to the neutral condition was not expected (e.g., Costafreda et al., 2008). Elevated response of the amygdala to neutral stimuli has been found in severe (Hendler et al., 2003) and mild post-traumatic stress disorder (Brunetti et al., 2010). Significantly higher activation during processing of neutral images has also been observed in schizophrenic patients, relative to controls (Lakis and Mendrek, 2013). However, psychiatric disorders were part of the exclusion criteria in our study. In Lakis and Mendrek's (2013) study, men showed enhanced responsiveness to the emotionally neutral material. In fact, sex could be a potential factor modulating emotional processing and its neural mechanisms (Hofer et al., 2006; Stevens and Hamann, 2012; Wrase et al., 2003), but our study does not account for such potential effects, as all participants were men.

Our finding regarding the neutral stimuli can thus result from specific meaning and/or somatosensory-inducing features of the contents of the neutral scenario, considering

that a similar pattern of higher brain activation for the neutral 3D scenario, when compared with the homologous 2D scenario, was also found in the left rolandic operculum and in the right postcentral gyrus. This explanation would concur with the functional roles of these latter brain structures, namely the involvement of the left rolandic operculum in the encoding of language elements and their somatosensorial importance. It is also possible that the observed patterns result from differences in the materials used. Visual features such as the contrast and light of the scenarios, as well as the presence/absence of human beings were not fully controlled. Although the exact same scenarios were presented in both 2D and 3D visualization modes, those elements varied across emotional valences. This could have introduced differences in the perception of neutral, pleasant, and unpleasant scenarios with eventual effects in amygdala activation. However, further studies and bigger samples are necessary to inspect these explanatory hypotheses.

In future studies we will also consider other brain areas such as early visual association cortices that process different aspects of 2D vs 3D stimuli, and the limbic cortex for emotion. Nevertheless, considering the existing literature, these results already contribute to highlight the potential benefits of using virtual scenarios, comprising 3D objects, in the research of emotions. Future studies could also further inspect the use of 3D scenarios to increase realism (Lourenço, Azeff, Sveistrup and Levin, 2008). This could be important for the purpose of inducing emotions (e.g., Coelho, Santos, Silvério and Silva, 2006; Insko, 2003; Riva et al., 2007). Our study suggests that, comparing with 2D, 3D can enhance amygdala response and that its role in enhancing emotions in realistic environments needs to be further explored.

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