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October, 2013

BIAL Fellowship Report: 170/10

BIAL FOUNDATION
À Av. Da Siderurgia Nacional
4745–457 S.Mamede de Coronado

Dear Sir/Madam,

Attached to this letter you may find the Scientific Report and the Finantial Report for the Bial Fellowship 170/10, which are sent in both electronic and paper format. The original documents related to costs and expenses (receipt and bill) can also be found in attachment. Finally, this cover letter contains a key points summary.

With best regards,

Gina Caetano, PhD

Key points Summary BIAL Fellowship 170/10:

1. Project Output

2. Communications/Posters/Conferences

3. Future Outputs

Scientific Report 2013: BIAL Fellowship 170/10

BIAL Fellowship: 170/10

"The role of fusion of multisensory percepts in dynamic facial/body expressions: an fMRI study"

The Scientific Report 2013, for BIAL Fellowship 170/10 has the following structure:
- 1) Manuscripts
- 2) Communications/Posters/Conferences
- 3) Future Outputs

1) Manuscripts

One manuscript is published, one will soon be submitted to a high level impact international peer reviewed Journal under the advice of Beatrice de Gelder (a leader in the field), and at least three more will be published (one concerning multimodal integration in affective perception, another on body/face affective processing and a final one on data driven validation of our cognitive models). A major finding of this project was the discovery of a direct involvement of the Pulvinar and the Superior Colliculus in affective processing, and in particular a role of the Superior Colliculus in event anticipation, whilst being able to pinpoint different dynamics in subcortical structures along the affective evaluation task.

1.1 – Published manuscript within the scope of BIAL Fellowship 170/10, as a contribution from Prof. Miguel Castelo-Branco:

=> Almeida I, van Asselen M, Castelo-Branco M. The role of the amygdala and the basal ganglia in visual processing of central vs. peripheral emotion content. Neuropsychologia 2013. http://www.ncbi.nlm.nih.gov/pubmed/23872141

The published manuscript can be found in attachment to this letter, both in electronic and paper format.

1.2 – Manuscript preparation for submission to the Journal of Neuroscience

Currently, and after a more thorough and refined analysis and look upon the data we have novel results and re-restructured the scope of the first manuscript. This will focus on explicit emotion recognition and on the role of subcortical structures. A brief summary is presented below:

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

Preliminary Title: Emotion event recognition within a dynamic audiovisual stream: the role of the superior colliculus, amydgala and basal ganglia

Authors: Gina Caetano[1,a], Beatrice de Gelder[2,3], Miguel Castelo-Branco[1,4]
[1] Institute for Biomedical Imaging and Life Sciences, Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal
[2] Faculty of Psychology and Neuroscience, Maastricht University, Maastricht Brain Imaging centre M-BIC, Oxfordlaan 55, 6229 ER Maastricht, The Netherlands
[3]Cognitive Neuroscience, Tillburg University, Tillburg, The Netherlands
[4] Brain Imaging Network, ICNAS, University of Coimbra, Coimbra, Portugal.

ABSTRACT/SUMMARY:

The role of subcortical structures in processing sensory information conveying emotional content is still highly debated. It is known that the amygdala (AMG) is the entryway to sensory processing of emotions, although its functional role at the interface between emotion and cognition remains to be clarified . This study aimed at underpinning neural correlates of emotion detection and recognition, irrespective of sensory modality, focusing on the role of subcortical structures (colliculus, amygdala, and basal ganglia). Dynamic information was presented as a stream of neutral stimuli with pseudo-randomized presentation of an embedded deviant stimulus conveying emotional content. These audio and/or visual miniblocks were interleaved by periods of rest. We found that the superior colliculus and thalamic nuclei were significantly recruited before the appearance of the deviant stimulus. At this time interval the AMG and putaminal areas, bilaterally, were deactivated in comparison to baseline activity. Importantly, right lateralized AMG activity increased once the emotionally loaded deviant stimulus was presented, in comparison to baseline and bilaterally in comparison to onset of neutral content presentation. Also, recruitment of bilateral putaminal regions was observed once emotional content is presented and thereafter, whereas activation of the pulvinar is identified at the last stages of the paradigm preceding the categorization task. These results suggest a differential role of subcortical structures in emotion processing: the superior colliculus in response anticipation, the amygdala in direct emotional content processing, and basal ganglia in integration of this information in motor behavioral planning after affective value has been computed.

INTRODUCTION/RATIONAL:

The field of affective neuroscience has shown novel insights onto the role of subcortical and cortical networks in explicit/implicit emotion recognition. Central attention has been given to the amygdala as a major player in appraisal of sensory

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

information, whereas a continuing debate exists on how, where, and when the sensory information conveying emotional content is processed. Most accurate information on afferent/efferent connections and timing of neuronal firing comes from animal studies. Albeit a direct link between neuroanatomical and neurofunctional models in rodents, non-human primates, and humans may not be direct, the field of animal neuroscience altogether with methodological advances in neuroimaging has enabled novel questions and experimental paradigms in affective neuroscience.

Since early 1980s studies, on fear conditioning in rodents and small animals (Bordi and LeDoux, 1992; LeDoux, 2000; Antunes and Moita, 2010), it has been retained that cortical pathways to the AMG are related to a slower learning on conditioning settings than the direct thalamo-AMG pathway (LeDoux, 2000) (see **Figure 1A**). A similar hypothesis was advanced for humans by Morris and co-authors (Morris et al., 1999), who showed correlation of AMG activity only with thalamic nuclei in similar context. Most recently, MEG recordings (Garrido et al., 2012) further suggests a dual route to the amygdala, unrelated to fear conditioning or perception, within a deviant auditory discrimination task.

Similarly, several pathways may lead to visual sensory input to the amygdala (Damasio et al., 2000; Rudrauf et al., 2008; Pessoa and Adolphs, 2010; de Gelder et al., 2011; LeDoux, 2012), comprising subcortical and cortical pathways (see **Figure 1B**). It remains to be clarified how different bottom-up and top-down processes modulate neural recruitment along this dynamic dual parallel route model. For example, studies in patients with striate cortex lesions (‘blindsight’) have shown superior colliculus (SC) and pulvinar (Plv) activations, and AMG activity modulation, to unconsciously perceived fear-related stimuli (Morris et al., 1999). But, extrastriate cortical regions can also process undetected emotion stimuli when sensory input bypasses striate visual cortex (de Gelder et al., 1999; Vuilleumier et al., 2002; Tamietto et al., 2009; Berman and Wurtz, 2010; Lyon et al., 2010; Schmid et al., 2010; Tamietto et al., 2012).

The retina-SC-Plv pathway subserves direct and fast relay of coarse visual information to the amygdala (Vuilleumier et al., 2003), besides the already assigned roles in saccadic eye movements, orientation to and detection of novel stimuli. In parallel, high frequency visual information has shown to be preferentially processed through the parvocellular input which decussates in the ventral stream. The former may have had early evolutionary predominance, as enabling fast preparation for action from coarse information on potential environmental threats. Within a readiness for action (appraisal of external stimuli), basal ganglia may relate to goal-directed behavioral planning after affective value has been computed.

Following evidence from animal neuroscience, amygdala has early-on been assigned the role of fear/threat perception. It has since then been complemented and

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

extended to a variety of affective functions, encompassing positive and negative valence, such as implicit/explicit emotion perception, emotional learning and memory, emotional modulation of memory, appraisal, attention, and social behavior (Davis and Whalen, 2001; Sah et al., 2003; Phelps and LeDoux, 2005; Costafreda et al., 2008; Sergerie et al., 2008). All in all, emotion perception is highly relevant in social contexts, intrinsically embedded within a continuously changing dynamic multisensory environment, comprising different levels of explicit expressions and subtleties. Only recently the study of emotion perception has integrated some of these variables through the use of film displays, semantic information, or tackling body contributions.

Our main goal consists in identifying neural correlates for the perception of fast explicit dynamic displays (unimodal or bimodal) and their differential recruitments in relation to successful recognition of deviant emotional content. We hypothesize the involvement of both cortical and subcortical structures, with modulation of the latter through attentional, appraisal, and sensorial-motor (goal-oriented) integration processes, respectively.

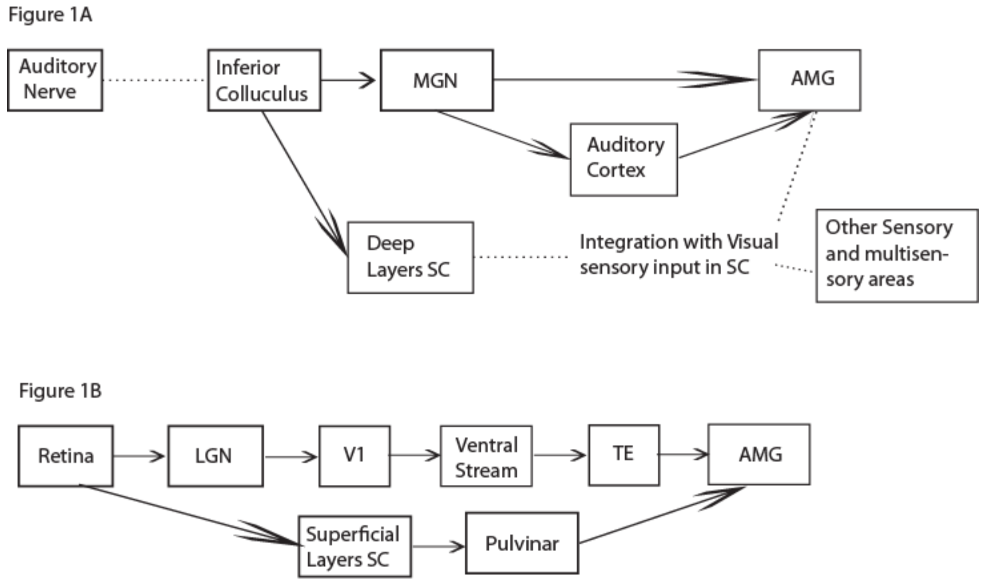

Figure 1 – Depiction of major subcortical and cortical pathways for sensory input to the amygdala. This key summary does not entail the complex subcortical-cortical-cortical connectivity, rather schematizing the major routes. A) Auditory Pathway. MGN = Medial Geniculate Nuclei; SC = Superior Colliculus; AMG = Amygdala. B) Visual Pathway. LGN = Lateral Geniculate Nuclei; V1, primary visual cortex; Ventral Stream includes: V2, (V3), V4, and the inferior temporal area TEO.

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

MATERIALS AND METHODS:

Participants

Sixteen healthy male volunteers (26.3 ± 3.1 years; one left-handed) participated in the experiment after providing written informed consent. All participants had normal or corrected-to-normal vision. The study was performed in accordance to the Declaration of Helsinki and was approved by the local medical ethical committee.

Stimuli and Paradigm

Stimulus construction – Nine male students and young researchers of the University of Tillburg were filmed while displaying fearlful, angry, happy or neutral faces accompanied by non-verbal vocal utterances, simultaneously recorded with the video-camera microphone. Auditory and video/video-image stimuli were extracted from the originally selected scenes, respectively ([600; 2000] ms).

Paradigm* –** We implemented an fMRI mini-block design of neutral stimuli with an embedded deviant stimulus, pseudo-randomly presented at the second or third instance. Each stimulation mini-block has a duration of 4-seconds, and is composed of 4 stimuli, 3 neutral and 1 conveying emotional content (happy, angry, or afraid), as described in ***Stimuli (see **Figure 2**). The same experimental paradigm was used for each set of stimuli (visual, auditory, or audiovisual), separately.
Participants were instructed to passively view and/or listen to the stimuli presented in each mini-block, and to answer to a categorization screen presented for 2 seconds immediately after each mini-block. The categorization screen had two possible configurations, pseudo-randomized across participants: *Happy, Angry, Afraid* OR *Afraid, Angry, Happy* (see **Figure 2**). Participants gave their answer via two response-pads comprising each two answer buttons, and correspondingly, the order of the category had two configurations – reversed order. In addition to the categories presented in the screen, the right-/left-most response button was assigned value NILL, corresponding to situations in which participants were unable to identify the emotional content displayed in the deviant stimulus.

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

Figure2 – Experimental Design. – An explicit emotion recognition paradigm, within a 4 seconds mini-block fMRI design, was presented. Participants had to identify the emotional content of a deviant stimulus (auditory, visual, or audiovisual) and explicitly identify the recognized emotion in the 2-seconds time window after stimuli presentation.

Data Acquisition

The experiment was divided into three non-consecutive days, one for each type of stimulus (visual, auditory, audiovisual). Visual or auditory sessions were presented within the first or second day, in a pseudo-randomized order across participants, whereas the audiovisual session was always the last. By this means we aimed at minimizing modality interference effects. Prior to each scanning session, the subject was trained at the perceptual-categorization task for each of the individual stimuli, aiming at minimizing novelty effects.

Participants underwent neuroimaging at the facilities of the Portuguese Brain Imaging Network on a 3T Siemens Magnetom Trio Tim system (Erlangen, Germany), with a standard 12 channel headcoil. Foam padding was used to minimize head movements, headphones were provided to minimize scanner noise and deliver auditory stimuli.

Data Analysis

Functional Data Analysis – fMRI data were analyzed with the software package BrainVoyager QX (version 2.6, Brain Innovation, Maastricht, The Netherlands). Former to the application of pre-processing stage, a Mean Intensity Adjustment was performed to check for possible signal fluctuations that may influence results.

PreProcessing stages included: slice scan time correction, spatial smoothing (Gaussian filter 6 mm FWHM), temporal high-pass filtering with removal of linear drifts (nonlinear drifts of 3 or fewer cycles were removed), and finally a 3D motion correction within session was applied.

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

Random Effects-Analysis was performed at group-level to pinpoint significant effects that can be generalized across sensory modalities and to the population level. Talairach stereotaxic space was chosen for display purposes, considering the focus on subcortical structures.

RESULTS:

Behavioral Results

We used the behavioral results to sort out the incorrect trials (in general < 10%).

General patterns of subcortical responses

Subcortical activation patterns show differential recruitment depending on whether one is looking upon time periods before/during/after the presentation of the deviant stimuli that convey explicit emotional content. (see **Figure 3**).

Brief summary of main results.

We would like to emphasize that this is one of the first studies showing a direct involvement of the Pulvinar and the Superior Colliculus in affective processing, the latter being involved in event anticipation and the former in the period of cognitive appraisal.

1. The superior colliculus (SC) shows an increased response that starts before the emotional deviant, while neutral content is being presented.

2. The amygdala and putamen, bilaterally, exhibit lowered responses to neutral content presented prior to emotional content.

3. The amygdala only responds once the deviant presenting explicit emotional content has appeared. Significant activation is observed on the right hemisphere, whereas on the left hemisphere lowered responses have ceased at least.

4. Bilateral putamen activation is identified during the neutral stimulus immediately following the emotion deviant content.

5. Amygdala lowered response, bilateral putaminal and pulvinar activations are observed during neutral displays immediately preceding the categorization period (therefore, when one neutral has appeared before this stimulus from the time of emotion deviant onset).

As expected, activation of LGN, MGN and other thalamic nuclei were identified, mostly during neutral stimuli preceding the emotion deviant.

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

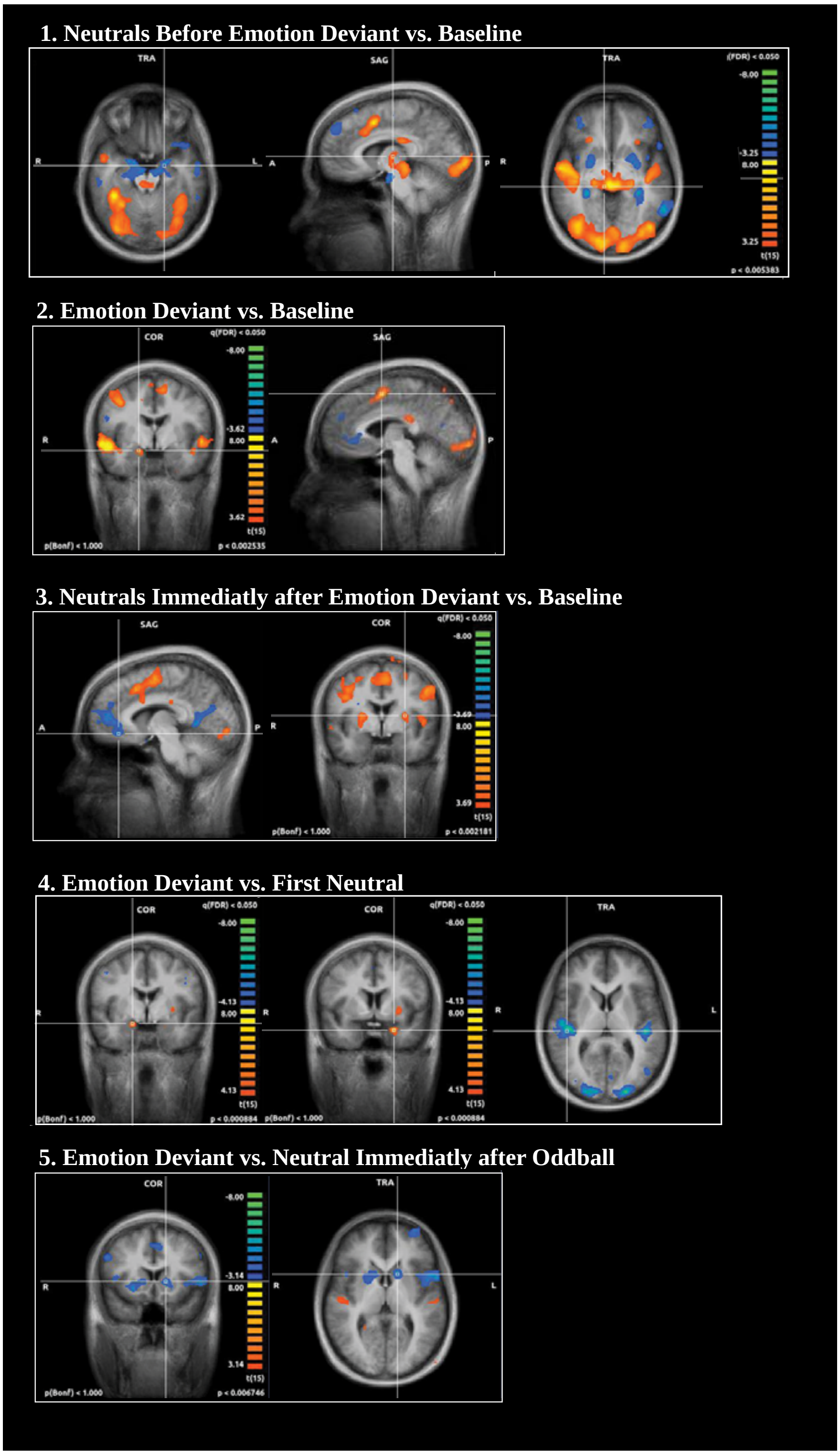

Figure 3 – BOLD contrasts [RFX, q(FDR) 0.05]. (1-3) –Contrasts: Neutral Before Deviant/ Emotion Deviant/ Neutral Immediately After Emotion Deviant > Baseline Activity, respectively; (4) Contrast: Emotion Deviant > First Neutral; (5) Contrast: Emotion Deviant > Neutral Immediately After Deviant.

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

	Neutrals Before Deviant	Deviant	Neutral After Deviant	Last Neutral [1]	Deviant vs. First Neutral Before	Deviant vs. Neutral After
Amygdala	Deactivation (L/R)	Activation (R)		Deactivation (R)	> (L/R)	
Putamen	Deactivation (L/R)		Activation (L/R)	Activation (L/R)	> (L)	< (L/R)*
Superior Colliculus	Activation (L/R)				< (R)	
Pulvinar				Activation (L/R)		
Caudate		Deactivation (L-head)				< (L/R)*
LGN	Activation (L/R)					
MGN	Activation (L/R)					
Subthalamic Nucleus	Activation (L/R)					
Medial Dorsal Nucleus	Activation (L/R)			Activation (L/R)		
Ventral Anterior Nucleus			Activation (R)	Activation (L/R)		

Table1 – Key summary of activation and deactivation patterns for the contrasts, from left to right, respectively: 1) Neutrals before deviant vs. Baseline; 2) Emotional Deviant vs. Baseline; 3) Neutral After Deviant vs. Baseline; 4) Emotion Deviant vs. First Neutral Before; 5) Emotion Deviant vs. Neutral After.

*Observed at $p<0.005$, cluster thresholding ($N>20$) uncorrected for multiple comparisons.[1] Activation of several thalamic nuclei is not reported yet in Table1.

CONCLUDING REMARKS:

Our study presents novel and complementary findings on subcortical activity once an individual has to attend to and identify short-explicit emotional content within a stream of dynamically presented neutral information. Besides recruitment of sensory-related thalamic relay nuclei, the superior colliculus is engaged before the appearance of targeted emotional content, whilst the amygdala and putamen show bilateral lowered responses. After the appearance of emotional content the amygdala responds, whereas only afterwards bilateral putaminal activation is present. At last, for neutral information that appears secondly after the emotional deviant, we identified amygdala deactivation on the right hemisphere, and bilateral pulvinar and putamen activations in between other subcortical structures. These finding confirm the active role of the amygdala in appraisal of sensory information, whilst identifying a direct role of the superior colliculus in attentional and anticipatory evaluation of sensorial input, which ceases once the target

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

content has emerged. Additionally, pulvinar responses have been unveiled, despite overt activation being solely identified at the end of the stimulation period. We suggest basal ganglia, and particularly the putamen, have an active integration role (after affective value has been computed) towards motor readiness preparation for the conscious recognition/categorization task.

2) Communications/Posters/Conferences

2.1. Oral Communications

2011 – Invited Talk **Caetano G** "Técnicas de NeuroImagem: Aplicação em Neurociências" including the section *"Multivariate Pattern Analysis Applied to Functional Magnetic Resonance Imaging: Classification of Auditory/Visual/Audiovisual perception and recognition of emotions"*, 16th of December 2011, Instituto Superior de Engenharia de Coimbra.

2012 – Invited Talk **Caetano G** "Técnicas de NeuroImagem: Aplicação em Neurociências" including the section on BIAL fellowship 170/10 "*The role of fusion of multisensory percepts in dynamic facial/body expressions: an fMRI study*". 17th December 2011, Instituto Superior de Engenharia de Coimbra.

2.2. Poster Presentations

2012 – From master thesis work
- Guerra C., De Gelder B., Castelo-Branco M., **Caetano G**. Multivariate pattern analysis applied to functional magnetic resonance imaging: classification of auditory perception and recognition of emotions. 2nd Portuguese Meeting of Biomedical Engineering, Coimbra Portugal, 23rd-25th February 2012

2012 – From master thesis work
- Guerra C, **Caetano G**, de Gelder B, Castelo-Branco M. IV Annual IBILI Meeting, IBILI Meeting 2012, Coimbra Portugal, December 6–7 **2012**,

2013 & 2014 – In 2013 and 2014, I plan to present the described results in the following meeting (with possible change in title):

1.=> **Caetano G**, de Gelder B, Castelo-Branco M. "*Emotion event recognition within a dynamic audiovisual stream: the role of the superior colliculus, amydgala and basal ganglia*". VI Annual Meeting of IBILI, IBILI Meeting 2013, Coimbra Portugal, December 12-13 **2013**.

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

2.=> **Caetano G**, de Gelder B, Castelo-Branco M. "*Emotion event recognition within a dynamic audiovisual stream: the role of the superior colliculus, amydgala and basal ganglia*". **2014 BIAL Foundation Symposium** "Behind and Beyond the Brain", 26th-29th March 2014, Casa do Médico, Porto Portugal.

3.=> Organization for Human Brain Mapping's 2014 Annual Meeting, June 8–12 Hamburg, Germany.

Additionally, I would intend to present these and additional results from the project in:

4.=> 2014 Annual Meeting of the Society for Neuroscience, November 15–19, Washington DC, United States of America.

2.3. Master Thesis Supervision

<u>*2012*</u> – A master thesis was completed under the direct supervision of Gina Caetano, PhD and Miguel Castelo-Branco, MD, PhD, and according to delineated ideas with Beatrice de Gelder for the BIAL Fellowship 170/10.

Title: "*Univariate Statistics and Multi-Voxel Pattern Analysis in fMRI: perception and categorization of emotions.*"

Master Student: Carla Guerra, *Mestrado Integrado em Engenharia Biomédica, Faculdade de Ciências e Tecnologia da Universidade de Coimbra.*

<u>*3. Future Outputs*</u>

I expect at least three additional articles from this project, besides a manuscript within the line of thought presented here, one concerning multimodal integration in affective perception, another on body/face affective processing, and a final on data driven validation of our cognitive models.

GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800

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GINA CAETANO, PHD
IBILI – FACULDADE DE MEDICINA,
AZINHAGA DE SANTA COMBA, CELAS
3000-548 COIMBRA, PORTUGAL
• E-MAIL: GCAETANO@FMED.UC.PT, GSM: +351 927 316 800