Inês Alexandra Teixeira de Almeida

Implicit processing of emotional faces using temporal and spatial constraints: A multimodal approach.

Tese de Doutoramento em Ciências da Saúde, no ramo de Ciências Biomédicas,
apresentada à Faculdade de Medicina da Universidade de Coimbra sob a orientação de
Miguel de Sá e Sousa de Castelo-Branco e Marieke van Asselen

2013

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UNIVERSIDADE DE COIMBRA

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Processamento implícito de faces emocionais usando limites temporais e espaciais:

Uma abordagem multimodal

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Thesis presented to obtain a Ph.D. degree in Health Sciemces, in the field of Biomedical Sciences,
at the Faculty of Medicine of the University of Coimbra

Tese de Doutoramento em Ciências da Saúde, no ramo de Ciências Biomédicas,
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2013

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Co-Superviser by: Marieke van Asselen, Ph.D

Para o Tonito, a Rosinha, a Sana e o Serafim.

"Perfection is not just about control. It's also about letting go...."
In *Black Swan*

CONTENTS

ABBREVIATIONS VIII
SUMMARY XI
SUMÁRIO XIII

INTRODUCTION

Introductory notes 1

CHAPTER 1

The Neural correlates of visual perception 7

METHODS

CHAPTER 2

Methods 27

RESULTS

CHAPTER 3

Introductory notes and Motivation 37
Temporal limits of Visual awareness and the role of skin conductance response in understanding emotional cognition 41

CHAPTER 4

How the visual position of emotionally loaded stimuli influences the activation of the amygdala: A systematic review 57

CHAPTER 5

The role of the amygdala and the basal ganglia in the visual processing of central vs. peripheral emotional content 69

CHAPTER 6

Fear-relevant animal Faces and Shapes: the role of central vs. peripheral processing in threat detection 91

CONCLUDING REMARKS

CHAPTER 7

Discussion and Conclusions 119

LIST OF PUBLICATIONS 131
AGRADECIMENTOS 133
CURRICULUM VITAE 137

ABBREVIATIONS

AC-PC	Anterior commissure - posterior commissure
ANOVA	Analysis of variance
BA	Brodmann area
BOLD	Blood-oxygen-level-dependent
cm	centimeters
CS+	Conditioned stimulus
CS-	Unconditioned stimulus
CRs	Conditioned responses
d	Dorsal
d'	d prime (sensitivity index)
DTI	Difusion Tensor Imaging
EDA	Electrodermal activity
EEG	Electroencephalography
EPI	Echo planar imaging
FA	Flip angle
FDR	False discovery rate
FFA	Fusiform face area
fMRI	Functional magnetic resonance imaging
FOV	Field of view
GLM	General linear model
HSF	High spatial frequency
IAPS	International Picture Affective System
ISI	Inter-stimulus interval
K	Koniocellular
KDEF	Karolinska Directed Emotional Faces
LGN	Lateral geniculate nucleus
LO	Lateral occipital
LSF	Low spatial frequency
M	Magnocellular
MEG	Magnetoencephalography
MPRAGE	Magnetization prepared rapid gradient echo
MR	Magnetic resonance
MRI	Magnetic resonance imaging
msec	miliseconds

NMR	nuclear magnetic ressonance
NS	Non significant
Nr	Number
n.-s.	Non-specified
P	Parvocellular
PET	Positron emission tomography
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RF	Receptive field
RFX	Random effects
RGC	Retinal ganglion cell
RMSE	Root mean squared error
ROI	Region-of-interest
RT	Response time
SC	Superior colliculus
SCR	Skin conductance response
SCL	Skin conductance level
SMI	SensoMotoric Instruments
SD	Standard deviation
SDT	Signal detection theory
SE	Standard error
sec	seconds
SNAQ	Snake Phobia Questionnaire
SPECT	Single photon emission computerized tomography
SPSS	Statistical Package for the Social Sciences
SOA	Stimulus onset asynchrony
STS	Superior temporal sulcus
TAL	Talairach
TE	Echo time
TI	Inversion Time
TMS	Transcranial magnetic stimulation
TR	Repetition time
v	Ventral

SUMMARY

Emotional cues in the environment give important information about a specific context or situation (e.g., fear recognition when a threat is present). Under threatening situations, relevant stimuli should be quickly detected to promote survival. However, these emotional cues are not always consciously perceived or fully recognized due to either temporal constraints or to less accurate perception. Two pathways are usually accounted for visual processing of emotional information: a 'fast' subcortical route to the amygdala, a brain structure crucial in the fear module, and a cortical 'slow' route for detailed object recognition. The two are thought to play different but not independent roles.

In the current project, we aimed to understand the temporal constraints that determine how emotional cues can be processed without awareness, and how the stimulus position in the visual field (central vs. peripheral and left vs. right) influence the processing of emotional items of distinct ecological significance. A multimodal approach that combined behavioural, psychophysiological and functional neuroimaging methods was used in order to understand implicit vs. explicit processing of emotional stimuli and its neural correlates.

We developed paradigms in which emotional faces/shapes were presented either below the limits for visual awareness (temporal constraints) or at peripheral locations (spatial constraints). Specifically, in the first study we used human emotional faces given their social relevant value, whereas in the remaining studies, we used animals as stimuli, both threatening and non-threatening animal faces or fear-relevant shapes such as snakes. The different paradigms were applied to normal subjects, enabling us to study either emotional cognition under different levels of awareness or spatially detailed vs. degraded forms of access to stimulus content.

In the first study, by manipulating temporal constraints combined with visual masking, we could directly assess the processing of emotional faces under graded levels of sensory awareness. To test if content was processed and its behavioural and psychophysiological implications, we measured skin conductance responses (SCRs) to emotional (angry, happy) and neutral faces with variable temporal durations, while asking our participants in a trial-by-trial basis if they were aware of the picture content, had seen a face, and if so whether they were able to discriminate an emotion. Additionally, arousal ratings of picture content were also collected. We tested if a dissociation between the two measures of arousal – subjectively reported and physiological measured (SCRs) – occurred. Moreover, we studied the effect of stimulus duration on SCRs while awareness of the emotional content of the stimulus was reported.

We found a specific effect of stimulus presentation duration on SCRs when using unconditioned angry faces, but not when using happy or neutral faces. Additionally, arousal ratings were also affected by stimulus presentation duration, in particular concerning for happy faces which yield higher ratings already at short durations.

In the second and third studies, by manipulating the spatial location of stimulus presentation (centre, left, right), we could test whether the processing of peripheral threat information is distinct from central visual processing, and if this distinct type of processing relies on different neural correlates. For this, we used functional neuroimaging (fMRI) to test if the amygdala was biased to the processing of peripheral (coarse, low spatial frequency) information or if in contrast responded more during central analysis of the stimulus. This allowed us to examine the relative role of foveally-biased

central vs. peripheral visual object recognition. Additionally, we investigated how stimulus processing demands (implicit or stimulus-driven approach vs. explicit or goal-oriented approach) influence the neural processing of threat cues.

In the second study, we found amygdala responses preferentially to animal faces presented at central locations, whereas the left amygdala responded preferentially to threatening animal faces in the implicit task. In addition, the right amygdala responded to both threatening and non-threatening animal faces during explicit appraisal of threat. Importantly, we found a twofold role of the basal ganglia in explicit threat evaluation dependent on spatial location: the caudate was specifically implicated during central processing, while peripheral processing recruited mainly the putamen.

In an ensuing (third) study, we asked if such a (central) bias was also present for other ecologically relevant objects, such as animal shapes as these do not require detailed processing and can therefore be analysed in the visual periphery. We found larger amygdala responses to centrally presented snake stimuli (body, face or fake) than for right peripheral presentations, independent of task and amygdala. For the contrast centre > left hemifield, these differences were found only for the left amygdala during the implicit snake identification task. During the implicit task this difference centre vs. right correlated positively with reported fear of snakes. Importantly, a strong hemispheric lateralization was found, with real shapes activating stronger the right hemisphere as compared to fake shapes, which is consistent with its dominance for stimuli with emotional content.

These results validate the ecological meaning of our stimuli, and the value of *central* appraisal of emotional information, although not disputing the role of preattentive, non-conscious, and peripheral, less accurate, processing. Future work should further elucidate how automatic attention mechanisms interact with explicit goal oriented emotional cognition.

SUMÁRIO

Pistas emocionais no ambiente acarretam informação relevante sobre situações/contextos específicos (ex: reconhecimento de faces de medo na presença de ameaça). Em situações de risco de vida, estas devem ser rapidamente detectadas, permitindo comportamentos de sobrevivência. No entanto, estas pistas nem sempre são percebidas ou reconhecidas, devido a limites temporais ou espacias da percepção. Foram propostas duas vias para o processamento emocional: um trajeto subcortical "rápido" para a amígdala (uma estrutura cerebral crucial no modelo de reconhecimento de medo), e um trajeto "lento", para o reconhecimento de objetos. As duas vias desempenham papéis diferentes mas não necessariamente independentes.

Esta tese visa o estudo das condições sob as quais as pistas emocionais podem ser processadas de forma implícita, bem como a influência que a posição dos itens no campo visual afecta o processamento dos mesmos. Optou-se por uma abordagem multimodal que integrou métodos comportamentais, psicofisiológicos e de neuroimagem funcional, de forma a melhor compreender o processamento implícito vs. explícito de estímulos emocionais e os seus correlatos neuronais.

Desenvolvemos paradigmas nos quais faces/formas emocionais são apresentadas tanto acima/abaixo dos limites para consciência visual (constrangimentos temporais da percepção) como no centro/periferia do campo visual (constrangimentos espaciais). Especificamente, no primeiro estudo usámos faces emocionais humanas, dado a sua relevância social, e nos restantes estudos usámos imagens de animais, quer faces de animais ameaçadoras e não-ameaçadoras, quer formas biologicamente relevantes (ex. cobras). Os diferentes paradigmas foram aplicados a participantes normais, permitindo-nos estudar diferentes níveis de consciência, bem como o acesso detalhado central ou impreciso da periferia ao conteúdo dos estímulos.

Primeiro, a combinação de limites temporais com o uso de técnicas de mascaramento visual permitiu avaliar o processamento de faces emocionais em condições onde não há necessariamente consciência sensorial do estímulo. Para testar se o estímulo foi realmente processado, bem como os seus paralelos comportamentais e psicofisiológicos, medimos a resposta da condutância da pele (SCR) a faces neutras e emocionais (raiva, alegria) variando a duração temporal do estímulo, enquanto perguntámos aos participantes, em cada ensaio, se eles percebiam o conteúdo da imagem, tinham visto uma face, ou eram capazes de discriminar a emoção. Recolheram-se também classificações de arousal relativamente ao conteúdo das imagens. Testámos se ocorria uma dissociação entre as duas medidas de "arousal": a psicofisiológica (SCR) e os relatos subjetivos, e estudámos o efeito da duração dos estímulos na SCR quando era reportada a consciência do conteúdo do estímulo.

Foi identificado um efeito específico de duração na SCR apenas para faces de raiva, mas não para faces neutras ou alegres, e apenas para durações longas. Adicionalmente, as classificações de arousal também foram afectadas pela duração do estímulo, em particular para as faces alegres, traduzindo-se estas em classificações de arousal elevadas mesmo para durações muito curtas.

Na segunda parte do nosso trabalho, manipulámos a posição dos estímulos no campo visual (centro, esquerda, direita) para testar se o processamento periférico de conteúdo ameaçador é diferente do central, e se isto tem por base diferentes correlatos neuronais. Para tal, usámos ressonância magnética funcional e testámos se a amígdala estava enviesada para o processamento de informação periférica (frequências espaciais baixas) ou se respondia mais à inspecção central do estímulo, re-

flectindo o enviesamento da visão central para reconhecimento de objectos. Adicionalmente, manipulámos o processamento do estímulo usando diferentes estratégias de atenção seletiva em pistas de ameaça (tarefa implícita – apenas reconhecer animal, vs. explícita – orientada para a detecção de ameaça).

No primeiro estudo identificámos uma preferência da amígdala direita para faces de animais apresentadas ao centro, enquanto que a amígdala esquerda respondeu preferencialmente para faces de animais ameaçadoras na tarefa implícita. A amígdala respondeu também para as faces em geral, durante a tarefa de avaliação explícita de ameaça. Encontrámos também um papel dos gânglios da base na avaliação explícita de ameaça, dependendo da localização espacial: o caudado esteve envolvido no processamento central e o putamen foi recrutado particularmente durante o processamento periférico.

O estudo seguinte avaliou se o enviesamento (central) encontrado estaria também presente para outros itens com validade ecológica (ex. cobras), uma vez que estes não requerem processamento detalhado e são mais facilmente analisadas na periferia visual. Identificámos respostas maiores da amígdala para estímulos de cobras (caras, formas ou cobras falsas) apresentados ao centro do que na periferia direita, independentemente da tarefa e da amígdala. Para o contraste centro>hemicampo esquerdo, estas diferenças apenas foram encontradas na amígdala esquerda para a tarefa implícita. Durante a tarefa implícita, a diferença centro vs. direita correlacionou-se positivamente com o grau de fobia a cobras. É de relevar uma forte lateralização hemisférica, com formas reais a ativar mais fortemente o hemisfério direito do que formas falsas, em acordo com a dominância direita para estímulos com conteúdo emocional.

Estes resultados confirmam o significado ecológico dos nossos estímulos, e o valor da avaliação *central* da informação emocional, sem no entanto descartar o papel do processamento mais automático, e menos preciso. Trabalhos futuros deverão elucidar a interacção de mecanismos automáticos com a cognição social guiada por objectivos.

"We do have a measure of control, but it is not true that we have full control and it is not true that when we are executing an action we are necessarily controlling it at that moment consciously."

— António Damásio

INTRODUCTORY NOTES

The ability to process information outside the limits of awareness has held a long standing debate. In fact, some controversy still persists concerning the possibility that stimuli which escape the boundaries of conscious visual perception can still contribute to decision making and influence behaviour. Since Sigmund Freud's suggestion that an unconscious division of the *self* could rule its conscious part (Freud, 1899) much has been added to the discussion. It is now widely accepted that a subdivision of the nervous system, the autonomous nervous system, can be fully devoted to automatic processes that are subordinated to basic physiological functions. However, there is more debate regarding its parallel in terms of cognitive and decision making bias due to non-conscious or preattentive modulation.

Nevertheless, as animals, humans are prone to survive. Mechanisms for automatic detection of threat signals would be particularly advantageous for situations in which awareness of a given stimulus might be narrowed, or when attentional resources are engaged in parallel tasks. Ideally, humans should be able to process biological or social relevant signals in order to best cope with a possible dangerous situation. This would be in accordance with a faster subcortical pathway to the human amygdala that facilitates more adaptive physiological, neural and behavioural outcomes in appropriate contexts (Lang, Davis, & Öhman, 2000). However, its existence has been under fire due to the existence of anatomical and electrophysiological inconsistencies, and also due to methodological issues that do not ensure that the processing of relevant stimuli happens completely outside awareness or attention (Adolphs, 2008; Cauchoix & Crouzet, 2013; Pessoa, 2005; Pessoa, & Adolphs, 2010). Additionally, although we know that our sensory systems (e.g. visual system) are limited in their capacity to process all the features in the environment (Marois & Ivanoff, 2005), complete knowledge about these limits is still lacking.

In the last decades, there has been increased knowledge regarding threat detection and enhanced processing of emotional information, especially of fear-relevant stimuli (e.g. Adolphs, 2008, 2002; Öhman & Mineka, 2001; Pessoa, 2005, 2010; Pessoa & Adolphs, 2010; Tamietto & De Gelder, 2010; Whalen et al., 1998).

This thesis focused on the processing of information when constraints are posed to visual perception: either temporal (stimulus duration) or spatial (stimulus spatial location / position). Although this is an issue with major implications for the understanding of visual perception, emotional information processing and survival behaviour, in both its ontogenetic and phylogenetic perspectives, no consensus has yet been found. Problems in the definition of visual awareness and lack of direct comparisons of information processing at different locations of our visual field have contributed to the delay of a more close and attainable conclusion.

Here we used different methods in order to study emotional processing at the central nervous system level (functional neuroimaging) and its consequences at the peripheral nervous system level (skin conductance response), since the latter is more related to output behaviour. It was our aim to contribute to disentangle the neural correlates of conscious and non-conscious visual perception, as well as to clarify the conditions under which implicit processing of emotionally relevant information can occur. We believe that this knowledge, even at a small degree, will contribute for the understanding of human behaviour, in its social and more primitive efforts.

The opening chapter will start by presenting the visual system and the state-of-the-art concerning the neural pathways that hold visual perception. First, we will make a brief overview of the visual system and its structures, with special emphasis on the characteristics and the visual pathways of central and peripheral visual processing. In the closing of this chapter, the current frame regarding the neural correlates of face and emotional perception will be presented, with a special issue dedicated to evidence regarding a subcortical pathway to the human amygdala. Clarification of key concepts and terminology used throughout the thesis will close the last section. Thereafter, the main goals of this thesis, an outline of its content and the specific aims of the following chapters will be provided.

> *"It's not what you look at that matters, it's what you see."*
>
> — Henry David Thoreau

Introduction

Chapter 1

The Neural correlates of Visual perception

Unlike the frog, who has a relatively uniform retina and a simple visual system with an emphasis on the connections from the retina to the colliculus (Lettvin, Maturana, Mcculloch, & Pitts, 1968), humans have a more heterogeneous retina, composed of several functional mosaics that define parallel visual pathways and convey visual information in an position dependent manner. Therefore, sensory items are processed according to where they fall within the visual field and the characteristics of the functional mosaic at that level. This separation of labour has implications in terms of both their spatial and temporal response properties.

1.1. Visual information processing from the retina to the cortex: Spatial and temporal features

The foveola is the region of greater visual acuity in the retina. It corresponds to an eccentricity of roughly 2º of visual angle and is responsible for central or foveal vision. It is located in the central part of the fovea *centralis*, which together with the parafovea and the perifovea, constitute the macular region. The fovea has a perimeter that ends around 5.2º, whereas parafovea refers to a region between 5º and 9º, and the perifovea beyond 9º until the limits of the macula, approximately at 17º. Strasburger, Rentschler and Jüttner (2011) referred to *central or foveal vision* when an object falls within 2º of eccentricity, and to *peripheral vision* anything beyond 2º of eccentricity, definitions that we will follow in this thesis. Importantly, beyond the foveola, visual acuity diminishes with increasing eccentricity (Strasburger, Rentschler, & Jüttner, 2011). This occurs due to the heterogeneous cell composition of the human retina, varying density and their different cell properties. Their distribution, density and size do indeed depend on their location in the retina (Dacey, 1994). Importantly, the different functional characteristics of these cells modulate information processing already at the retinal level, and have impact in the type of information which travels to the cortex.

Cells in the retina may be distinguished based on their morphology, physiology and connectivity (Kaplan, 2003). The photoreceptors are involved in the stage in transduction of light stimuli into neural signals. Two major types of photoreceptors have been found in the human retina (but see Schmidt, Chen & Hattar, 2011). Cones (blue or short, S; green or medium, M; and red or long, L, types, according to their wavelength sensitivity) range around six to seven million in a human eye. They are abundant in the macula region, especially within the fovea *centralis* where they are densely packed, but quickly reduce in number towards the periphery of the retina. They can detect rapid light fluctuations and respond mostly under bright light conditions, and underlie colour and sharp vision. Rods, by its turn, are more numerous, around 120 million, and are found mainly beyond the perifovea, in the peripheral retina. They are specially tuned to dim light signals and slowly varying brightness, enabling high contrast sensitivity, and are therefore responsible for vision under low light or more degraded conditions (Kaplan, 2003; Masland, 2001) (Figure 1.1).

Even though rods outnumber cones in a large scale, most mammalian retinas have 8 to 10 cone-driven cells as compared to each cell associated with the rod pathway. In fact, more rods converge into one rod bipolar cell compared to the number of cones that connect with one cone bipolar cell. This trade-off in number contributes to the fact why vision in the centre is spatially more accurate, whereas vision conveyed by the peripheral retina is more sensitive to light (Masland, 2001).

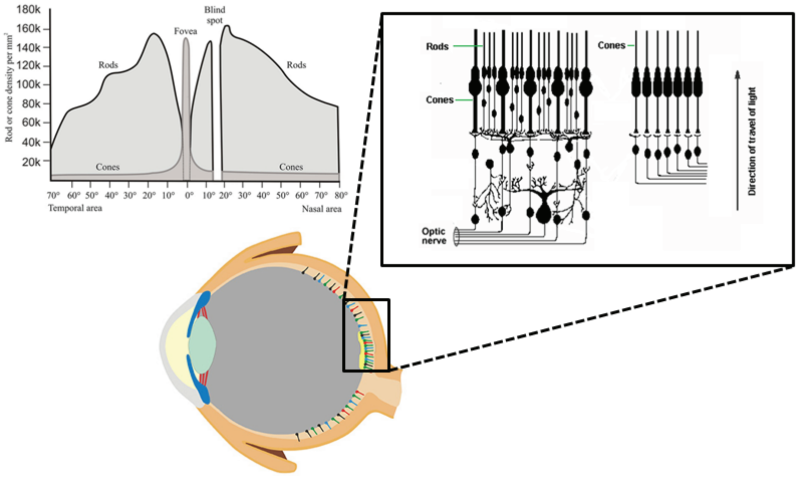


Figure 1.1 – Schematic composition of the human retina. *The fovea (central vision) contains mainly one type of photoreceptor (cones), whereas the peripheral retina contains mainly rods, besides cones (top left).*

Information from the photoreceptors is passed to the bipolar cells in the subsequent retinal layer through the amacrine and the horizontal inter-neurons, which then pass the neural signals to the retinal ganglion cells (RGCs) (Figure 1.2). Importantly, contrary to other mammals, primates have an atypical higher number of cone bipolar cells (Masland, 2001), which emphasizes their bias for central focused vision.

More than a dozen types of RGCs exist in the retina. These are specialized in the encoding of different aspects of the spatial, temporal and spectral composition of the image in the retina. Messages conveyed by these cells play an important role in visual perception as they allow conscious visual perception but also unconscious aspects of vision, such as attentional guidance and control of eye movements (Martin & Grünert, 2003). The two major types of RGCs include, the midget and the parasol ganglion cells, non-midget, non-parasol and another type, the small bistratified RGC types have also received recent attention. As the photoreceptors, their distribution depends of their location in the retina, with the number of midget ganglion cells progressively increasing relative to the parasol and small bistratified types from peripheral to central retina. In the fovea, midget cells constitute about 90% of all neurons, parasol cells about 5%, and the small bistratified cells, about 1%. In the periphery, midget cells decrease to about 40-45%, and both parasol cells and small bistratified cells increase in number to about 20% and 10%, respectively (Dacey, 1994).

RGCs can be characterized morphologically in terms of their dendritic field size and spatial density (Dacey, 1994). The receptive field (RF) can be identified as the region of the retina where the action of light alters the firing of the neuron, corresponding to a region in the visual field. This receptive field is constituted by antagonic subregions with opposite effects in the discharge rate of the cell, and they are known as "centre" and "surround" regions. The firing rate is determined by the balance between excitatory and inhibitory influences in the receptive filed (Martin & Grünert, 2003). This is the basis for the known ON- and OFF-type responses which shape the firing rate output of the retinal cells (Kaplan, 2003; Purves et al., 2004). Their responses might be broad-band, non-opponent, meaning in this case that although the centre and the surround regions of these cells might respond to bright vs. shadowed regions, they are nevertheless unresponsive to colour. In addition, RGCs might respond to light within a range of preferred wavelengths (that can be specified in terms of S, M, or L relative cone contrast units), originating ON- and OFF-type responses related with colour opponency (Kaplan, 2003).

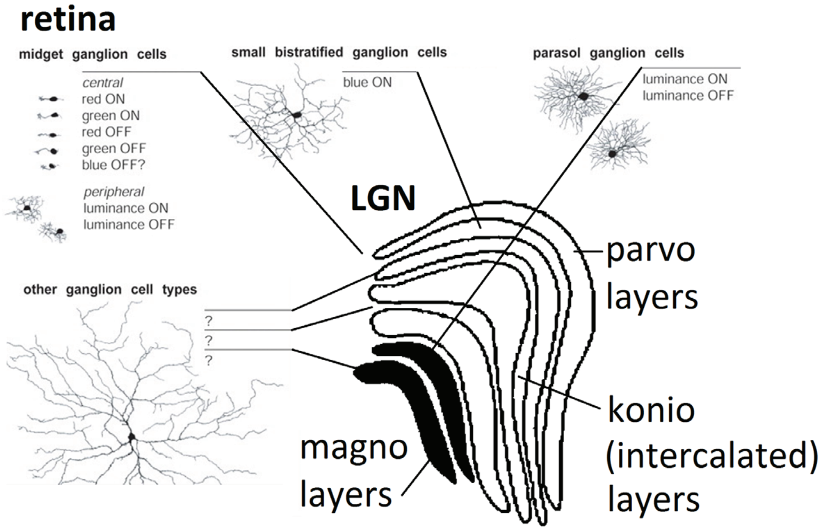


Figure 1.2 – Cell types in the retina and their projection to the LGN layers.

Receptive field within distinct retinal mosaics differ and determine the visual field they sample, and the type of information that is extracted from the visual scene (Martin & Grünert, 2003). Large receptive fields correspond to an increased area in the visual field at the expense of visual acuity (Masland, 2001). Cell morphology dependence on eccentricity factors is quite conspicuous, and explains how the distance from the fovea affects the size of receptive fields. Dendritic field diameter of both types, midget and parasol, increases with increasing retinal eccentricity, but parasol ganglion cells are consistently larger than midget ganglion ones at each eccentricity (Famiglietti & Kolb, 1976; Kaplan, 2003). These distributions have important consequences in terms of spatial vision, giving to central and peripheral vision quite different information processing properties. In fact, these different types of RGCs constitute functional arrays that project to specific visual pathways.

Interestingly, the visual system is composed of several visual asymmetries, already at the early visual level in the retina, with naso-temporal asymmetries (Perry & Cowey, 1985; Sylvester, Josephs, Driver, & Rees, 2007), and at the hemispheric level, with some evidence pointing to differences in spatial frequencies preference for the right and left hemisphere (for a review see Karim & Kojima, 2010).

The reticulogeniculate pathway

Cones output to midget ganglion cells dominate in daily vision, and project to the parvocellular (P) pathway, which also receives some parallel input from the small bistratified cells. In night vision, parasol ganglion cells dominate in conveying information from rod input, and form their dominant projection to the magnocellular (M) pathway (Callaway, 2005; Dacey & Brace, 1992; Martin & Grünert, 2003). An additional pathway, the koniocellular (K) pathway, conveys input sensitive to blue-on-yellow contrast with Blue ON-center input coming from the blue or S cones, through the small bistratified cells (Kaplan, 2003; Isbell, 2006).

RGCs axons bundle together to form the optic nerve which exits the retina through the optic disk. Fiber decussation is organized in such a way that information presented to one visual hemifield activates preferentially the contralateral hemisphere (Purves et al., 2004).

Concerning contralateral and ipsilateral projections, approximately 60% of the fibres coming from each eye cross at the optic chiasm. Furthermore, although much of the fibres arrive to the lateral geniculate nucleus (LGN) of the thalamus, in the diencephalon, around 10% are directed to the superior colliculus (SC), in the mesencephalon, which is part of the brainstem. Importantly, these fibres remain functionally segregated according to the pathways they represent, being projected to specific LGN layers, until they arrive to the primary visual area (V1) in the occipital cortex (Kaplan, 2003).

The following summarizes differences and convergences between the 3 major pathways which travel from the retina to the LGN. Although some part of these also project to the SC, we will describe this pathway later on. Cells projecting to the dorsal (P-cellular) layers in the LGN, the midget ganglion cells, have small receptive fields and, in trichromatic primates, have wavelength opponency, showing red-green colour sensitivity; have a low luminance contrast gain, with a tonic type of response to light steps; have low contrast sensitivity; have low axonal velocity, and have afferent input from a high number of ganglion cells (high ganglion cells/mm^2 ratio). Finally, they constitute about 80% of the LGN cell population. Cells associated to the ventral (M-cellular) layers have relatively large receptive fields, and high achromatic contrast sensitivity, as the M pathway is classically considered as having no colour opponency (although some recent studies suggest that a small subset might show a red-green opponency (Lee & Sun, 2009). Additionally, M-cells have a high luminance contrast gain, receive input from less number of ganglion cells than the P pathway, with a high conduction velocity and respond in a phasic manner. They constitute around 10% of the cell population in the LGN (Kaplan, 2003). Finally, a third (K-cellular) division of the LGN was more recently described, as compared with the other two pathways. It comprises diffuse layers of mostly small cell bodies (Szmajda, Grünert & Martin, 2008), and refer to about 10% of the cells in the LGN (Kaplan, 2003). The K pathway shares some characteristics with both the P and the M pathway. In fact, as the P-path, is characterized by having partial colour opponency, their luminance contrast gain is similar to the one of the M pathway, having a large receptive field, although its spatial resolution is more variable, as the cell size. It receives projections mostly from the bistratified RGCs, (Kaplan, 2003) (for a general schematic drawing, see Figure 1.2).

In humans, input from the central (within 10º) part of the visual field is overrepresented in the LGN, being associated to around 73% of the total LGN volume (Schneider, Richter, & Kastner, 2004).

Importantly, complete segregation of specific cell types to specific pathways is only partially achieved. Although traditionally a complete segregation between the three main visual LGN pathways has been defined, one must be careful when taking conclusions based on this assumption. In fact, as pointed by Kaplan (2003) this might not be the case, as cells in the M pathway might show some colour opponency characteristics in contrary to what was previously thought (Lee & Sun, 2009). In fact, although the M-layers form the LGN seem to rely only on parasol cells input, P-layers also receive input from a non-midget ganglion cell type that convey information from the S cones, classically related to the K pathway (Dacey, 1994).

Additionally, beyond the LGN, it was previously postulated that M-cell pathway projects mostly to the dorsal stream, whereas the P-cell pathway projected preferentially to the ventral stream (which also receives M input). However, this division of labour might not be as linear as initially thought, as parvocellular input is also present in the MT motion parietal visual area (Nassi & Callaway, 2006, 2009). These facts carry important evidence about partial segregation of functions along the different visual pathways, as experimental hypotheses and their testing might rely on strict separation of function. So, attention must be given when trying to ascribe a specific and independent function to one stream, or when designing visual stimuli that try to selectively excite or bias the processing in one pathway at the expense of other, as conclusions might be erroneous or at least biased.

The retinotectal pathway through the superior colliculus

Much what is known about the SC comes actually from studies in the animal model. In fact, functions of the SC have not been exhaustively studied in primates, but mostly in the rodent model (e.g. Schneider, 1969). His function in the human brain has been inferred from pathological conditions such as blinsight (see section 1.1.2.1.). Methodological issues related with neuroimaging techniques might explain why studies in humans have been hard to perform, specifically the small size of this structure, its deep location, and its proximity to vascular structures that cause physiological noise (Schneider & Kastner, 2005).

The SC is composed of superficial and deeper layers. The superficial layers receive inputs from the retina, striate cortex, extrastriate cortex and the frontal eye fields, responding to broader visual stimulation irrespective of stimulus orientation, size, shape, or movement, whereas the deeper layers, receive input from the prefrontal and parietal cortices and frontal eye fields, and rely much less on primary sensory or motor cortices input. In the monkey, activity in these layers is found primarily before a saccade is executed or when shifts of attention occur (Berman & Wurtz, 2008; for a review see Schneider & Kastner, 2005).

As we said earlier, although approximately 90% of the fibres of the optic nerve travel from the retina to the LGN, around 10% reach the SC. These fibres arrive mostly from the K cellular division (Kaplan, 2003) and convey information only from the contralateral visual field (Schneider & Kastner, 2005). Although this pathway is linked with colour-opponent S cells, evidence from studies in new world monkeys show that the SC is insensitive to colour related with S cones (Tailby, Cheong, Pietersen, Solomon, & Martin, 2012; Schiller, & Malpeli, 1977). This would suggest the S cone signals would reach cortical regions through the K division of the LGN, whereas the SC would convey information only from the broad-band colour-blind cells.

In the macaque, parasol cells project to the SC only extremely rarely (Perry & Cowey, 1984; Rodieck & Watanabe, 1993). The M but not P layers of the LGN project to the SC indirectly through a corticotectal pathway (Hoffmann, 1973). In the squirrel monkey, the superficial layers of the SC have been reported to project to both the koniocellular (K) layers, also known as the interlaminar zones, with less consistent evidence that the SC projects to the M layers of the LGN (Harting, Casagrande, & Weber, 1978; Harting et al., 1991).

Regarding the retinotopic representation of the visual field and the effect of eccentricity on the SC, in the macaque, the size of the receptive fields changes with increasing eccentricity, and so as in the cat for which the central part of the visual field (within 10º) are overrepresented in the SC. However, recent neuroimaging data from humans using stimuli that mapped from the centre to the periphery of the visual field showed that although the SC maintains retinotopic position conservation, evidence of a consistent eccentricity map was difficult to find. Also, the human SC exhibited high sensitivity to low stimulus contrast and also responded well to stimulus motion (Schneider & Kastner, 2005).

A pathway for motion detection trough the SC and the pulvinar

A specific pathway for the detection of motion was proposed to involve the pulvinar, a thalamic structure in the top of the brainstem and part of the diencephalon. In the macaque, after arriving from the retina, the fibres go through the SC to the pulvinar, and then to MT, the motion area located in the parietal cortex (Berman, & Wurtz, 2008, 2011; Villeneuve, Thompson, Hess, & Casanova, 2012). Nevertheless, in the macaque the pulvinar also receives input from the MT area (Standage, & Benevento, 1983; Ungerleider, Desimone, Galkin, & Mishkin, 1984). Projections from the pulvinar to the superior colliculus were found in primates (Weller, Steele, & Kaas, 2002) and in humans (Leh, Chakravarty, & Ptito, 2008).

Finally, the pulvinar neurons which receive input from the SC share properties with neurons in the superficial layers of SC, responding to visual stimulation but no presaccadic activit (Berman & Wurtz, 2008).

Evolutionary pressures in the visual system

Knowledge about the visual system comes mostly from studies using the cat (Hubel & Wiesel, 1959, 1962), the rodent (Schneider, 1970), and the primate (Livingstone & Hubel, 1988; Ungerleider & Mishkin, 1982) models. Only recently, high resolution imaging techniques have allowed studying the visual system in humans (e.g. Schneider & Kastner, 2005, 2009).

However, besides the inherent interspecies differences, disparate evolutionary pressures might have shaped the evolution of visual systems in distinct manners. In fact, a recent conceptualization (Isbell, 2006) theorizes about the fact that the visual systems of different classes of primates (e.g. New World and Old World monkeys) might have been coined by the environments in which they lived. Development of better strategies for predator (snakes) detection and evasion, besides food attainment ones, might have been in the origin of modifications such as orbital convergence, visual specialization and brain expansion.

Magnification factor in the fovea and in the peripheral retina

Magnification factor refers to the linear extent in the cortex devoted to per degree of visual angle in the retina (Daniel & Whitteridge, 1961). Differences in the ganglion cell density already at the retina level seem to account for the cortical magnification factor. In fact, whereas a higher ratio of ganglion to cone cells exist in the foveal region, this ratio is severely reduced towards the peripheral retina (Dacey, 1994; Perry & Cowey, 1985; Wässle, Grünert, Röhrenbeck, & Boycott, 1990).

In humans there is some evidence that the P/M ratio in the fovea can achieve 30/1 (Dacey & Peterson, 1992; Kaplan, 2003). A reduction of the number of neurons for a given area of the visual field implies an increase of the size of the receptive fields of the neurons, since each neuron has to cover a larger part of the visual field. As a consequence, visual performance (e.g. visual acuity) is best in the centre and worse in the periphery, matching the fact that foveal input is overrepresented in the primary visual cortex (V1) (Daniel & Whitteridge, 1961).

Spatial resolution can be defined as the ability to respond to small patterns and is much affected by the RGCs density in the retina. As the density of the parasol cells decrease their dendritic field sizes increase, which explains the lower spatial resolution of the M system compared to the P system at each eccentricity (Kaplan, 2003). Importantly, M and P cells have a similar coverage of the visual field. However, due to the lower density of the parasol M cells, the resolution of the M system is much lower than the one form the P system.

The role of the LGN and of the SC, and the pulvinar in attention and awareness

Previous studies suggest that the SC plays a role in attention processes, especially in the guidance of eye movements to relevant positions in the visual field (Goldberg & Wurtz, 1972; Schneider & Kastner, 2009). It was first hypothesized that some layers in the SC contain a map that represents the visual field, whereas other layers contains a motor map related with saccadic behaviour. These two maps seem to have direct correspondence and therefore control the direction of gaze, allowing the shift of the highest acuity area of the retina to the relevant region (Lee, Helms, Augustine, & Hall, 1997; Purves et al., 2004).

Importantly, the SC also seems to play a role in covert attention mechanisms and in the control of eye movements, as it has been shown to participate in the process of target selection prior to eye movements have been performed (Ignashchenkova, Dicke, Haarmeier, & Thier, 2004; Krauzlis, Lovejoy, & Zénon, 2013; Wurtz & Goldberg, 1971). Additionally, since the appearance of a target in the visual field does not guarantee a necessary and sufficient saccadic behaviour, and training might prevent saccades to the target, a direct and automatic connection between the visual and the motor maps was questioned, and replaced by the hypothesis that connections between corresponding regions of the two maps might be indirectly modulated by other cortical regions (Purves et al., 2004).

Recent experiments using fMRI have found that both saccadic eye movements and spatial attention can modulate activity in the SC. However, the SC showed a higher magnitude of attentional modulation compared to the LGN, suggesting complementary but distinct roles of these structures (Schneider & Kastner, 2009).

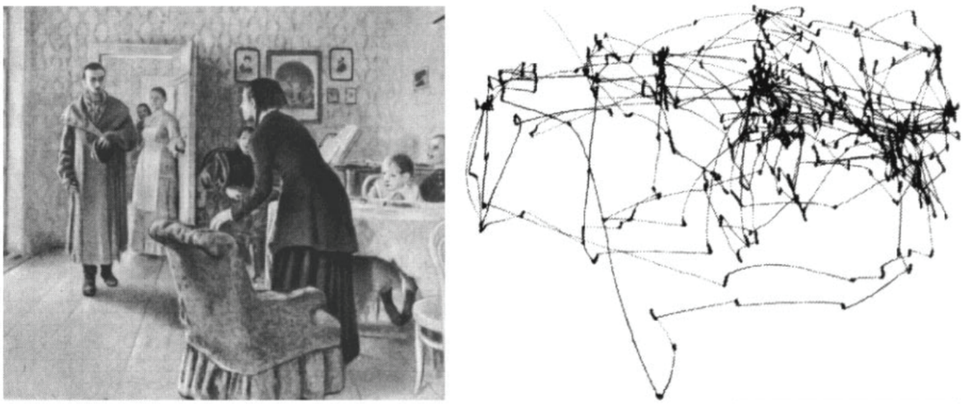
Regarding the role of the pulvinar, it has been suggested to play a role in residual visual functions in disease conditions in which awareness (Weizkrantz, 1996) and attention (Karnath, Himmelbach, & Rorden, 2002) are affected. However, a significant part of the literature directly relates the role of the pulvinar with visual attention (Padmala, Lim, & Pessoa, 2010; Pessoa, & Adolphs, 2010; for a review see Baluch & Itti, 2011), and with regulation of cortical synchrony and information transmission across the visual cortex (Saalmann, Pinsk, Wang, & Kastner, 2012; Shipp, 2003, 2004).

Final notes on early visual processing

All these factors co-work together to explain that in primates, spatially accurate vision, following conscious perception of a stimulus, relates to central processing. In this manner, visual mechanisms (controlled or automatic) for the detection of stimuli falling in the peripheral visual field require special adaptations, in particular saccadic control for foveation, if one wants the access the detailed content of an item. Nevertheless, automatic or preattentive mechanisms should exist to account for all the processes for which we cannot be aware of, or attend to. Importantly, cell and related pathways properties make them more prone for specific types of processing. The M pathway would be more relevant for processing fast transient stimuli, or for stimuli that only require coarse processing, whereas the P and the K pathways would be especially entailed when high detailed processing is required. Understanding the link between cell properties in each pathway and functions of the pathways should disentangle the way by which the visual system analyses and represents the external visual world (Kaplan, 2003). In the next subsection, we will shortly review what is currently known about the neural mechanisms for facial expression recognition and more general processing of emotional signals.

1.2. PATHWAYS FOR FACIAL RECOGNITION

Faces can be considered as a special class of objects which are generally capable of grabbing our attention as compared with other classes of stimuli (e.g. Hershler, Golan, Benti, & Hochstein, 2010; Johnson, 2005; Morand, Grosbras, Caldara, & Harvey, 2010) (Figure 1.3). However, differences should be considered regarding *identity* (invariant elements of a face) or *facial expressions* (changeable elements of a face), as lesion studies seem to separate the two recognition systems (Bruce & Young, 1986; Posamentier & Abdi 2003; Vuilleumier & Pourtois, 2007).



Figures 1.3 – Faces attract attention. *Example of a scene displaying faces (left) and corresponding eye movements while seeing it (right). Adapted from Chun, 2000.*

Recognizing emotional expressions is essential for social communication, enabling us to understand the emotional state of others and their intentions (Adolphs, 2008). Under certain circumstances, emotion recognition might even be essential for our survival, for example by alerting us to a possible threat (i.e. fear recognition). This underlines the importance of quickly processing these emotions in order to react to a possible life-threatening situation.

Two main cortical pathways are known to arise from the occipital pole after V1: the dorsal and the ventral streams. The dorsal pathway receives input both from the magnocellular layers of the LGN and from the retinotectal pathway through the superior colliculus (Kato, Takaura, Ikeda, Yoshida, & Isa, 2011; Kravitz, Saleem, Baker, Ungerleider, & Mishkin, 2013), and travels to the parietal cortex. It is especially devoted to functions such as spatial localization, visually guided action, and grasping behavior (Isbell, 2006; Milner & Goodale, 2008; Ungerleider & Mishkin, 1982). In fact, whereas this stream is known as the "where" or "vision for action" pathway, the ventral stream, which travels to the inferior temporal cortex, is known as the "what" pathway.

This is nevertheless a classical vision, as recent reformulations have proposed a much more complex and interactive network of connections, with several feedforward and feedback projections at several points in these networks (for a review see Kravitz, Saleem, Baker, Ungerleider, & Mishkin, 2013).

Previous lines of research have suggested that, besides the cortical or 'slow' cortical route, enabling detailed processing of emotional information, a 'fast' route, presumably subcortical, would convey information more quickly and without requiring attention or awareness (Liddell et al., 2005; Morris, Öhman, & Dolan, 1999). Importantly, a dissociation between the 'slow' and the 'fast' route should be reflected in distinct neural networks. It has been proposed that under normal conditions emotional expressions are processed by the geniculostriate cortical pathway (see above section 1.1.) involving the retina, lateral geniculate nucleus, and striate cortex (V1), then projecting to extrastriate (V2, V3, V4) areas along the visual ventral stream involving occipital-temporal regions: the occipital gyrus, the lateral occipital complex, the superior temporal sulcus, and the fusiform gyrus, specially devoted the object and face meaning extraction (Grill-Spector, Knouf, & Kanwisher, 2004; Haxby, Hoffman, & Gobbini, 2002; Kanwisher, McDermott, & Chun, 1997; Pitcher, Charles, Devlin, Walsh, & Duchaine, 2009).

Still, less is known about the neural structures involved in an alternative subcortical 'fast' pathway.

A SUBCORTICAL PATHWAY TO THE AMYGDALA

A subcortical pathway has been proposed to be preferentially engaged in the involuntary recognition of emotional cues, especially cues related with threat. This pathway relies on a central structure in emotional processes, the amygdala. At this point, we should make a short descriptionof the function and structure of the amygdala.

Structure and connectivity

The amygdala is a complex structure of several nuclei in the base of the temporal lobe. It was first described in the beginning of the 19th century as a mass of grey matter by Burdach (*cit. in.* Swanson, & Petrovich, 1998), and received its name due to its almond shape. Nevertheless, the exact extend of its structure and borders remain controversial (Swanson & Petrovich, 1998).

It has been mostly studied in primate and rodent models, but direct translation to the human is not always achieved. Previous reports on amygdalar connections are vast, complex and sometimes contradictory (Swanson & Petrovich, 1998). Given that in the current work we did not explore the role of the different nuclei, we will not present here a detailed review on this topic. Nevertheless, a broad picture will be presented, particularly related with the fear response (detection of threat).

There is some consensus relatively to its composition into central, medial, lateral, basal, accessory basal nuclei (Figure 1.4). It has several subcortical connectivity with structures such as the brainstem, the hypothalamus, the thalamus, the basal forebrain, the basal nucleus of the stria terminalis, the hippocampal formation, the striatum, and the olfactory system. It is particularly involved in the regulation of visceral and autonomic components of the escape, fear related response. In addition, it connects with all the major divisions of the cortex (Freese & Amaral, 2009). We focus particularly the insular, the cingulate, and the orbitofrontal cortex, due to their role in affective networks, the parietal cortex due to its role in selective attention, and the temporal and occipital cortex due to their role in visual processing. In general, projections to other subcortical structures originate from the central nucleus, whereas projections to cortex and the striatum originate from the basal, accessory basal, and in some cases the lateral nuclei (Salzman & Fusi, 2010). Importantly, it receives most visual input from the occipitotemporal ventral stream to the lateral nucleus, conceived as "evaluator" nucleus. Interestingly, these do not project heavily to the central nucleus, which are conceived as the "effector" nucleus, i.e., the first stage in the fear response. The basal nuclei are thought to play a role on this, by carrying the input from the lateral nucleus to the central nucleus through modulation of other cortical structures such as the orbitofrontal cortex (Freese & Amaral, 2009).

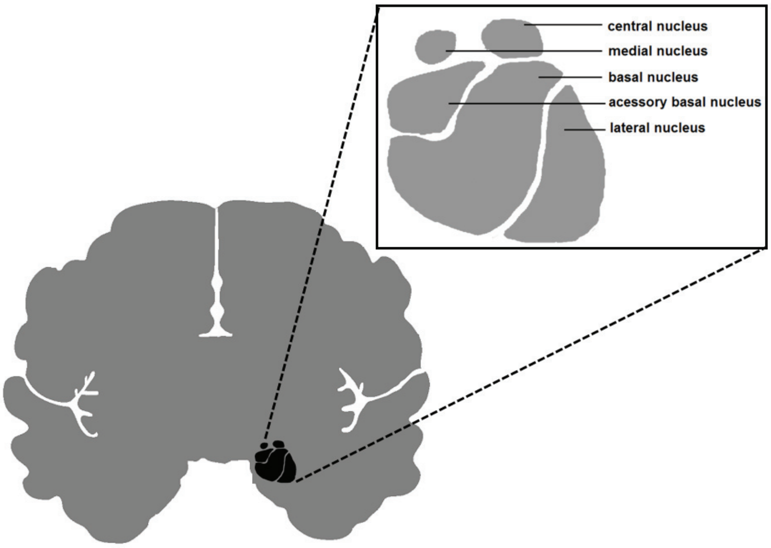


***Figure 1.4 – A schematic view of the human amygdala nuclei**. Central, medial, basal, accessory basal, and lateral nuclei.*

Function

The amygdala is referred as being majorly implicated in the detection of fear-relevant signals, such as spiders, snakes and angry faces, due to selectivity of evolutionary shaped mechanisms (Öhman & Mineka, 2001). Others authors propose that its role is more generally implicated in the processing of ambiguous information, such as fearful or surprised faces, because they carry insufficient information about the source of threat, in the sense that those faces are more context dependent than angry ones, acting as a "bell" that calls for enhanced attentional mechanisms in order to solve ambiguity (Kim et al., 2004). Furthermore, this structure is also referred as a relevance detector although not being circumscribed to fear-related information but being also implicated in a wider category of biologically relevant stimuli (Sander, Grafman, & Zalla, 2003).

The evidence of a subcortical route to process emotional expressions has thereby raised controversy. Some studies report amygdala activation with and without awareness (Anderson, Christoff, De Rosa, & Gabrieli, 2003; Williams et al., 2006). However, other studies do not support this evidence. Accordingly, amygdala activation was not found for fearful faces when presented in task-irrelevant locations (Pessoa et al. 2002) nor when it was part of covert attention conditions (Phillips et al., 2004). Nevertheless, under conditions of limited attention, such as suppressed phases of binocular rivalry, emotional face detection still occurs but a coarse form, supporting the existence of a fast route of processing that leaves behind detailed representations (Williams, Morris., McGlone, Abbott, & Mattingley, 2004).

It should be stated that most of the previous work regarding the amygdala and implicit emotional processing has studied fearful faces, although angry faces also signal significant threat. By displaying emotional expressions, faces can constitute such signals and provide us with important information about individuals' intentions (Adolphs, 2002). However, some faces would be more relevant than others in their capacity to activate fast mechanisms of defense. For example, both fearful and angry faces have been proposed to be preferentially processed at preattentive levels due to their negative valence, the first suggesting an eminent but unknown threat (Whalen et al., 1998) while the later constituting the direct source of danger (Mineka & Öhman, 2002).

Importantly, conceptual and methodological differences between studies might explain different findings. Pessoa and colleagues (2005) referred that a distinction should be made between subjective and objective measures of awareness. Objective measures use performance in recognition tasks, while subjective measures rely on self-reports (Merikle, Smilek, & Eastwood, 2001). It seems that the amygdala is only activated in conditions of subjectively rated unawareness. Furthermore, attentional load might also explain activation patterns. Using similar paradigms, Vuilleumier and colleagues (2002) found amygdala activation for faces during unattended conditions, whereas Pessoa and colleagues (2002) did not.

Information is hypothesized to enter the retina directly to the superior colliculus, passing then to the pulvinar nucleus of the thalamus and from here to the right amygdala (Adolphs, 2002, 2008; Lang, Davis, & Öhman, 2000; Liddell et al., 2005; Morris, Öhman & Dolan, 1999). However, conflicting evidence for the relevance of afferent connections from the pulvinar to the amygdala has extended the debate (Pessoa, 2005; Pessoa & Adolphs, 2010; Tamietto, Pullens, De Gelder, Weiskrantz, & Goebel, 2012).

HISTORICAL EVIDENCE, ANIMAL STUDIES AND DISEASE MODELS OF AMYGDALA FUNCTION

Much of our current knowledge that relates the amygdala with the fear module (Öhman & Mineka, 2001) arises from the animal model back in the 80's (LeDoux, Sakaguchi, & Reis, 1984). By inducing lesions in the geniculate, in the colliculi and in the auditory cortex of mice, LeDoux and colleagues were able of study the mechanisms of fear conditioning and their dependence on the amygdala and these afferent structures. They found that lesions of geniculate and subcortical auditory centres, but not lesions of the auditory cortex, suppressed the autonomic and behavioural conditioned emotional responses. Two routes for emotional processing were then proposed, a 'low' road through subcortical structures, and a 'high' road using cortical regions (LeDoux & Phelps, 2008).

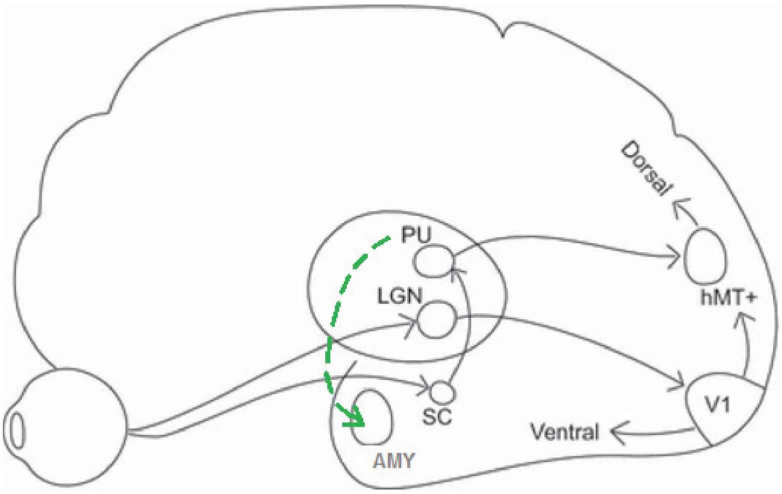


Figure 1.5 – Schematic view of the human visual pathways. *A cortical "slow" route through the laterate geniculate nucleus (LGN) to the primary visual cortex (V1) and then to the ventral stream (this route conveys information mainly from the parvocellular layers coming from the retina), and a subcortical "fast" route through the superior colliculus (SC) to the pulvinar (PU) nucleus of the thalamus. This pathway, usually involved in visual motion, carrying information to the dorsal parietal stream, is thought to project also to the amygdala. Adapted from Strand-Brodd et al., 2011.*

In 1974, Weiskrant and colleagues wrote a seminal paper about blindsight (unawareness for stimuli presented at the contralesional hemifield, due to visual cortex damage, associated with above chance performance). They described a patient who had lost part of his striate cortex, namely V1, and reported her still residual visual ability. Previous evidence (Schneider, 1969; Trevarthen, 1968) from the primate and rodent animal model suggested two main routes for visual processing but with different properties: the retinotectal and the retinogeticulate pathways (see section 1.1). Studies in the monkey (Old World monkey) supported the notion that the retinotectal pathway could play an important role in residual vision after V1 lesion, therefore mediating non-conscious vision in blindsight patients (Kato, Takaura, Ikeda, Yoshida, & Isa, 2011). However, other authors (Zeki, 1995) suggested that a direct geniculo-extrastriate path, bypassing V1, could account for the same phenomenon. In fact, feedforward projections from V2 to other extrastriate areas, as well as a poorer direct geniculocortical innervation of those other areas, could well give the K layers of the SC a central role in blindsight (Hendry & Reid, 2000). However, contrary support for this hypothesis came from a lesion study in the monkey which suggested that the LGN is required for residual vision (Schmidt et al., 2010).

Importantly, primary evidence for the role of this pathway in blindsight came from the use of stimuli such as moving dots, which likely involves processing in the retinotectal pathway to the motion MT area (see section 1.1) (Saharaie et al., 1997; Weiskrantz, Barbur, Sahraie, 1995; Weiskrantz, 1996). The use of affective stimuli such as facial expressions of emotion extended the notion that this pathway and its projections to the amygdala could also support additional visual capabilities related with emotional processing. In fact, a patient, when presented with fearful faces in his blind hemifield, was able of discriminate above chance level when presented emotional faces (De Gelder, Vroomen, Pourtois, & Weiskrantz, 1999). The process was then labeled as "affective blindsight", given evidence that it could also occur in healthy humans when visual awareness was limited through paradigms of visual masking (Tamietto & De Gelder, 2008).

Additional support for this pathway came from recent imaging studies. First, increased right SC, bilateral pulvinar and amygdala activation was found both to fearful (Morris, De Gelder, Weiskrantz, & Dolan, 2001) and to angry (Van den Stock et al., 2011) faces presented in the blind hemifield of a blindsight patient. Second, through means of a technique that maps white matter connections, Difusion Tensor Imaging (DTI), revealed direct connections between the 3 'low road' structures, the amygdala, the SC and the pulvinar (Tamietto, Pullens, de Gelder, Weiskrantz, & Goebel, 2012) (Figure 1.5).

Nevertheless, the controversy still persists (Cauchoix & Crouzet, 2013; De Gelder, Van Honk, & Tamietto, 2011; Pessoa, & Adolphs, 2011) and is now centred on the temporal signatures of cortical and subcortical processing. In fact, some emphasize that the cortical processing is sufficiently fast to account for the functions attributed to the subcortical 'fast low road' to the amygdala (Cauchoix & Crouzet, 2013; Pessoa, & Adolphs, 2011). Importantly, a recent proposal argues that affective processes is neither modular, nor operating in a strictly feedforward manner. Instead, the authors propose that the role of the amygdala in the processing of affective visual information comes from a broad functional connectivity organization encompassing both cortical and other subcortical structures (Pessoa & Adolphs, 2011; Kravitz et al., 2013).

1.3. Terminological issues and conceptual definitions

Here we provide definition of core concepts that will be used throughout the rest of thesis, in order to clarify what we mean when using them.

First, a distinction should be made between *attention* and *awareness*. In fact the two concepts have been often confused. *Attention* is the process by which a sensory input is prioritized for action, memory or thought (Lamme, 2003; Posner, 1994). This selective process allows that sensory items pass from the state of *phenomenal awareness* (being merely experienced as sensory item) to *access awareness* (allowing for direct control of reasoning, reporting and action) (Block, 1996; Lamme, 2003). Furthermore, attention might be stimulus-driven (based on stimulus sensory features, implicit process) or goal-oriented (based on knowledge, expectation and current goals, explicit process), and the two seem to rely on different brain networks (Corbetta & Shulman, 2002). Goal-oriented (top-down), is dependent on the dorsal posterior parietal and frontal cortex, while stimulus driven (bottom-up) recruits both the temporoparietal and the ventral frontal cortices, and it seems to be lateralized to the right hemisphere.

Attentional unawareness is an interesting concept that illustrate that visual perception of items that lie outside the focus of attention is attenuated or abolished. S*ensory unawareness*, reflects conditions in which short temporal duration or insufficient stimulus energy prevents the stimulus of generating a conscious percept (Tamietto & De Gelder, 2010).

By *subliminal*, we refer to the processing of a stimulus under short or weak presentations, thereby not allowing awareness of the sensory item. *Automatic* or *preattentive* emphasizes that a stimulus is processed before and independently of attentional selection, thereby not relying on top-down factors. Finally, the term *implicit* is used in a more broad manner, generally referring to task-irrelevant or unintentional processing (Tamietto & DeGelder, 2010).

For the following, when using the term *aware* or *conscious*, we will be referring to *access* awareness (Lamme, 2003) or *sensory* awareness (Tamietto & De Gelder, 2010). We will refrain from using the term *unconscious* and instead we will replace it by *non-conscious*, given the traditionally psychoanalytic view to which the former is linked (Tamietto & De Gelder, 2010). So, by *non-conscious* we refer to stimuli that remain inaccessible to conscious processing, even when attentional resources are allocated to the stimulus. In this manner, even when not perceived, these items are nevertheless represented in same manner in neural activity (Lamme, 2003).

For the sake of coherence, we will use the words *aware* and *unaware* or *non-conscious* only when considering temporal constraints in perception (Chapter 3). When referring to the limits posed by spatial constraints in particular in the visual periphery (Chapters 5 and 6), we will not use the terms *conscious*, *aware* and *non-conscious*, *unaware*, since the process underlying is different in nature from when using temporal limits. In fact, in this case the stimulus is consciously perceived, although in a degraded and less accurate form. In addition, accurate access to picture content due to good spatial resolution is intrinsically connected with differences between central (foveal) and peripheral (>2º) vision. In this manner, the study of processing of emotional cues is be made through task manipulation: either stimulus driven (implicit threat) or goal-oriented (explicit threat signals). The terminology *implicit* and *explicit* will be used when referring to cognitive demands that are imposed by particular instructions as a function of task type (Chapters 5 and 6).

As a final note, we must emphasize that all the tasks included in this thesis required the participants to pay attention to the stimulus. However, in the spatial location manipulation, attention was allocated to different features: threat (explicit emotional content) or identity (implicit emotional content). Prior evidence shows that this types of instruction modulates the way that the participant accesses the information (Lamme, 2003; Van den Stock et al., 2011).

AIMS AND OUTLINES

In the current project, we aimed to understand the temporal constraints that determine how emotional cues can be processed without awareness (Chapter 3), and how the stimulus position in the visual field (central vs. peripheral and left vs. right) influence the processing of emotional items of distinct ecological significance, such as animal faces, threatening and non-threatening (Chapter 5) or snake-related stimuli (Chapter 6). A multimodal approach that combined behavioural, psychophysiological and functional neuroimaging methods was used in order to understand implicit vs. explicit processing of emotional stimuli and its neural correlates.

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METHODS

Chapter 2

Methods

In this section will not describe procedures of preparation, acquisition, and recording, as these are better described in the respective methods section of each study, presented in the following chapters. The main goal of this chapter is thereby to give an overview on the methods we used for the elaboration of this thesis, particularly their (psycho)physiological basis, but also their advantages and limitations. Additionally, we focused in more detail some of the procedures of data analysis that we have performed.

2.1. Skin Conductance Response (SCR)

The skin conductance response (SCR) is a measure of change in sympathetic arousal generally implicated in situations that require 'fight' or 'flight' responses. SCRs have been taken as a measure of directed attention or when subjective salience is present (Sequeira, Hot, Silvert, & Delplanque, 2009) besides being also considered as an indicator that fear learning have occurred (Öhman & Mineka, 2001).

Physiology

The study of the electrical changes in the human skin began in the elaboratory of Jean Charcot. It was there that one of his collaborators, Féré, discovered that by presenting external stimuli the passage of an electrical current through the skin was momentarily facilitated (better conductance, decreased resistance: following Ohm's Law, Conductance is the reciprocal of Resistance, thereby Conducatnce is Current divided by Voltage). Later on, it was discovered that this momentarily changes were due to variations in the production of sweat. These variations are related with the activity of the sweat glands in the layers of the skin, which are responsible for the increase and decrease of sweat in the sweat ducts (Dawson, Schell, & Fillion, 2007). Therefore, it is included under the term of electrodermal activity (EDA). The SCR correspond to the phasic, more transient component of EDA that occur on top of the tonic, more sustained component – the skin conductance level (SCL).

Neural correlates

Major brain regions involved are the posterior hypothalamus, brainstem and ventrolateral pons, which have afferent and efferent connections with the amygdala (Sah, Faber, Lopez De Armentia, & Power, 2003), and at a higher level the anterior cingulate gyrus, the right inferior parietal region and the dorsolateral prefrontal region (Tranel & Damasio, 1994).

Measures

The SCR *amplitude* is probably the more used measure in the literature, concerning EDA measurements, and corresponds to the peak of the phasic increase in conductance following an event (is thereby an event-related response). This measure is intrinsically related with the *latency* and the *rise time* measure, which correspond to the temporal interval between stimulus onset and SCR initiation, and between the SCR initiation and the SCR peak, respectively (see Figure 2.1).

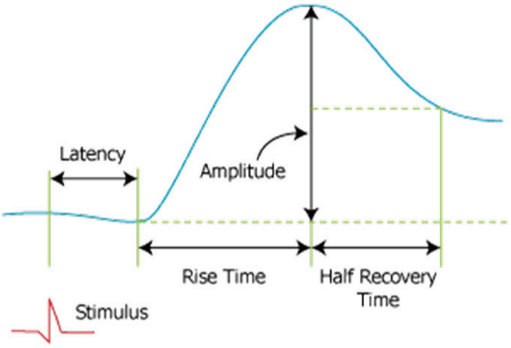

Figure 2.1 – Example of a skin conductance response (SCR) and the measures associated. *The image is courtesy of www.adinstruments.com.*

DISADVANTAGES

Although the SCR can be considered as a complementary method to study higher mental processes (Sequeira et al., 2009)the electrodermal activity (EDA, its major disadvantage is to be a slow response, given that the latency of its response is between 1 and 4 seconds. Additionally, it also has a higher rate of habituation as the stimulus is repeated (Dawson et al., 2007). This in fact constitutes also a measure of the significance of the stimulus.

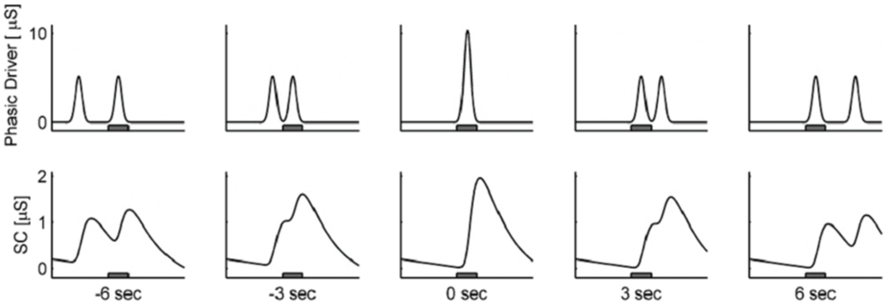

Figure 2.2 - Simulation of the amplitude of a fixed SCR*. Examples of how the SCR can become biased by a second SCR. The grey box corresponds to the response window. Adapted from Benedek & Kaernbach, 2010.*

DATA ANALYSIS

Instead of the traditional method given by softwares such as the AcqKnowledge (Biopac systems, www.biopac.com), we relied on an automatic method of scoring using Ledalab V3.1.1, a Matlab-based software developed specifically for the analysis of skin conductance (Benedek & Kaernbach, Kiel, www.ledalab.de). This tool is based on a two-compartment diffusion that addresses the process of sweat diffusion and, additionally, the process of pore opening in the sweat ducts (Benedek & Kaernbach, 2010). It uses a nonnegative deconvolution to decompose the signal into its tonic and phasic components and to reconstruct the SCR data into a segment of non-overlapped SCRs from which several parameters can be estimated. Importantly, it gives a very reliable response to some disadvantages of more traditional methods, as the well-known problem of the distortions into SCR measures (underestimation of the true amplitude) caused by overlapping SCRs (Dawson, Schell, & Filion, 2007; Benedek & Kaernbach, 2010) (see Figure 2.2).

2.2. Functional Magnetic Resonance Imaging (fMRI)

Functional magnetic resonance imaging (fMRI) is a recently (Ogawa et al., 1993) and exciting imaging technique to measure and localize specific functions of the human brain (Amaro & Barker, 2006; Casey, Davidson, & Rosen, 2002). Shortly, it begun first with the nuclear magnetic resonance (NMR), followed by the magnetic resonance imaging (MRI). Whereas the MRI analyses the structure of the brain, the fMRI, as the name says, evaluates its functioning while in resting state or when performing specific tasks (Casey et al., 2002).

Physiology

The fMRI method relies on changes in oxygen levels of the blood in the brain, which in turn are thought to reflect changes in the neuronal activity. This method is based on the magnetic properties of the haemoglobin in the blood, and measures differences between its oxygenated and deoxygenated state. During brain activation, localized increases in blood flow increase blood oxygenation and consequently reduce deoxygenated haemoglobin. In fact, when energy is required, oxygen is used to break down glucose and supply it to the brain. However, increases in levels of oxygen far exceed the blood flow and glucose consumption, and this results in increased amounts of oxygen in the blood. In its deoxygenated state, hemoglobin in the blood becomes strongly paramagnetic. As a outcome, highly oxygenated brain regions produce a larger magnetic resonance (MR) signal than less oxygenated areas. These differences constitute the basis of the blood-oxygenated-level-dependent (BOLD) signal, which is measured by fMRI (Casey et al., 2002).

Hemodynamic BOLD response

The change in the MR signal caused by neural activity is named hemodynamic response (HDR) and comprises three known phases: the *Initial dip*, which consists of a short-term decrease in the MR signal immediately after the onset of neuronal activity, before the main component of the hemodynamic response, and it is caused by a brief increase in oxygen consumption that is not immediately accompanied by a change in the blood flow; the *Positive BOLD response*, which corresponds to the maximal amplitude of the hemodynamic response, occurring typically about 4 to 6 sec following the stimuli presentation onset (if the neuronal activity is extended in time, the peak may be similarly extended into a plateau, typically with slightly lower amplitude than the peak); and finally, the *Undershoot*, which occurs upon cessation of the stimulus, before BOLD returns to the baseline level and has been suggested to occur due to biophysical and metabolic effects (Goebel, 2007).

Disadvantages

Although the fMRI method is considered to have good spatial resolution, in the order of the 3 mm, which allows the study of both cortical and subcortical structures, it has nevertheless a poor temporal resolution, given that the HDR is a slow response (Casey et al., 2002). Therefore, in term of spatial acuity is better than other techniques such as positron emission tomography (PET) and single photon emission computerized tomography (SPECT). In terms of temporal resolution, is worse than electroecephalography (EEG) and magnetoencephalography (MEG), which have resolution in terms of milliseconds (Dale et al., 2000).

fMRI EXPERIMENTAL DESIGNS AND ISSUES

The major goal in fMRI is to assess and locate sensory, motor and cognitive function. To this end, careful paradigm choice and experimental design is crucial.

Paradigm choices relates to the problem of isolating the task or process for which a brain map is intended. This generally involves a comparison between the activity patterns elicited by at least two different experimental conditions: a condition of interest, and a control condition. For instance, if a researcher wants to isolate the neural correlates of object perception, subjects inside the MR scanner need to see images of objects but also simple images of meaningless textures or patterns, so that the activity elicited by simple image viewing (any image) can be subtracted. This comparison or subtraction is called a contrast and constitutes the basis of most fMRI studies.

Regarding design, two main formats can be used in fMRI experiments: block-designs and event-related designs. In a *block design*, each condition is presented continuously for an extended period (one block lasting from 16 sec to a minute, on average) and blocks of different conditions are usually interchanged. The signal from one given condition is then contrasted with blocks of other conditions which, as mentioned above, typically differed only in the factor of interest. Alternatively, the signal from one condition can be compared against rest, as to reveal the whole network responsible for the execution of a given task.

In turn, in the event-related designs the stimuli are presented one at a time (trials) instead of being sequentially presented in a block. In this type of design, each event is separated from the subsequent event by a period named inter-stimulus interval (ISI). In contrast with what occurs in the block design, here the different conditions are usually randomly presented which avoids cognitive adaptation strategies of the subjects (Goebel, 2007). Event-related designs are generally better suited for estimation, and block design for detection. Estimation is the measurement of the time course within an active voxel in response to the experimental manipulation and does not require an *a priori* model. Such information is especially used when making inferences about the relative timing of neuronal activity, about processes occurring in different parts of the trial and about functional connectivity. Detection is the determination of whether activity of a given voxel (or region) changes in response to the experimental manipulation (Huettel, Song, & McCarthy, 2009). Block designs thus exhibit superior detection power and are less sensitive to differences in the shape and timing of the hemodynamic models.

To finalize this characterization of fMRI, some key concepts should be mentioned. Usually each volunteer participant undergoes a single experimental session. Each session includes collection of anatomical images and one or more functional runs. A run (4D volume composed information on space and time) consists of a set of functional images collected during the experimental task. Within each run, the functional data are acquired as a time series of volumes which consist of a single image of the brain made up of multiple slices. Slices, in turn, are acquired at a different point in time within the repetition time (TR – time interval between successive excitation pulses) and contain thousands of voxels (three-dimensional volume element) that together form an image of the brain (Huettel et al., 2009).

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RESULTS

Chapter 3

Temporal limits of Visual awareness And the role of Skin conductance response in understanding emotional cognition

PART I

INTRODUCTORY NOTES AND MOTIVATION

One of the most used methodologies to study the role of awareness levels in processing emotional information outside awareness is to present stimuli below, near and above a given temporal threshold of visual perception that may unfortunately vary (subliminal, near and supraliminal presentation). However, this approach has some technical and conceptual limitations since it does not fully ensure that the stimulus is not perceived. These limitations are generally tackled by the combination with another technique, *visual masking*.

VISUAL MASKING AS A METHOD OF LIMITING STIMULUS AWARENESS

Unawareness have mostly been inferred from the use of short presentation times (e.g. 30 msec) under the context of masking paradigms, mainly backward (Whalen et al., 1998). This type of paradigms has been used to prevent stimuli from reaching awareness, by presenting a mask immediately after the target in order to discontinue its conscious perception as sensory icon (see Kouider & Dehaene, 2007 for a review).

Lamme and Roelfsema (2000) stated that sufficiently prolonged stimulus durations allow for iterative and recurrent processing, as opposed to shorter durations that allow only for feedforward projections and no visual awareness. For example, neurons in the inferior temporal cortex first respond to if a face is present or not, and only at additional delays respond to facial expression and identity. Greater latencies of neuronal responses demonstrate higher cognitive and behavioural commitment, but this might be interrupted with masking techniques (Kouider & Dehaene, 2007). In fact, backward masking prevents the recruitment of feedback connections, allowing for processing dominance of feedforward ones. Feedback or recurrent processing has been proposed as a requisite, although not sufficient, for visual awareness to occur (Lamme, 2003).

THE PROBLEM OF DEFINING AWARENESS: SUBJECTIVE AND OBJECTIVE MEASURES

The major difficulty of studying the processing of emotional cues without awareness is to be sure that the stimulus is not perceived. This question has raised an important debate in the literature with some studies finding threat processing and significant differential brain activity in conditions of assumed unawareness (e.g. Morris, Öhman, & Dolan, 1999; Whalen et al., 1998), while others (Pessoa, 2005) suggest that this differentiation can be explained by the way that awareness is defined.

Öhman and colleagues (see Öhman & Mineka, 2001, for a review) have inferred unconscious processing from the use of stimulus onset asynchronies (SOAs) below 30 msec. Indeed, in a study of Esteves and Öhman (1993) it was shown that although there were some individual variability, the 30 msec was well below the threshold for recognition, either using subjective (verbal reports) or objective (force-choice detection) measures of awareness. In another study, Szczepanowski and Pessoa (2007) presented subjects with fearful versus neutral faces using 4 different durations (17, 25, 33 and 41 msec). Using the signal detection theory (SDT), they set two different thresholds for their subjects: an *objective awareness* threshold at 17 msec, in which subjects could correctly detect fearful faces above chance level, and a *subjective awareness* threshold at 24 msec above which subjects correctly discriminate fearful with high confidence ratings. Later on, and using the same SDT procedure, they demonstrated that activity in subcortical structures such as the amygdala does not necessarily depend on duration but that depends instead on awareness, and different subjects might have different awareness thresholds (Pessoa, Japee, Sturman, & Ungerleider, 2006). These authors criticized the use of subjective measures to define awareness, as these reflect response bias due to individual differences in confidence ratings to report awareness. Additionally, they pointed that the thresholds for objective and subjective awareness may relate to different awareness levels, with objective relating to phenomenal (experiential content) awareness and subjective to access awareness. However, some authors argue that we should not disconnect objective from subjective reports, defending instead that awareness should not be defined based on direct relations with performance above chance level. Doing that, a very conservative criterion to define awareness would be used, disregarding the fact that increased performance might reflect unconscious influences, as suggested by studies with Blindsight patients (Kouider & Dehaene, 2007).

Emotionally loaded stimuli: how to ensure affective significance?

To perform emotional studies, it is important to ensure that the stimuli used carry affective significance. Two major lines of research can be pointed out based on the way that they infer and attribute relevance to stimuli used in the experimental paradigms.

Studies using fear conditioning procedures

A large line of research considers that responses to fear-relevant stimuli become less likely if no negative outcome arises in the course of their presentation (e.g. in real life environments we expected angry faces accompanied of costs) and thereby the processing of affective information might be altered in the course of task performance. In fact, although faces are *a priori* conditioned stimuli, under these conditions extinction induced by behavioural patterns might occur. To overcome this limitation, some authors (e.g. Critchley, Mathias, & Dolan, 2002; Flykt, Esteves, & Öhman, 2007; Lim & Pessoa, 2008; Lim, Padmala, & Pessoa, 2008; Morris, Öhman, & Dolan, 1998; Öhman & Mineka, 2001; Wong, Shevrin, & Williams, 1994) have used fear conditioning procedures to enhance the stimulus affective meaning, by combining an initially neutral stimulus with a negative outcome. This is in fact one of the most used methods in the literature. It assumes an increasing line of relevance: conditioned aversive (CS+) faces would be more likely processed than unconditioned (CS-) aversive faces, and this is tested by analysing how resistance to extinction is traduced in differential responses (CRs) when CS+ with CS- are directly compared. Fear learning is one of the most consistent processes linked with the function of the amygdala (Ledoux, 2003).

Studies without fear conditioning

Another line of research implicitly assumes that faces, especially emotional and negative ones, are a priori relevant stimuli and thereby studies specific measures (e.g. behavioural, physiological or neural) by presenting emotional facial expressions versus neutral ones. This is based in studies with infants and adults (e.g. Johnston, 2003; Nelson, 2001), but arrives also from clinical studies with Blindsight (e.g. Morris, DeGelder, Weiskrantz, & Dolan, 2001) and Spatial neglect patients (e.g. Vuilleumier, 2000, 2002; Vuilleumier & Schwartz, 2001) that report increased detection of emotional versus neutral faces in the absence of concomitant subjective awareness. This relates with the above indicated line of relevance: faces are processed over non-face fear-irrelevant stimuli (e.g. Vuilleumier, 2000), with emotional faces being more likely processed as compared to neutral (e.g. Somerville, Kim, Johnstone, Alexander, & Whalen, 2004; Vuilleumier & Schwartz, 2001), and with negative emotions (e.g. fearful) over positive (e.g. (happy) faces (e.g. Tamietto & De Gelder, 2008). It assumes that faces are already "naturally" conditioned stimuli since in real environments they predict biologically relevant outcomes. In this manner, they predict that differential responses to more relevant stimuli would be at least initially evident. But they also assume response habituation due to the fact that presentations of emotional faces in the context of experimental designs due not carry the same consequences in the lab as in external environments (Whalen et al. 1998). Within this group of studies, responses in the amygdala seem to be more consistent or stronger to fearful facial expressions (Whalen et al., 2001) as compared to other negative faces such as faces displaying anger. Nevertheless, both seem to implicate this structure, at least under conscious processing (Hariri, Bookheimer, & Mazziotta, 2000; Whalen et al., 2001).

Skin conductance response Measurements: a method to study fear-relevance

As we referred before, awareness can be prevented by using backward masking paradigms. Fear conditioning studies that employ this technique have generally found that CRs (e.g. SCRs) to fear-relevant CS+ (e.g. snakes, angry faces) provoke larger SCRs and are more resistant to extinction than both fear-relevant CS- and fear-irrelevant (e.g. happy faces), either CS+ or CS-, independent of awareness (Öhman & Mineka, 2001). When relying on more social stimuli, such as faces, angry faces have been used as fear-relevant stimuli assuming that, when conditioned, angry faces would show the above pattern, while happy and neutral faces will not (Öhman & Mineka, 2001).

Morris, Öhman and Dolan (1998) contrasted angry CS+ faces with angry CS- faces under backward masked (using the neutral face as the mask) and unmasked conditions (using the neutral face as the target). They report larger SCRs to angry CS+ relative to angry CS-, irrespective of condition. However, they did not report direct contrasts with neutral faces for the SCR measure. In fact, the magnitude of SCR for CS- (happy, neutral) faces is similar either when presented masked or unmasked, but the essential question is if, when no awareness is possible, unconditioned (CS-) angry faces can be, albeit to a lower degree than angry CS+, more likely processed than neutral faces. In fact, most of the studies using angry, happy and neutral faces do not report results for SCRs using angry CS+ vs. neutral or, most interestingly, angry CS- vs. neutral faces (Boschen, Parker, & Neumann, 2007; Pischek-Simpson, Boschen, Neumann, & Waters, 2009). Although this was often outside their research goals, this type of contrasts might have shown the capacity of angry faces outside conditioning procedures to elicit differential responses, even when no awareness exists.

This is a particularly relevant question, as other fear-relevant facial expressions (e.g. fearful) have so readily been used without fear conditioning to demonstrate subliminal processing (e.g. Whalen et al., 1998; Williams et al., 2004). This question motivated the study presented in this chapter.

Remarks considering the experimental study of temporal constraints in affective processing

Some controversy is still present in which concerns the temporal limits of affective processing. First, most visual masking studies which study facial expression of emotions (fearful or angry) with functional imaging methods refrain from using fear conditioning procedures (but see Morris, Öhman, & Dolan, 1999). This implies that emotional signals in the stimuli are expected to be sufficient to engage involvement of brain areas related with automatic emotional processing at short time scales. However, this assumption might not hold regarding the outcome of SCRs. Second, although some studies find amygdala responses under conditions of unawareness others do not, arguing that amygdala responses might depend on variable thresholds of individual awareness which might not have been carefully measured during the experiment (Pessoa, Japee, Sturman, & Ungerleider, 2006). However, is not clear how awareness manipulation might affect the SCR measure, particularly outside fear conditioning studies.

In this chapter we will address these questions by employing a visual masking procedure to test how emotional faces of happiness and anger are affected by stimulus duration, but having in consideration a trial-by-trial definition of awareness into 3 levels: unawareness of content, face detection and emotional discrimination.

PART II

A SPECIFIC EFFECT OF STIMULUS DURATION ON SKIN CONDUCTANCE RESPONSES TO UNCONDITIONED ANGRY FACES

ABSTRACT

The role of unconscious vs. conscious processes in emotional face perception, and their relevance to the expression of autonomic changes reflected in skin conductance responses (SCR) have been widely studied, but the relevance of other factors such as stimulus duration need to be considered. Here we investigated this issue using face stimuli displaying neutral, happiness and anger related features. Three perceptual awareness response levels were defined: unawareness of any content (faces or emotions in faces), detection of faces and discrimination of emotions. We found that the SCR was modulated by stimulus duration and that this effect was only found in response to unconditioned angry faces. In contrast higher awareness-dependent arousal responses were modulated sooner by unconditioned happy faces, compared to angry and neutral faces. These results suggest that distinct mechanisms underlie processing of angry and happy unconditioned faces.

Abbreviations: skin conductance response, *SCR*, stimulus onset asynchrony, *SOA*.

Keywords: arousal, awareness, emotion, faces, detection, discrimination, skin conductance response, subjective and objective measures, duration.

3.1. INTRODUCTION

Faces represent important social stimuli in threat detection and may therefore be processed in a fast, automatic manner (Adolphs, 2008; Whalen, 1998; but see also Pessoa, 2005). Accordingly, fearful and angry faces can probably be processed at preattentive levels due to their negative valence, the first suggesting an eminent but unknown danger while the later constituting the direct source of threat (Whalen, 1998).

Mechanisms for subliminal detection may be ecologically advantageous and a subcortical pathway has been proposed to be preferentially engaged in the fast recognition of emotional cues (Adolphs, 2008; Morris, Öhman & Dolan, 1999). Others have further suggested that given the ambiguous nature of such information, additional attentional resources are needed in order to solve ambiguity (Kim et al., 2004; Whalen, 1998).

The existence of a fast subcortical pathway processing emotional information in a subliminal manner has been challenged by methodological issues that do not ensure that the processing of relevant stimuli happens completely outside awareness (Adolphs, 2008; Pessoa, 2005). In fact, unawareness has mostly been inferred from the use of short presentation times (e.g. 30 msec) within backward masking paradigms. Since it has been shown that very short presentations (e.g. 25 msec) can allow for awareness of picture content if presented alone (Calvo & Lundqvist, 2008), masking paradigms have been used to prevent stimuli of reaching awareness. A mask is immediately presented after the target in order to prevent its conscious perception as a sensory icon (for a review, see Kouider & Dehaene, 2007). Double "sandwich" masking (forward and backward) may nevertheless be more effective than the mere presentation of a single mask (Kouider, Dolan, & Henson, 2009).

Additionally, masking effectiveness depend on variables such as the nature of the mask (Loffler et al., 2005), stimulus duration, stimulus onset asynchrony (SOA) between target and mask and trial by trial individual fluctuations in awareness thresholds (Pessoa, Japee, Sturman, & Ungerleider, 2006). Accordingly, Pessoa and colleagues (2006) reported amygdala activation as a function of individual awareness even for fixed target durations. In fact, significant amygdala activity was found for overachiever subjects that could perceive the fearful faces at 33 msec but not for other individuals that could not discriminate fearful from neutral faces at the same duration. Interestingly, Szczepanowski and Pessoa (2007) have shown that objective awareness thresholds can be as low as 17 msec (detection of fearful faces above chance) and that even subjective awareness thresholds (correct discrimination of fearful faces with high confidence) can be as low as 24 msec. Subjective reports are important because, by definition, they imply awareness unlike objective threshold measures (Cheesman & Merikle, 1986).

Under circumstances that require 'fight' or 'flight' responses, changes in sympathetic arousal reflected by physiological responses such as the skin conductance response (SCR) are likely to occur (Sequeira, Silvert & Delplanque, 2008). Although increased SCRs have been proven to arise as a result of directed attention or when subjective salience is present (Sequeira et al., 2008), other studies have suggested that increased SCRs might occur even in the absence of awareness of content (e.g. Esteves, Dimberg, & Ohman, 1994a; Esteves, Parra, Dimberg & Ohman, 1994b). In effect, it has been shown that increased SCRs to angry versus happy faces can occur as a result of previous fear conditioning, with the acquisition phase being done either within (Esteves et al., 1994a) or outside (Esteves et al., 1994b) awareness states. This suggests that when the information presented is relevant for the individual, it can be processed and combined subliminally.

Importantly, the intrinsic perceived value of angry faces is better extracted when the conditioned stimulus is changed. Accordingly, Esteves and colleagues (Esteves et al., 1994b) have found a SCR difference for CS+ (conditioned to an aversive stimulus) angry vs. unconditioned happy faces, but not vice-versa, after learning had occurred. The former effect was true for both short (e.g. 30 msec SOA) and large (e.g. 500 msec SOA) stimulus presentations. Since this difference was not observed before the acquisition of fear learning, the results were taken as reflecting the higher probability of processing stimuli with higher relevance to the individual, such as angry faces. These observations are in good agreement with the association between fear conditioning and SCRs (e.g. Globisch, Hamm, Esteves, & Öhman, 1999; Öhman, Esteves, & Soares, 1993).

Taken together, these findings suggest different physiological responses of unconditioned vs. conditioned stimuli. In our study, we have focused on responses to unconditioned stimuli. A few studies using unconditioned stimuli have found evidence for SCR differences in fearful (emotional) vs. neutral for higher stimulus durations (e.g. 170 msec) with clear awareness (Williams et al., 2004). Evidence for differential subliminal processing has not been found under similar stimulus conditions (Williams et al., 2006). It is unclear whether prior susceptibility or preconditioning may be important. In fact, Globisch and colleagues (1999) tested high fearful and non-fearful participants to pictures of snakes and spiders shown for 150 msec and found that although the SCR was higher to negative versus neutral pictures for both groups, this difference was smaller for the no-fearful group. Additionally, Esteves and colleagues (1994a) have reported that higher SCRs to conditioned angry than to happy faces were cancelled under masking conditions when the subjects were instructed to pay attention to the (neutral) mask. We argue that generality of findings should not depend on preconditioning and therefore opted to use unconditioned stimuli in this experiment.

In the current study we aim to explore the role of stimulus duration in the modulation of the skin conductance response to unconditioned emotional faces displaying anger, as compared to positive (happy) and neutral facial expressions.

A "sandwich" masking paradigm was employed using scrambled faces as masks. To assess the effectiveness of the masking procedure together with the temporal manipulations, we took an 'on-line' measure of subjective awareness. We asked the participants to report their awareness by discriminating 3 levels in a trial-by-trial assessment - unawareness of content, detection of faces and discrimination of emotions. Importantly, we do not make assumptions concerning specific durations to define subliminal and supraliminal conditions. Subjects also rated their arousal level following each picture presentation. This measure reflects the perceived intensity of either a positive or negative disposition (valence) and it has been shown to co-vary positively with the SCR magnitude measure (Lang, Greenwald, Bradley & Hamm, 1993).

We predict that the SCR that is associated with processing of unconditioned angry faces and happy faces will be modulated differently by stimulus presentation duration. The idea that duration may affect the SCR magnitude measure in an emotion dependent manner is consistent with the observation that viewing time correlates positively with magnitude increase of the SCR (Lang et al., 1993).

3.2. Methods

3.2.1. Participants

Seventeen subjects were included in this study (8 males, mean [SD] age of 24.71 [3.08]); mean [SD] number of years of education: 16.88 [1.65]). Exclusion criteria were (a) relevant clinical history or (b) bad electrodermal responsiveness. All subjects were right handed except one and all had normal or corrected-to-normal vision. All gave written informed consent, with the experimental protocol being approved by the ethics committee of the Faculty of Medicine of the University of Coimbra.

3.2.2. Stimuli and apparatus

Pictures of faces and scenes were used as stimuli. Faces consisted of 8 identities (4 females, 4 males) taken from the Karolinska Directed Emotional Faces database (Karolinska Institutet, Sweden, www.facialstimuli.com; Lundqvist, Flykt & Ohman, 1998) and displaying coloured neutral, happy and angry facial expressions with directed gaze. Additionally, and as a way of reducing the habituation to faces and to keep the arousal level high, pictures of scenes taken from the International Affective Picture System (IAPS[1]) set (CSEA-NIMH, USA, csea.phhp.ufl.edu; Lang, Bradley & Cuthbert, 2008) were also included. They were chosen to have negative (mean [SD] =2.53[0.57], range: 0 to 9) and positive (mean [SD] =7.11[.44], range: 0 to 9) valence, but to always elicit a high arousal rating (mean [SD] =6.48[.63], range: 0 to 9).

Each face was presented within a grey ellipsoidal frame of size 23.03 x 29.79 cm (737×1000 pixels) to hide hair and clothes and yielding a visual angle of 13.15ºx 16.94º. IAPS pictures had a size of 25 x 17.87 cm (800×600 pixels), yielding a visual angle of 14.25º x 10.21º. Both were displayed at the centre of the screen. Stimuli were presented using Presentation software (Neurobehavioral Systems, USA, www.neurobs.com) on a 40×30.5 cm (1280×1024 pixels) monitor with a 85Hz refresh rate, that was placed at a viewing distance of 100 cm.

3.2.3. Task design and procedure

The task was divided into 4 different scenarios applied on different days in order to minimize habituation across testing sessions. Each scenario was composed of 5 practice trials followed by the 38 testing trials. All scenarios had the same structure. Scenarios 1, 2, 3 and 4 were composed by the same stimuli but the faces were presented in a pseudorandom balanced manner.

Participants were seated in front of a monitor in a dimly lit room, with a constant temperature set to be around 23ºC, and the electrodes were attached to the hypothenar eminence of the non-dominant hand (Dawson, Schell, & Filion, 2007). They were asked to remain as still as possible, minimize deep breaths, and limit speaking and sudden movements during the testing session. It was emphasized that this would be important in order to minimize data artefacts. After a variable period taken to achieve a baseline condition, the session started with 5 practice trials displaying pictures of houses during 2000 msec each. Immediately after, the test pictures were presented. The test trials (Figure 3.1) consisted of 30 face pictures (2 female identities, 2 male identities) displaying the 3 possible facial

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[1] The International Affective Picture System identification numbers for unpleasant, negative valence pictures were 2691, 6212, 6313, 6350, 6520, 6550, 6560, 6562, 6825,9403, 9410, 9414, 9424, 9425, 9426, 9427; for pleasant, positive valence pictures: 5621, 5629, 8030, 8080, 8158, 8163, 8178, 8179, 8180, 8185, 8186, 8206, 8370, 8400, 8490, 8492.

expressions (neutral, happiness and anger) and 8 additional IAPS pictures (4 negative, 4 positive, high arousing). While face pictures were presented at five possible durations - 12ms (1 refresh rate), 24 msec (2 refresh rates), 35 msec (3 refresh rates), 165 msec or 2000 msec - the IAPS were presented only at the higher duration, 2000 msec. The order of presentation was randomly generated for each subject.

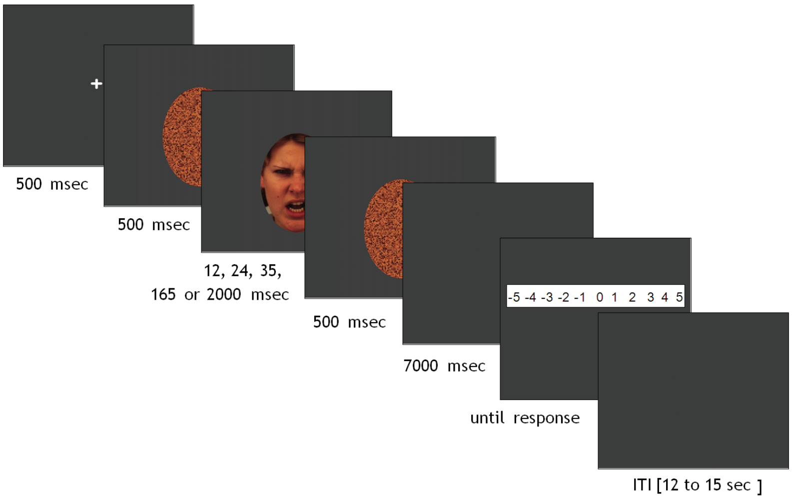

Figure 3.1 – Task design displaying a stimulus presentation trial. *Following a fixation period (500 msec), the target picture was presented between two noise masks (500 msec each). A blank screen interval was presented for 7 seconds, before the target picture could be rated (valence, arousal). The subsequent trial started after a randomly defined ITI of 12 to 15 seconds.*

To prevent awareness of stimuli for the shorter durations, the faces were always presented within a backward + forward ("sandwich") masking procedure. The masks consisted of 8 scrambled pictures (see Figure 3.1 for an example) of faces. They were created using MATLAB (MathWorks, USA, www.mathworks.com/products/matlab/) in such a way that no face parts (e.g. eye, nose) could be detected, in order to generate neutral pictures that had the same low level visual information of the face stimuli.

Each trial started with a fixation cross (500 msec) followed by the target picture displayed between 2 masks (500 msec each), in the case of face trials. A blank screen of 12 msec (1 refresh rate) occurred between the target and the backward mask stimulus. IAPS pictures were presented without masks. After a period of 7 seconds, set to record the SCR signal without motor interference, a rating scale appeared and participants had to decide the valence (negative or positive) that they attributed to the picture. Additionally, they also had to rate the arousal elicited by the stimulus on a scale of 0 to 5. They did this by using 2 buttons (right, left) that allowed them to move forward and backwards in the visual scale. The subjective detection/discrimination threshold was assessed with 3 additional buttons that subjects had to press after making their valence/arousal ratings: they were asked to report if they could (a) detect a face only, (b) discriminate an emotional expression, or (c) see nothing else than a scrambled picture. After the choice was made, the scale disappeared and a randomly defined ISI of 12 to 15 seconds was used to allow the SCR to go back to baseline.

3.2.4. PHYSIOLOGICAL DATA ACQUISITION AND ANALYSIS

The SCR was acquired with a SC5 system from PsychLab (Contact Precision Instruments, UK, www.psychlab.com) supplying a constant voltage of 0.5V DC and recording with an absolute accuracy of +/- 0.1 microSiemens (μS) and a relative accuracy of 5.96e-6 μS. Ag/AgCl electrodes with 8mm contact area were filled with K-Jelly and attached to the hypothenar eminence of the non-dominant hand (Dawson, Schell, & Filion, 2007). Data was sampled at 250 Hz, except for one subject (JL) that was sampled at 200Hz (this was taken into account in the time line computation of his data and subsequent downsampling).

After acquisition, data was processed using Ledalab V3.1.1, a Matlab-based software developed specifically for the analysis of skin conductance (Benedek & Kaernbach, Kiel, www.ledalab.de) and described elsewhere (Benedek & Kaernbach, 2010). After downsampling the raw data to 50Hz, the best fitting for our data was explored using the default initial parameters of the model but using the slow tonic increase correction whenever it showed to improve the resulting fit of data (goodness of fit statistics: average of the *root mean squared error*, RMSE, was 0.028 [SD=0.02], using the RMSE values from the best fitting for each of the files).

A specific SCR was considered to be a single response with an increase of at least 0.02 μS in amplitude, with onset within a latency window of 1 to 3 seconds post-stimulus.

3.2.5. STATISTICAL ANALYSIS

The statistical analyses were performed with IBM SPSS 19.0 software using non-parametric tests - Related-Samples Friedman's Two-Way Analysis of Variance by Ranks (χ^2F) and Wilcoxon Sign Rank (Z) tests - whenever the distribution was not normal. When applicable, we used ANOVA Repeated Measures and Paired samples *t*-tests. Bonferroni corrections were performed whenever required. For the recognition performance analysis we used the Chi-Square (χ^2F) test to test for differences in trial frequencies.

Additionally, subjects had to have at least two valid scenarios (with multiple SCR responses) to be included in the analysis. Two participants had 3 instead of 4 scenarios, while one participant had only two valid scenarios (11.11% of trials were excluded).

3.3. RESULTS

3.3.1. MAIN EFFECT OF EMOTION AND STIMULUS DURATION ON SKIN CONDUCTANCE MAGNITUDE

We analysed the effects of Emotion and Stimulus Duration on SCR responses. We found a main effect of Stimulus Duration on the magnitude of SCR ($\chi^2F_{(4)}$= 11.906, p<.05). Post-hoc comparisons for pairs 12-2000 (p<.005), 35-2000 (p<.05) and 165-2000 (p<.01) showed statistically significant differences, while not for any of the other contrasts. Additionally, no main effect ($\chi^2F_{(2)}$ = 1.882,) was found for the Emotion factor (when considering all three emotion categories).

3.3.2. EMOTION TYPE MODULATES STIMULUS DURATION EFFECTS: SPECIFIC EFFECTS FOR UNCONDITIONED ANGRY FACES

We have then specifically tested whether the Stimulus Duration effect was contingent on Emotion type, using non-parametric tests. We hypothesized that emotion type induced distinct SCR modulation patterns. Accordingly, when performing a Wilcoxon test, we found a significant difference for the contrast Anger vs. Neutral at 2000 msec (Z=-2.391, p=.015, corrected for multiple comparisons), but not for the contrast Happy vs. Neutral for the same stimulus duration (Z=-2.201, *n.s.*, corrected for multiple comparisons).

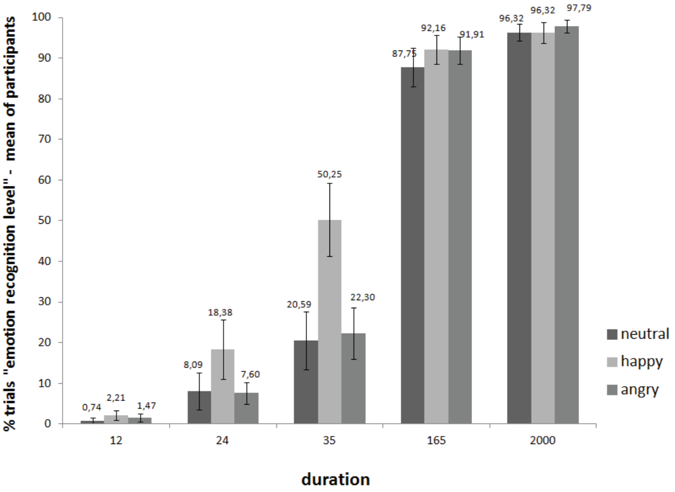

***Figure 3.2** – **Percentage of trials for which the stimulus was fully recognized** ('emotion discrimination' level) split by emotion type (happiness, anger, neutral) and stimulus duration (12, 24, 35, 165 and 2000 msec).*

Moreover, we found a main effect of Stimulus Duration when using only the emotion Anger ($\chi^2F(4)=10.777$, $p<.05$; Figure 3.3B, top left), but neither for Happiness ($\chi^2F(4)=3.198$, n.s.; Figure 3.3B, middle left) nor for faces displaying Neutral expressions ($\chi^2F(4)=3.693$, *n.s.*; Figure 3.3A, bottom left). Pairwise comparisons for the emotion Anger revealed significant results again for pairs 12-2000 ($p<.005$), 35-2000 ($p<.05$) and 165-2000 ($p<.05$).

3.3.3. Influence of awareness level on SCR

Taking the overall means for each participant, we found no significant difference in SCR when comparing the three levels of awareness ($\chi^2F(2)=1.882$, *n.s.*).

3.3.4. Behavioural analysis of Arousal effects: higher Arousal responses for unconditioned Happy faces

A Repeated Measures ANOVA revealed a main effect of Emotion ($F_{(2,32)}=45.212$, $MSE=.056$, $p<.0001$). Post-hoc analyses indicated a significant effect between all 3 pairs of emotions (happiness > neutral: $t(16)= -7.515$, $p<.001$; anger > neutral: $t(16)= -7.088$, $p<.001$, happiness > anger: $t(16)=4.023$, $p<.005$). A similar non-parametric approach using the Friedman test showed a main effect of Stimulus Duration ($\chi^2F(4)=65.520$, $p<.0001$). Pairwise comparisons revealed significant effects for pairs 12-35 ($p<.005$), 12-165 ($p<.0005$), 12-2000 ($p<.0005$), 24-35 ($p<.05$), 24-165 ($p<.0005$), 24-2000 ($p<.0005$) and 35-2000 ($p<.0005$) msec.

In contrast to the SCR measure, when we performed separate analysis for each emotion, we found main effects of Stimulus Duration for both Happiness ($\chi^2F(4)=62.767$, $p<.001$) and Anger ($\chi^2F(4)=62.700$, $p<.001$) emotions, as well as for Neutral ($\chi^2F(4)=43.019$, $p<.001$) faces.

Again differently from the SCR results, Happy faces received higher arousal ratings beyond the 24 msec stimulus duration, compared to Neutral and Angry faces. In fact, we found main effects of Emotion type when comparing Neutral, Happy and Angry face trials for the stimulus durations 24 msec ($\chi^2F(2)=8.867$, $p<.05$), as well for higher stimulus durations (35 msec: $F(2,32)=15.439$, $MSE=.192$, $p<.0001$; 165 msec: $F(2,32)=37.601$, $MSE=.216$, $p<.0001$; and 2000 msec: $F(2,32)=33.570$, $MSE=.267$, $p<.0001$). Planned comparisons showed that, in general, Happy faces were rated higher than both Neutral and Angry faces (24 msec: happy > neutral, $Z=-2.442$, $p<.05$, happy > angry, $Z=-2.038$, $p<.05$; 35 msec: happy > neutral, $t(16)=-4.828$, $p<.0005$, happy > angry, $t(16)=2.945$, $p<.01$; 165 msec: happy > neutral, $t(16)=-7.406$, $p<.0005$, happy > angry, $t(16)=3.005$, $p<.01$; and 2000 msec: happy > neutral, $t(16)=-6.605$, $p<.01$, happy > angry, $t(16)=1.792$, *n.s.*).

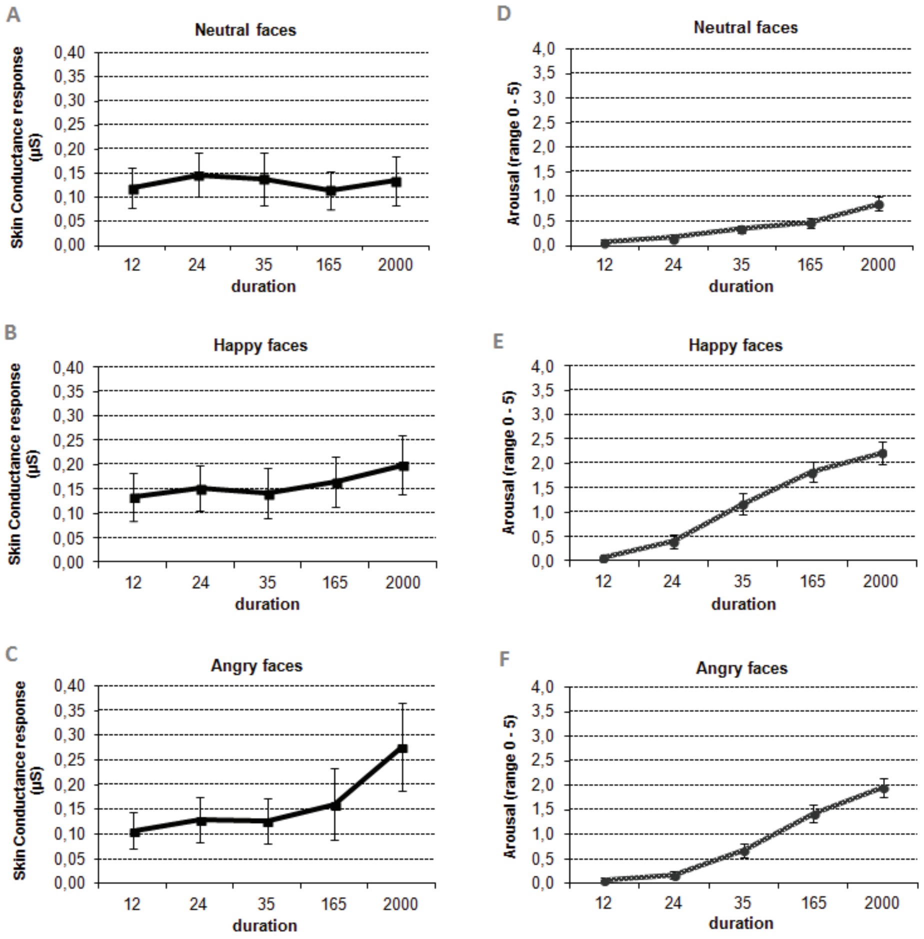

Figure 3.3 – Skin conductance and arousal ratings displayed for Emotional type by Duration. *Left panels: Skin conductance response magnitude (µS) evoked by the three emotion (A: neutral, B: happiness, C: anger) categories for each stimulus duration (12, 24, 35, 165 and 2000 msec). Right panels: Arousal ratings split by facial emotion type (D: neutral, E: happiness, F: anger) and stimulus duration. Error bars display standard errors of the mean.*

3.3.5. THE EFFECT OF STIMULUS PRESENTATION DURATION ON VISUAL AWARENESS

Using non-parametric tests, we analysed the effect of Stimulus Duration on the mean percentage of trials for which our participants have reported full awareness of content (Emotion discrimination level). A main effect of Stimulus Duration ($\chi^2F(4)=63.430$, $p<.0005$) was found. Post-hoc tests revealed differences for pairs 12-165 ($p<.0005$), 24-165 ($p<.0005$), 35-165 ($p<.01$), 24-35 ($p<.05$), 12-2000 ($p<.0005$), 24-2000 ($p<.0005$), 35-2000 ($p<.005$). No other differences were found.

Figure 3.2 suggests that participants reported awareness of both faces and their respective emotion even at very brief presentation durations, with differences in awareness rate depending on Emotion type. In fact, although there was already a trend for differences the 24 msec stimulus duration ($\chi^2F(2)=4.846$, $p=.089$), a main effect of Emotion type emerged only for the stimulus duration 35 msec ($\chi^2F(2)=20.333$, $p<.0005$), but not for other presentation durations where asymptotic values were reached. Planned comparisons across emotion types revealed that Happy faces were more easily recognized than Neutral ($p<.0005$) and Angry ($p<.005$) faces. No other significant differences were found.

3.3.6. No correlation between SCR magnitude and Behavioural Arousal

Given the fact that emotion type (Angry or Happy faces) differentially modulated SCR and arousal responses, we predicted that behavioural Arousal and SCR magnitude should only be weakly correlated. In fact, using the means of our participants, the correlation between the two measures was not statistically significant (Spearman rho, $\rho=.071$, *n.s.*).

3.4. Discussion

The present study sought to investigate how emotional faces affected SCR and Arousal ratings in relation to stimulus presentation duration. We used a “sandwich” masking paradigm to study responses to unconditioned faces, displaying happy, angry or neutral emotion.

3.4.1. SCR and Behavioural Arousal responses dissociate in relation to Emotion type

We found an interesting dissociation between SCR and Arousal ratings to angry vs. happy faces. Arousal responses were significantly larger for happy faces, but only the angry emotion induced significantly larger SCRs. The SCR effect is consistent with the notion that angry faces represent fear–relevant information, so they should be preferentially processed over more positive or neutral stimuli.

Previous work (Williams et al., 2004) also failed to find differences in SCR magnitude at 10 and 30 msec presentation durations for the contrast fearful vs. neutral faces, with differences in SCR magnitude appearing as soon as the percept became conscious (170 msec under their *a priori* defined “aware” conditions). For the same contrast, Williams and colleagues (2006) showed a tendency for higher SCRs at subliminal presentation durations (16.7 msec) that became only evident in the supraliminal condition (500 msec), which is also consistent with our findings (but see Esteves et al., 1994b).

Our results are surprising because it has been suggested that emotional faces carrying negative value are sufficient to induce enhanced SCRs, even under subliminal presentations (Ohman, Esteves, & Soares, 1993). The dissociation pattern is supported by the observation that subjective arousal showed quite early differences (e.g. 24 msec).

The fact that for some presentation durations (e.g. 35, 165 msec) a difference in arousal occurred already as a function of Emotion displayed, while no such difference (significant) occurred for the SCR measure, suggests that awareness at very small presentation durations does not predict differential SCR to unconditioned angry faces. Indeed, the fact that no SCR differences for Emotion arose even when awareness was clearly reported (e.g. 165 msec, recognition rate: above 90%) implies that SCR magnitude depends mostly on the duration of stimulus presentation (see also Lang et al., 1993). It is possible that longer exposure times to reliable emotional pictures, such as angry faces, enhance SCRs. This also suggests that SCR depends on available attentional resources and the load of cognitive processing (Esteves et al., 1994a), which increase with presentation duration.

3.4.2. Different physiological processing of threat related stimuli: Does Anger activate a distinct processing mechanism?

Fear conditioning methods increase the significance of the stimulus through the learned association with an aversive outcome (e.g. Esteves et al., 1994a; Esteves et al., 1994b). This might explain why previously conditioned fear-relevant stimuli (e.g. angry faces) are more likely to maintain increased SCRs than unconditioned or even conditioned fear-irrelevant stimuli (e.g. happy faces) that were associated to an aversive outcome (Esteves et al., 1994b). The study of Esteves and colleagues (Esteves et al., 1994a) using conditioning has found a difference between SCR angry vs. happy responses at 30 msec SOA. In our study using unconditioned stimuli at a similar SOA we did not find such difference even when awareness was reported, suggesting that the presence vs. absence of conditioning is critical.

The literature supporting subliminal processing refers mainly to fearful (Whalen et al., 1998) and conditioned angry faces (Williams et al., 2006). Other studies have further suggested that physiological responses to angry faces are distinct with differences being reported in the processing of fear and anger facial related features (Williams et al., 2005).

3.4.3. Implications for the definition of Awareness levels and Subliminal processing: the importance of trial-by-trial based classification

Studies that report differences in SCRs when contrasting aware vs. unaware perception usually establish subliminal and supraliminal stimulus durations *a priori*. In that sense, awareness is mostly inferred from the (short) stimulus duration used, even though it has been shown that some subjects can discriminate emotion at very short presentation durations (Szczpanowski & Pessoa, 2007). Here we defined awareness levels using the trial-by-trial reports of our participants. Comparisons across studies should take this critical methodological point into account. It has been referred that using short stimulus durations (e.g. 33 msec) does not ensure stimulus unawareness, suggesting that awareness of the stimulus content could explain why previous studies find increased SCR to angry faces. Since in most of these studies, a trial-by-trial classification was not pursued, the question remains unanswered. However, in the current study, we show that although increased SCRs occurred specifically for the angry faces (at long stimulus duration, 2000 msec), effective emotion recognition was not sufficient to induce a SCR. In fact, no differences were found neither at 12, 24 nor 35 msec, durations for which the literature usually expects unawareness of content. This differences appeared only at 2000 msec, and occurred only as an effect of stimulus duration. A specific limitation of this study is that we cannot ensure that under fear conditioning procedures, awareness of the face or of the emotion is not sufficient to enhance the SCR measure as we used unconditioned faces. In any case, our case challenges the general significance of SCR measures, because they show they are rather non sensitive even when the subject is able to perform some level of subjective categorization. In this respect, the results of Esteves and collegues (1994a) contribute to the controversy, showing that attention to the mask (but not to the target face) can abort differences between conditioned angry faces compared to unconditioned happy faces. And this should not be the case if SCR is caused by automatically driven processes.

3.4.4. HAPPY FACES: HIGHER AROUSAL AND EARLIER EMOTION RECOGNITION

Finally, as expected, recognition performance increased with increasing stimulus duration, what is in accordance with previous reports (Calvo & Lundqvist, 2008; Grill-Spector, Kushnir, Hendler & Malach, 2000; Quiroga, Mukamel, Isham, Malach, & Fried, 2008). Additionally, happy faces were easier to recognize, with higher reports of full awareness (emotion discrimination) already at 24 msec compared to neutral and angry faces. This explains why happy faces received higher arousal ratings sooner than any other class of faces (neutral and anger) and it is in accordance with previous reports (Calvo & Lundqvist, 2008; Esteves & Öhman, 1993; Palermo & Coltheart, 2004) that show that happy faces reach ceiling levels of recognition accuracy faster than other emotions. It was suggested that this is a result of their distinctiveness due to fewer overlapping features with other emotions (Calvo & Lundqvist, 2008; Esteves & Öhman, 1993). However, the study of Calvo and colleagues (2008), in which increasing durations of presentation were also used, shows that both neutral and happy faces share similar recognition accuracy already at 25 msec, what does not occur in our data for the neutral faces. But we should point that they have not used backward masking and this might have increased stimulus visibility and allowed increased emotion discrimination. In fact, apart from happy faces, they point that neutral faces were the only category that was not misperceived with other emotions, what supports the higher accuracy already at short times.

In sum, our results show very clearly how behaviourally rated arousal depends on perceived awareness of content. Thus, it further underlines the importance of assessing awareness in a trial-by-trial basis (Szczpanowski & Pessoa, 2007), and not merely depend on the use of short durations to allege unawareness.

3.5. Conclusion

To conclude, we showed a specific effect of stimulus presentation duration on skin conductance responses when using unconditioned angry faces, but not when using happy or neutral faces. Additionally, arousal ratings were also affected by stimulus presentation duration, in particular concerning for happy faces for which subjective ratings of arousal showed differences as soon as subjects were able of discriminate the emotion.

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CHAPTER 4

HOW THE VISUAL POSITION OF EMOTIONALLY LOADED STIMULI INFLUENCES THE ACTIVATION OF THE AMYGDALA: A SYSTEMATIC REVIEW[2]

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[2] Almeida, I., Patrício, M., & Castelo-Branco, M. How the visual location position of emotionally loaded stimuli influences the activation of the amygdala: A systematic review. (in preparation)

ABSTRACT

Perception of stimuli in the surrounding environment occurs using either central or peripheral visual pathways. Given the attentional allocation constraints to more eccentric parts of the visual field, fast and automated processing of peripherally presented stimuli constitutes an adaptive behaviour. A subcortical pathway involving the amygdala for fast and coarse processing of relevant emotional information has been proposed, being particularly sensitive to peripheral information channelled by particular cell-types in the retina. However, this hypothesis is still controversial.

This review aimed to evaluate if the amygdala shows a biased pattern of response to either peripheral or central visual field positions when processing emotionally loaded visual stimuli. Both the response amplitude and its temporal characteristics, when available, were analysed. Following the PRISMA statement guidelines, a systematic review of original research articles was conducted. Articles were retrieved from the PubMed and ScienceDirect databases on February 2013, respecting a predefined search strategy. Both the methods reported and bias risk factors were assessed.

Twenty six articles with studies presenting stimuli in the visual periphery were identified. These show that the literature employ a wide range of visual angles to study emotion processing in peripheral vision, with a bias for far versus near periphery being encountered in behavioural/EEG versus neuroimaging studies, respectively. Out of these articles, only five allowed an explicit comparison between central and peripheral processing. The results point to lack of consensus regarding an increased (amplitude of) amygdala activity to peripherally presented emotional stimuli. Nevertheless, amygdala responses occur faster (speed of processing) for peripherally presented emotional stimuli.

To date, few studies have evaluated the response of the amygdala by performing a direct comparison between central and peripheral processing. Differences concerning experimental factors are analysed and potential bias by concurrent factors are further discussed. Future directions for research on emotion perception are also presented.

Keywords: amygdala, implicit / explicit, central / peripheral, fMRI, spatial location.

4.1. INTRODUCTION

The amygdala is a key structure in the processing of emotional stimuli such as fearful faces and snakes (Öhman, 2005, 2009; Pessoa & Adolphs, 2010). Visual cues in the surrounding environment give important information carrying emotional meaning which can be processed in a conscious way, if attentional resources are available or, alternatively, in a pre-attentive manner in which stimulus characteristics guide visual attention to the relevant object. The former type of processing is usually achieved through detailed central vision, whereas the latter is believed to be largely routed by the peripheral vision magnocellular pathway, especially tuned to low spatial frequencies (Palermo & Rhodes, 2007). Accordingly, a subcortical pathway to the amygdala through the SC and the pulvinar, bypassing the primate visual cortex, has been proposed to process emotional signals in a fast and coarse manner (Tamietto & de Gelder, 2010; Pessoa & Adolphs, 2010). This pathway receives major input from peripheral vision, given its bias for low spatial frequency information (Vuilleumier, Armony, Driver, & Dolan, 2003). However, studies directly addressing this issue have been sparse. To date, most of available reports in the literature showing amygdala responses to emotional stimuli (e.g. facial expressions of fear or anger, snakes, spiders, emotional scenes) have used central presentations in the middle of the visual display, thus rendering impossible the evaluation of whether a different response pattern would occur if the stimulus appeared in the periphery (Whalen et al., 2001; Whalen et al., 1998; Morris, Dolan, & Öhman, 1999; Vuilleumier, Armony, Driver, & Dolan, 2003).

Besides the location of the stimuli, other factors have been shown to modulate the amygdala response and should therefore be taken in consideration. For instance, the type of task has been shown to differentially recruit the amygdala depending if the task requires an implicit or an explicit emotional label (Habel et al., 2007; Hariri, Bookheimer, & Mazziotta, 2000; Smith, Stephan, Rugg, & Dolan, 2006). The use of different types of stimulus might also account for some potential differences as faces seem to activate the amygdala at a higher degree than emotional scenes (Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002). The duration of the stimulus, combined with the direction of gaze (averted / directed) may also play a relevant role (Adams et al., 2011; Boll, Gamer, Kalisch, & Büchel, 2011; Van Der Zwaag, Da Costa, Zürcher, Adams, & Hadjikhani, 2012), given the suggestion that the amygdala might respond to the amount of white sclera displayed in the eyes (Whalen et al., 2004). Additionally, concerning studies using visual masking, in which the awareness of a target stimulus is compromised, the entailed process might differ from the one relying on conscious processing of the event stimulus. Finally, the use of magnification factors to compensate the stimulus mismatch (size) in central and peripheral vision is also controversial. Some authors point that the compensation for size is not sufficient to compensate a parallel crowding effect (Strasburger, Rentschler, & Jüttner, 2011). Moreover magnification factors at the amygdala input level and within this structure are hard if not impossible to accurately determine, suggesting that alternative approaches are also required.

The aim of this review was to evaluate if the amygdala shows a biased pattern of response for stimuli presented in the peripheral or in the central visual field, when processing emotionally loaded visual stimuli. Moreover, the authors of the review also wanted to revisit the notion of visual location, by identifying the definition and the range of visual angles used in the literature to assess processing of visual emotional stimuli in the periphery. The review is based studies comparing available objective outcome measures in both central and peripheral spatial locations. These outcomes depend on the amplitude of the amygdala response, and also on its temporal characteristics whenever possible. This review aims to facilitate the interpretation of seemingly contradictory findings in the literature.

The main results of the reviewed studies will be summarized in two Tables, highlighting the two-stage election process for studies described in the Methods section. First, both behavioural and neuroimaging results will be summarized with a focus on visual position (spatial location) and the range of used angles (Table 4.1). Then, a Table including only the final set of studies in which is possible to compare amygdala responses to central vs. peripheral stimuli will be presented. This time, in addition to the eccentricity values used, other factors which might contribute for the understanding of the outcome measures, will be summarized as well: type of task, use of magnification factors, stimulus duration, stimulus type, direction of gaze, centre vs. periphery comparison and amygdala lateralization patterns (Table 4.2). The review concludes with a critical discussion of these factors and an evaluation of their impact on past and future research.

4.2. Methods

4.2.1. Data sources and literature search

A systematic review was performed adhering to the principles of the PRISMA statement (Liberati et al., 2009). Two preliminary searches were conducted in order to properly define the search terms. The final search reported herein was undertaken in February 2013. Publications were searched on two databases, notably on MEDLINE, via PubMed (http://www.ncbi.nlm.nih.gov/pubmed), and on Science Direct (Elsevier, http://www.sciencedirect.com/) using the search string "(vision or visual) AND amygdala AND (peripher* OR central) AND (emot* OR fear OR angry OR threat*)", with no further filters or restrictions being imposed. References included in the articles deemed appropriate for full-text revision were hand-searched for retrieving other relevant publications. Articles suggested by authors contacted to provide access to their publications were also assessed.

4.2.2. Eligibility criteria

A two-stage study selection process was adopted. For a study to be considered as eligible in the first stage, it had to meet the following criteria: (1) be written in English language; (2) involve non-medicated, healthy participants (animal studies were excluded); (3) involve original research articles (review studies were excluded); (4) use the aforementioned search terms in the context defined for this review (studies in which the "peripheral" term referred to peripheral body parts, systems [e.g. peripheral nervous system] or measures [e.g. SCR] were excluded); and (5) present stimuli to assess normal performance, without introducing sources of disturbance such as transcranial magnetic stimulation (TMS). Finally, the second stage was reserved for the studies that were found to satisfy criteria 1 to 5 and also met the following conditions: (6) the experimental design included presentation of stimuli both in the centre and periphery of the visual field; (7) the study allowed brain imaging using either functional neuroimaging (fMRI) or magnetoencephalography (MEG); (8) direct measurements were made in the amygdala, with activation reported; (9) the activity in the amygdala had to be specifically and separately reported (e.g. without being included in a general "medial temporal lobe" label).

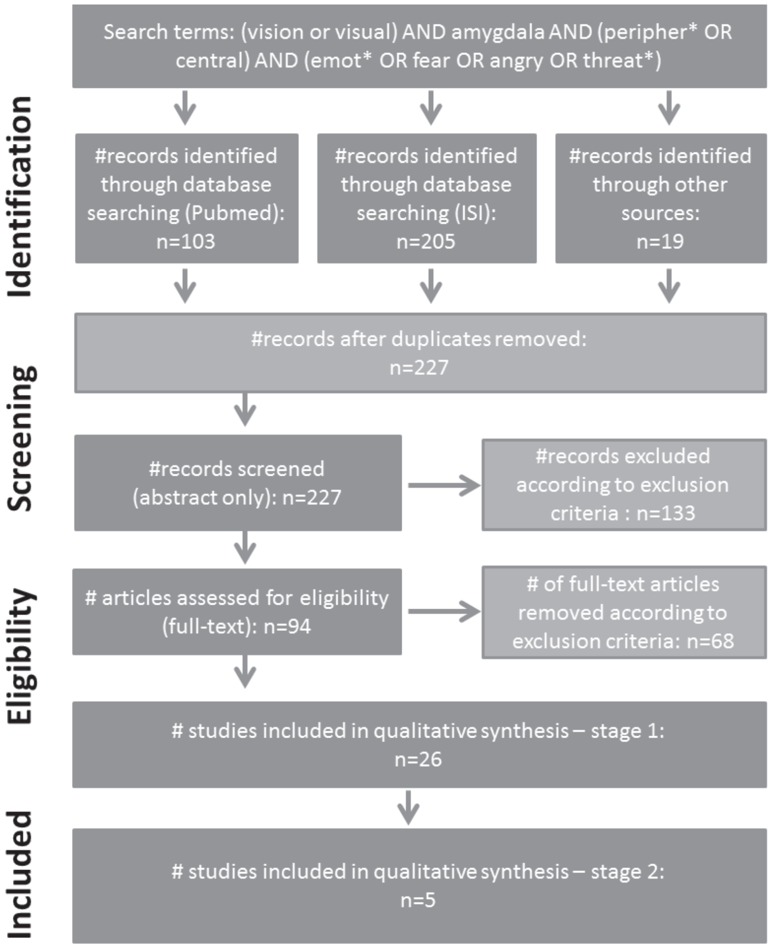

Figure 4.1 *– Flow of information describing the different phases of the systematic review.*

4.2.3. STUDY SELECTION AND DATA EXTRACTION

The selection of eligible studies was performed by two authors (I.A. and M.P.). The reasons for rejecting the inclusion of a paper, both at this step and throughout the process of paper selection, were discussed between the authors and registered. The data was collected and duplicates were eliminated (identification phase). The titles and abstracts of the remaining records were then screened independently by the two authors (screening phase) and assessed for eligibility (see Section 4.2.2 and Figure 4.1). All records which were deemed potentially eligible for criteria (1) to (5) by at least one of the reviewers were included for further full paper assessment (eligibility phase). These were studies (behavioural, electrophysiology, neuroimaging) presenting stimuli at both central and peripheral locations, or only in the periphery. They were included for data extraction regarding eccentricity values used in the literature. The articles retrieved in the first stage were then further assessed for inclusion in the second stage. Studies which allowed direct comparison between central and peripheral presentation of emotionally loaded stimuli were then elected for further detailed analysis. In Table 4.1, studies reporting the eccentricity values used are reported. The Table 4.2 presents the following variables that were extracted. Notably, the following factors of interest were registered: (1) the type of task (implicit or explicit; and labelling, matching, signal detection, gender categorization, masked presentation, or passive viewing), (2) use of magnification factors (yes, no), (3) stimulus duration, (4) stimulus type (faces, scenes), (5) direction of gaze (directed, averted), (6) the nature of stimuli presentation (static pictures or dynamic videos), (7) the eccentricity angles used, (8) the task design (event-related, block design), (9) the neuroimaging statistical analysis (whole brain; regions of interest, ROI), (10) the type of neuroimaging results: contrasts of interest that represent the spatial location effect outcomes (centre vs. periphery), and (11) amygdala lateralization (if mentioned).

4.2.4. Data analysis

The review provides a qualitative summary of behavioural, electrophysiology (EEG, MEG) and neuroimaging (fMRI, MEG) findings.

4.2.5. Risk of bias (across studies, within studies)

As mentioned before, the response of the amygdala depends on several factors. To properly assess the risk of bias, potential confounding variables were extracted and reported in Table 4.2 (e.g. visual angle / eccentricity; type of stimuli; emotional expression; type of task; task design; and use of magnification factors).

4.3. Results

The Flow Diagram displayed in Figure 4.1 reflects the selection process. Our review of the literature using search items as described above identified 327 potential target studies [103 were identified via PUBMED database, 205 through ScienceDirect and 19 through other sources: 10 were sent by authors, whereas 9 were identified hand-searching the reference list of articles meeting criteria (1) to (5)], out of which 100 were duplicates. During the screening phase, 133 records were excluded, based on the information displayed in the abstracts, taking into account criteria (1) to (5). A total of 94 publications were carried to full text assessment. From here, 68 records were excluded. The remaining 26 studies underwent "stage 1" data extraction (e.g. eccentricity values used) (see Tables 4.1 and 4.2). Finally, our systematic review of the literature identified 5 studies as target studies that additionally met the criteria (6) to (9). Of these studies, 3 were functional magnetic resonance imaging (fMRI) studies (Morawetz, Baudewig, Treue, & Dechent, 2010, 2011; Preibisch, Lanfermann, Wallenhorst, Walter, & Erk, 2009) and 2 used magnetoencephalography (MEG) (Bayle, Henaff, & Krolak-Salmon, 2009; Liu & Ioannides, 2010). None of the studies reported effect sizes. All were published in the last 5 years, which suggests that this is a still growing field and further studies are required.

4.3.1. Stage 1: Visual angles (Eccentricity)

From the 26 studies which presented emotional stimuli both in the centre and in the periphery, or only at peripheral locations, the eccentricity values employed were extracted. The visual angles used to test the visual periphery differed mainly regarding the type of study performed. In fact, whereas the neuroimaging studies typically used visual angles ranging up to 14º, behavioural/EEG studies made use of far more eccentric visual angles - up to 40º (see Table 4.1).

4.3.2. Stage 2: centre vs. periphery

The main aim of this study was to identify articles in the literature which directly compare responses of the amygdala to centrally and peripherally presented emotional stimuli. The core findings regarding the final set of 5 studies are described and summarized in Table 4.2.

Table 4.1 *– Eccentricity values reported in the literature.*

Studies	*Range of angles*	*Type of study*
Bayle, et al., 2009	*0º, ±8º*	*behavioural / MEG*
Morawetz, et al. 2010	*±1.68º, ±5.6º, ±11.25º*	*Behavioural / fMRI*
Morawetz, et al. 2011	*±1.7º, ±9.5º*	*Behavioural / fMRI*
Preibisch, et al.	*n.s.*	*Behavioural / fMRI*
Liu, & Ioannides, 2010	*0º, ±10º*	*Behavioural / MEG*
Bayle, et al., 2011	*±0º, ±10º, ±15º, ±20º, ±25º, ±30º, ±35º, ±40º*	*behavioural*
Calvo, et al., 2007	*±2.5º*	*behavioural*
Calvo, et al., 2008	*0º, ±5.2º*	*behavioural*
Calvo, et al., 2011	*0º, ±5.2º*	*behavioural*
Nummenmaa, et al., 2006	*±8.5º*	*behavioural*
Nummenmaa, et al., 2009	*±10.5º*	*behavioural*
Nummenmaa, et al., 2010	*0º, ±2.5º*	*behavioural*
Silvert , et al., 2007	*±6.5º*	*Behavioural / fMRI*
Williams, et al., 2005	*±11.7º*	*Behavioural / fMRI*
Coy, et al., 2012	*±5.57º; ±5.77º; ±15.57º*	*behavioural*
Doallo, et al., 2006	*±5.7º*	*Behavioural / EEG*
Friesen, et al., 2011	*±0.5º, ±10º*	*behavioural*
Gutiérrez, et al, 2009	*0º, ±5.2º*	*behavioural*
Kouider, et al., 2011	*±18º*	*behavioural*
Faivre, et al., 2012	*±14º*	*Behavioural / fMRI*
Liu, et al., 2008	*0º, ±6.8º*	*EEG*
Weaver, et al., 2011	*±7.9º, ±9.7º*	*behavioural*
Rigoulot, et al., 2008	*0º, ±30º*	*behavioural / EEG*
Rigoulot, et al., 2011	*±15º, ±30º*	*behavioural / EEG*
Rigoulot, et al., 2012	*±15º, ±30º*	*behavioural / EEG*
Mohanty, et al., 2009	*±5.5º*	*Behavioural / fMRI*

4.3.2.1. Outcome measures

The aim of this study was to identify studies in the literature which directly compare responses of the amygdala to centrally and peripherally presented emotional stimuli. Below, the main findings can be found regarding the final set of 5 studies.

4.3.2.1.1. Amygdala preferential role in central vs. peripheral spatial vision: inference based on response amplitudes

Amygdala preference to central or peripheral locations was not unanimous. In fact, while 2 studies (1 fMRI, 1 MEG) (Morawetz et al, 2010; Liu & Ioannides, 2010) found higher amplitude of response to central presentations (depending on the visual angle), 1 (fMRI) study found no differences between

Table 4.2 – Summary of results for the "stage 2" 5 elected studies which directly compared central versus peripheral emotional stimuli.

Study	It mentions eccentricity angles?	Range of angles (minimum to maximum)	Task	Magnifi-cation	Duration	Stimuli type / emotional expressions	Centre > periphery?	Amygdala lateralization
fMRI; Preibisch *et al.*, 2009	*Yes, but does not provide definition*	*n.s.*	*implicit: press button to indicate location of stimulus presented (centre, left, right)*	*n.s.*	*1 sec*	*FEEST/KDEF; neutral, fearful human faces; houses*	*interaction: neutral: > centre; fearful: > periphery*	*left*
fMRI; Morawetz *et al.*, 2010	*yes*	*1.68°, 5.6°, 11.25°*	*implicit (match digits / gender)/ explicit (match emotion)*	*yes*	*550 msec*	*neutral, fearful, happy human faces*	*centre (> 5.6° : match digits low; match emotion)*	*no difference (left+right) (ROI analysis)*
fMRI; Morawetz, *et al.*, 2011	*yes*	*1.7°, 9.5°*	*implicit (indicate on which side the face appeared)*	*yes*	*500 msec*	*fearful (happy and neutral faces only for the behavioural results) human faces; LSF/HSF/normal*	*no differences*	*no difference (left+right) (ROI analysis)*
MEG; Liu & Ioannides, 2010	*yes*	*0°, 10°*	*explicit: emotion recognition (verbally)*	*yes*	*500 msec, block design*	*neutral, fearful, happy (FEEST) human faces*	*centre > all locations (10°; UL, UR, LL, LR)*	*left and right*
MEG; Bayle *et al.*, 2009	*yes*	*0°, 8°*	*"explicit (but masked): Subjects*	*no*	*33 msec (masked fearful faces) + 150 msec target faces (neutral, happy)*	*masked fearful + target (neutral, happy) human faces*	*periphery (no amygdala response in central displays)*	*right*

n.-s., non-specified.

the spatial locations (Morawetz et al., 2011) and 1 (MEG) found stronger amygdala response stimuli presented at peripheral locations (Bayle et al., 2009). Finally, one of the studies (fMRI) found an interaction between the response of the amygdala and the combination of type of facial expression and its spatial location There was preferential response to neutral faces located at central locations but for fearful faces it was the peripheral locations that elicited a greater response (Preibisch et al., 2009). Unfortunately, this study did not report the eccentricity used.

4.3.2.1.2. Lateralization of the amygdala function

No consensus was found regarding lateralization of the amygdala responses as a function of spatial location. In fact, 2 studies (fMRI) did not find lateralization differences analysing the amygdala as a whole (Morawetz et al., 2010, 2011). However, one study (fMRI, supraliminal, implicit task) found preferential activity within the left amygdala (Preibisch et al., 2009) and another (MEG, subliminal, explicit task) found right lateralization (Bayle et al., 2009). Finally, one (MEG) publication reported finding increased responses at both left and right amygdala but no direct comparison was performed between the two (Liu & Ioannides, 2010).

4.3.2.2. BIAS FACTORS

4.3.2.2.1. Visual angles/Eccentricity

All studies used both central (0 to < 2º) and peripheral (> 2º) spatial locations. One of the studies did not report the angle of eccentricity used (Preibisch, *et al.*, 2009). For the other, the peripheral eccentricities used were below 11.25º, ranging between 5.6º, 8º, 9.5º and 10º.

4.3.2.2.2. Use of magnification factors

In 3 of the studies, a magnification factor was reported (Liu, & Ioannides, 2010; Morawetz, *et al.*, 2010, 2011), while in one no magnification of peripheral stimuli was made (Bayle, et al., 2009). One of the studies did not report if it was used or not (Preibisch, *et al.*, 2009).

4.3.2.2.3. Task (implicit, explicit) and Stimulus duration

One of the studies (fMRI) resorted to both explicit and implicit tasks (Morawetz, *et al.*., 2010). In 2 of the studies (fMRI) only implicit performance emotional processing (subjects had to indicate location of presentation) was required (Preibisch et al., 2009; Morawetz et al., 2011), while in the other 2 (MEG) explicit tasks were performed (Bayle et al., 2009; Liu & Ioannides, 2010). However, the explicit tasks differed in nature, as in one of the studies the participants had to explicitly label the facial expression displayed (Liu & Ioannides, 2010), whereas in the other they had only to detect the putative happy face content among the neutral faces, with the target fearful face being presented in a masked manner (Bayle et al., 2009). All the other studies used supraliminal stimuli durations of 500-550 msec or 1 sec.

4.3.2.2.4. Type of stimuli, emotional expression, and direction of gaze and nature of stimuli presentation

All stimuli presented were faces with directed gaze. All the studies made a comparison between static neutral and fearful faces, with some using additional happy faces as an emotional control. None of them tested for other (e.g. angry) facial expressions or made use of dynamic video presentations.

4.3.2.2.5. Methods (event-related/block-design; ROI/whole-brain)

Two of the 5 studies used event-related designs, whereas three (Liu & Ioannides, 2010; Morawetz et al., 2010, 2011) resorted to block-design paradigms. Only two studies analysed the amygdala as a region-of-interest (ROI) (Morawetz et al., 2010, 2011).

4.4. Discussion

The main goal of this systematic review was to evaluate the response of the amygdala to emotionally loaded stimuli, unravelling if a bias occurs regarding spatial location (central vs. peripheral). Twenty six articles with studies presenting stimuli in the visual periphery were identified. These show that the literature provides a wide range of visual angles to study emotion processing in peripheral vision, with a bias for far versus near periphery being encountered in behavioural/EEG versus neuroimaging (fMRI/MEG) studies, respectively. In fact, visual angles range from 0º to 40º in behavioural studies, form 0º to 30º in EEG studies and from 0º to 14º in neuroimaging (fMRI, MEG) tasks directly assessing the impact of central vs. peripheral spatial manipulation in amygdala response.

Of the 26 neuroscience studies regarding the range of visuals angles tested for visual processing of emotional information, the present review identified 5 articles which fulfilled all the inclusion criteria, and allowed a direct comparison between central and peripheral stimuli, with both fMRI and MEG yielding reliable results regarding amygdala responses. All of these were published in the last 5 years, which suggest that this is a field still growing and further studies are required.

Regarding the main goal, the searched literature does not support a preferential response of the amygdala (amplitude) to peripherally presented stimuli. In fact, the results cover almost all the available hypothesis: no differences, preferred central, preferred peripheral. Nevertheless, amygdala responses occur faster (speed of processing) in the presence of peripherally presented emotional stimuli (Bayle et al., 2009; Liu & Ioannides, 2010).

Finally, and importantly, all of these studies were performed using fearful faces, with none of this making use of angry faces, or any other type of emotionally relevant stimuli (e.g. snakes).

4.5. Conclusions

This review provides an overview of 26 neuroscience studies regarding the range of visual angles tested for visual processing of emotional information. Out of these articles, only a subset of 5 offer answers to the critical question of whether the human amygdala displays a location (central or peripheral) bias concerning the processing of emotion loaded visual information. Both fMRI and MEG yielded reliable results regarding amygdala responses. Nevertheless, the small number of studies prevented us from performing meta-analysis on the data.

We conclude that a wide range of visual angles is used in the literature, with the higher eccentricities angles being used in EEG/behavioural studies. It would be important to investigate far peripheral vision using neuroimaging techniques as well, namely taking advantage of the MEG possibilities. In any case, the available literature does not support the notion that the amygdala shows a (amplitude of response) bias for processing peripheral information. Nevertheless, an important trade-off between amplitude of response and speed of processing should be taken into account when assessing the impact of central vs. peripheral spatial manipulation in the amygdala response (Liu & Ioannides, 2010).

Finally, it is worth to notice that, to date, few studies have evaluated the response of the amygdala by performing or allowing a direct comparison between the processing of central and peripheral information. We suggest that future studies should evaluate both central and peripheral processing of emotional stimuli. It would also be important to test for other types of stimuli, with a particular emphasis placed on those with an ecological relevance (e.g. fear-relevant animal shapes) (Öhman, 2009). This further strengths and supports the two studies that we have performed, and which will be described in the following Chapters, 5 and 6.

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CHAPTER 5

THE ROLE OF THE AMYGDALA AND THE BASAL GANGLIA IN THE VISUAL PROCESSING OF CENTRAL VS. PERIPHERAL EMOTIONAL CONTENT [3]

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[3] Almeida, I., van Asselen, M., & Castelo-Branco, M. (2013). The role of the amygdala and the basal ganglia in the visual processing of central vs. peripheral emotional content. *Neuropsychologia*. In press.

ABSTRACT

In human cognition, most relevant stimuli, such as faces, are processed in central vision. However, it is widely believed that recognition of relevant stimuli (e.g. threatening animal faces) at peripheral locations is also important due to their survival value. Moreover, task instructions have been shown to modulate brain regions involved in threat recognition (e.g. amygdala). In this respect it is also controversial whether tasks requiring explicit focus on stimulus threat content vs. implicit processing differently engage primitive subcortical structures involved in emotional appraisal. Here we have addressed the role of central vs. peripheral processing in the human amygdala using animal threatening vs. non-threatening face stimuli. First, a simple animal face recognition task with threatening and non-threatening animal faces as well as non-face control stimuli was employed in naïve subjects (implicit task). A subsequent task was then performed with the same stimulus categories (but different stimuli) in which subjects were told to explicitly detect threat signals.

We found lateralized amygdala responses both to the spatial location of stimuli and to the threatening content of faces depending on the task performed: the right amygdala showed increased responses to central compared to left presented stimuli specifically during the threat detection task, while the left amygdala was better prone to discriminate threatening faces from non-facial displays during the animal face recognition task. Additionally, the right amygdala responded to faces during the threat detection task but only when centrally presented. Moreover, we have found no evidence for superior responses of the amygdala to peripheral stimuli. Importantly, we have found that striatal regions activate differentially depending on a peripheral vs. central processing of threatening faces. Accordingly, peripheral processing of these stimuli activated more strongly the putaminal region, while central processing engaged mainly the caudate. We conclude that the human amygdala has a central bias for face stimuli, and that visual processing recruits different striatal regions, putaminal or caudate based, depending on the task and on whether peripheral or central visual processing is involved.

Keywords: amygdala, basal ganglia, implicit / explicit, central / peripheral, threat, faces.

5.1. INTRODUCTION

Much of what we know regarding the functional anatomy of neural pathways connecting to the amygdala comes from auditory fear conditioning studies in the rat animal model (LeDoux & Phelps, E., 2008; Whalen, Davis, Oler, Kim, Kim, & Neta, 2009). A large difference between rodents and primates can be recognized in the processing of social stimuli such as faces (Buchanan, Tranel & Adolphs, 2009). In primate visual and affective processing, faces can be considered as a special class of objects (Critchley et al., 2000; Hershler, Golan, Bentin, & Hochstein, 2010; Johnson, 2005). Faces are preferentially processed in central vision, where they are screened for high-resolution foveal information (Kanwisher, 2001; Levy, Hasson, Avidan, Hendler, & Malach, 2001). Studies in humans suggest the existence of foveally-biased specialized regions along the occipito-temporal ventral visual pathway to extract meaning from faces: the occipital gyrus, the lateral occipital (LO), the superior temporal sulcus (STS), and the fusiform gyrus (Grill-Spector, Knouf, & Kanwisher, 2004; Kanwisher, McDermott, & Chun, 1997). Accordingly, regions in the fusiform gyrus, such as within the FFA complex, are tuned to a broad category of faces (Tong, Nakayama, Moscovitch, Weinrib, & Kanwisher, 2000), especially when these are presented in central vision, but they do nevertheless also respond to peripherally presented faces (Faivre, Charron, Roux, Lehéricy, & Kouider, 2012; Kanwisher, 2001; Morawetz, Baudewig, Treue, & Dechent, 2010).

Subcortical regions such as the amygdala are also involved in face meaning extraction (Atkinson & Adolphs, 2011; Gothard, Battaglia, Erickson, Spitler, & Amaral, 2007). This structure, which has been implicated in the detection of external threats (e.g. snakes) (Öhman, 2005) and other ecologically relevant stimuli categories (Sander, Grafman, & Zalla, 2003), receives direct input from temporal visual areas (Lori, Akbudak, Shimony, Cull, Snyder, Guillory, & Conturo, 2002; Rolls, 2007; Stefanacci, & Amaral, 2002) such as the fusiform gyrus (e.g. Faivre et al., 2012), which in turn receive significant input from occipital visual areas (McDonald, 1998), both biased for central vision (Kanwisher, 2001; Strasburger, Rentschler, & Jüttner, 2011). In any case, the role of the amydgala in processing social aspects of emotion such as in recognition of facial expressions is undisputed (Buchanan et al., 2009; Whalen et al., 2009). In line with this view both invasive and non-invasive studies have previously shown that it responds strongly to human and even animal faces (Mormann et al., 2011; Blonder et al., 2004).

Unsurprisingly, most studies of emotional processing have used central presentation of faces (e.g. Heutink, Brouwer, de Jong, & Bouma, 2011; Morris, Öhman, & Dolan, 1999; Padmala, Lim, & Pessoa, 2010; Vuilleumier, Armony, Driver, & Dolan, 2003; Whalen et al., 2001). However, relevant stimuli that require a rapid response also arise from the visual periphery (e.g. snakes, threatening animals) (e.g. Thorpe, Gegenfurtner, Fabre-Thorpe, & Bülthoff, 2001). In this case, visual input is limited by receptive field properties in the peripheral retina, which are fast but convey mainly coarse information, from the magnocellular pathway (Dacey & Petersen, 1992). Crowding effects and reduced acuity thus weakens the discrimination of fine-grained details (e.g. facial expressions of fear and surprise expressions share many facial features) (Strasburger et al., 2011). Peripheral processing often requires the superior colliculus (SC) and the pulvinar – two structures thought to be involved in a subcortical pathway to the amygdala for fast and often implicit emotional processing (Morris et al., 1999; Vuilleumier et al., 2003), although this is still debated in humans (but see Tamietto, Pullens, de Gelder, Weiskrantz, & Goebel, 2012). Due to its role in threat detection, connections with SC and sensitivity to coarse information, it has been suggested that the amygdala might have a bias for peripheral faces (Bayle, Henaff, & Krolak-Salmon, 2009; Palermo & Rhodes, 2007; Preibisch, Lanfermann, Wallenhorst, Walter, & Erk, 2009). In a MEG study, early onset amygdala responses to fearful faces have accordingly been found preferentially at peripheral locations compared to central ones (Bayle et al., 2009). However, such peripheral preference was not found in recent fMRI work (Morawetz et al., 2010, 2011), and is not consistent with the known major connections with central vision input regions described above (e.g. fusiform gyrus).

Only a few studies have addressed the neural correlates of central and peripheral processing of facial expressions (Bayle et al., 2009; Preibisch et al., 2009). The pattern of results suggested a complex interaction between facial expression type and spatial location across multiple brain regions (e.g. Preibisch, *et al.*, 2009). A magnetoencephalography (MEG) study performed by Liu and Ioannides (2010) found faster peripheral responses but stronger central amplitudes, which is slightly at odds with the study of Bayle and colleagues (2009). Some of these inconsistencies might be related to differences between tasks. Preibisch and colleagues (2009) required passive viewing of the emotional faces only, whereas Bayle and colleagues (2009) masked fearful face stimuli and asked the participants to detect happy faces. Morawetz and colleagues (2010) manipulated both attentional load (high, low) and task type (implicit or explicit emotion). Finally, Liu and Ioannides (2010) explicitly required participants to verbally name the emotion displayed.

Studies suggesting the existence of specific pathways for implicit and explicit emotional processing have also generated another longstanding debate, with no consensus if the amygdala is preferentially involved when implicit processing of threat is required, or when this emotional information is the focus of attention (explicit processing). Some studies have suggested that explicit labelling recruits cortical temporal and frontal regions thus inhibiting activity of subcortical structures such as the amygdala, which are more prone to respond when the task requires only matching of faces (Hariri, Bookheimer, & Mazziotta, 2000), gender classifications (Critchley et al., 2000) or passive viewing (Morawetz et al., 2010; Taylor, Phan, Decker, & Liberzon, 2003; for a review see Costafreda, Brammer, David, & Fu, 2008). Nevertheless, some studies have found significant engagement of the amygdala during both during implicit and explicit tasks (Winston, O'Doherty, & Dolan, 2003), or even enhanced activity of the left (Gorno-Tempini et al., 2001) or bilateral amygdala (Habel et al., 2007; for a review see Fusar-Poli et al., 2009) when explicit emotional processing is required.

Subcortical structures beyond the amygdala such as the basal ganglia have not been as widely studied as the amygdala in terms of its role in visual processing of affective information. However, they have been implicated in affective processing (Arsalidou, Duerden, & Taylor, 2012), namely but not exclusively in the processing of disgust in faces (Sprengelmeyer et al., 1997). These regions connect with the amygdala in both monkeys (Fudge, Kunishio, Walsh, Richard, & Haber, 2002) and humans (Kim & Whalen, 2009), and show parallel activations with the amygdala in human reward and goal-oriented behaviour studies (O'Doherty, 2004). Our recent study in a clinical model of basal ganglia dysfunction also suggests a contribution of the basal ganglia in general face emotion recognition (van Asselen et al., 2012).

Concerning explicit vs. implicit processing activity within the basal ganglia seems to be modulated by task, with the left putamen showing stronger responses to fearful than to neutral faces during passive viewing, but to neutral than to fearful during explicit emotion judgments (Lange et al., 2003), although another study suggested its involvement both during explicit or implicit discrimination of angry and happy faces (Critchley et al., 2000). In addition, the right neostriatum (putamen and caudate) was activated when subjects made explicit judgements of disgust, with the right caudate (head) differentiating between disgusted and happy faces (Gorno-Tempini et al., 2001) or being generally involved in explicit judgements (Fusar-Poli et al., 2009).

In sum, the role of amygdala in emotion processing does remain controversial (for reviews see Öhman, 2009; Pessoa & Adolphs, 2010; Tamietto & de Gelder, 2010) and the link with basal ganglia function remains also intriguing. Here we studied animal face recognition and threat detection using stimuli presented either at foveal regions or at near-periphery locations (<10°), although we will refer here to the near-periphery as peripheral vision (see also Strasburger et al., 2011).

The main goals of this study were to investigate the neural correlates underlying central and peripheral processing of threat relevant stimuli, and in particular test the peripheral bias hypothesis with stimuli that are ecologically relevant for human emotional cognition (animal faces). We hypothesize that different regions may be recruited for central and peripheral processing of faces, given the likely reorganization of amygdala input from foveally-biased areas. Since in primates, faces are preferentially processed in the fovea, we also hypothesize amygdala preference for faces presented at central locations. Additionally, we aimed to study the dissociation between automatic/implicit vs. more controlled/explicit processing of threat relevant information and the role of the amygdala and other regions, such as the basal ganglia, in those processes.

5.2. Materials and Methods

5.2.1. Participants

Twenty healthy participants (age range 19-34, mean [SD] age = 26.30[4.54], 10 males) took part in the study. All subjects were right handed except 1 (ambidextrous) and all had normal or corrected-to-normal vision. All gave written informed consent, according to the Declaration of Helsinki, and the experimental protocol was approved by the ethics committee of the Faculty of Medicine of the University of Coimbra.

5.2.2. Stimuli and apparatus

Pictures of animal faces and natural displays were used as stimuli. Two types of animal faces were used: *threatening animal faces* (e.g. wolves, bears, dogs, sharks, tigers, leopards) displaying the mouth open and showing their teeth; and *non-threatening animal faces* (e.g. horses, sheep, rabbits, cows), displaying a neutral facial expression and mouth closed. A third set of stimuli, *control non-faces*, displaying non-facial natural scenes (e.g. grass, plains, meadows, flowers), was used as a baseline size matched control set. The images were taken both from the internet and the International Affective Picture System (IAPS) set (CSEA-NIMH, USA, csea.phhp.ufl.edu), and were manipulated in such a way that the animal face was centred in the picture display. Each picture was presented within a squared shape, yielding a visual angle of 6.84ºx 6.84º (W x H), and presented at one of three possible locations: centre, 0º, right or left, 7.71°. A prior validation study was performed for stimulus selection. A total of 110 pictures (55 containing animal faces, 55 containing control non-face stimuli) were presented at peripheral locations (both right and left) during 150 msec. Twelve participants responded if they could recognize an animal in the picture, and were requested to rate the pictures in terms of valence (positive, negative or neutral) / arousal (5-point scale) ratings. A final set of 48 pictures were selected. Threatening faces were rated as negative (valence mean[SD]=-0.49[.42], range: 0, 1, -1) and with mean [SD] arousal ratings of 1.57[.38], range: 0 to 5), while the non-threatening animal faces were rated as positive (valence mean [SD] = 0.68[.23], range: 0, 1, -1), and having a mean [SD] arousal rate of 1.38[.56], range: 0 to 5). 24 baseline size matched control stimuli were also used. Inside the scanner, the stimuli were back projected using an AVOTEC (www.avotec.org) projector on a 20(w) x 15(h) (1024 x 768 pixels) screen pad that was placed at a viewing distance of 50.5 cm by means of a head coil mounted mirror. The tasks were presented using Presentation software (Neurobehavioral Systems, USA, www.neurobs.com), and originally displayed on a monitor with a 60Hz refresh rate. Responses were given by means of a response box (Cedrus Lumina LP-400 response pad for fMRI, www.cedrus.com).

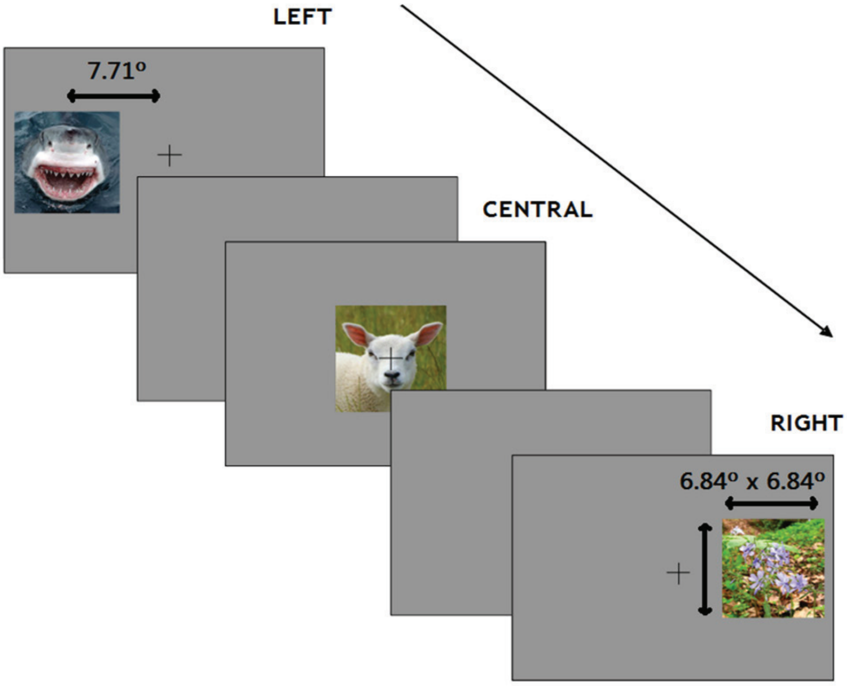

***Figure 5.1** – **Experimental design** (slow event related paradigm; stimulus duration: 150 msec; ITI: 7500, 10000 or 12500 msec). Examples of left, central and right hemifield presentations using three types of categories: neutral (control non-threatening) and threatening animal faces, and natural scenes/landscapes without animal categories.*

5.2.3. Task design and procedure

An fMRI slow event-related design was performed with 4 sequential runs of 54 trials each (4 x 217 volumes). Each trial started with a fixation cross (500 msec) followed by a picture (150 msec) presented in central, left or right locations of the screen. The participants had to press one of two buttons, according to the task to perform. An inter-trial interval (ITI) matched with the Repetition Time (RT, 2500 msec) followed the picture presentation and varied randomly (7.5, 10, 12.5 sec) (see Figure 5.1). Participants were asked to remain as still as possible during the testing session. It was emphasized that this would be important in order to minimize data artefacts. Importantly, different tasks were performed while fixating a central cross: (a) to report presence of an animal face (task 1: *'implicit threat' animal face recognition*, first 2 runs) or (b) to detect threat signals (task 2: *'explicit threat' detection*, last 2 runs) by means of a 2-button (Yes/No) response box. Picture duration was kept short to prevent visual saccades and eye movements were recorded (MR compatible AVOTEC/SMI systems) to ensure central fixation.

5.2.4. Imaging data acquisition and preprocessing

Functional images were acquired in a 3T Siemens TimTrio scanner using BOLD contrast echo planar imaging (EPI, TR 2.5 sec, TE 49 msec, 29 4 mm-thick-slices with no inter-slice gap, with an in-plane matrix of 128 x 128 voxels) covering the entire brain. The scanning session also included a high resolution T1 weighted anatomical scan (MPRAGE sequence, 1 x 1 x 1 mm3 voxel size, TR 2.3 sec, TE 2.98 msec, 160 slices) to help in the transformation of the functional images into standard space. The data were preprocessed and analysed using BrainVoyager QX v2.4 32-bit (Brain Innovation, www.brainvoyager.com). Preprocessing included slice scan time corrections, temporal filtering and motion correction. Before group analysis the images were spatially smoothed using a 4-mm full-width-half-maximum Gaussian kernel and then transformed into Talairach space.

5.2.5. Statistical analyses

All the statistical analyses were performed using IBM SPSS Statistics 19 and 20 (IBM, USA, http://www.ibm.com/software/analytics/spss/) and the Brain Voyager v2.6 software. The computation of effect sizes and power was performed with G*Power 3.1.6 (Faul, Erdfelder, Lang, & Buchner, 2007).

5.2.5.1. Behavioural data

Data from behavioural reports were considered to classify the trials where correct responses occurred. Therefore, trials corresponding to misses and false alarms (e.g. trials with: no response, threatening and non-threatening faces not recognized, or non-threatening faces and natural displays considered threatening) were excluded from the present analysis, but included in the design model of the functional data analysis as confound predictors. Accuracy measures, observer's *d* prime measures (*d'*) and reaction times (RTs) were obtained. The Accuracy was computed in order to have a measure of correct performance, whereas the *d* prime measure being a measure of response sensitivity was computed in order to see if there was a bias towards one type of response (Stanislaw, & Todorov, 1999; Provost, & Fawcett, 1997). Both the Accuracy and the index *d'* measures were computed for each task and spatial location. For the Accuracy measure we used hits, false alarms, misses and correct rejections in the following formula: Accuracy = [hits + correct rejects] / [hits + false alarms + misses + correct rejects]. For the index *d'* we used the subsequent formula: Z(hits) – Z(false alarms), using the idf.norm function of the IBM SPSS software. For the RT measure, we compared between tasks ('implicit threat' animal face recognition, 'explicit threat' detection), spatial locations (centre, left, right) and stimulus types (threatening animal face, non-threatening animal face, control non-face). One participant was excluded from the behavioural analysis due to lack of data regarding response time. Due to the non-normal distribution of data, non-parametric tests were used in all the analyses (Friedman and Wilcoxon signed rank tests for related samples).

5.2.5.2. FUNCTIONAL DATA

Statistical analyses were performed using a random effects general linear model (GLM) approach. Event duration was set to 4 sec beginning in the stimulus onset. Both *spatial location* (centre, left, right) and *stimulus type* (threatening animal faces, non-threatening animal faces, and control non-faces) were manipulated, with 9 predictors being included in each single-subject's design matrix (spatial location x stimulus type). A box car function was defined for each predictor and convolved with a canonical hemodynamic response function.

Two different analyses were then carried: region of interest and whole brain analyses. First, two regions of interest (ROIs) were defined in the left and right amygdalae of each participant based on anatomical landmarks (Duvernoy, 1999) (see Figure 5.2). Parameter estimates (z-normalized beta weights) were computed for each ROI and each task, with ANOVAs random effects (RFX) and post-hoc *t*-tests being performed using the IBM SPSS software. When applicable, corrections of Greenhouse-Geisser were reported together with tests of sphericity. Planned RFX-GLM contrasts analyses were performed using BrainVoyager.

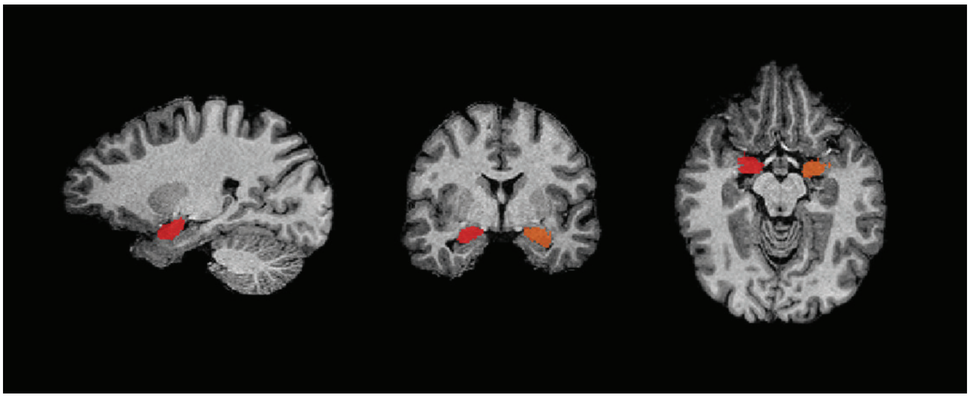

***Figure 5.2** – Defined Regions of Interest in the right and left amygdala of the participant AP. Mean coordinates (mean x [SD],mean y [SD],mean z [SD]) of all Regions Of Interest defined for the 20 participants were 20.6[1.1],-2.2[1.8],-13.4[1.2] for the right amygdala (number of voxels = 1553), and -20.9[1.2],-3.7[1.8],-13.7[1.0] for the left amygdala (number of voxels = 1460).*

Second, whole brain analyses were performed for each task separately and for direct comparison of both tasks. The statistical maps display specific contrasts after a whole brain RFX-GLM analysis being computed with brain mask restriction (53842 voxels). Corrections for multiple comparisons were made through the Cluster Threshold plugin (BrainVoyager) using 1000 Monte Carlo simulations. Minimum cluster sizes corresponding to significance at a threshold of $p < .01$ were computed for each contrast.

5.3. RESULTS

5.3.1. BEHAVIOURAL DATA

The participants performed two different tasks. In the first task (*'implicit threat' animal face recognition task*), they were asked to report (yes/no) if the picture presented contained an animal face, while in the second task (*'explicit threat' detection task*) they were required to report an yes/no answer regarding the detection of threat signals in the picture. For the Accuracy and Sensitivity index (*d'*) analyses, we compared performance between *tasks* ('implicit threat' animal face recognition or 'explicit threat' detection) and *spatial locations* (centre, right, and left). For the reaction time analysis, we further included *stimulus type* (threatening animal face, threatening animal face, and control non-face) as a factor.

5.3.1.1. Accuracy

Accuracy across participants was above 98%, for task 1 ('implicit threat' animal face recognition task), and above 97% for task 2 ('explicit threat' detection task), with differences in Accuracy not reaching significance (Wilcoxon paired test, W=90.500, Z=-.182, *n.s.*; 2-tailed).

The participants were able to recognize an animal face (task 1) presented in the centre (mean[SD]=.98[.03]) or in peripheral locations (left: mean[SD]=.98[.02]; right: mean[SD]=.97[.04]) with a high level of accuracy. Likewise, they were able to accurately detect threat in threatening animal faces (task 2) independently of location of presentation (centre: mean[SD]=.97[.04]; left: mean[SD]=.97[.04]; right: mean[SD]=.97[.04]). Friedman tests performed separately for each task showed that were no differences for *spatial location* when discriminating between stimulus type (task1: χ^2F(2) =1.192, *n.s.*; task2: χ^2F(2) = 2.333, *n.s.*). To see if a difference between tasks occurred as a function of *spatial location*, we performed Wilcoxon paired tests between task 1 and task 2 at each location. These turned out non-significant for all of the spatial locations (centre: W=58.500, Z=-.495, *n.s.*; left: W=53.500, Z=-.759, *n.s.*; right: W=53.000, Z=.032, *n.s.*; 2-tailed tests).

In this manner, we can conclude the participants responded to the pictures as expected, with no dissimilar performances neither between tasks nor spatial locations being found.

5.3.1.2. Sensitivity index (d')

In order to see if the accuracy of performance was due to an increased/decreased willingness (bias) to respond "yes", we have further tested matched accuracy across tasks by using the bias free classical *d* prime measure. This measure computes the observer's sensitivity to detect a signal having in consideration the false alarm rate (e.g. animal face, threat).

The results indicated no differences in *d'* measures between task 1 and task 2 (task 1 > task 2: Wilcoxon paired test, W=126.000, Z=.784, *n.s.*, 2-tailed). Again, Friedman tests performed separately for each task displayed no differences for *spatial location* (task1: χ^2F(2) = .724, *n.s.*; task2: χ^2F(2) = 4.651, *n.s.*). Additionally, Wilcoxon paired tests showed no differences between *tasks* at each location (centre: W=54.500, Z=-.698, *n.s.*, 2-tailed; left: W=46.000, Z=-1.140, *n.s.*, 2-tailed; right: W=64.000, Z=.227, *n.s.*; 2-tailed tests).

Therefore, the accuracy data is not better explained by a response bias, given the results from the sensitivity index *d'*. We can conclude that the performance was globally matched in what concerns task type and spatial location.

5.3.1.3. Reaction time (RT)

For the RT measure, Friedman tests showed neither differences between tasks (mean[SD] RT task 1 = 804.40[132.44] msec; mean[SD] RT task 2 = 882.50[179.99] msec; W=143.000, Z=1.932, *p*=.053; although a trend was found for higher RT during task 2) nor an effect of *spatial location* (task1: χ^2F(2) = 2.842, *n.s.*; task2: χ^2F(2) = 2.632, *n.s.*). However, a main effect of *stimulus type* was found in both tasks (task1: χ^2F(2) = 7.895, *p*=.019; task2: χ^2F(2) = 7.053, *p*=.029). Post-hoc paired sample test revealed differences in the contrasts 'non-threatening faces > threatening face' (W=-.789, Z=-2.433,

p=.045, Cliff's delta=.197; corrected for multiple comparisons) and 'non-threatening face > control non-faces' (W=.789, Z=2.433, p=.045, Cliff's delta=.197; corrected for multiple comparisons) during the 'implicit threat' animal face recognition task, and for the contrast 'threatening faces > control non-faces' (W=.842, Z=2.596, p=.028, Cliff's delta=.263; corrected for multiple comparisons) during the 'explicit threat' detection task.

5.3.2. Functional MRI data

Region of interest (amygdala) and whole brain random effects general linear model (RFX-GLM) analyses were performed.

5.3.2.1. Region of interest (ROI) analysis: The amygdala

We performed 3x3 ANOVAs RFX for each *task* ('implicit threat' animal face recognition or 'explicit threat' detection) in each amygdala ROI. *Spatial location* (centre, right, and left) and *stimulus type* (threatening animal face, threatening animal face, and control non-face) were taken as factors (Figures 5.3, 5.4 and 5.5).

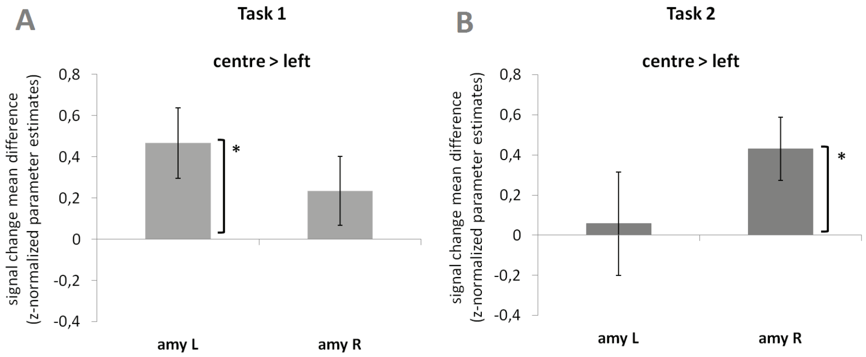

***Figure 5.3** – Effect of spatial location: responses of the amygdala for centrally presented stimuli are larger than for left peripheral presentations in a task dependent manner. The left amygdala is preferentially involved during the implicit threat animal face recognition (task 1), while the right amygdala is more engaged during the explicit threat detection task (task 2). ROI RFX-GLM contrasts: mean differences in parameter estimates (z-normalized beta-values) for the contrasts centre > left, task 1 (**A**) and 2 (**B**) are displayed. Legend: amy L, amygdala left; amy R, amygdala right; * p<0.05. The bars display the standard error of the mean (SE).*

5.3.2.1.1. Main effects

For the '*explicit threat' detection* task (task 2), a main effect of *spatial location* was found for the right amygdala ($F_{(2,38)}$= 3.533, p=.039, Cohen's d=.432, power(1-β)=.999). During the *'implicit threat' animal face recognition* task (task 1), a main effect of *stimulus type* was found for the left amygdala ($F_{(2,38)}$= 4.103, p=.024, Cohen's d =.465, power(1-β)=1.000), as well as a marginal effect of *spatial location* ($F_{(2,38)}$= 3.194, p=.052, Cohen's d =.410, power(1-β)=.999).

Posthoc analyses showed the differences in the right amygdala ROI emerged from the contrast 'central > left' (t(19)=2.733, p=.013, Cohen's d =.611, power(1-β)=.839), the same contrast was also underlying the marginal effect of spatial location in the left amygdala during task 1 (t(19)=2.694,

p=.042, corrected for multiple comparisons, Cohen's d =.552, power(1-β)=.769) (Figure 5.3). Regarding the effect of stimulus type, the left amygdala differences were found for the contrast 'threatening animal faces > control non-face: $t(19)=2.375$, $p=0.028$, Cohen's d =.465, power(1-β)=.999) (Figure 5.4).

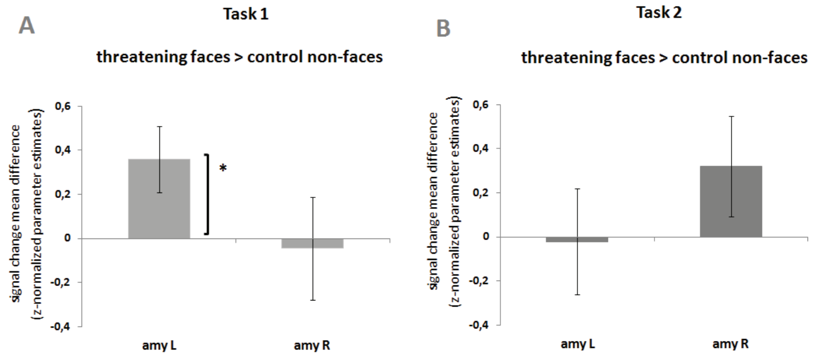

***Figure 5.4** – Effect of stimulus type: responses of the amygdala to the threatening animal faces are larger than to the non-facial stimulus. The left amygdala is preferentially involved in the discrimination of threatening and non-threatening stimuli during the animal face recognition (task 1). ROI RFX-GLM contrasts: mean differences in parameter estimates (z-normalized beta-values) for the contrasts threatening faces > non-threatening faces, task 1 (**A**) and 2 (**B**) are displayed. Legend: amy L, amygdala left; amy R, amygdala right; * p<0.05. The bars display the standard error of the mean (SE).*

5.3.2.1.2. Interaction effects

An interaction effect between *spatial location* and *stimulus type* was found in the right amygdala ($F_{(2,850; 54,144)}= 3.180$, $p=0.033$, Cohen's d=.409, power(1-β)=.999, Greenhouse-Geisser correction, Mauchly's W(9)=.263, p=.006, ε=.712) for the '*explicit threat' detection* task.

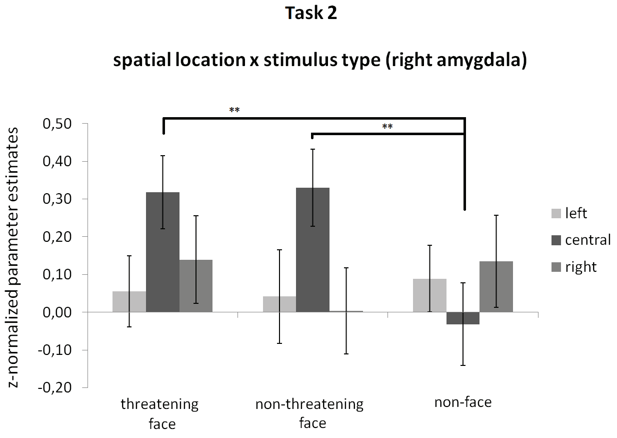

***Figure 5.5** – Interaction effect of stimulus type X spatial location: responses of the (right) amygdala to both threatening and non-threatening animal faces are larger than to the non-facial stimulus, and occur only for centrally presented stimuli during the explicit threat detection task (task 2). ROI RFX-GLM contrasts: z-normalized parameter estimates (beta-values) for the contrasts 'centre: threatening faces > non-faces' and 'centre: non-threatening faces > non-faces', task 2, are displayed. * p<0.01 and ** p<0.005. The bars display the standard error of the mean (SE).*

Posthoc tests revealed differences between animal faces and control non-faces only for centrally presented stimuli (centre: threatening animal faces > control non-faces: $t(19)=3.701$, $p=.001517$, Cohen's $d=.828$, power(1-β)=.973; non-threatening animal faces > control non-faces: $t(19)=3.341$, $p=.003432$, Cohen's $d=.747$, power(1-β)=.942) (Figure 5.5).

5.3.2.2. Whole brain RFX analysis

We performed whole brain RFX contrast analyses to identify brain regions involved in task and spatial location effects (brain regions, peak voxel coordinates and statistics are presented in Table 5.1).

5.3.2.2.1. Task: 'implicit threat' animal face recognition vs. 'explicit threat' detection

Differences among tasks become apparent in the right fusiform gyrus, right cuneus, left lingual gyrus, left medial frontal gyrus, left putamen, left middle temporal gyrus and left cerebellum, with increased activity during the 'explicit threat' detection task (see Figure 5.6 and Table 5.1 – contrast a).

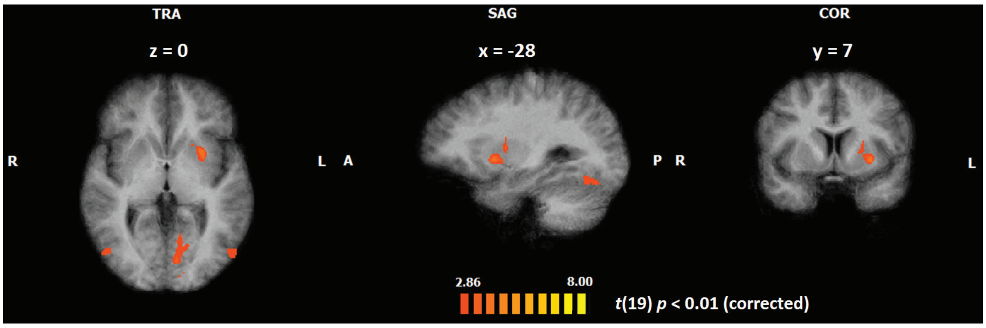

Figure 5.6 *- Activated regions yielded by the RFX group analysis for the contrast explicit threat detection task (task 2) > implicit threat animal face recognition (task 1). Cluster threshold correction was set at p<0.01 with a minimum cluster size of 15*9 voxels.*

5.3.2.2.2. Spatial location: centre vs. periphery

Whole brain RFX planned contrasts performed for the effect of *spatial location* revealed increased activity in the right fusiform gyrus, left superior frontal gyrus and left middle temporal gyrus in the centre compared to peripheral stimulation. In the contrary, the right posterior cingulate gyrus responded more to peripherally presented stimuli (Table 5.1 – contrast b).

5.3.2.2.4 Task x Spatial location

We identified a surprising difference in striatal activation patterns across tasks. We directly compared performance across spatial locations between task 1 and task 2 (n=20).

Central representations: concerning areas that activate more strongly for task 2 (threat detection) we observed that when stimuli were presented centrally, significant differences were in seen the *right caudate head of the basal ganglia* and in the left lingual gyrus, (Figure 5.7 - top, and Table 5.1 – contrast c).

Peripheral representations: for peripheral presentations, between task differences showed stronger activity during task 2 in the *left putamen*, right fusiform gyrus, right posterior cingulate and bilateral cerebellum (Figure 5.7 - bottom, and Table 5.1 – contrast d).

The data shows a task dependent centre vs. periphery bias in visual and importantly, also in striatal regions. Given the pattern found in the basal ganglia, planned post-hoc tests were then performed. Therefore, first we contrasted only the task-relevant conditions. The contrast 'threatening animal faces (task 2)' > 'animal faces (threatening + non-threatening) (task 1)' for central presentations yielded differences in the right caudate (*t*(19)=4.521, *p*=.000234; x=11, y=7, z=3) and in the right (*t*(19)=4.993, *p*=.000081; x=18, y=-4, z=7) and left putamen (*t*(19)=4.260, *p*=.000423; x=-24, y=-1, z=10), matching the original pattern of results. For peripheral stimuli the contrast 'threatening faces' (task 2) > 'animal faces (threatening + non-threatening)' (task 1) returned a difference only in the left putamen (*t*(19)=3.638, *p*=.001749; x=-16, y=10, z=6). Most important is that the contrast 'non-threatening (face + non-face)' (task 2) > control non-face' (task 1) did not yield any significant pattern, suggesting that the basal ganglia findings are specific for task-relevant stimuli.

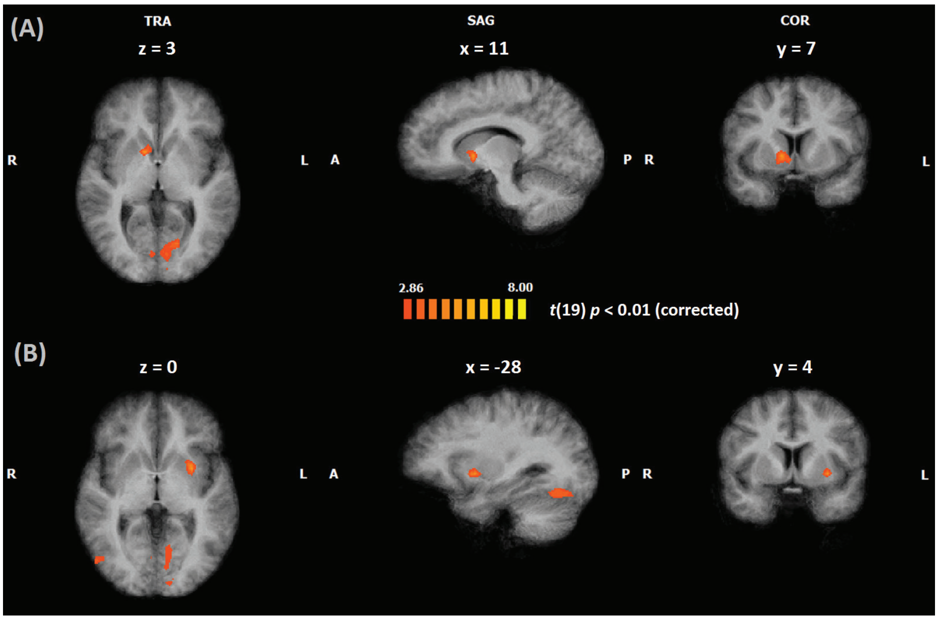

Figure 5.7 *- Two different striatal circuits are differentially activated depending on task type and spatial location: (top, A) central processing recruits the right caudate, (bottom B) peripheral processing recruits the left putamen. Peak voxel coordinates (TAL) are displayed. Cluster threshold correction was set at p<0.01 with a minimum cluster size of 15*9 voxels, for peripheral comparisons (B), and of 13*9, for central ones (A).*

5.4. DISCUSSION

The main goals of this work were twofold: first, to investigate the hypothesis of a potential central bias in the amygdala for processing of facial stimuli, given that its major input comes from foveally-biased ventral visual areas. Secondly, we studied a possible dissociation in the neural correlates of central and peripheral threat processing, and how task instructions can modulate information streaming and brain regions involved. Although we focused on the role of amygdala as a region of interest, we also performed whole-brain analyses to understand face recognition and threat processing at a more general level.

We identified both in ROI and whole brain analysis task related activity differences (animal face recognition vs. threat detection) on a spatial location (central vs. peripheral emotional) dependent basis. Accordingly, we have found evidence for distinct regions being involved in explicit vs.

Table 5.1 - *Whole brain analyses: summary of random-effects (RFX)-GLM contrasts, outputs and statistics.*

Region	Peak X (TAL)	Peak Y (TAL)	Peak Z (TAL)	Nr of voxels	*t*	*p*
Contrast a : explicit threat detection > implicit threat animal face recognition						
r occipital fusiform gyrus	38.0	-71.0	-9.0	806	4.809	0.000122
r cuneus	14.0	-77.0	15.0	429	3.577	0.002012
l lingual gyrus	-7.0	-77.0	3.0	2248	4.458	0.000270
l medial frontal gyrus	-10.0	55.0	15.0	467	6.045	0.000008
l putamen	-28.0	7.0	0.0	851	4.817	0.000120
l cerebellum	-28.0	-62.0	-18.0	456	4.050	0.000683
l middle temporal gyrus	-55.0	-68.0	12.0	535	3.948	0.000863
Contrast b : centre > periphery						
r lateral occipital gyrus	23.0	-89.0	-12.0	59312	8.625	0.000000
r posterior cingulate gyrus	20.0	-14.0	42.0	921	-6.331	0.000004
l superior frontal gyrus	-19.0	46.0	39.0	779	3.886	0.000994
l middle temporal gyrus	-40.0	-74.0	24.0	1640	4.602	0.000195
Contrast c : centre: explicit threat detection > implicit threat animal face recognition						
r caudate head	11.0	7.0	3.0	426	5.312	0.000040
l lingual gyrus	-10.0	-77.0	6.0	1126	4.492	0.000250
Contrast d : periphery: explicit threat detection > implicit threat animal face recognition						
r occipital fusiform gyrus	38.0	-71.0	-9.0	721	5.523	0.000025
r cerebellum	20.0	-62.0	-12.0	420	3.868	0.001036
r posterior cingulate gyrus	2.0	-68.0	12.0	3428	4.369	0.000330
l putamen	-28.0	4.0	0.0	485	5.291	0.000042
l cerebellum	-25.0	-71.0	-18.0	741	4.252	0.000431

All contrasts were performed at $p<.01$ using cluster threshold correction. X, Y and Z represent Talairach coordinates. r, right; l, left.

implicit processing, with an emphasis on the amygdala and the striatum. In the amygdala, no response bias to peripheral locations was found using face stimuli, a finding that was only apparently surprising (see below). Moreover we found that these responses were task (implicit vs. explicit) and hemisphere dependent. In the basal ganglia structures we also identified strong task and location dependence. Indeed, one of the most important findings of this work was that central and peripheral 'threat vs. animal face' task differences recruited different basal ganglia regions: central information involved the caudate head and peripheral information engaged mainly the putamen. These findings are interesting in the way they may contribute for the scarce but stimulating body of evidence that implicates basal ganglia in affective aspects of visual processing.

This study bears implications on the understanding of implicit vs. explicit processing of emotional information as a function of spatial position (central or peripheral).

5.4.1. The amygdala

5.4.1.1. A central bias for faces in the amygdala

Different object categories have specific eccentricity biases, with face stimuli being preferentially processed within central vision (Levy et al., 2001). The amygdala receives major input from foveally-biased ventral areas. For this reason, we hypothesized that it might show increased activity for face-objects presented at foveal locations, in spite of the conventional view (Palermo & Rhodes, 2007). Our results are in line with our prediction, with central compared to left visual field face presentations eliciting stronger activation in particular in the right amygdala during the explicit threat task.

Some studies (Bayle et al., 2009; Preibisch et al., 2009; Palermo & Rhodes, 2007) have nevertheless suggested that, due to its potential connections with the SC and the pulvinar, which are more related with magnocellular pathways and processing of low spatial frequency information, a bias might arise for peripheral processing of negative (e.g. fear) facial expressions. It must be pointed however that although the ratio parvocellular/magnocellular projections is high for stimuli processed in the fovea, in the periphery both magnocellular and parvocellular processing are significant (Azzopardi, Jones, & Cowey, 1999). In any case, in our study no peripheral bias was found. This is at least partially consistent with previous results using fearful faces (Morawetz et al., 2011), which found a lack of modulation concerning spatial location, and in substantial agreement with another study (Morawetz et al., 2010) which found a difference central > periphery during the performance of relatively low attentional load tasks, regardless of whether they were implicit (matching digits) or explicit (matching emotion).

Two factors should be discussed here. First, different eccentricities were used across studies, with the difference between central and peripheral locations arising at 5.6° of visual angle, but not at 11.25° (Morawetz et al., 2010) nor at 9.5° (Morawetz et al., 2011). In our study, we used an intermediate visual angle, 7.71°, which is more close to the study reporting a central bias. Secondly, magnification factors (used in Liu & Ioannides, 2010; Morawetz et al., 2011, 2010; Preibisch et al., 2009 but see Bayle et al., 2009) may be an issue. We addressed this issue by using control non-face stimuli that were scale matched to the face stimuli. The former did not show the central bias found with faces. This approach showed that stimulus type was more relevant than scaling in explaining our pattern of results. Moreover, task and hemispheric dependencies are not easily explained by magnification differences. Previous studies confirmed the possibility to study early amygdala activity at peripheral locations even when no scaling is used (Bayle et al., 2009), an approach that we also followed (for a review on the role of low level properties see Strasburger et al., 2011).

The central face bias in the amygdala might be explained by centrally-biased inputs from areas along the occipital-temporal cortex belonging to the face network (e.g. Rolls, 2007). Accordingly, our whole brain data showed increased activity in the right lateral occipital (LO) during central presentations. Moreover, the contrast of explicit over implicit threat seemed to engage more strongly the right occipital gyrus. Together, these findings provide some insight to understand why the right amygdala showed a specific response at central spatial locations to both threatening and non-threatening faces in particular during the explicit threat task. In contrast, the left amygdala, although showing a difference between threatening animal faces from non-faces, this happened irrespective of spatial location (as no left occipito-temporal areas showed a spatial location bias). These observations support the notion major involvement of right hemispheric specialized areas in foveal face processing (Kanwisher et al., 1997).

5.4.1.2. The amygdala responds to the threatening content of animal faces

We found an overall increased response of the left amygdala to threatening animal faces compared to control non-faces, irrespective of spatial location, during the ‘implicit threat’ animal face recognition task, whereas during the ‘explicit threat’ detection task, the right amygdala differentiated between faces and non-faces only when centrally presented.

In our study we made use of threatening animal faces whereas most of the previous studies have used fearful human faces. Two points should be addressed, in this context. First, some studies have suggested that the amygdala responds differently to fearful and angry emotional faces. Overall angry faces elicit stronger responses in specific amygdala nucleus (corticomedial), being positively correlated with increased behavioural reports of perceived threat (Boll, Gamer, Kalisch, & Büchel, 2011; but see opposite findings in Whalen et al., 2001). As a second point, threatening signals in animal and human face are most likely species-specific (e.g. Gothard et al., 2007). Therefore, direct comparisons between previous studies with human faces may not be feasible. In fact, we decided to use animal instead of human faces because both have distinct ecological value and since direct recordings suggest the amygdala responds surprisingly stronger to animal faces than to human (irrespective of facial expression) faces (Mormann et al., 2011), as they might have an increased survival value.

It has been shown that activity within the amygdala declines with repeated presentations, an effect attributed to stimulus familiarity (Wilson & Rolls, 1993). However, we have reasons to believe this was not an issue in our study because stimuli were not repeated. It might also be pointed that the amygdala shows a preferential response to the ‘threatening’ animals simply because these act as ‘new’ stimuli, in comparison with non-threatening stimuli. This is unlikely given the nature of our sampled population. Moreover our data shows that this region of the brain responds to both animal categories, in agreement with previous results (Mormann et al., 2011).

5.4.1.3. THE AMYGDALA SHOWS A LATERALIZED RESPONSE DEPENDING ON TASK INSTRUCTIONS

Differences regarding task related activity were related to a lateralized central vs. peripheral amygdala preference for threatening faces. In fact, our results point to a central preference in the right amygdala during the explicit threat task and to a left lateralized amygdala response during the implicit threat (animal face recognition) task. This might at first glance seem counterintuitive, as right amygdala has been originally more related with automatic and implicit processing (e.g. Morris et al., 1999), unlike the left amygdala (Gorno-Tempini et al., 2001). However these views can be reconciled if one considers that our study took into account how central vs. peripheral responses in the amygdala may interact with task instructions.

The controversy regarding the role of the amygdala in implicit processing is well recognized (e.g. Hariri et al., 2000). Other authors (Öhman, 2009; Tamietto & de Gelder, 2010) have hypothesized a prominent role of the amygdala in automatic emotional processing, in relation to coarse recognition of relevant information routed through the pulvinar and the superior colliculus (Tamietto et al., 2012). It has been recognized that task demands might modulate the amygdala response (Costafreda et al., 2008; Pessoa, McKenna, Gutierrez, & Ungerleider, 2002), in addition to the fact that the pulvinar has also been related to conscious attentional processes (Padmala, Lim, & Pessoa, 2010). Our work provides a new perspective on this view by showing that the amygdala may also show task dependent responses to both explicit and centrally processed stimuli. Our findings are therefore in agreement with a recent review which has pointed to major involvement of the amygdala in explicit processes (Fusar-Poli et al., 2009).

In any case, our findings add to the ongoing discussion on the left/right amygdala lateralization patterns. It has been proposed that the right amygdala responds when the emotional property of the stimulus is visual and directly obvious to the subject, while the left would show preference for verbally learned stimuli (Phelps et al., 2001; see also Gläscher & Adolphs, 2003). Also, the intriguing study of Heutink, Brouwer, de Jong & Bouma (2011) have found that absence of the right amygdala impairs the overt, explicit, identification of fear, while covert, implicit, recognition of fear and aversive conditioning may still occur following lesion of the right amygdala. Notably, stimulus type (face vs. control non-faces) and task seem to influence patterns of activity and lateralization in the amygdala (but see Baas, Aleman, & Kahn, 2004).

5.4.2. THE BASAL GANGLIA

To our knowledge there are very few studies addressing directly the relation between the amydgala, basal ganglia and central vs. peripheral emotion processing. Morawetz and colleagues (2010) addressed the question of how spatial location, and attentional load modulate particular brain regions by using a ROI-based approach centred only in the amygdala and the fusiform gyrus, which did not allow them to explore the functional role of other regions.

5.4.2.1. DIFFERENT NEURAL CORRELATES FOR CENTRAL AND PERIPHERAL VISUAL EMOTION RECOGNITION

In our study, we presented angry and neutral animal facial expressions both in the centre and in visual periphery. We found that peripheral and central processing of visual threat signals do correspond to different brain networks. Our results showed that peripheral processing recruited mainly the putamen, which is known to be dominantly related to implicit processing (Rauch et al., 1997), whereas the caudate was only involved during central stimuli appraisal. This region is relatively more involved in explicit goal oriented processing (Brown, Redondo-Verge, Chacon, Lucas, & Channon, 2001; Ruge & Wolfensteller, 2010).

One MEG study, with inherent limitations in the interpretation of activity in deep structures and their subparts, has partly addressed this issue by suggesting an involvement of the thalamus, amygdala and basal ganglia in the rapid detection of threat (Luo, Holroyd, Jones, Hendler, & Blair, 2007). However, this pattern was found for fearful but not for angry or neutral expressions. Furthermore, faces were only presented centrally. To our knowledge, only one study showed striatum activity with peripheral presentation of static (happy > neutral) faces (Faivre et al., 2012). However, several methodological differences with our study were present, as the caudate was specifically found in our study to be modulated by the difference between explicit threat detection and simple animal face recognition tasks, whereas Faivre and colleagues (2012) studied only implicit processing of happy vs. neutral faces.

Importantly, the caudate head seems to receive and project for several areas along the visual cortex, in particular inferotemporal (Baizer, Desimone, & Ungerleider, 1993; Saint-Cyr, Ungerleider, & Desimone, 1990). This might in part explain why it plays a major role at central spatial locations. Different functions have been attributed to the caudate head and the putamen, with the caudate more engaged in emotional (Arsalidou et al., 2012) and goal-oriented processes, whereas the putamen appears to subserve more automatic cognitive functions (Grahn, Parkinson, & Owen, 2008).

5.4.2.2. An explicit > implicit bias goal-oriented response in the basal ganglia

Our findings are in agreement with reports of increased right caudate activity for explicit compared with implicit emotional processing (for a review, see Fusar-Poli et al., 2009). In fact, in our study the caudate part of the striatum was consistently found to respond more to threatening animal faces during the explicit task than to neutral (non-threatening) animal faces (bilateral caudate) or non-facial displays (right caudate), which is consistent with its role in conscious emotional processes. The fact that the putamen activated more strongly for the explicit threat task might however challenge its preferential involvement in implicit processes (at least when spatial central-periphery constraints are not taken into account). Nevertheless, other authors have found increased left putamen for explicit emotional tasks (Critchley et al., 2000; Sugiura et al., 2000), with bilateral putamen responding to implicit tasks (Critchley et al., 2000). It is possible that the right and the left putamen play different roles in emotional processing. Here we found an interaction of basal ganglia structures with spatial location, with the left putamen activating preferentially to explicit threat mainly in the periphery.

5.4.3. Limitations

Potential differences between our study and others (Liu & Ioannides, 2010; Bayle et al., 2009) might arise from the methodologies used. In fact, the temporal resolution of MEG is much higher than the one currently used in our fMRI study, although the latter has better spatial resolution. The former point is nevertheless an important point, as the lack of amygdala responses for peripheral stimuli as measured in fMRI does not mean that this type of processing does not occur. In fact, one might argue about detection sensitivity: the peripheral response might occur earlier and faster, and/or with diminished amplitude as compared to more central and explicit processing. Moreover, the differences in the left amygdala for responses to threatening vs. non-face stimuli might suggest an automatic role of the amygdala, adding to the evidence for a role on conscious emotional processing.

Although different amygdala subnuclei were proposed to be involved in the processing of angry and fearful expressions (e.g. Whalen et al., 2001), the spatial resolution (voxel dimension) chosen for our study did not allow us to individuate the contributions of each. However, recent work (e.g. Boll et al., 2011) offers promising opportunities to study the role of different amygdala nuclei in different affective functions.

5.5. Major conclusions

We found a lateralized response of the amygdala as a function of task instructions, with a bias for central processing of faces occurring specifically in the (right) amygdala during the explicit threat processing.

Furthermore, we found a dual striatal contribution preferentially tuned for central (caudate) or peripheral (putamen) processing of threat content information, the former being more related to goal directed processing and the later with automatic processing.

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Chapter 6

Fear-relevant animal Faces and Shapes: the role of central vs. peripheral processing in threat detection

ABSTRACT

In humans, ecologically relevant stimuli (e.g. faces) are processed in central vision. Since central vision processes high-resolution details, it is better suited for identification processes that require accuracy. Peripheral vision is more likely to process objects that do only require coarse stimulus identification, such as animal shapes (e.g. snakes), due to its preference for low spatial frequencies and degraded colour information. In fact, snakes seem to have been agents in evolutionary changes in primate visual system, and might therefore have acquired a phylogenetic value. The amygdala is a core structure in the subcortical pathway for threat detection, and it seems to be preferentially entailed during automatic, preattentive detection of fear-relevant (e.g., angry faces, snakes) as opposed to fear-irrelevant (e.g., flowers, rabbits) stimuli.

In a previous study we found that the amygdala showed a central bias for animal faces. In this study we asked if such a (central) bias was also present for other ecologically relevant objects, such as ecologically relevant shapes. To test this hypothesis, we used snake-related stimuli: snake faces, snake shapes, and control fake snakes, and manipulated both the spatial location and the allocation of attention to threat (implicit and explicit tasks).

We found larger amygdala responses to centrally presented snake stimuli (body, face or fake) than for right peripheral presentations, independent of task and amygdala. For the contrast centre > left hemifield, these differences were found only for the left amygdala during the implicit snake identification task. During the implicit task this difference centre vs. right correlated positively with reported fear of snakes. Importantly, a strong hemispheric lateralization was found, with real shapes activating stronger the right hemisphere as compared to fake shapes, which is consistent with its dominance for stimuli with emotional content.

These results point to the role of central vision in primates, although not disputing the role of and peripheral, less accurate, processing. In fact, left hemifield/right hemisphere (visual and emotional dominant) asymmetries found specifically for the snake shapes in the amygdala suggest that these stimuli have phylogenetic value.

Keywords: amygdala, snakes, right hemisphere, visual asymmetries, central / peripheral, implicit / explicit, threat, fear, faces.

6.1. Introduction

It has proposed that the amygdala responds preferentially to peripheral menacing stimuli (Palermo & Rhodes, 2007) although experimental evidence is still scarce (for a review see Chapter 4). However in human cognition, most relevant stimuli such as faces are processed in central vision. This is consistent with the fact that the face processing network is foveally-biased, with face-related regions associated with center-biased representations (Kanwisher, 2001; Levy, Hasson, Avidan, Hendler, & Malach, 2001). Previously, we have studied the affective processing of faces, showing that faces activate the amygdala at a larger degree when presented at central locations compared with peripheral (e.g. left) spatial locations (Almeida et al., 2013, Chapter 5). This unexpected observation may be explained by the above mentioned bias to process detailed stimuli in central vision, and in particular faces. Accordingly, the amygdala seeks information from the eye region in human faces (Adolphs, Gosselin, Buchanan, Tranel, Schyns, & Damasio, 2005; Atkinson & Smithson, 2013; Gamer & Büchel, 2009; Kennedy & Adolphs, 2010; Morris, deBonis, & Dolan, 2002; Whalen et al., 2004). Moreover the amygdala receives direct input from ventral areas (Lori et al., 2002; Rolls, 2007; Stefanacci & Amaral, 2002) which are known to be biased to foveal input. In sum, critical information about the social meaning of faces, in particular their expressions requires detailed visual analysis and central vision.

An outstanding question is whether such bias is also present for other ecologically relevant objects, such as animal shapes (e.g. snakes, spiders). Alternative, they could be processed by peripheral visual systems which connect with the amygdala, in line with the view that favours peripheral processing for stimuli that are potentially threatening (Bayle, Henaff, & Krolak-Salmon, 2009). This hypothesis is consistent with the idea that shapes do not require much detail to be processed and can therefore be analysed in the visual periphery. A peripheral bias in medial regions such as the anterior collateral sulcus in the parahippocampal gyrus has also been for non-ecological stimuli that usually appear in our peripheral visual field such as buildings (Levy et al., 2001), which only require low spatial frequency (LSF) coarse processing. The question remains whether biological forms such as snake shapes preferentially require peripheral coarse processing. This was the main question of our study.

Ancient sensory mechanisms with an origin in organisms with primitive brains evolved for rapid detection of what could turn out life-threatening events, on the basis of a "quick and dirty" analysis, calling on an attentional shift in order to monitor the environment for potential hazardous stimuli (Öhman & Mineka, 2003). Although the visual detection of fear stimuli is an essential adaptive ability, the capacity to apprehend different kinds of stimuli in the environment decreases with the degradation of the visual performance associated with retinal eccentricity (e.g. Atkinson & Smithson, 2013; Jüttner & Rentschler, 2000). This question has not been analysed in detail because most fMRI/MEG studies have used emotional face stimuli in the central visual fields (e.g., Padmala, Lim, & Pessoa, 2010). Even the ones that compared centre vs. periphery have mainly used face stimuli (reviewed in Chapter 4). In general, a central bias was found for human and animal faces (Almeida, van Asselen, & Castelo-Branco, 2013 - see Chapter 5; Morawetz, Baudewig, Treue, & Dechent, 2010).

Based on an evolutionary claim, it is reasonable that individuals must capitalize on automatic attention mechanisms to potentially threatening events to allow safe avoidance or escape, which could have shaped human peripheral vision to allow rapid identification of such stimuli (Isbell, 2006). Congruently, peripheral processing involves the subcortical circuit centered in the amygdala (LeDoux, 1996; Öhman, 1993) via the superior colliculi (SC) and the pulvinar nucleus of the thalamus (Isbell, 2006; Tamietto & De Gelder, 2010), which then results in rapid threat detection (Morris, Öhman, & Dolan, 1999; Vuilleumier, Armony, Driver, & Dolan, 2003). This route is based on magnocellular pathways from the retina and is served by large rapidly conducting neurons, which mediates gross, LSF information directly to the amygdala for rapid threat detection (Liddell et al., 2005; Morris, De Gelder, Weiskrantz, & Dolan, 2001; Vuilleumier et al., 2003). In spite of the acuity limits of peripheral spatial vision, behavioural studies with peripherally presented emotional stimuli do nevertheless suggest relatively good performance with parafoveal and peripheral locations both in recognition (Calvo, 2006; Calvo & Lang, 2005) and categorization tasks (Rigoulot et al., 2008; Thorpe, Gegenfurtner, Fabre-Thorpe, & Bülthoff, 2001).

It is also under debate whether humans are biased to respond with amygdala activation during implicit tasks processing (Baas, Aleman, & Kahn, 2004; Fusar-Poli et al., 2009). Because the detection advantage of threatening stimuli is likely to be evolutionary ancient, originating in creatures having primitive visual systems, it must rest on features that require minimal capacity for higher visual processing. Thus, some bias should be expected concerning implicit processing. However, controversy surrounds the automaticity of the amygdala response to emotional stimuli and the role of task demands in such processing (see Pessoa, & Adolphs, 2010; Tamietto & De Gelder, 2010). Human attention has evolved with a biased category-specific criterion to monitor animals (Drewes, Trommershäuser, & Gegenfurtner, 2011; New et al., 2007; Thorpe et al., 2001), with accurate animal detection being performed even at far peripheral locations. Accordingly, a recent study intended to investigate the interaction between eccentricity and the nature of task (implicit vs. explicit) with ecological relevant face stimuli (animal faces) (Almeida et al., 2013). The results showed a bias for central faces, specifically during the explicit threat detection task.

The present study aims at further investigating such interaction while using stimuli holding a high evolutionary relevance, as it is the case with snakes (see Öhman, Soares, Juth, Lindström, & Esteves, 2012). Classic studies in primates showed that learning of fear by observation of conspecifics behaviour occurred more easily when the object of fear were reptiles, namely snakes, compared with other type of animals (e.g. rabbits) (Cook & Mineka, 1989). In fact, it has been proposed that predation pressure from snakes is ultimately responsible for the superior vision and large primate brains, which represented a critical facet of human evolution (see Isbell, 2006, 2009; Öhman, 2007). Thus, such stimulus may represent an ideal candidate for resolving the conflicting debate regarding the role of eccentricity. Additionally, the nature of task requirements (implicit vs. explicit) might influence amygdala processing. However, it is not known in what way using central or peripheral vision (manipulated by simply changing the stimulus spatial location) interacts with task related modulation.

Since the processing of snakes seems to be carried out independently of available resources (Öhman et al., 2012; Soares, 2012; Soares & Esteves, 2013), we hypothesize that their processing should occur outside the known eccentricity bias for object recognition. Additionally, it should also be relatively immune to implicit or explicit task requirements, concerning threat detection, since we expect that relevant shape processing should be more based on bottom-up, stimulus driven, processes (Öhman, Soares, Juth, Lindström, & Esteves, 2012). A hemispheric asymmetry is also predicted to occur, due to the known right hemispheric preference for threat detection in a wide category of animals (Vallortigara, & Rogers, 2005). To test for these hypotheses we conducted an fMRI event-related design manipulating stimulus type, spatial location and nature of task.

6.2. Materials and Methods

6.2.1. Participants

Twenty participants with normal or corrected-to-normal vision (7 males, mean [SD] age = 25.2[5.1]; education level range: 7-20) took part in the study. All subjects were right handed except one. Participants were selected from a pool of 94 individuals according to their scores on a Portuguese version of the Snake Phobia questionnaire (SNAQ) (Klorman et al., 1974; Soares, 2012). They were recruited either in classes at the Faculty of Medicine, University of Coimbra, Portugal, or from a voluntary participants database created for research purposes (http://voluntariosibili.pt.vu/). The mean rate of the fear scores in the snake phobia questionnaire was 13.65[8.83] (mean[SD]) (median = 9.5), ranging between 1 and 29 points (maximum score = 30).

All participants gave written informed consent, according to the Declaration of Helsinki, and the experimental protocol was approved by the ethics committee of the Faculty of Medicine of the University of Coimbra.

6.2.2 Stimuli and apparatus

Pictures of snakes (e.g. faces and shapes) and stimuli resembling snake shapes (e.g. cables, strings, hoses, bracelets, trunks) were used as stimuli. The images were taken both from the internet and from the International Affective Picture System (IAPS) set (CSEA-NIMH, USA, csea.phhp.ufl.edu), and were manipulated in such a way that the face or shape (real or fake) was centred in the picture display. The final set was constituted by 32 snake faces, 32 snake shapes and 32 fake snake control stimuli, based on a pilot study for stimuli selection.

Each picture was presented within a squared shape, yielding a visual angle of 6.84°x 6.84° (W x H), and presented at one of three possible locations: centre, 0°, right or left, 7.71°. Inside the scanner, the stimuli were back projected using an AVOTEC (www.avotec.org) projector on a 20(w) x 15(h) (1024 x 768 pixels) screen pad that was placed at a viewing distance of 50.5 cm by means of a head coil mounted mirror. The task was presented using Presentation software (Neurobehavioral Systems, USA, www.neurobs.com), and originally displayed on a monitor with a 60Hz refresh rate. Responses were given in a response box (Cedrus Lumina LP-400 response pad for fMRI, www.cedrus.com).

6.2.3 Task design and procedure

An fMRI slow event-related design was performed with 8 sequential runs of 36 trials each. Both stimulus type (snake faces; snake whole bodies; fake snake shapes) and spatial location (central; peripheral left and right) were manipulated. Importantly, different tasks were performed while fixating a cross at the centre: the participants were asked to report (a) if the picture presented referred to a real snake (either face or body) (task 1, snake identification, first 4 runs) or (b) detection of threat signals (task 2, last 4 runs) by means of a 2-button (Yes/No) response box. Each trial started with a fixation cross (500 msec) followed by a picture presented in central, left or right locations of the screen. Picture duration was kept short (150 ms) to prevent visual saccades and eye movements were recorded (MR compatible AVOTEC/SMI systems) to ensure central fixation. The inter-trial interval (ITI) matched with the Repetition Time (RT, 3 sec) followed the picture presentation and varied randomly (3 or 6 sec) (Figure 6.1).

Participants were asked to remain as still as possible during the testing session. It was emphasized that this would be important in order to minimize data artefacts.

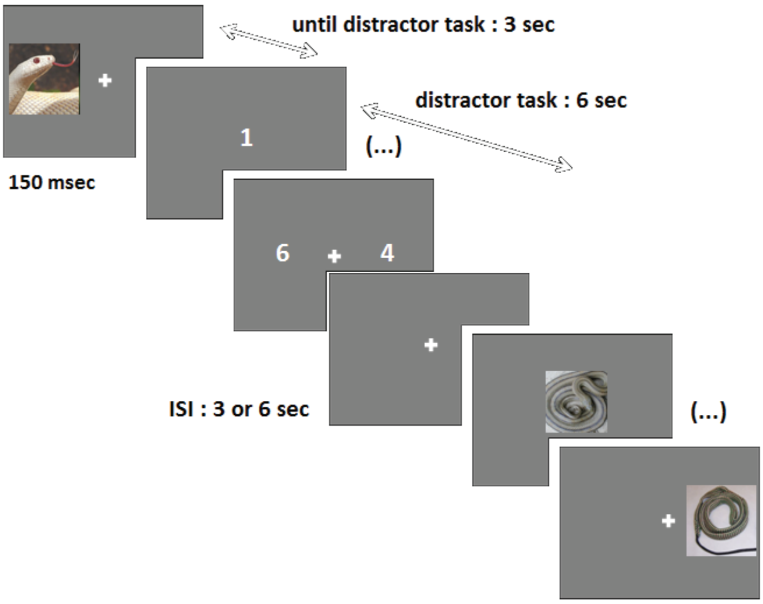

***Figure 6.1** – **Experimental design.** Each trial starts with the presentation of a picture which can be of 3 different types, snake face, snake shape, or fake snake shape, and presented at 3 different locations in the visual display: centre, left or right. Following the picture presentation (150 msec), time is given for a response according to the current task. After 3 seconds from stimulus onset, a distracter task starts to ensure the participants' attention: counting the number of times the number "1" appears and then selecting the right answer in a forced choice display. The inter stimulus interval (ISI) can be of 3 or 6 seconds until a new picture is displayed.*

6.2.4 IMAGING DATA ACQUISITION AND PREPROCESSING

Functional images were acquired in a 3T Siemens TimTrio scanner using BOLD contrast echo planar imaging (EPI, TR 3 sec, TE 30 msec, 40x3 mm-thick-slices, in-plane matrix 84x84 voxels, 165 volumes) covering the entire brain. The scanning session also included a high resolution T1 weighted anatomical scan (MPRAGE sequence, 1x1x1 mm3 voxel size, TR 2.3 sec, TE 2.98 msec, 160 slices) to help in the transformation of the functional images into standard space. The data were preprocessed and analysed using BrainVoyager QX v2.6 (Brain Innovation, www.brainvoyager.com). Preprocessing included slice scan time corrections, temporal filtering and motion correction. Before group analysis the images were spatially smoothed using a 6-mm full-width-half-maximum Gaussian kernel and then transformed into Talairach space.

6.2.5 STATISTICAL ANALYSES

All the statistical analyzes were performed using IBM SPSS Statistics 20 (IBM, USA, http://www.ibm.com/software/analytics/spss/) and Brain Voyager v2.6 software. The computation of effect sizes and power for the parametric analyses was performed with G*Power 3.1.6 (Faul, Erdfelder, Lang, & Buchner, 2007). For the non-parametric tests, effect sizes were computed based on the standardized statistics scores (Field, 2009) using the following formula:

$$r = Z/\sqrt{N}$$

6.2.5.1 BEHAVIOURAL DATA

Data from behavioural reports were considered to classify the trials where correct and incorrect responses occurred. Trials lacking response were excluded from all analyses. Trials corresponding to misses (e.g. snake faces or shapes not identified, snake faces or shapes not considered threatening) and false alarms (e.g. fake snake shapes identified as true snakes, or fake snake shapes considered threatening) were excluded from the analysis but considered in the design model of the functional data analysis as confound predictors.

Observer's *d* prime measures (*d'*) and reaction times (RTs) were obtained. The d prime, as a measure of response sensitivity, was computed in order to test if there was a bias towards one type of response (Provost & Fawcett, 1997; Stanislaw & Todorov, 1999). It was computed for each task and spatial location, by means of the subsequent formula:

$$d' = Z(\text{hits}) - Z(\text{false alarms})$$

We used the idf.norm function of the IBM SPSS 20 software to compute the Z normalized scores for hits and false alarms. Due to the non-normal distribution of data, non-parametric tests were used for the *d* prime measure (Friedman [χ^2F] and post-hoc Pairwise comparisons [t.s. stands for tests statistic], or Wilcoxon signed rank [T] tests - whenever more specific hypothesis were being tested - all of this for related samples). For the RT measure we performed a Three-factor Repeated Measures ANOVA 2x3x3 using task, spatial location and stimulus type as factors. When applicable, corrections of Greenhouse-Geisser were reported together with tests of sphericity. T-tests were used to identify statistically different pairs. All significant *p* values resulting from post-hoc tests were corrected for multiple comparisons.

6.2.5.2 FUNCTIONAL DATA

Statistical analyses were performed using a RFX general linear model (GLM) approach. Event duration was set to 4 sec beginning in the stimulus onset. Both spatial location (central; peripheral left and right) and stimulus type (threatening animal faces; non-threatening animal faces; natural displays without animals) were manipulated, with 9 predictors being included in each single-subject's design matrix (spatial location x stimulus type). A box car function was defined for each predictor and convolved with a canonical hemodynamic response function.

Two different analyses were then carried: *region of interest* and *whole brain* analyses. First, two regions of interest (ROIs) were defined in the left and right amygdalae of each participant using FreeSurfer v5.0.0 64-bit (http://surfer.nmr.mgh.harvard.edu/) automated segmentation of subcortical structures (http://surfer.nmr.mgh.harvard.edu/fswiki/SubcorticalSegmentation/). Anatomical brain FreeSurfer's processing includes removal of non-brain tissue, automated Talairach transformation, and segmentation of the subcortical white matter and deep gray matter volumetric structures. Amygdala ROIs in nifti (.nii) format where then imported to BrainVoyager v2.6 (plugin: nifticonverter_v106.dll) and transformed into Talairach space (Figure 6.2) (mean[SD] coordinates, x, y, z, and number of voxels: left amygdala, -21.27[1.24], -3.23[1.33], -13.27[.92], N= 3055 voxels; and right amygdala: 22.06[1.17], -3.90[1.50], -13.46[.95], N = 3091 voxels).

Parameter estimates (z-normalized beta weights, using specific volume segments to compute the baseline) were computed for each ROI (subject-based) and each task, with ANOVAs RFX being performed using the IBM SPSS software. Planned RFX-GLM contrasts analyses were performed using BrainVoyager v2.6.

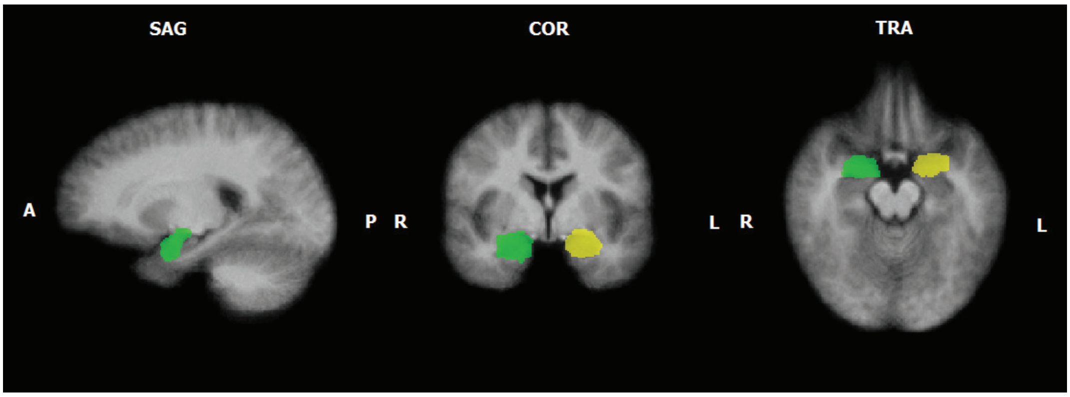

Figure 6.2 *– Defined Regions of Interest in the right and left amygdala of the average brain. Mean coordinates (mean x [SD], mean y [SD], mean z [SD]) of all Regions Of Interest defined for the 20 participants were 22.06[1.17], -3.90[1.50], -13.46[0.95]for the right amygdala (number of voxels = 3091), and -21.27[1.24], -3.23[1.33], -13.27[0.92] for the left amygdala (number of voxels = 3055).*

Second, whole brain analyses were performed first for each task separately and then by directly comparing both tasks. The statistical maps display specific GLM contrasts using mask restriction (51377 voxels). Corrections for multiple comparisons were made using the Cluster Threshold plugin (BrainVoyager) using 1000 Monte Carlo simulations. In this case, minimum cluster sizes corresponding to significance at a threshold of $p < .01$ were computed for each contrast.

6.3. RESULTS

6.3.1 BEHAVIOURAL DATA

The participants performed two different tasks. In the first task (snake identification task), they were asked to report (yes/no) if the picture presented contained a true snake (either face or shape), while in the second task (threat detection task) they were required to report an yes/no answer regarding the detection of threat signals in the picture.

6.3.1.1 SENSITIVITY INDEX (D')

In order to test if accuracy of performance was due to an increased/decreased bias to respond "yes" (see Table 6.1), we have tested matched accuracy across tasks by using the bias free classical *d* prime measure. This measure computes the observer's sensitivity to detect a signal having in consideration the false alarms rate (e.g. true snake, threat).

Differences were neither found significant between *tasks* (T=132.000, Z=1.008, *p*=.313) nor between *tasks* at each *spatial location* (task 1> task 2: centre, T=96.000, Z=-.336, *p*=.737; left, T=122.000, Z=.635, *p*=.526; right, T=141.000, Z=1.344, *p*=.179; 2-tailed tests).

However, the sensitivity to detect a signal was found to depend on *spatial location* (task 1: $\chi^2F(2) = 20.447$, *p*=.000; task 2: $\chi^2F(2) = 12.274$, *p*=.002). Post-hoc tests revealed better performances for central presentations compared with peripheral ones (task 1: centre > left, t.s.=.975, Z=3.083, *p*=.006, r=.488; centre > right, t.s.=1.350, Z=4.269, *p*=.000, r=.675; right vs. left, t.s.=.375, Z=1.186, *n.s.*; task 2: centre > left, t.s.=.800, Z=2.530, *p*=.034, r=.400; centre > right, t.s.=1.000, Z=3.162, *p*=.005, r=.500; right vs. left, t.s.=.200, Z=.632, *n.s.*; 2-tailed tests).

Therefore, we conclude that performance is matched when corrected for response bias, given the results from the sensitivity index *d'* to task and interaction task X spatial location. However, differences still occurred within spatial location.

Table 6.1 *– Sensitivity index (d') (mean[SD]) for the "implicit threat" snake identification task and for the "explicit threat" detection task as a function of Spatial location and Stimulus type.*

Task	Left (%) mean[SD]	Centre (%) mean[SD]	Right (%) mean[SD]	*total*
Snake identification (implicit task)	***2,64***[2,44]	***5,01***[3,47]	***1,72***[1,69]	***2,09***[1,65]
Threat detection (explicit task)	***3,84***[2,99]	***4,90***[3,14]	***3,17***[3,05]	***2,80***[2,44]
total	***3,24***[1,74]	***4,96***[2,44]	***2,44***[2,02]	

6.3.1.2 Response time analysis

The paragraph below describes analysis only for the correct responses (i.e. hits and correct rejections), which were considered for the RFX functional analyses. We performed separate analyses for the two tasks (task 1: n=20; task 2: n=18) (Table 6.2).

Table 6.2 *– Response time (mean[SD]) for the "implicit threat" snake identification task and for the "explicit threat" detection task as a function of Spatial location and Stimulus type (for hits and correct rejections only).*

Task	Left (%) mean[SD]	Centre (%) mean[SD]	Right (%) mean[SD]	
Snake identification (implicit task)				***total***
Snake faces	***916,26***	***820,31***	***955,67***	***897,42***
	[237,93]	[200,75]	[224,48]	[207,60]
Snake shapes	***928,32***	***858,08***	***983,10***	***923,17***
	[248,26]	[212,81]	[225,01]	[216,91]
Fake snakes	***1004,64***	***969,35***	***1014,00***	***996,00***
	[178,56]	[176,57]	[205,15]	[173,71]
total	***949,74***	***882,58***	***984,26***	
	[200,56]	[180,57]	[193,85]	
Threat detection (explicit task)				***total***
Snake faces	***971,99***	***901,23***	***929,27***	***909,48***
	[349,06]	[275,48]	[207,36]	[235,30]
Snake shapes	***992,53***	***866,08***	***977,37***	***929,53***
	[306,85]	[159,19]	[221,43]	[187,61]
Fake snakes	***865,87***	***868,56***	***879,72***	***888,36***
	[142,47]	[138,89]	[151,06]	[184,83]
total	***945,27***	***872,46***	***931,07***	
	[230,26]	[171,54]	[169,74]	

A repeated measures 3-way ANOVA revealed a main effect of *spatial location* ($F_{(2,34)}$=9.222, *p*=.001, Cohen's *d*=.737, power(1-β)=.817). Interaction effects arose only between *task* and *stimulus type* ($F_{(1.306,\ 22.194)}$=9.671, *p*=.003, Cohen's *d*=.755, power(1-β)=.573, Mauchly's W(2)=.468, *p*=.002, ε=.653).

Post-hoc paired samples t-tests showed the participants responded faster to *centrally* presented stimuli as compared to peripheral ones (mean[SD] RT central = 866.95[150.67] msec; mean[SD] RT left = 916.52[169.70] msec; mean[SD] RT right = 944.71[162.19] msec; central < left: $t_{(17)}=3.204$, $p=.015$, Cohen's $d=.755$, power(1-β)=.923; central < right: $t_{(17)}=4.474$, $p=.000$, Cohen's $d=1.055$, power(1-β)=.996).

For the interaction between *task* and *stimulus type*, a difference was found only for the fake-snake (control) stimulus (task 1 > task 2: $t_{(19)}=4.426$, $p=.000$ Cohen's $d=.982$, power(1-β)=.995), with the participants being faster when judging its threatening content as compared to deciding if the stimuli was a snake or not. Within task response time differences between snake faces and fake snake stimuli were found only during task 1 (snake face < fake snake: $t_{(19)}=3.080$, $p=.018$, Cohen's $d=.689$, power(1-β)=.907).

6.3.1. Functional MRI data

Region of interest (amygdala) and whole brain random effects general linear model (RFX-GLM) analyses were performed in this study.

6.3.2.1. Region of interest (ROI) analysis: The amygdala

Our primary hypothesis pointed to an interaction between spatial location (factor) and stimulus type (factor): we were interested in testing if the amygdala responded strongly to (snake) faces presented in the centre and/or to (snake) shapes in the periphery. Additionally, we aimed to assess how amygdala activity would be modulated by task type (factor) and if the snake phobia scores in the SNAQ questionnaire influenced activation levels.

First, we performed 3x3 ANOVAs RFX for each *task* ('implicit threat' snake identification or 'explicit threat' detection) in each amygdala ROI. *Spatial location* (centre, right, and left) and *stimulus type* (snake face, true snake shape, and fake snake "control" shape) were taken as factors. Second, we performed correlation analyses between the SNAQ scores and the significant contrasts given by the ANOVAs.

Below, we describe main effects and post-hoc analyses by performing ANOVAs 3x3 for each task and amygdala. All the post-hoc testes are corrected for multiple comparisons.

6.3.2.1.1. Main effects

During the *'implicit threat' snake identification* task (task 1), a main effect of *spatial location* was found both for the *left* ($F_{(2,38)}= 6.594$, $p=.004$) and for the *right* amygdala, ($F_{(2,38)}= 5.090$, $p=.011$). As for the '*explicit threat' detection* task (task 2), a main effect of *stimulus type* was found for the *left* amygdala ($F_{(2,34)}= 3.623$, $p=.038$). Additionally, a marginal effect of *spatial location* was found for the *left* ($F_{(2,34)}= 3.027$, $p=.062$) and for the *right* amygdala ($F_{(2,34)}= 3.266$, $p=.050$). Figure 6.3 summarizes the post hoc results.

Responses of the amygdala for *centrally* presented stimuli were larger than for *right* peripheral presentations, independent of task and amygdala, while for the contrast '*centre > left*', these differences depend both on task and amygdala, with significant differences being only found for the *left* amygdala during the *implicit snake identification task* (task 1). In fact, post-hoc analyses following the ANOVAs main effects showed that during *task 1*, the differences in the *left* amygdala ROI emerged both from the contrasts 'central > left' ($t_{(19)}=2.655$, $p=.047$) and 'central > right' ($t_{(19)}=3.396$, $p=.009$), while for the *right* amygdala, the effect of *spatial location* was stronger for the contrast ‘central > right’ during task 1 ($t_{(19)}$=3.472, *p*=.008). Regarding the effect of *stimulus type* during *task 2* (Figure 6.4), the *left* amygdala differences were found for the contrast ‘snake faces > fake snake shapes’ ($t_{(17)}$=-2.722, *p*=.045).

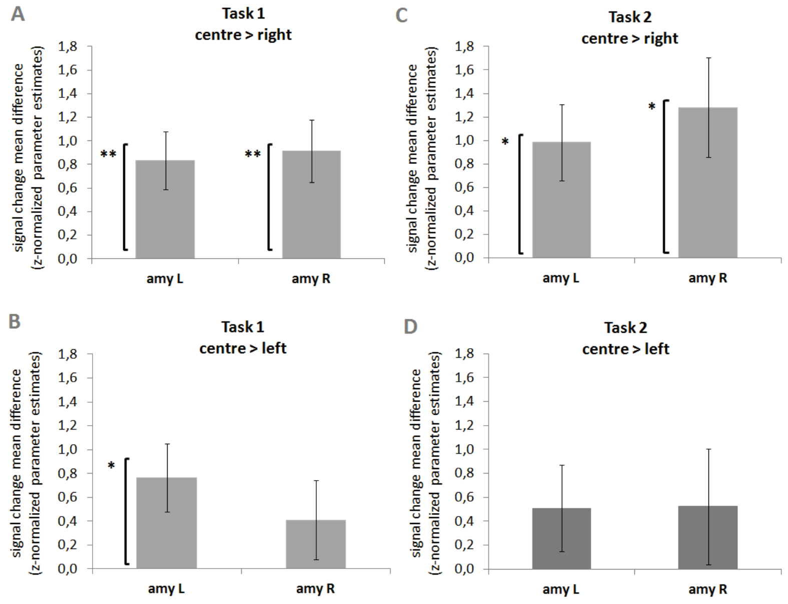

Figure 6.3 *– Effect of spatial location: responses of the amygdala for centrally presented stimuli are larger than for right peripheral presentations, independent of task and amygdala. However, for the contrast centre > left, these differences depend both on task and amygdala, with significant differences being only found for the left amygdala during the implicit snake identification task (task 1), ROI RFX-GLM contrasts: mean differences in parameter estimates (z-normalized beta-values) for the contrasts centre > right, task 1 (A) and 2 (C), and for the contrasts centre > left, task 1 (B) and 2 (D), are displayed. Legend: amy L, amygdala left; amy R, amygdala right; * p<0.05, ** p<0.01. The bars display the standard error of the mean (SE).*

6.3.2.1.2. Correlations between SNAQ and significant contrasts

The range of scores (1-29) of our participants in the snake phobia questionnaire (SNAQ), enabled us to test if it was related to the activity patterns observed in the amygdala. For the *“implicit threat” snake identification* task, the contrast ‘central > right’ in the *right* amygdala revealed a significant correlation with the SNAQ scores (r=.55, *p*=.012).

6.3.2.1.3. Planned contrasts testing hemispheric asymmetries as a function of stimulus type

Given the evidence for the ecological (survival) role of stimulus type and hemispheric asymmetries in modulating emotional responses, we hypothesized the presence of hemispheric asymmetries as a function of stimulus type. These results are summarized in Figure 6.5.

Hemispheric asymmetries: the specificity of snake shapes

We found a systematic effect of lateralization that was specific for the snake shapes stimuli but not for the snake faces or the fake snakes. In fact, for the snake shapes, differences between the centre and the right visual hemifield (left hemisphere) occurred for both the left (*t*(17)=3.522; *p*=.003) and the right (*t*(17)=3.595; *p*=.002) amygdalae, whereas this was not true for the contrast centre > left (which maps to the visually dominant right hemisphere) in none of the amygdalae (left amygdala: *t*(17)=0.911; *n.s.*; right amygdala: *t*(17)=-.356; *n.s.*). Importantly, the same was neither true for the snake face (left amygdala: centre > left, *t*(17)=1.720, *n.s.*; centre > right, *t*(17)=1.843, *n.s.*; right amygdala: centre > left, *t*(17)=1.503, *n.s.*; centre > right, *t*(17)=2.554, *p*=.063) nor for the fake snake stimuli (left amygdala: centre > left, *t*(17)=1.507; *n.s.*; centre > right, *t*(17)=.608; *n.s.*; right amygdala: centre > left, *t*(17)=1.520; *n.s.*; centre > right, *t*(17)=1.143; *n.s.*).

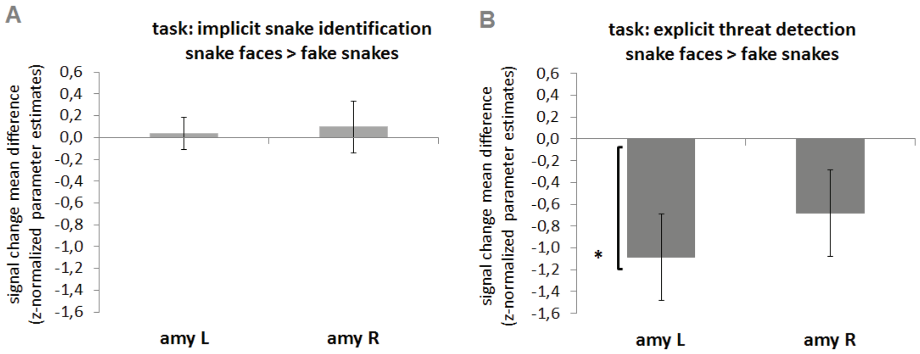

Figure 6.4 *– Effect of stimulus type: responses of the amygdala to snake faces are smaller than to fake snake stimuli in a task and amygdala dependent manner. The left amygdala is preferentially involved in the discrimination of threatening and non-threatening stimuli during the threat detection task (task 2). ROI RFX-GLM contrasts: mean differences in parameter estimates (z-normalized beta-values) for the contrasts snake faces > fake snake, task 1 (**A**) and 2 (**B**) are displayed. Legend: amy L, amygdala left; amy R, amygdala right; * p<0.05. The bars display the standard error of the mean (SE).*

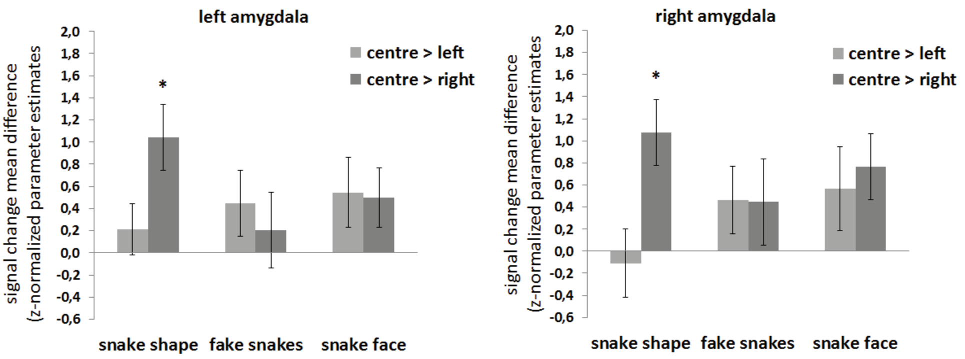

Figure 6.5 *– Effect of visual field: Hemispheric asymmetries are found in a stimulus type dependent manner, as response difference between the centre and each of the visual hemifields (left, right) are only found for the snake shapes. ROI RFX-GLM contrasts: mean differences in parameter estimates (z-normalized beta-values) for the contrasts centre > left, centre > right, for the right and the left amygdala ROI. * p<0.01, corrected for multiple comparisons. The bars display the standard error of the mean.*

This suggested the occurrence of hemispheric asymmetries specific for the snake shape stimuli. As this effect seem to be independent of the amygdala side, we concatenated the two ROIs into one, and we tested the effect of hemispheric asymmetry, by comparing the amygdala response for left and right stimuli presentations. A lateralization was confirmed for the snake shapes, with the amygdala responding significantly more to the (visually dominant) left than to the right visual hemifield, compared to the control fake snake stimuli (snake shapes: left > right, *t*(17)=2.617, *p*=.036; fake snakes: *t*(17)=-.416, *n.s.*; 2-tailed, corrected for multiple comparisons) (see Figure 6.6).

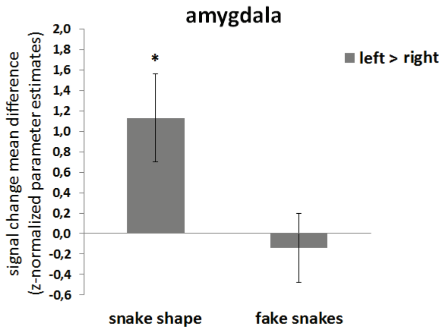

***Figure 6.6** – Effect of visual field: Hemispheric asymmetries are found in a stimulus type dependent manner: the right hemisphere (left visual field) responds more to snake shapes, but the same in not found for the fake snakes (control stimuli). ROI RFX-GLM contrasts: mean differences in parameter estimates (z-normalized beta-values) for the contrasts left > right visual fields, for the amygdala (left and right ROI). * p<0.05, corrected for multiple comparisons. The bars display the standard error of the mean.*

6.3.2.2. Whole brain RFX analysis

We performed whole brain RFX contrast analyses to identify brain regions involved in task and spatial location effects (brain regions, peak voxel coordinates and statistics are presented in Tables 6.3, 6.4 and 6.5).

6.3.2.2.1. Task

The contrasts performed between the explicit threat detection task (task 2) and the implicit snake identification task (task 1) revealed stronger activations for the explicit task compared to the explicit task. These were found in the cerebellum (anterior lobe) and pons (and Table 6.3 – contrast b).

***Table 6.3** - Summary of random-effects (RFX)-GLM contrasts, outputs and statistics for the factor Task.*

Region	Peak X (TAL)	Peak Y (TAL)	Peak Z (TAL)	Nr of voxels	*t*	*p*
Contrast b : explicit threat detection > implicit threat snake identification						
r cerebellum (anterior lobe)	38.0	-53.0	-42.0	10382	3.943	0.00105
r pons (solitary tract nucleus)	5.0	-38.0	-33.0	799	4.352	0.000434

All contrasts were performed at p<.01 using cluster threshold correction. X, Y and Z represent Talairach coordinates. r, right; l, left.

6.3.2.2.2. Spatial location: centre vs. periphery

Whole brain RFX planned contrasts performed for the effect of spatial location revealed mainly regions with increased activity for central presentations. Importantly, we found increased bilateral amygdala, and also more extended bilateral parahippocampal activity, bilateral insula, the left hippocampus, the right medial dorsal nucleus of the thalamus, and regions in the basal ganglia such as the right putamen, the right caudate (tail), and the left lateral globus pallidus. As expected, regions in the occipito-temporal 'ventral' stream activated more for central presentations, namely the inferior occipital gyrus and the cuneus (BA 18), both bilaterally, the left middle occipital gyrus (BA 19), the left fusiform (both occipital and temporal regions) and the right fusiform gyrus (BA 37) in the temporal lobe. Additionally, we found the left superior (BA 38) and the right middle (BA 21) temporal gyri, the left inferior (BA 47) and the right middle (BA 46) frontal gyri, the right precentral gyrus and the right pons and cerebellum.

We found only one region which activated more for the periphery than for the centre: the precuneus (BA 7), bilaterally (see Figure 6.7 and Table 6.4 – contrast a, for peak voxel coordinates and statistics of some of these regions).

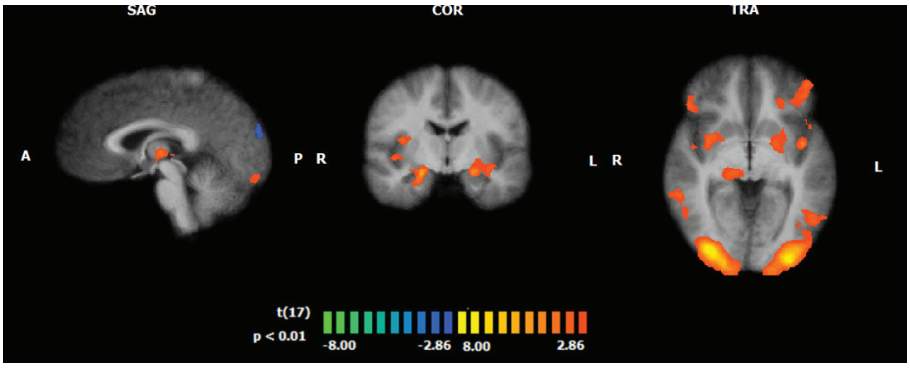

***Figure 6.7** – Activated regions yielded by the RFX group analysis for the contrast centre > periphery. Cluster threshold correction was set at p<0.01 with a minimum cluster size of 54 voxels.*

***Table 6.4** - Summary of random-effects (RFX)-GLM contrasts, outputs and statistics for the factor Spatial location.*

Region	Peak X (TAL)	Peak Y (TAL)	Peak Z (TAL)	Nr of voxels	*t*	*p*
Contrast a : centre > periphery						
r amygdala	20.0	-8.0	-12.0	1162	6.049	0.000013
l amygdala	-19.0	-5.0	-12.0	1376	6.114	0.000011
r parahippocampal gyrus (BA 34)	29.0	1.0	-15.0	170	4.403	0.000389
l parahippocampal gyrus (BA 35)	-28.0	-29.0	-21.0	4174	11.190	0.000000
r thalamus (medial dorsal nucleus)	2.0	-14.0	6.0	1739	4.450	0.000351
r putamen	29.0	4.0	-6.0	1753	5.203	0.000072
r caudate (tail)	35.0	-14.0	-12.0	67	3.514	0.002660
l lateral globus pallidus	-19.0	-5.0	-7.0	1315	5.379	0.000050
r fusiform gyrus (BA 37)	48.0	-47.0	-12.0	1080	4.644	0.000232
l fusiform gyrus (BA 37)	-19.0	-92.0	-18.0	11843	8.859	0.000000
r insula (BA 13)	35.0	-8.0	15.0	306	4.165	0.000649
l insula (BA 13)	-40.0	-2.0	-3.0	602	5.749	0.000024
r middle frontal gyrus (BA 46)	50.0	28.0	24.0	4497	6.700	0.000004
l inferior frontal gyrus (BA 47)	-43.0	16.0	-6.0	2547	7.040	0.000002
r pons	11.0	-20.0	-30.0	317	4.996	0.000111

The table displays a selection of some of the relevant regions found. For the complete list, please see section 6.3.2.2.2. All contrasts were performed at p<.01 using cluster threshold correction. X, Y and Z represent Talairach coordinates. r, right; l, left.

6.3.2.2.4 Task x Spatial location

We directly compared performance across spatial locations between task 1 and task 2 (n=18). Differences between tasks at each location, revealed only small and very specific regions.

Central representations: no regions in the cerebrum were found differentiating the explicit and the implicit tasks. In fact, increased activity was only found in the pons and the cerebellum for the task 2 (explicit threat detection) (Figure 6.8 - top, and Table 6.5 – contrast c).

Peripheral representations: interestingly, for peripheral presentations, the right medial frontal cortex (extending to the dorsal anterior cingulate) was found to be preferentially engaged during task 1 (implicit snake identification task), as the left inferior parietal lobe (Figure 6.8 – bottom, and Table 6.5 – contrast d).

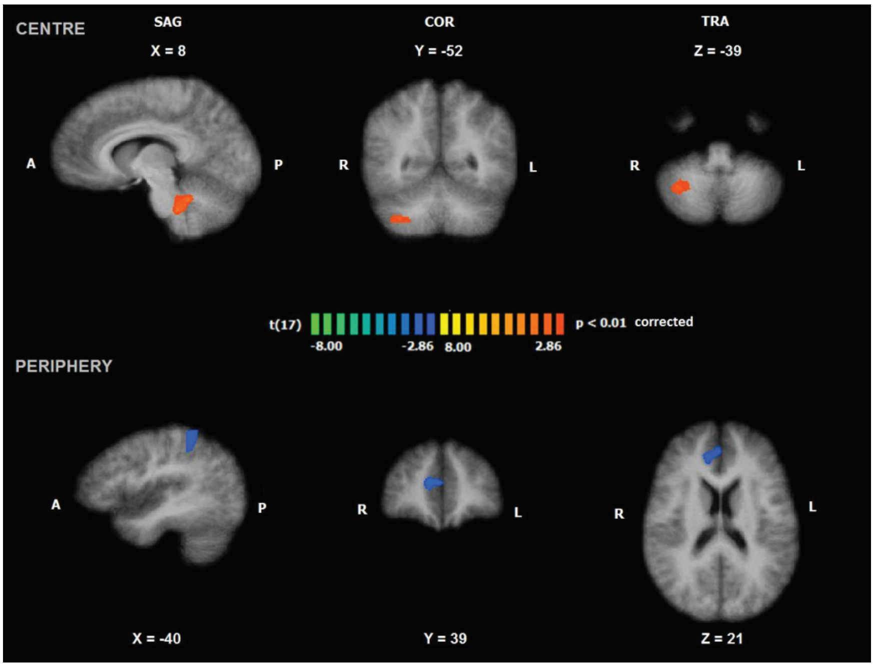

Figure 6.8 *– Different regions are activated depending on task type and spatial location: in the centre (top), cerebellum and pons (stronger for the explicit threat detection task, task 2); in the periphery (bottom), right medial frontal cortex (aMFC) and inferior parietal lobe (stronger for the implicit snake identification task, task 1). RFX-GLM contrasts for 'task 2 > task 1'. Cluster threshold correction was set at p<0.01 with a minimum cluster size of 25*9 voxels, for peripheral comparisons (B), and of 29*9, for central ones (A).*

Table 6.5 - *Summary of random-effects (RFX)-GLM contrasts, outputs and statistics for the interaction Task x Spatial location.*

Region	Peak X (TAL)	Peak Y (TAL)	Peak Z (TAL)	Nr of voxels	*t*	*p*
Contrast c : centre: explicit threat detection > implicit threat snake identification						
r cerebellum (anterior lobe)	38.0	-53.0	-42.0	933	3.863	0.001248
r pons (solitary tract nucleus)	8.0	-41.0	-24.0	1013	4.598	0.000256
Contrast d : periphery: implicit threat snake identification > explicit threat detection						
r medial frontal gyrus	11.0	40.0	21.0	816	-4.465	0.000340
l inferior parietal lobe	-43.0	-38.0	54.0	1093	-5.280	0.000061

All contrasts were performed at p<.01 using cluster threshold correction. X, Y and Z represent Talairach coordinates. r, right; l, left.

6.6. Discussion

The present study aimed to investigate whether the amygdala response to stimuli holding a high evolutionary relevance, such as snakes, shows the same central bias as previously observed for threatening (fear-relevant) faces (Almeida et al., 2013). Moreover we aimed to find whether such evolutionary special stimuli recruit dedicated circuitry. In this study the main findings were:

(1) Responses of the amygdala for snake stimuli (shape, face or fake) were larger in the *centre* for both amygdalae for at least one contrast, contradicting the idea that these ecological stimuli are more effective in the periphery. Specifically, amygdala responses to centrally presented snake stimuli were larger than for *right* peripheral presentations, independent of task and amygdala. However, for the contrast centre > *left* hemifield (which corresponds to the visually dominant hemisphere), these differences depended both on task and amygdala, with significant differences being only found for the *left* amygdala during the *implicit* snake identification task;

(2) Stimulus related effects in the amydgala were found only during *explicit* threat detection (task 2) in particular for the *left* amygdala, which is a biologically plausible finding as explicit emotional processing was required. Surprisingly however, larger activity was found for *fake* snakes compared to snake faces;

(3) *Snake shapes* (true) showed a specific *central* bias, for both amygdalae, unlike the other stimulus types, providing confirmation that these ecological stimuli are special. However the fact that *shape* stimuli showed a *central* bias is somewhat at odds with our original prediction. Our interpretation is that snake shapes are ecologically much more relevant than snake faces and require detailed central processing. Our brains are automatically prepared to analyse snake shapes but not snake faces. This seems to require detailed analysis in foveal regions.

(4) A strong hemispheric *lateralization* was found between real and fake snake shapes. *Real* shapes activated stronger the *right* hemisphere, which is consistent with its dominance for stimuli with loaded emotional content.

(5) In the periphery, the *implicit* snake identification task recruited more the right (anterior) medial frontal cortex and the inferior parietal lobe than the explicit task, specifically for peripheral presentations, suggesting that the role of frontal lobe is more important for explicit real vs. fake stimulus separation in the *periphery*. This may be due to the involved task load.

(6) Snakes phobia questionnaire (SNAQ) cores correlated positively with the *central* vs. right hemifield stimulus presentation in the right amygdala. These results further confirm the ecological meaning of our stimuli, and the unexpected value of *central* presentation. This does not mean that peripheral processing is not important. We hypothesize that peripheral mechanisms are more important to trigger automatic attention mechanisms, irrespective of stimulus type.

6.6.1. VISUAL ASYMMETRIES

6.6.1.1. CENTRE VS. PERIPHERY

We predicted that fear-relevant shapes should be processed in a bottom-up, stimulus driven manner. This stimulus-driven mechanism is related to automatic orienting, also termed “exogenous” attention (Posner & Cohen, 1984; Corbetta, Kincade, & Shulman, 2002). Based on this hypothesis we had therefore expected some peripheral bias for the snake shapes stimuli, however this was not observed in the imaging data of the amygdala.

These results are puzzling, since we were not able to find a central bias for snake faces as well, reproducing previous findings for faces (human, other animals) with threat related (fear, threat) features (Morawetz et al., 2010; Almeida et al., 2013; see Chapter 5). Nevertheless, one must be careful about doing strict generalizations. In effect, contrary to human and other animal faces, snake faces are not stimuli which we are used to foveate. In fact, they display much less (emotional transient) information than other animal faces (Almeida et al., 2013; see Chapter 5). Accordingly, we are not used to extract information from snake faces, because the stimulus shape displays sufficient information concerning its potential fear-relevant meaning (Isbell, 2006). This is not the same with other animal categories for which the expression in the face provides important information about its future behaviour, hostile or not.

Another point one must consider, are task related differences, and their relation with spatial location. In the current study, an implicit task was employed and it required the subject to identify a true snake, among stimuli that resembled but were not snakes (e.g. ropes). Thus, the participant had to rely on coarse features to correctly discriminate the real and the fake snake. This turn out to be a particularly difficult task, particularly at peripheral locations. In fact, although an increased response time was found for the visual periphery irrespective of task, a higher false alarm rate occurred particularly during the implicit snake identification task. In addition, participants took more time to respond to the fake stimuli than to faces only during the implicit task. Increased response times were expected with eccentricity increment (Thorpe et al., 2001).

Discrimination of fine detail in the periphery is harder, suggesting that differences among the explicit and the implicit tasks occurred due the attentional load required to correctly perform both. This differs from our previous study in which the participant was only required to detect an animal (Almeida et al., 2013, Chapter 5). The activation within the left inferior parietal lobe is consistent with this interpretation, as a role for this region in sustained attentional processes was described (Fink, Dolan, Halligan, Marshall, & Frith, 1997; Singh-Curry & Husain, 2009). Additionally, co-activation in the right medial frontal cortex and anterior dorsal cortex also fits this approach, with fronto-parietal circuits playing a role in spatial attention orientation (Taylor, Rushworth, & Nobre, 2008) and monitoring of ongoing behaviour for performance improvement (Cardinal, Parkinson, Hall, & Everitt, 2002; Sheth et al., 2012; but see Grinband et al., 2011).

6.6.1.2. Left vs. right asymmetries

Visual orienting is related to mechanisms of spatial attention, and is often associated with right hemispheric dominance (Corbetta et al., 2002; Fink et al., 1997a; Thompson et al., 2005), which we did observe. Additionally, although we did not find a peripheral bias for the ecologically relevant shapes (snake shapes), stimulus-driven automatic processing was nevertheless suggested by a hemispheric asymmetry irrespective of task (implicit, explicit). This effect was specific for the snake shapes, and demonstrated by a difference between central and right visual hemifield presentations. We found an increased response of the amygdala (right or left) specifically to left hemifield presentations (right hemisphere) of snake shapes compared to when the same stimuli was presented to the right hemifield (left hemisphere). Conversely, the same did not occur neither for the fake snakes nor for snake face stimuli. This contrast showed that when required to recognize a snake in the periphery, the left amygdala engages more strongly the right hemisphere than the left one. This is consistent with previous research in animal behaviour, showing the existence of hemispheric asymmetries for threat detection and launching of appropriate responses (Bisazza, Rogers, & Vallortigara, 1998; Vallortigara, Rogers, Bisazza, Lippolis, & Robins, 1998; Vallortigara, & Rogers, 2005). Evidence shows that attention to global cues and predator detection is (right) lateralized for instance in toads, fishes, birds (Andrew, & Rogers, 2002; Vallortigara, & Rogers, 2005), dogs (Siniscalchi, Sasso, Pepe, Vallortigara, & Quaranta, 2010), and humans, for whom a right hemisphere bias for processing emotional items has been reported (Spence, Shapiro, & Zaidel, 1996), particularly of negative pictures (Canli, Desmond, Zhao, Glover, & Gabrieli, 1998; Merckelbach & Van Oppen, 1989). This is consistent with the proposal of an increased tuning of the right hemisphere to low spatial frequency (LSF) information (Hellige, 1996; Howard & Reggia, 2007; Karim & Kojima, 2010; Merckelbach & Van Oppen, 1989; Peyrin et al., 2005) using a preferential coarse-to-fine (low-to-high) SF, which accommodates our results. In fact, snake shapes require only LSF to be identified (Delplanque, N'diaye, Scherer, & Grandjean, 2007), in contrast with faces which are stimuli for which we have a foveal bias (Levy et al., 2001).

6.6.2. The role of Fear and of Fear-relevant stimuli

A difference 'centre vs. right' presentations for the right amygdala during the implicit task was the only significant contrast which correlated positively with the score in the SNAQ. This correlation suggests that with increased reported fear of snakes, the amygdala responds more strongly for central compared to right (left hemisphere) presentations, but does not explain the difference centre – left (right hemisphere). This might be accounted in two ways, both related to the role of hemispheric dominance. First, asymmetries centre > right and centre > left occur differently in the amygdala, with right presentations not being so efficient as left presentations to elicit a response in the amygdala, particularly for (true) snake shape stimuli. These differences are accommodated by left > right asymmetries suggesting a right hemispheric dominance in fear-relevant stimulus detection (see above section 6.6.1.2). Therefore, the correlation of the difference centre vs. right (left hemispheric dominance) with the SNAQ score suggests a less influent role of the left hemisphere in fear related behaviour (Davidson, Marshall, Tomarken, & Henriques, 2000; Johnsen & Hugdahl, 1993). Second and related with this, central presentations elicit stronger amygdala responses than presentations to the right hemifield (left hemisphere), and this is correlated with snakes fear in a positive manner. Although previous research has suggested that the amygdala is specially involved under non-conscious appraisal of fear-relevant stimuli, and inhibition of the fear module may be supressed by prefrontal networks during conscious appraisal (Öhman, 2005; Pessoa, Kastner, & Ungerleider, 2002), our results are not necessarily incompatible with this view, but extend it. In fact, it seems natural that objects that we fear (e.g. snakes, spiders) elicit stronger anxiety when in our focus of gaze. Accordingly, direct gaze towards the feared stimulus elicits stronger anxiety and is in the base of phobic avoidance of the feared stimulus (Fredrikson et al., 1993; Huijding, Mayer, Koster, & Muris, 2011; Pflugshaupt et al., 2005; Rinck & Becker, 2006; Rohner, 2002). Additionally, since this occurred particularly during the snake identification task, the participants had to more effortfully focus on detailed snake-related information.

6.6.3. Implications for central vs. peripheral visual mechanisms involved in emotional processing

Different views regarding the role of the amygdala in emotional processes have coexisted in the literature. Whereas some defend the preferential role of this structure in non-conscious emotional processing through a subcortical pathway (Öhman, 2005; Tamietto & De Gelder, 2010), others propose its role in both preattentive and conscious appraisal processes through rich subcortical and cortical connections (Pessoa & Adolphs, 2010). The major difference relies on the emphasis on the pathways for emotional processing. In this manner, we can hypothesize that if the role of the amygdala was particularly outside awareness, or preattentive, the detection of fear-relevant stimuli, such as snakes, in the periphery would be preferentially made through a coarse, LSF pathway, eliciting stronger amygdala responses. In fact, more conscious and explicit appraisal of the stimuli in central vision would inhibit amygdala response (Öhman, 2005). However, increased fear and anxiety towards fear-relevant stimuli (e.g. snakes) would diminish a potential prefrontal emotional regulation of the amygdala response (Bishop, Duncan, & Lawrence, 2004; Öhman, 2005) as it has been consistently shown that anxiety levels modulate behavioural performance (Soares, Esteves, & Flykt, 2009) and amygdala responses (Bishop et al., 2004). In our study, we show that central presentations of snake-related stimuli elicit stronger responses in the amygdala. Nevertheless, some of these differences regarding the lateralization (right vs. left) of presentation are correlated with reported fear of our participants, which might be interpreted as reduced prefrontal modulation of the amygdala response. We must point nevertheless that increased (inferior and middle) frontal activity was also observed in our data to central presentations, concomitant with the increased amygdala responses to central presentations.

We have pointed before that the amygdala receives strong input from ventral stream areas (Lori et al., 2002; Rolls, 2007; Stefanacci & Amaral, 2002), which show a bias for certain types of objects such as faces (Levy et al., 2001). Our hypothesis was that, similarly as other stimuli which are based in LSF cues, snakes shapes could be preferentially processed in areas which are devoted to peripheral processing (Levy et al., 2001). However, two final points should be taken. First, we show here that amygdala responses to snake-related stimuli benefits as much of central vision as other stimuli which have a foveal bias, such as faces (Almeida et al., 2013). Second, in contrast to the "building-type" stimuli reported by Levy and colleagues (2001), snakes are a fear-relevant stimulus (Öhman & Mineka, 2001; Öhman, Soares, Juth, Lindström, & Esteves, 2012), with high ecological value (Isbell, 2006, 2009), which may then be prioritized for central processing thereby eliciting a complete different pattern of responses as compared to non-emotive stimulus types (e.g. buildings).

Our data is consistent with a previous magnetoencephalography (MEG) study which, although using magnified faces in the periphery, was nevertheless able to show increased response amplitudes in the amygdala for central vs. peripheral presentations. They do however point out that early onset response of the amygdala was faster for stimuli in the periphery than for centrally presented ones (Liu & Ioannides, 2010). Accordingly, another MEG study has been able to demonstrate a bias to peripheral masked fearful faces by early onset amygdala responses (Bayle et al., 2009), although neither early nor late response were found during central presentations. Differences in the stimulus awareness might account for the differences found. Importantly, MEG studies, givn their ability to study not only the amplitude of response but also its temporal pattern, might help to solve the apparent incongruence in the literature, although spatial localization issues are more questionable with this technique, because they rely on a solution to the inverse problem. Therefore, although we have found stronger response for snake–related stimuli in the centre of the visual field, fMRI does not have enough resolution to uncover early temporal signatures of this processing. Nevertheless we can speculate that given the hemispheric asymmetry found specifically for this stimulus, some kind of pre-attentive processing might have occurred, with early onset responses.

In our view, mechanisms of central and peripheral vision serve different purposes, with central vision being entailed with more accurate processing of information, aiding the process of achieving more accurate decision-making and performance. In contrary, peripheral vision relies on less accurate mechanisms which benefit mainly of coarse LSF information, in the trade-off between speed and accuracy. Subcortical structures such as the superior colliculus (White & Munoz, 2012) and the pulvinar (Pessoa & Adolphs, 2010), and cortical structures such as the frontal eye fields (Thompson et al., 2005) are implicated in mechanisms of covert attention and guidance of eye movement towards relevant stimuli in the visual environment and are likely pivotal in solving such speed vs. accuracy trade-offs that are of high survival value.

6.7. Conclusions

Our results show that snake stimuli are evolutionary relevant stimuli that are processed in a characteristic but unexpected manner. We found a surprising absence of a peripheral bias and stronger amygdala activity for central stimuli in particular when compared with stimulus presentations to the right hemifield (corresponding to the visual non-dominant hemisphere). In addition we found a right hemispheric asymmetry specific for the snake shapes stimuli vs. non-real (fake) snake shapes. These results suggest that snake shapes are indeed processed in a different manner compared to face snakes or fake snake stimuli. However, responses of the amygdala to this fear-relevant are stronger under central vision, suggesting that like other stimulus categories such as faces, a ventral stream bias also exists toward this type of stimulus.

Our study has implications in terms of the debate in the literature concerning automatic vs. conscious processing in the amygdala, in relation to the role of central and peripheral processing. Here, we defend different roles for central and peripheral vision in a way that we believe to reconcile the debate in the literature. Central vision is more detailed and serves the purpose of accurate processing of information, whereas peripheral vision uses more automatic, preattentive attentional mechanisms (stimulus-driven, bottom-up, "exogenous") in order to capture attention towards relevant stimuli. This might explain the peripheral right hemispheric dominance for real snake stimuli.

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"We find comfort among those who agree with us, growth among those who don't."

— Frank A. Clark

DISCUSSION

Chapter 7

Discussion and Conclusions

In the current project, we aimed to study the nature of processing of emotional information as a function of temporal and spatial constraints. Different types of paradigms were applied to normal subjects, enabling us to study the processing of emotional information under different levels of awareness (Chapter 3), or under accurate (central vision) vs. degraded (peripheral vision) forms of access to stimulus content (Chapters 5 and 6). A multimodal approach that combined behavioural, psychophysiological and functional neuroimaging methods was used in order to compare implicit and explicit processing of emotional stimuli and its neural correlates.

7.1. A specific effect of stimulus duration on skin conductance responses to unconditioned angry faces (temporal constraints of emotional cognition)

By imposing temporal presentation limits combined with visual masking (Chapter 3), we could directly assess the processing of emotional faces under graded levels of sensory awareness. To test if emotional content was processed and its behavioural and psychophysiological implications, we measured skin conductance responses (SCRs) to emotional (angry, happy) and neutral faces with variable temporal durations. In addition, we asked participants to rate the perceived arousal in order to compare it with the psychophysiological arousal measure given by the SCR. Moreover, we studied the effect of stimulus duration on the SCR when awareness of the emotional content of the stimulus was reported, given the wide assumption that short durations are sufficient to prevent the stimulus from achieving awareness. We focused on the facial expression of anger, since it signals a direct source of threat, instead of using fearful face expressions (which in fact has been mostly studied).

Our results showed a specific effect of stimulus presentation duration that occurred unexpectedly only for long durations on SCRs when using unconditioned angry faces. These effects were absent when using happy or neutral faces. Additionally, arousal ratings were also affected by stimulus presentation duration, increasing already during short durations. This occurred in particular for happy faces, which received increased arousal ratings as soon as awareness of the stimulus was reported (which happened at short durations).

Trial-by-trial awareness measurements allow to establish a more direct link with physiological measures

Studies that report differences in SCRs as a function of awareness have contrasted conditions that arise from the use of different durations: one labelled as subliminal and the other as supraliminal. In that sense, in those studies, awareness is directly related to the duration used. However, some subjects can discriminate emotion at very short durations which define thresholds for discrimination that are temporally very short (Szczpanowski & Pessoa, 2007).

Importantly, we did not make any assumptions that specific durations define subliminal and supraliminal conditions. Indeed, to assess the effectiveness of the masking procedure together with the temporal manipulations, we took an 'on-line' measure of subjective awareness by asking the participants to report their awareness level in a trial-by-trial basis. Analyses of subjective arousal and of SCRs followed this subjective discrimination in 3 levels: unawareness of content (neither face nor emotion), detection of faces and discrimination of emotions. This is a major point in our study, as we made the division into awareness levels *a posteriori* and not before, thus not assuming any threshold before responses were collected. This procedure has before proven to better fit the patterns of response found (Quiroga, Mukamel, Isham, Malach, & Fried, 2008) by accounting for individual differences in awareness thresholds.

ANGRY FACES AND THEIR EFFECTIVE PROCESSING OUTSIDE AWARENESS

Two major lines of research have used SCR to test unconscious versus conscious processing of relevant stimuli. One line of research seems to implicitly assume that faces, especially emotional and negative ones (e.g. fearful), are a priori relevant stimuli (e.g. Tamietto & De Gelder, 2008; Vuilleumier & Schwartz, 2001; Whalen et al., 1998), whereas the other considers that responses to fear-relevant stimuli (e.g. angry faces, snakes, spiders) become less likely if no negative outcome arises, and thereby use fear conditioning procedures to enhance affective meaning (e.g. Lim, Padmala, & Pessoa, 2008; Morris, Öhman, & Dolan, 1998; Wong, Shevrin, & Williams, 1994).

One important point to make is that enhanced SCRs to angry faces have only been indirectly tested in studies that contrast conditioned with unconditioned angry faces. These studies find larger and higher resistance to extinction to angry faces than to the later ones, independent of awareness (e.g. Boschen, Parker, & Neumann, 2007; Morris, Öhman, & Dolan, 1998; Pischek-Simpson, Boschen, Neumann, & Waters, 2009). Increased SCRs to angry faces at very short durations (e.g. 30 msec) have thus been reported, but only if fear conditioning procedures are previously applied, suggesting that affective learning during the task might improve the processing of a stimulus (e.g. Esteves et al., 1994a; Williams et al., 2004; Williams et al., 2006). This emphasizes the association between fear learning and SCRs, and not so much the preattentive processing of angry faces. The exact type of processing of fear-relevant angry faces presented in the absence of prior fear conditioning remains therefore to be determined.

Nevertheless, higher resistance to extinction (after learning has occurred) for conditioned angry compared to conditioned happy faces give clues about the intrinsic fear-relevant value of angry faces (Esteves et al., 1994b), and suggest that these faces have an a priori increased stimulus value. However, the failure in our data to find increased SCRs to unconditioned angry faces when no awareness of the stimulus was reported, or at least as soon as awareness (of face or emotion) was reported can be interpreted in two non-independent manners. First, it shows that increased SCRs to non-conscious fear-relevant stimuli such as angry faces require previous fear learning. Second, the fact that differential SCRs for unconditioned angry faces occurred in our study only at long exposure durations (2000 msec) suggests that the SCR might dominantly depend on conscious processing of the stimuli, and challenges its role as a measure of non-conscious processing of fear-relevant stimuli, at least for unconditioned angry faces (Williams et al., 2005).

SUBJECTIVE AND PSYCHOPHYSIOLOGICAL AROUSAL

Our data shows dissociation between the subjective arousal measure and the psychophysiological measure of arousal (SCR). The increasing of subjective arousal with both awareness and duration, added to the fact that SCR amplitude only showed major changes at the highest duration, thereby not reflecting reported awareness of the emotion, seem to suggest that subjective arousal is not a simple measure of SCR amplitude (autonomic arousal). The weak correlation between both seems to further support this hypothesis (but see Lang et al., 1998). Overall, our data suggests that SCR amplitude does not depend merely on awareness of content, at least for angry faces, but mostly on how long is duration of stimulus presentation.

FACTORS AFFECTING THE SKIN CONDUCTANCE RESPONSES

Conflicting views in the literature remain regarding the capability of the SCR measure to reflect fear learning and the role of awareness on this issue (Dawson, Rissling, Schell, & Wilcox, 2007; Klucken, Kagerer, Schweckendiek, Tabbert, Vaitl & Stark, 2009; Mineka & Öhman, 2002; Öhman & Mineka, 2001). Differences between studies might in part be due to the category of stimuli used. Fear learning, measured by increased SCRs, to masked conditioned fear-irrelevant (e.g. flowers, mushrooms and happy faces) stimuli seem to require awareness of the contingency between the unconditioned and the aversive stimuli for learning to occur, whereas fear learning to evolutionary fear-relevant stimuli (e.g. spiders, snakes and angry faces) measured through increased SCRs can occur outside awareness (Isbell, 2006; Mineka & Öhman, 2002; Öhman & Mineka, 2001; but see Dawson et al., 2007). This 'stimulus-type' effect would account for some part of the discrepancy, as in anxiety disorders such as phobias fear learning seems to occur automatically. In fact, some stimuli are more likely to be feared due to the fact that they constitute inputs to which evolutionarily shaped behavioural systems are more selective and primed to respond (Isbell, 2006; Mineka & Öhman, 2002; Öhman & Mineka, 2001; but see Dawson et al., 2007), and these are the ones that actually constitute major phobic categories. In contrast, other fear-irrelevant stimuli require association to an aversive stimulus during the ontogenetic story of the individual to become feared. Nevertheless, increased SCRs to masked fear-relevant stimuli have only been shown under fear conditioned paradigms, with unconditioned fear-relevant stimuli (e.g. fearful faces) being only processed at suprathreshold levels of awareness (Williams et al., 2004). This suggested that autonomic responses to emotional pictures of happiness and anger require at least conscious perception, which is consistent with our data. In contrast, awareness level itself was not sufficient to explain SCRs, as in our data only long stimulus durations accounted for the difference found for unconditioned angry faces.

As a possible explanation, it was shown that within a pool of autonomic responses such as electromyography and heart rate, the SCR was the only parameter that had a high correlation with viewing time (Lang et al., 2003). Although this measure was taken as a measure of interest in the picture, it is possible that longer exposition times to reliable emotional pictures, such as angry faces, enhances SCRs, supporting the suggestion that higher cognitive factors have a role in the SCR (Dawson et al., 2007; Maia & McClelland, 2004). Using electroencephalography (EEG) measurements, it has been shown that non-conscious perception of fear was distinguished by an early negative component, while conscious perception entailed a later component. This later component seems to reflect detailed processing of the stimulus affective content, with its integration in working memory (Williams et al., 2004), thereby reflecting more detailed emotional appraisal. This is also consistent with previous models which state that prolonged durations allow for iterative and recurrent processing, as opposed to shorter durations that allow only for feedforward projections and no visual awareness (Lamme & Roelfsema, 2000). Greater latencies demonstrate higher cognitive and behavioural commitment, as long as the processing is not interrupted (Kouider & Dehaene, 2007).

Relation between psychophysiological and neuroimaging measures

Importantly, the fact that we did not found differential SCRs to the smallest durations does not mean that the processing of the angry faces was not reflected in brain activity in structures such as the amygdala. In fact, in this study (Chapter 3) we did not use concomitant central nervous system direct measures. Nonetheless, it has been suggested that appraisal of fearful faces with concomitant SCRs showed amygdala and medial frontal activity, whereas in the absence of SCRs only hippocampus-lateral frontal activity was found (Williams et al., 2001). However, this does not reveal the causality between SCR and amygdala-medial frontal activity. If this were the case in our study, activity of these structures would not be enough to engage automatic sympathetic outcomes in terms of differential SCRs (see also Williams et al., 2005) .

Our findings in the first study (Chapter 3) do not support the hypothesis that awareness of the picture content itself, in particular for short stimulus durations, is sufficient to amplify the SCR. Instead, and in partial accordance with previous findings in the literature for unconditioned fear-relevant stimuli, it seems that SCR enhancement to unconditioned angry faces requires not only awareness of the stimulus (e.g. Dawson et al., 2007; Critchley et al., 2002; Williams et al., 2004) but also enough time for detailed processing and conscious appraisal. This type of processing, which more detailed, is particularly susceptible to variations in the stimulus position within the visual field, and was therefore tested in the other two studies of this thesis. In fact, whereas in the first study we presented emotional faces only at central positions, varying its temporal duration, in the following studies (Chapter 5 and 6) we kept the duration constant and manipulated the spatial location (centre, left, right).

7.2. Fear-relevant animal Faces and Shapes: the role of central vs. peripheral processing in threat detection

The main goal of this part of the project was to understand how the stimulus position in the visual field (centre, left, right), which is an ecologically relevant variable, influences the processing of emotional items (Chapters 5 and 6), and how brain activity in the affective network is modulated by this factor. Additionally, we studied the role of task requirements and attentional allocation to threat signals by using an implicit task, which required directing the attention toward features other than affective signals, or an explicit task, which imposed directing attention to threatening signals which had to be reported.

We used functional neuroimaging (fMRI) to study the role of the hypothesized subcortical pathway to the amygdala, to elucidate which mechanisms regulate amygdala activation, and in particular if this structure was biased to the processing of peripheral information or, at least, of more coarse and degraded information. Other neocortical and subcortical structures involved in the processing of emotions were also examined, to uncover evidence for a distinction between explicit and implicit emotional processing. We tested if central vs. peripheral threat recognition in the amygdala mirrors the central bias in visual object recognition that is known to be present in foveally-biased occipitotemporal cortical regions.

ANIMAL FACES VS. ANIMAL SHAPES IN CENTRAL VS. PERIPHERAL VISION

To study the role of object features, such as shapes and faces in affective processing, we used animal faces displaying threatening and non-threatening content (Chapter 5) to test a central bias for faces and well as other biological shapes in the amygdala response, given its direct connections with foveally-biased occipitotemporal regions. According to this logic, we first used animal faces as stimuli, given their biologically relevant value.

Specifically, we found that the right amygdala responses preferentially to animal faces presented at central locations, whereas the left amygdala responded preferentially to threatening animal faces in the implicit task. In addition, the right amygdala responded to both threatening and non-threatening animal faces during explicit appraisal of threat. Importantly, we found a twofold role of the basal ganglia in explicit threat evaluation dependent on spatial location: the caudate was specifically involved during central processing, while peripheral processing recruited mainly the putamen.

Overall, our results point to a central processing bias for animal faces in the amygdala, paralleling the central bias for object devoted areas in the occipitotemporal stream. Given this pattern of results, we asked if the central bias found in the first study on this topic (Chapter 5) was specific for faces or if it was generalized for other type of ecologically relevant emotional stimuli, such as fear-relevant shapes - snakes (Chapter 6). In fact, snakes do not require so much detail to be processed and can therefore be potentially analysed in the visual periphery (Delplanque et al., 2007).

Surprisingly, we found larger amygdala responses to centrally presented snake stimuli (body, face or fake) than for right (visually non dominant) peripheral presentations, independent of task and amygdala. For the contrast centre > left hemifield (visually dominant), these differences were found only for the left amygdala during the implicit snake identification task. During the implicit task this difference centre vs. right correlated positively with reported fear of snakes. Importantly, a strong hemispheric lateralization was found, with real shapes activating stronger the right hemisphere compared to fake shapes, which is consistent with its dominance for stimuli with emotional content (Spence et al., 1996) and visual orienting.

Although our results were expected for the animal face stimuli, the same was not predictable regarding the snake shapes. In fact, faces are usually processed in central vision, for which ventral occipitotemporal regions are biased (Levy et al., 2001), and the amygdala is interconnected with areas in the inferior temporal lobe (Amaral, Behniea, & Kelly, 2003; Rolls, 2007). Conversely, coarse shapes are usually processed in the periphery, attracting our attention when perceived preattentively as relevant (Corbetta, Kincade, & Shulman, 2002; Ignashchenkova, Dicke, Haarmeier, & Thier, 2004; Krauzlis, Lovejoy, & Zénon, 2013).

LEFT VS. RIGHT ASYMMETRIES

One of the main novelties of our study is that it elucidates the neural pathways involved in the processing of evolutionary relevant stimuli, such as snakes (Isbell, 2006). In fact, our pattern of results reconcile previous findings regarding the role of central and peripheral-biased vision (Levy et al., 2001), in addition to hemispheric specialization for specific aspects of visual, emotional and cognitive behaviour (Corballis, 2003; Pisella et al., 2011; Spence et al., 1996; Vallortigara & Rogers, 2005).

Importantly, we found a central bias in the amygdala for both animal faces and snake-related stimuli, emphasizing the role of central / foveal vision in detailed processing. This would be in accordance with the special role that foveal vision has in primates (Barton, 2004; Trevarthen, 1968).

Nevertheless we can speculate that some kind of preattentive processing might have occurred, at early onset times. Accordingly, the hemispheric asymmetries specifically found for the snake shapes (but neither to the fake snakes nor the snake faces), supports preserved peripheral responses for the dominant visual hemisphere in agreement with old phylogenetic roots and evolutionary pressures (Isbell, 2006).

Additionally, in the present thesis, we found lateralized responses in the amygdala, depending on the task and stimulus type. For instance, during the first fMRI study (Chapter 5), the left amygdala was more prominently involved during the implicit animal face recognition task, whereas the right amygdala responded more consistently during the explicit threat detection task. Conversely, in the second study (Chapter 6), we could not observe this separation. Both studies (Chapters 5 and 6) found stimulus type discrimination relying on the left amygdala, however with different task dependence (study 1 – implicit; study 2 – explicit). Finally, considering the contrast centre vs. periphery, the contrast centre vs. left visual field in both amygdalae was found to be significant during the animal faces study, whereas the study using snake-related stimuli identified a more effective contrast centre vs. right irrespective of amygdala. This apparent complex pattern of lateralization in the amygdala may help explain why a review failed to found a significant interaction between laterality of amygdala activation with task and with stimulus type (Baas, Aleman, & Kahn, 2004). These differences are better accounted by having in consideration specific stimuli task demands.

DIFFERENCES BETWEEN EXPLICIT (GOAL-ORIENTED) VS. IMPLICIT (STIMULUS-DRIVEN) PROCESSING

The second study (animal faces, Almeida et al., 2013, Chapter 5) showed a striking pattern in the basal ganglia, with caudate (head) vs. putamen preferential involvement depending of the central vs. periphery asymmetry. Here, we found both the caudate (tail) and the putamen more strongly involved during central compared to peripheral presentations, but with no differences found between tasks.

As we stated before, differences between the explicit and the implicit task in the snake study (Chapter 6) cannot be strictly compared with the same contrast in the first study using animal faces (Chapter 5). In fact, for the animal faces study, we have used threatening and non-threatening faces, but during the 'implicit threat' animal recognition task, the participants were requested to simply report the presence or the absence of an animal in the picture. We predict that the performance in this task was based on global features, given that the control study was a natural display with no animals. Therefore, the task did not require focus in detailed information. Conversely, the second study requested that true and fake snakes were discriminated, with the fake snakes strongly resembling the true snake stimuli. This increases perceptual ambiguity, and possibly task demands, even if performance is preserved. The pattern of response in the amygdala during the implicit task, namely increased response of the amygdala to central than to right hemifield presentations, was correlated with reported fear. This suggests that, although not requested, the snake identification task implied increased attention to threat or fear-relevant details. This might explain reduction of task type (implicit, explicit) dependence in the third study. Accordingly, the pattern observed suggested increased involvement of the basal ganglia during central presentations, compared to peripheral ones, with no differences between tasks. Furthermore, we found both the caudate and the putamen during central presentations, what does not contradict our previous results. In fact, some contrasts (see Chapter 5, results section) revealed also increased response of the putamen during centrally presented animal faces during explicit threat, whereas the caudate was never preferentially involved during peripheral presentations.

Overall, our results suggest an increased role of the basal ganglia in threat exlicit appraisal processing (Arsalidou et al., 2012; Critchley, et al., 2000), with different involvement of its nuclei. Both the putamen and the caudate respond to central presentations of threat, but the caudate responds specifically to foveal and explicit threat appraisal, which is in accordance with previous meta-analyses (Fusar-Poli et al., 2009; but see Faivre et al., 2012).

7.3. FUTURE STUDIES

In our project we used both psychophysiological (SCR) and functional neuroimaging (fMRI) measures. Both methods, relying on different physiological outcomes (electrical, BOLD), are characterized by slow time-to-peak responses, having a low temporal resolution (Amaro, & Barker, 2006; Casey, Davidson, & Rosen, 2002; Dawson, Schell, & Fillion, 2007). Although previous studies (Morris et al., 1998; Morris & Dolan, 1999; Whalen et al., 1998) have found evidence of involvement of the amygdala and increased SCRs during non-conscious processing of fear-relevant faces, supporting the evidence for a subcortical pathway, our study intended to test differences in amygdala activation concerning central and peripheral presentations, or whether differences in SCRs reflected non-conscious vs. conscious emotional faces.

We hypothesized that, if the amygdala was preferentially involved in automatic processing of emotionally relevant information, peripheral emotional stimuli would trigger stronger responses in the amygdala, whereas increased SCRs would occur preferentially to masked non-conscious angry faces. However, the SCRs to angry faces arose during long durations, and the amygdala response was overall higher during central presentations. These results are focused on analyses of *amplitude* changes of the signal. As pointed before, longer duration of stimulus appraisal and central presentations allow recurrent processing in widespread neural networks to occur (Lamme & Roelfsema, 2000), and thereby fit the current pattern in our data. We thus emphasize the role of both SCRs and BOLD signal in the amygdala in conscious stimulus appraisal.

Nevertheless, this does not mean that both SCRs and the amygdala are not involved in non-conscious and less accurate/peripheral processing of emotional information, but that these responses have higher amplitudes during conscious and accurate/foveal processing. A potential difference between processing speed and amplitude might account for this. In fact, we suggest that magnetoencephalography (MEG) studies might give a fresh view on this topic (see Bayle, Henaff,

& Krolak-Salmon, 2009; Liu & Ioannides, 2010; Luo, Holroyd, Jones, Hendler, & Blair, 2007), given their better temporal resolution (relative to fMRI) and relatively good spatial resolution (relative to electroencephalography (EEG)) measures. In fact, this method promises important input in the understanding of the dichotomy of visual / emotional processing under short vs. long-time windows.

One potential interesting study in the future would be to test whether processing of fear-relevant shapes such as snakes (Isbell, 2006; Öhman et al., 2012) have indeed different (faster) temporal signatures compared to other animals – shapes (e.g. spiders) and faces (such as the ones studied in Chapter 5), given that the processing of this stimulus type seems special (Soares, Esteves, Lundqvist, & Ohman, 2009). In addition, it would be interesting to see if hemispheric asymmetries in the temporal pattern would occur as a measure of stimulus position in the visual field (Liu & Ioannides, 2010). Furthermore, MEG studies, by accounting for processing speed and temporal signatures of different structures, may provide an important conceptual input about the role of cortical and subcortical structures in visual pathways for emotional recognition (Kravitz et al., 2013; Pessoa & Adolphs, 2010; Rudrauf et al., 2008).

Finally, in the studies reported in this thesis, we carefully used the behavioural reports of our subjects on a trial by trial basis to investigate psychophysiological (SCR) and neuroimaging (BOLD) measures. This point was particularly important to ensure that our created categories (e.g. face detection vs. emotion discrimination; threatening vs. non-threatening; animal/true snake vs. non-animal/fake snake) matched their perceptual meaning on a trial by trial basis. Future work should further elucidate how automatic attention mechanisms interact with explicit goal oriented emotional cognition. New analyses, on such trial-by-trial based sorting might further help disentail the role of stimulus-driven and goal-oriented processes in emotional appraisal.

7.4. Concluding remarks

In this thesis we explored the following dichotomies in affective processing: central vs. peripheral, explicit vs. implicit, automatic vs. non-automatic, left vs. right dominance in visual orienting as well in emotion processing, and short vs. long-time windows for processing. We found that all these dichotomies are relevant in explaining neural responses to emotional stimuli, with different weights depending on the task.

Here, we defend different roles for central and peripheral vision, with implications on the ongoing debate in the literature concerning preattentive/automatic vs. conscious processing in the amygdala. Central vision is more detailed, and serves the purpose of accurate processing of information, aiding the achievement of decision-making and performance. In a complementary manner, peripheral vision relies mainly on coarse LSF information, uses more automatic, preattentive attentional mechanisms (stimulus-driven) in order to capture attention towards relevant stimuli. This has consequences in terms of the trade-off between speed and accuracy (when it is better to respond fast regardless of whether an object is correctly recognized or not).

Our project substantiates the importance of taking into account the value of ecological variables in affective information processing, and the value of central appraisal of emotional information, while not disputing the role of preattentive, non-conscious, and peripheral, less accurate, processing.

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"Rabbit's clever," said Pooh thoughtfully.
"Yes," said Piglet, "Rabbit's clever."
"And he has Brain."
"Yes," said Piglet, "Rabbit has Brain."
There was a long silence.
"I suppose," said Pooh, "that that's why he never understands anything."
— A.A. Milne, *Winnie-the-Pooh*

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CURRICULUM VITAE

Inês Alexandra Teixeira de Almeida was born on July 6, 1984, in Belém, Lisboa, Portugal. Her primary and basic education were made at Externato Júlio Dinis, with the completion of her secondary school education at Escola Secundária Padre Alberto Neto, both at Queluz, Sintra, Portugal. In 2001, she went to the University of Coimbra to study Psychology. For her final B.Sc. (5 years) project she spend one year doing research at the Institute for Biomedical Imaging and Life Sciences (IBILI), in the Visual Neuroscience Laboratory, IBILI, at the Faculty of Medicine of the University of Coimbra, where she continued as a PhD student.