Neural substrates of 2D/3D object perception: a combined EEG/fMRI approach

Tese de doutoramento em Ciências da Saúde do ramo de Ciências Biomédicas, orientada por Prof. Dr. Miguel Castelo-Branco e apresentada à Faculdade de Medicina da Universidade de Coimbra.

2014
Neural substrates of 2D/3D object perception: a combined EEG/fMRI approach

Investigação dos mecanismos de decisão e categorização perceptual utilizando técnicas de imagem multimodal

João Miguel Seabra Castelhano
2014
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Cover Design: João Castelhano

Cover Legend: Upright/Inverted Mooney face (two-tone image) used to investigate the role of high frequency oscillatory activity during perceptual decision processes (the coherent perception of incomplete pictures). Mooney faces are difficult to perceive when inverted.


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Neural substrates of 2D/3D object perception: a combined EEG/fMRI approach

Dissertation presented to obtain a Ph.D. degree in Health Sciences at the Doctoral Programme in Health Sciences of the Faculty of Medicine, University of Coimbra.

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João Miguel Seabra Castelhano

2014

Supervised by: Miguel Castelo-Branco, Ph.D.
Para a Madrinha, a Avó e a restante família mais chegada

“...There's more to see than can ever be seen...”

Tim Rice
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<td>2D</td>
<td>Two-dimensional</td>
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<tr>
<td>3D</td>
<td>Three-dimensional</td>
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<tr>
<td>4D</td>
<td>Four-Dimensional</td>
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<tr>
<td>A</td>
<td>Anterior</td>
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<tr>
<td>AAC</td>
<td>Anterior Cingulate Cortex</td>
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<td>ADHD</td>
<td>Attention Deficit and Hyperactivity Disorder</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>ASD</td>
<td>Autism Spectrum Disorders</td>
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<td>BA</td>
<td>Brodmann Area</td>
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<td>BCG</td>
<td>Ballistocardiogram</td>
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<td>BEM</td>
<td>Boundary Element Method</td>
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<td>BESA</td>
<td>Brain Electrical Source Analysis</td>
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<td>BOLD</td>
<td>Blood-Oxygen Level Dependent</td>
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<td>CDR</td>
<td>Current Density Reconstruction</td>
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<td>CSD</td>
<td>Current Source Density</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<td>Control</td>
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<td>dB</td>
<td>Decibel</td>
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<td>DLPFC</td>
<td>Dorsolateral Prefrontal Cortex</td>
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<td>ECoG</td>
<td>Electroencephalography</td>
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<td>EKG</td>
<td>Electrocardiogram</td>
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<td>Electromyography</td>
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<td>EOG</td>
<td>Electrooculogram</td>
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<td>EPI</td>
<td>Echo planar imaging</td>
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<td>EPSPs</td>
<td>Excitatory Postsynaptic Potentials</td>
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<td>ERO</td>
<td>Event Related Oscillations</td>
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<td>ERP</td>
<td>Event Related Potential</td>
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<td>FDR</td>
<td>False Discovery Rate</td>
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<td>FFA</td>
<td>Fusiform Face Area</td>
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<td>FFT</td>
<td>Fast Fourier Transform</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>fMRI</td>
<td>functional Magnetic Resonance Imaging</td>
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<td>GABA</td>
<td>Gamma-Aminobutyric Acid</td>
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<td>GLM</td>
<td>General Linear Model</td>
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<td>Hb</td>
<td>Deoxyhaemoglobin</td>
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<td>HbO2</td>
<td>Oxyhaemoglobin</td>
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<td>HEOG</td>
<td>Horizontal Electrooculogram</td>
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<tr>
<td>HF</td>
<td>High Frequency</td>
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<td>HRF</td>
<td>Haemodynamic Response Function</td>
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<td>ICA</td>
<td>Independent Component Analysis</td>
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<td>ImCoh</td>
<td>Imaginary Coherence</td>
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<td>IPSPs</td>
<td>Inhibitory Postsynaptic Potentials</td>
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<td>ISI</td>
<td>Interstimulus Interval</td>
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<td>L</td>
<td>Left</td>
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<tr>
<td>LAURA</td>
<td>Local Autoregressive Average</td>
</tr>
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<td>LF</td>
<td>Low Frequency</td>
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<tr>
<td>LIBSVM</td>
<td>Library for Support Vector Machines</td>
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<tr>
<td>LOC</td>
<td>Lateral Occipital Complex</td>
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<tr>
<td>LORETA</td>
<td>Low Resolution Electromagnetic Tomography</td>
</tr>
<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
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<tr>
<td>MNE</td>
<td>Minimum Norm Estimate</td>
</tr>
<tr>
<td>MPRAGE</td>
<td>Magnetization-Prepared Rapid-Acquisition Gradient Echo</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic Resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MT</td>
<td>Middle Temporal</td>
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<tr>
<td>MUSIC</td>
<td>Multiple Signal Classification</td>
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<tr>
<td>n.s.</td>
<td>non-significant</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>O2</td>
<td>Oxygen</td>
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<td>OFA</td>
<td>Occipital Face Area</td>
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<td>OFC</td>
<td>Orbitofrontal Cortex</td>
</tr>
<tr>
<td>P</td>
<td>Posterior</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>PLV</td>
<td>Phase Locking Value</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PPA</td>
<td>Parahippocampal Place Area</td>
</tr>
<tr>
<td>R</td>
<td>Right</td>
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<tr>
<td>RF</td>
<td>Radio Frequency</td>
</tr>
<tr>
<td>ROIs</td>
<td>Regions of Interest</td>
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<tr>
<td>ScZ</td>
<td>Schizophrenia</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SE</td>
<td>Standard Error of the mean</td>
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<tr>
<td>SFM</td>
<td>Structure-from-motion</td>
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<td>SIFT</td>
<td>Source Information Flow toolbox</td>
</tr>
<tr>
<td>sLORETA</td>
<td>Standardized Low Resolution Brain Electromagnetic Tomography</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-noise Ratio</td>
</tr>
<tr>
<td>SPMs</td>
<td>Statistical Parametric Maps</td>
</tr>
<tr>
<td>SR</td>
<td>Sampling Rate</td>
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<tr>
<td>STFT</td>
<td>Short-term Fourier Transform</td>
</tr>
<tr>
<td>STS</td>
<td>Superior Temporal Sulcus</td>
</tr>
<tr>
<td>SVM</td>
<td>Support Vector Machines</td>
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<tr>
<td>TE</td>
<td>Echo Time</td>
</tr>
<tr>
<td>TF</td>
<td>Time-frequency</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition Time</td>
</tr>
<tr>
<td>Tv</td>
<td>Time volume</td>
</tr>
<tr>
<td>VEOG</td>
<td>Vertical Electrooculogram</td>
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<td>WS</td>
<td>Williams Syndrome</td>
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Summary

Perceptual decision making is defined as the choice of possible interpretations of the world based on the incoming sensory evidence. The role of temporal coding in this process and coherent perception, defined as hierarchical grouping of local elements, remains controversial. Oscillatory processes in the gamma frequency range (>30 Hz) have been proposed to play a role in signaling emerging object percepts in the brain. Studies using Electroencephalography and Magnetoencephalography (EEG and MEG) have suggested that gamma-band oscillations are related to the integration of information and the ability to form coherent gestalts as well as attention and working memory processes. It is accepted that gamma-band synchrony reflects binding of information across different brain regions leading to the emergence of a coherent percept. There are also reports that correlate gamma activity with many other cognitive processes. Hence, a wide variety of gamma-band patterns and sources were reported for different tasks. In this line, both animal and human studies have suggested that understanding oscillatory activity patterning can be important to understand normal and abnormal cognitive function. However, it remains unclear whether distinct patterns across the gamma frequency range related to different cognitive modules do coexist in the same task.

We investigated visual perceptual recognition moments based on EEG analysis with ambiguous Mooney stimuli (black and white incomplete pictures). We departed from classical paradigms which are based on contrasts between stimuli conditions that are fixed in time, and adopted a paradigm whereby the moment of perception of an emergent global pattern was variable. Therefore we could directly compare perception vs. no perception states for the same stimuli and separate sensory and motor processing components. We found a direct link between gamma-band temporal patterns (in two distinct sub-bands: ~40 Hz and ~60 Hz) and the presence versus absence of emerging holistic perception of variable onset. These findings were confirmed in a data driven manner with a support vector machine classification approach based on time-frequency features.

Unimodal studies do not have enough resolution to test for non-unitary sources of these sub-bands and to establish their spatial distribution. Using a simultaneous Electroencephalography and functional Magnetic Resonance Imaging (EEG/fMRI) approach we provided new evidence for separable gamma activity patterns reflecting holistic perception. We found that distinct gamma frequency sub-bands reflect different neural substrates and cognitive mechanisms when comparing object perception states vs. no
categorical perception. Accordingly, at least two separate neural modules are involved in holistic perceptual decision, one in the visual cortex (∼60 Hz) and the other in the anterior insula (∼40 Hz). These findings showed that current neuronal models of gamma-band spatial distribution need to consider the duality by separating low and high sub-bands. This provides a step forward in understanding the functional specialization of decision-making networks and the role of gamma frequency range sub-bands in signaling their different neural and cognitive components. This may shed new light on the role of gamma-band response in normal cognition and in neuropsychiatric disorders such as autism and schizophrenia, where both visual and decision making circuits may be impaired.

Importantly, it remains unclear whether oscillation amplitude is relevant for encoding global stimulus properties or, alternatively, it is neural synchrony that plays a pivotal role in gestalt formation. In this study, we addressed this question by studying Williams Syndrome (WS), a well characterized model of impaired central coherence, using EEG and a set of experimental tasks requiring visual integration. It has been hypothesized that neural synchrony underlies central coherence that is a well-known model for cognitive dysfunction in autistic spectrum disorders. WS patients show markedly disrupted visual perceptual coherence and holistic integration. Using this human model of loss of coherence, we showed for the first time that neuronal synchrony is reduced across stimulus conditions and this is associated with increased amplitude modulation at 25-45 Hz. This combination of a dramatic loss of synchrony despite increased oscillatory activity represents strong evidence that synchrony underlies central coherence.

To directly identify the sources of those specific sub-bands within gamma range and clarify their roles, we used Electrocorticography (ECoG) with the added value of greater spatial and temporal resolution. We used the unique opportunity provided by functional mapping in epilepsy and tested an epileptic patient. Interestingly, we identified a stimulus dependent graded posteroanterior sharpening of frequency responses. Lower frequencies dominated in the anterior ventro-temporal areas and higher frequency modulations in occipital regions.

In summary, this set of works addressed several critical points to understand the role of oscillatory activity in perceptual decision mechanisms. We conclude that separable gamma sub-bands reflect different cognitive mechanisms. A distinct spatial source map is present for different gamma sub-bands activity during visual holistic perception. Low gamma (40 Hz) activity is related to the decision making network and High gamma (60
Hz) is localized to early visual processing regions. Moreover, we showed that synchrony underlies central coherence.

These demonstrations of a clear functional topography for distinct gamma sub-bands within the same task shows that distinct gamma-band modulations (amplitude and synchrony) underlie sensory processing and perceptual decision mechanisms. These results have potential implications for the development of new diagnostic biomarkers and therapeutic targets.

**Keywords:** Perceptual decision; EEG; Simultaneous EEG/fMRI; Brain Oscillations; Gamma-band activity; Object perception; Categorization.
Sumário

A decisão perceptual representa o processo de escolha de possíveis interpretações do mundo com base na evidência sensorial externa. O papel dos ritmos cerebrais neste processo e na emergência da percepção holística de objetos, a partir do processamento hierárquico de elementos locais, permanece controverso. No entanto, tem sido proposto que as oscilações num intervalo de frequências conhecido como a banda gama (> 30 Hz), estejam envolvidas neste processamento, com relevância particular na identificação de objetos a partir de estímulos ambíguos. Vários estudos de EEG e MEG (Eletroencefalografia e Magnetoencefalografia) sugeriram que as oscilações nesta banda de frequências estão relacionadas com a integração de informação proveniente de diferentes áreas cerebrais e a capacidade de tomar decisões perceptuais. Outros processos cognitivos, como a atenção ou a capacidade de memória de trabalho, também parecem ter por base mecanismos análogos. Neste sentido, compreender os mecanismos de emergência de oscilações em relação com processos cognitivos bem como as suas bases neurais é importante para compreender a função cognitiva normal e/ou em doenças neuropsiquiátricas. Apesar do crescente interesse nesta área de estudo, ainda não é claro se a multiplicidade de padrões encontrados está relacionada com diferentes módulos cognitivos que coexistem na mesma tarefa.

Neste estudo, utilizámos EEG para estudar os momentos de decisão perceptual em tarefas visuais com estímulos ambíguos (estímulos Mooney, imagens compostas de fragmentos negros e brancos sem interpretação perceptual imediata). Dado que o momento da percepção do objecto era variável foi assim possível separar os componentes sensoriais e motores daqueles relacionados com a decisão perceptual. Assim, construímos novos paradigmas para comparar directamente estados de percepção vs. não percepção do mesmo estímulo físico. Foi possível identificar actividade em duas sub-bandas distintas (40 Hz e 60 Hz) com importância para a percepção holística. Estes resultados foram confirmados com classificadores automáticos usando como entradas as características do sinal obtidas no domínio das frequências.

Para podermos identificar as fontes (em termos da distribuição espacial no cérebro) destas sub-bandas, recorremos a uma técnica multimodal com melhor resolução espaço-temporal que o EEG. Usando electroencefalografia e Imagem por Ressonância Magnética funcional em simultâneo (EEG/fMRI), descobrimos que aquelas sub-bandas da banda gama reflectem diferentes substratos neurais e mecanismos cognitivos. Neste
sentido, pelo menos dois módulos estão envolvidos na rede da percepção holística. Um sediado no córtex visual (60 Hz) e outro na ínsula anterior (40 Hz). Estes resultados permitem compreender melhor a especialização das redes de tomada de decisão e mostram que os actuais modelos neuronais da localização espacial da banda gama devem considerar a sua dualidade, separando-a em diferentes sub-bandas, com diferentes funções. Estes dados podem trazer novas perspectivas sobre o papel funcional das diferentes sub-bandas na cognição normal, assim como em doenças como o autismo ou a esquizofrenia, onde vários circuitos (quer visuais, quer de decisão) parecem estar afectados.

Uma questão de interesse científico considerável, é se é a amplitude das oscilações o factor relevante para a codificação dos estímulos como um todo (percepção holística) ou, por outro lado, se é a sincronização entre áreas cerebrais que desempenha o papel chave. Para responder a esta questão estudámos uma população com síndrome de Williams (WS). Esta condição é caracterizada por dificuldades na integração visual e processamento holístico (“como se não vissem a floresta, mas apenas as árvores”). Como a sincronia está relacionada com a coerência central, esta deveria estar afectada neste modelo de disrupção da percepção holística. Pela primeira vez, mostrámos que a sincronização neuronal está reduzida neste grupo ao mesmo tempo que há um aumento da amplitude das oscilações na mesma banda de frequências (25-45 Hz). Esta combinação de uma dramática perda de sincronia mesmo na presença de um aumento concomitante da amplitude representa uma forte evidência de que a sincronia está subjacente à coerência central.

Com o objectivo de melhorar a resolução espacial e identificar directamente as fontes destas oscilações, aproveitámos a oportunidade única proporcionada pelo mapeamento funcional de um doente com epilepsia usando electrocorticotografia (ECoG). Neste caso, foi possível identificar um padrão posterior-anterior de actividade que é consistente com a noção de que existem bandas representativas de diferentes processos cognitivos. As frequências mais baixas dominam nas áreas anteriores ventro-temporais (<100 Hz) e modulações de frequência numa banda mais alta dominam nas regiões occipitais.

Em suma, estes estudos permitiram contribuir para esclarecimento do papel das oscilações nos mecanismos de decisão perceptual. Conclui-se assim que é possível separar diferentes sub-bandas dentro da banda gama e que estas refletem diferentes mecanismos.
cognitivos. Estas têm uma função específica na decisão perceptual e uma origem distinta no córtex. A actividade na banda mais baixa (40 Hz), está relacionada com a rede de tomada de decisão. Por outro lado, a banda dos 60 Hz está essencialmente localizada em regiões de processamento visual primário.

A demonstração de uma topografia funcional específica para sub-bandas específicas, dentro da mesma tarefa, mostra que diferentes modulações das mesmas (amplitude e sincronia) estão na base do processamento sensorial e dos mecanismos de decisão perceptual. Estes resultados, em conjunto com o estudo dos mecanismos moleculares que dão origem às oscilações, têm implicações para a compreensão dos fenómenos perceptuais na saúde e na doença, bem como no possível desenvolvimento de novos biomarcadores de diagnóstico e alvos terapêuticos.

(escreto sem recurso ao novo acordo ortográfico)
CHAPTER I

General Introduction
In this introductory chapter a brief overview of the perceptual decision making processes and their neuronal correlates will be described. At the end of this chapter the thesis aims and outline are presented.

**Perceptual decision making**

Perceptual decision making is defined as the choice of one option from a number of options based in the evaluation of information gathered from sensory systems. On the other hand, high-level decision making is associated to decisions that involve reward, motivation and complex cognitive context. The later include decisions concerning buying or not new shoes, or deciding if to spend more time doing a boring task or not, etc.. In fact, any interpretation of sensory information requires in itself an implicit decision process. This is used to influence our behavior \(^1\). For example, in a face/object categorical perception task, subjects are presented with images of faces and objects and for each image have to decide if a face is present and then report their choice by pressing a button. In this case, images reaching the brain are interpreted and decision is translated into a button press. Using similar paradigms, the underlying cognitive mechanisms that control decisions can be studied \(^2\). Decision can be fast (<100 ms) and is made by swift integration of incoming information \(^3\). This is a process that involves distinct brain regions, from early visual areas to frontal decision-related areas such as dorsolateral prefrontal cortex (DLPFC) \(^4\). Decision-making is an area of very active research and both animal \(^5\) and human studies have addressed perceptual decision processes \(^6\). Studies in animals focused on the visual domain and showed that information from low-level brain areas is integrated in higher level brain regions until the decision is reached \(^7\). Evidence for a similar process in humans was recently provided \(^8\)-\(^11\). Simple holistic perceptual decision processes start in low-level visual areas but require a highly distributed neural network \(^12\) including motor-planning and affective processing regions as well as the reward or conflict monitoring system \(^13\).

**Holistic visual perception**

General cognitive responses underlying visual perception are based on distinct holistic and part-based processes. The processes of recognizing faces or discriminating non-face categories \(^14\) may be solved using a configural strategy (e.g. the relations among facial
features within a face), a holistic (face as a gestalt or a whole) or part-based analysis (the primary processing of object components) \(^{15-17}\). As a first step of the process, faces might first be processed as wholes and only thereafter in a piecemeal manner \(^{18-20}\), which would imply a global precedence mechanism and that its parts are processed independently. But this is not the case for inverted faces or non-face objects \(^{15,16,21}\) which are processed piecewise before the whole. Holistic processing of faces can be defined as the fact that facial features are integrated, rather than being represented independently from each other \(^{16,20,22}\).

**Mooney Stimuli Paradigm – a tool to study holistic processing**

In the first half of the last century, Gestalt psychology showed that features with common characteristics (proximity, similarity, continuity or closure) tend to be automatically bound together as a coherent percept.

Mooney faces (incomplete black and white photographs of faces \(^{23}\); see Figure 1-1) are used to study the specific process of holistic visual perception since they are processed as a whole \(^{18,24}\). Their perception involves integration of the fragmentary parts into coherent percepts, based on the Gestalt principle of closure \(^{23,25}\).

![Figure 1-1. Mooney Face Stimuli. A) Upside-down human face (Mooney face). B) Upright Mooney Face. Note that this is the same picture rotated, and the inverted version is harder to perceive. These are used to investigate the role of high-frequency gamma-band activity during perceptual decision processes.](image)
Although Mooney faces are easily identified as faces when presented in the upright orientation, they are difficult to recognize when inverted. These stimuli are useful to understand perceptual decision processes because they evoke momentary events of perceptual discovery. By leading to sudden perceptual interpretation they create an “ah-ah” or so-called “eureka” perceptual moment. At this moment, the component face features (eyes, nose or mouth) are not really identified separately. As a consequence, a holistic perception mechanism is instantly activated 26–31. Mooney stimuli are thus used to study holistic visual perception that leads to face perception because faces cannot be parsed or segmented by bottom-up processes in order to recognize the face 32. Some authors did nevertheless suggest that this processing is preceded by an initial “structural encoding” mechanism integrating features in a not fully identified face 33 with faces being identified at a global level 34. This step might occur simultaneously at multiple locations of the visual field 35. Given the cortical hierarchy, the perceptual grouping process might also be viewed as a succession of steps (implicit or explicit) that are specialized and dependent on the input from previous stages.

Kanwisher and colleagues showed that a specific region in the fusiform gyrus, the so-called fusiform face area (FFA 36), was activated in response to the upright Mooney faces 24,37. Several studies have investigated the specialness of face processing 38,39 and the expertise theory (the specialized processing of faces), based on the ecological importance of faces in our environment 40,41. Moreover, intracranial and functional imaging studies revealed brain regions that are involved specifically in face detection 42–44. These were shown to be lateralized to the right hemisphere 45,46. Accordingly, previous studies showed a specific impairment of holistic perception of individual faces when a brain lesion is located in the right hemisphere but this was not the case for object recognition 27,47.

The visual system solves a giant combinatorial problem

Although a large body of knowledge has been accumulated concerning the face/object processing network little is known regarding the contribution of each brain region to the specific components of perceptual face analysis. Furthermore, even the separation of low-level sensory components from high-level perceptual decision is poorly understood and the mechanisms of feature integration remain to be clarified. This may be due to the
complexity of the visual system and its large capacity for the recognition of a vast array of object subordinate categories.

The visual system is capable of solving a giant combinatorial problem. For example, a given image is composed by a collection of features that may, or may not, be integrated to identify one object or multiple objects. Moreover, the object within a scene has to be separated from the whole image and from other objects 48. This is an everyday visual segmentation problem, e.g. each time you want to find a certain object within a shelf or to identify a friend in a crowd. This process involves identifying which features belong to which object or to integrate face features for example to recognize your friend. The problem becomes daunting because objects may appear in different spatial locations and orientations. Thus, these mechanisms are required to be flexible. However, the visual system has solved this problem since it has no problem in segmenting any new image within a fraction of a second 35. Identifying the relationships among features in an image needed for object recognition is dependent on the integration of information from functionally linked brain regions. This perceptual grouping of features into an object representation by a Gestalt criteria instantiates the “binding problem” 49.

Object processing involves a large population of cells distributed over several brain regions but it is not clear which neuronal mechanisms solves that computational problem. Hence, the way the brain solves this integration of information is still under debate.

**Brain Oscillations and feature integration – The binding by synchrony hypothesis**

One salient feature of neuroelectric brain signals is that they oscillate in various frequencies. Oscillatory activity in the brain refers to periodic fluctuations in the activity of a neuronal structure 50. It has been suggested that brain oscillations are of fundamental importance for mediating “higher-level” processing over widespread and functionally specialized areas in the human brain requiring neuronal communication 51-53. Castelo-Branco et al., 1998 54 showed that cortical assemblies display a broad-band oscillatory activity spanning multiple frequencies in response to visual input. The role of neural synchrony (cell assemblies representing grouped features which “work” in phase 55) and oscillatory activity in cortical networks, has been conceptually expanded to provide a general mechanism for the coordination of distributed neural activity patterns (Figure1-2;
for review see Uhlhaas et al., 2009 and Velik, 2012 [56,57]. Previous studies suggested that integration of anatomically distributed processing (coupling and uncoupling of distributed functional networks responding to features of the same object) occurs by Synchronization of oscillatory activity [58-60] or that neurons forming a functional assembly are bound together by synchronization of their action potentials [51,61].

Figure 1-2. Temporal Binding by synchrony. A) Visual information is processed in distributed brain regions (e.g. shape, color, motion). Nonetheless the grouping of different features pertaining to distinct objects in a visual scene is achieved (the firing to the dog or to the woman). B) Neurons from different areas coding for different features of a same object become bound by synchronized oscillations [62]. E.g., spiking of neurons responding to a square, blue and downward motion are synchronized. This is a more “economic” mechanism that accounts for the role of inhibitory interneurons (mainly GABAergic; gamma-aminobutyric acid) without the need of extensive learning of possible objects that would be a combinatorial problem the brain could not deal with. C) Synchronization of functionally linked but distributed brain regions is assumed to be crucial to top-down and bottom-up processes [48,62-64]. (Adapted from Fries, 2009 and Velik, 2012 [53,57])
It has accordingly been proposed, that the discharges of neurons undergo particular temporal patterning and become synchronous if they participate in the encoding of related information. In a general sense, binding is the process of combining stimulus features to form an object representation. Synchronization occurs at a millisecond time scale and can be sustained over periods ranging from tens to thousands of milliseconds and spanning a broad frequency range. It arises between cells in the same cortical column, different columns, different cortical areas, and the two hemispheres or even between sensory and motor areas. Synchronization across a set of neurons depends on the features preference of the individual cells and on the configuration of the stimuli (e.g., common motion, orientation, color, contrast, duration and size). Neuron synchronization tends to reflect gestalt criteria: features (having similar properties) in images tend to be grouped together into objects, in the same manner neurons responding to similar properties in the image tend to synchronize (Figure 1-2).

The coding synchronization hypothesis provided a solution for the binding and superposition problems. By conceptualizing the emergence of dynamic neuronal assemblies, it provided a solution for the integration of features into a coherent representation while distinguishing different representations when they appear simultaneously. Long-range inter-area communication can occur between neuronal assemblies belonging to the same functional network firing synchronously in a certain frequency band. This synchronization pattern correlates with animal’s performance in a perceptual discrimination task.

Synchronization of oscillatory activity plays an important role in perceptual grouping of features into larger coherent units and it is likely important in brain maturation. However, which particular regions engage in this pattern, at which frequency and which are the roles of specific narrow-bands within the broad-band of all oscillatory activity are still open questions.

Although no single theory provides a complete answer to the complexity of neuronal interactions, synchronization of functionally linked but distributed brain regions is assumed to be important for integration of information from local or distinct areas in the visual system, being crucial to top-down and bottom-up processes. In line with this notion, we have studied brain oscillations (amplitude and synchrony) and their potential contribution to holistic visual perception.
Neural Oscillations: the distinct frequency bands

Oscillations were first described in early Electroencephalographic (EEG) experiments (for a review see: Uhlhaas et al., 2009, Ahmed & Cash, 2013 or Engel & Lopes da Silva, 2012) mainly through animal studies obtained from low-level visual areas. In humans, oscillatory activity has been described in the auditory, visual, motor and somatosensory modalities. Rhythmic activity of electrical data acquired during EEG recordings discriminate between different sensory and cognitive processes. Typically, oscillatory activity is classified accordingly to their oscillatory frequency (see Table 1-1).

### Table 1-1. Neural Oscillations in Cortical networks (Adapted from Uhlhaas et al., 2010)

<table>
<thead>
<tr>
<th>Delta  (0.5-4 Hz)</th>
<th>Theta (4-7 Hz)</th>
<th>Alpha (8-12 Hz)</th>
<th>Beta (13-30 Hz)</th>
<th>Gamma (30-200 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anatomy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n.a.</td>
<td>Hippocampus, prefrontal cortex, sensory cortex</td>
<td>Thalamus, sensory cortex, motor cortex, hippocampus</td>
<td>All cortical structures, hippocampus, basal ganglia</td>
<td>All brain structures, retina, olfactory bulb</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>present during normal sleep, large-scale integration</td>
<td>Memory, synaptic plasticity, top-down control</td>
<td>Inhibition, attention, awareness, consciousness, top-down control, visual idle state</td>
<td>Sensory gating, attention, perception, motor control, long-range synchronization</td>
<td>(Gestalt) Perception, attention, memory, consciousness, synaptic plasticity, motor control, motor idle state</td>
</tr>
</tbody>
</table>

Delta oscillations (0.5-4 Hz), present during normal sleep are important for large-scale integration. Theta oscillations (4-7 Hz) mainly correlate with attention and working memory processes. Alpha oscillations (8-12 Hz), the most prevalent oscillation in the brain, related to the state of arousal in individuals. Alpha desynchronization implies larger visual processing. Local desynchronization of beta-oscillations (12-30 Hz) have largely been associated with movement preparation and execution in the motor cortex. Gamma-band oscillations are a phenomenon of fast neuronal oscillatory activity. Activity in this range occurs at frequencies over 30 Hz, although a precise definition of the lower end of this band is unclear. It is originated in inhibitory interneuron networks and can be detected as local field potentials (see Bartos et al., 2007 and Buzsáki & Wang, 2012).
for review on the origins of gamma oscillations). Recent years have brought a substantial interest in this frequency range due to their suggested role in higher cognitive functions.

**Gamma-band and perception of a Gestalt**

Neural oscillations and synchronization in the gamma frequency range support perceptual and value-based decision making. To clarify the role of neural oscillatory activity and synchrony in response to perceptual decision, we focused in this high frequency (>30 Hz) oscillatory activity band. This was described to correlate with many stimulus-induced cognitive functions, including attention, memory, perceptual binding, object recognition, and language perception. Gamma oscillations have also been reported to be involved in visual perception and to possibly subserve the more general function of feature binding reflecting local processing. In particular, previous studies reported a high correlation between oscillatory activity (amplitude or phase) in the gamma-band and Gestalt perception with high correlation with stimulus type, coverage and motion (for a review see Uhihaas et al., 2011 and Güntekin & Başar, 2014).

**Evoked or induced oscillatory activity**

Oscillations at this frequency range are distinguished into three main types: 1) spontaneous activity, 2) evoked oscillations, time-locked to an event and 3) induced oscillations, time-locked but not phase-locked to an event. These non-stimulus locked oscillations underlie feature binding and the formation of meaningful visual neuronal object representations. Evoked activity was reported to contribute to the formation of early object representations of well-known stimulus categories and to be sensitive to task difficulty. Taken together, both evoked and induced gamma-band activity reflect high-level stimulus features and perceptual grouping, are related to attention and modulated by memory.

Gamma oscillations may represent an attractive solution to the binding problem because they occur in a millisecond time scale and were shown to synchronize distinct neuronal assemblies, possibly grouping features together to be processed as a coherent whole.
Sub-bands within Gamma-band: their role and origins

The Gamma-band is a broad-band that includes frequencies up to 100 Hz (in EEG) but even higher frequencies have been reported for magnetoencephalography (MEG) or electrocorticographic (ECOG) recordings (up to 250 Hz). It is still controversial whether stimulus modulations are mediated at different sub-bands within this range (which have very different properties, and likely roles) and if different brain regions have different contributions to distinct frequency sub-bands \(^{88}\). However, some evidence has been reported in favor of this hypothesis particularly concerning a few specific visual perception paradigms \(^{88,94,96,121}\).

Two clearly separated sub-bands have been reported in MEG studies, in response to visual inputs, one around 40 Hz and another between 70-80 Hz with its sources laying in the calcarine sulcus \(^{90,122,123}\) closely related to the blood-oxygen level dependent (BOLD) response \(^{124}\) in primary visual areas \(^{122}\) and dependent on the local concentration of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) \(^{125}\). Others have shown visual (object recognition and motion perception) high-gamma activity in a slightly different range (60-90 Hz) localized to the primary visual areas \(^{96}\), lateral occipital-temporal and inferior temporal cortical areas \(^{118}\). Cortical sources of gamma oscillatory activity have also been studied with an auditory paradigm \(^{126,127}\), demonstrating similar frequency sub-bands but with sources located in the inferior anterior cingulate and adjacent inferior frontal gyrus for the low gamma-band (30-55 Hz) and in the superior part of the middle frontal gyrus for the high gamma-band (65-85 Hz) activity. Other neuroimaging studies have reported attentional effects in visual cortex at lower gamma frequency range \(^{93,104,128}\). Moreover, an ECoG study by Ray et al., 2008 \(^{34}\) reported enhanced high-frequency gamma oscillatory activity over the auditory and somatosensory cortex of the monkey.

Care must be taken with the frequency band range definition since thresholds for separating these bands have been somewhat arbitrary and vary between studies. However, these results further suggest a close association between low and high gamma and cognitive processes \(^{129}\). Another crucial distinction may be made concerning the definition of narrowband and broadband gamma. The former is centered around 40 Hz \(^{90}\). The latter, typically starts at 30 Hz and extends up to >150 Hz \(^{130}\). Accordingly, an human ECoG study of Gestalt perception showed that the activity in the narrow frequency band was increased for the perception trials in early visual areas while increases in later visual areas were more broadband \(^{30}\). This was also reported in a recent MEG study \(^{96}\).
In spite of these findings for visual and auditory tasks, the role of low and high (or narrow and broad-band) gamma activity concerning visual perceptual decision paradigms are still controversial and in need of clarification, although it is likely that they may serve distinct roles depending on the visual area in which each occurs.

**Gamma oscillations in pathological conditions**

Abnormalities of gamma oscillations have been found in several pathological conditions. Thus, establishing the role of cortical gamma oscillations may be potentially helpful in diagnosis or definition of therapeutic targets. For a review regarding the potential role of changes in neuronal dynamics as pathophysiological mechanisms in brain disorders that can help to understand the origins of neuropsychiatric disorders and the development of effective therapies see Uhlhaas & Singer, 2012, Yener & Başar, 2013 and Başar et al., 2013.

High gamma oscillations (>60 Hz) have been found to be impaired in pathological conditions such as epilepsy, schizophrenia or Autism Spectrum Disorders (ASD). Uhlhaas and colleagues reported abnormal synchronization in the beta-band and mainly intact gamma-band activity in schizophrenia patients relative to controls during a Mooney face perception task (Figure 1-3). This was more evident for the long-range synchronization between frontal, temporal and parietal electrodes during Gestalt perception, than for local-synchronization.

![Figure 1-3. Disrupted phase synchronization during Gestalt perception in schizophrenia (ScZ). The time-frequency panels show the average phase synchrony (indicated by the colored scale) over time for all electrodes. In patients with schizophrenia, phase synchrony between 200–300 ms was significantly reduced relative to controls (SD stands for standard deviations). (Adapted from Uhlhaas et al., 2010)](image_url)
Grice et al., 2001 \(^{138}\) showed that in Williams syndrome (WS) and ASD populations an enhanced amplitude of N170 to inverted faces correlated with a decrease of gamma-band activity around 40 Hz as recorded by EEG. WS is a genetic neurodevelopmental disorder and a well-known model of impaired coherence \(^{139,140}\). It has been studied to probe whether gamma-band activity reflects changed holistic perception \(^{121}\). In fact, the application of a three-dimensional (3D) structure-from-motion (SFM) stimuli to WS patients resulted in higher oscillatory activity within the low gamma-band (\(\sim 40\) Hz) in comparison to controls while performance in the face recognition task employed was preserved \(^{121}\). The task employed requires integration of local perceptual features and motion cues to achieve coherent perception \(^{141}\). In a sense, this is a form of perceptual binding that involves integration across dorsal and ventral visual pathways by inferring 3D object structure based on correlated local motion \(^{142}\).

Such a clinical model may constitute a relevant key in the understanding of the neuronal underpinnings of oscillatory gamma-band activity and synchrony, to holistic visual perception during perceptual decision making tasks.

**Face/object perception networks**

The processing of face/object stimuli and the process of deciding which type of object category is present and what are its spatial properties, is carried out in the visual pathways that are physiological divided in the dorsal and ventral visual streams (Figure 1-4). The former, lying in the parietal lobe is important for spatial localization (‘where’ an object is) and motion processing. The latter, in inferotemporal areas, has an important role on object recognition and identification (‘what’ it is) \(^{143}\).

EEG studies from Bentin et al., 1996 \(^{144}\) described in detail the above mentioned negative potential 170 ms after stimulus onset that was elicited by human faces mainly for the ventro-temporal regions. It was shown that the N170 precedes within-category identification \(^{17}\) and that it is significantly larger and delayed for inverted faces \(^{21,144,145}\). This enhancement in the N170 amplitude reflects the difficulty of holistic processing to encode the upside-down face \(^{144,145}\). Accordingly, the holistic processing of faces is disrupted with inversion \(^{147}\). This signal was associated with parieto-occipital and temporal sources and thus correlated to the two main visual pathways (for a review refer to Rossion, 2014 \(^{148}\)).
**Figure 1-4. Schematic representation of Ventral and Dorsal visual streams.** Ventral stream projects to the ventro-temporal areas while Dorsal stream projects to the dorso-parietal areas.

Regarding functional magnetic resonance imaging (fMRI) studies, in terms of the ventral areas involved in the ‘what’ system, the areas (Regions of Interest, ROIs) typically reported include the fusiform face area (FFA), the occipital face area (OFA) and the superior temporal sulcus (STS). Also well described is the object-selective region called lateral occipital complex (LOC).

Concerning high-level mechanisms, the important roles of anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) have been established and related to the decision-making process. The OFC is described as underlying processing of social and emotional information and ACC would be involved in selection and control of appropriate behavior. These two areas act in concert with other prefrontal areas (DLPFC and posterior medial frontal cortex) to make appropriate choices. Moreover, other functional connections involving fronto-parietal or operculo-insular networks and thalamo-cortical circuits are also associated with perceptual decision making suggesting that it involves a comprehensive network of multiple brain regions (see Figure 1-5 for schematic representation of these object-selective and perceptual decision related regions).

Defining the underlying processes that integrate all the information of these distributed areas is a complex problem but novel discoveries are revealing new concepts beyond the ‘tip of the iceberg’. The accumulating evidence that face processing in the human brain is subserved by distinct neural (visual and decision) circuits in which both
whole-face configurations and face parts are processed raises the question of how is holistic visual feature integration over multiple networks performed in the brain? To understand the mechanism by which individual features are combined together to form a coherent (holistic) percept is a high topic of research.

**Figure 1-5. Representation of object-selective and perceptual decision related regions.** Face selective ROIs (FFA) were defined as regions that respond more to faces than houses. LOC and parahippocampal place area (PPA) respond more strongly to objects and places, respectively. Middle temporal area (MT) responds strongly to motion stimuli. Frontal regions (DLPFC, ACC) as well as the insula are activated by perceptual decision-making tasks. Note that early visual areas (color dark blue) also contain the OFA. Labeling of brain areas is provided in just one view but the color-code stands the same for all the views. Adapted from Grill-Spector et al., 2004.
Imaging Brain activity during object categorization

fMRI studies have been used to study face/object perception networks. Studies applying fMRI to investigate perceptual decision in an ambiguous face categorization task revealed brain regions in the ventral visual cortex with high spatial resolution \(^6\). Moreover, results from subjects performing those tasks were highly consistent with intracranial recording studies in monkeys \(^{15}\). In addition, fMRI has the benefit of providing information on whole-brain activity. Nonetheless, fMRI lacks the temporal resolution of EEG and MEG techniques also covering the whole cortex and provide accurate temporal information about the underlying neuronal processes \(^8\). In spite of the fact that obtained temporal resolution is very good, the spatial information of these techniques is limited.

To overcome the lack of spatial resolution from EEG and MEG, several techniques were developed to study the neuronal sources of these data and to analyze the interactions between brain regions in visual perceptual decision processes (for a review please refer to Heekeren et al., 2008 and Philiaastides & Heekeren, 2009 \(^{1,155}\)). However, to localize the brain activity, fMRI is still the best option. In line with this notion, many studies started to integrate information from different imaging modalities taking advantage of the strengths of each technique, including invasive recordings \(^{156-158}\).

The simultaneous recording of complementary imaging data helped to clarify those perceptual processes. In particular, the use of multimodal approaches such as simultaneous EEG/fMRI measurements and EEG-informed fMRI analysis techniques \(^{158}\) allowed to characterize the spatiotemporal neuronal dynamics of underlying perceptual decision making in humans \(^{12}\).
General outline and aims of the thesis

The neuronal mechanisms underlying perceptual decision processes and the putative relation between gamma-band activity and holistic perception have been widely studied, but many aspects remain either largely unknown or controversial.

In this thesis we aimed to separate low and high-level cognitive components of perceptual decision using a multimodal approach that combined EEG and fMRI with respective relatively high temporal and spatial resolution to help clarify the origin of the gamma oscillations.

In the field of basic and clinical neuroscience this can be done in different ways. One can use fMRI and EEG to study brain activity, either in clinical or research settings, and oscillations may also be observed by using intracranial EEG, or MEG recordings. MEG has improved sensitivity (in comparison with EEG) in detecting sources of high frequency oscillations. Nonetheless, sensitivity can be improved, through simultaneous recordings and analysis of EEG and fMRI, which resulted in EEG/fMRI being now widely accepted as a predominant tool to study brain oscillations (both in terms of amplitude and synchrony). This thesis aimed to study the holistic perceptual decision-making process through state-of-the-art brain imaging techniques and multimodal approaches, and thus help to clarify their neuronal correlates.

In Chapter 2 – Methods, we provide an overview of the methodological approaches used in this thesis.

Chapter 3 to 6 present the results obtained using the chosen experimental tasks. Chapter 3 aims to provide insights into the putative role of gamma-band activity in encoding unpredictable holistic visual perception (with static and dynamic Mooney stimuli) as recorded by high density EEG. A critical issue to understand how perception is predicted from neuronal activity patterns is to separate sensation and perception in time and to separate activity at the time of recognition from activity related to the behavioral output. These tasks take advantage of the well-known bias in holistic processing of Mooney stimuli, by delaying the moment of global integration, through stimulus inversion. As a result it was possible to dissociate percepts from the visual input using physically similar but perceptually distinct trials and to probe the functional role of brain oscillations and synchrony in the emergence of those perceptual decision states.

Chapter 4 explores the advantages of simultaneous EEG/fMRI measures to map the networks causing perceptual decision and object categorization at defined perceptual
time points. The findings of this study may help elucidate the regions critically involved in generating distinct oscillatory activity patterns within the gamma-band and their contribution to visual cognition processes.

Chapter 5 provides a non-invasive EEG study of neuronal oscillatory activity in a clinical model of impaired coherence. While the role of low and high gamma-band activity is still matter of debate, this chapter provides a clue for understanding their putative role in the coherent perception of object 2D/3D representations. Moreover, it provides new evidence for a specific role of synchrony in visual integration.

In order to establish the sources of brain oscillations in perception, it is critical to assess the neural correlates of oscillations with high temporal and spatial resolution. Chapter 6 describes an experimental task using invasive recordings. An epilepsy patient submitted to pre-surgical invasive electrocorticographic assessment provided an opportunity to study the electrophysiological correlates of functional brain activation and face/object perception in detail with direct relationship with behavior. We acquired data from perceptual decision-related tasks and this was useful to validate our findings of spatiotemporal networks identified with EEG/fMRI with extremely high spatial and temporal resolution.

The study of the neuronal mechanisms underlying gamma-band activity is an active area of research. Localization of gamma-band activity in frequency, time and space (their brain generators) should help clarify their roles in perceptual decision and may help to better understand mechanisms of fragmented perception in the healthy and diseased brain. In the last chapter of the thesis (Chapter 7), we provide a general discussion regarding how holistic perception is encoded in distinct neural activity patterns and the putative role of distinct gamma sub-bands in this process.
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METHODS
Brain Imaging Methods

Unimodal and multimodal approaches for mapping brain function
Electroencephalography and Electrocorticography, as well as functional magnetic resonance imaging provide unique contributions to the study of specific aspects of neuronal activity. Due to their distinct advantages and pitfalls they have been used as complementary techniques for studying brain function. In this chapter, details about data acquisition and analysis are described for unimodal and multimodal approaches using those techniques. As a note, in this work experimental paradigms and other specific analysis procedures were implemented and they are described in the respective chapters in the results section.

EEG: origin and measurement

The electrical measurement of extra-cellular neuronal currents and activity in the brain as recorded by non-invasive electrodes placed on the scalp is known as electroencephalography (EEG) \(^1\). Human EEG was first recorded by the German psychiatrist Hans Berger in 1920s and, since then, its interest in both basic and clinical research has increased exponentially \(^2\). This is due to the fact that EEG provides an online measure of brain function during cognitive, perceptual, sensory and motor tasks in health and disease \(^3\). With the methodological improvements applied to electrodes types, recording and analysis tools, high-density EEG is nowadays a powerful non-invasive brain-imaging technique which provides excellent temporal resolution in the sub millisecond range.

Neurons are excitable cells with intrinsic characteristic electrical properties driven by changes in membrane permeability to specific ions. For more details on membrane potentials and the generation of these neuronal and extracellular currents please refer to Mulert & Lemieux, 2010 \(^4\). Briefly, there are two distinct kinds of postsynaptic potentials: 1) those which depolarize the membrane of the output neuron, known as excitatory postsynaptic potentials (EPSPs), and 2) those which act on the membrane in the opposite manner known as inhibitory postsynaptic potentials (IPSPs). The EPSP and IPSP lead, respectively, to a current sink and a current source generated in the extracellular medium at the level of active synapses that gives rise to recordable extracellular potential differences, and thus this process can be compared to and modelled by a tiny electrical dipole inside the brain \(^5\). However, the electric potentials generated by single neurons are too small and desynchronized to be recorded by EEG. Thus, EEG yields the measurement along the scalp of summed electrical activity produced by the synchronous firing of
thousands of neurons within the brain (see Figure 2-1) with spatial radial orientation\(^1,2,4\). Since the magnitude of the signal falls off with the square of the distance, activity from deep sources is harder to detect and register than currents near the skull.

![Diagram of brain with EEG electrode, scalp, neural activity, dipole sources, and EEG labels](image)

**Figure 2-1. Schematic representation of the generation of scalp potentials.** Measured EEG signal reflects the summation of neural activity of many synchronously active neurons in the cortical layers. It is dependent on the nature and location of their dipole sources (summed activity within a small area of cortex) and on the resistive properties of the brain, skull and scalp as well as on the location of the recording electrodes\(^1\).

**Procedure and practicalities**

EEG scalp recordings are performed with caps providing electrodes positioned accordingly to the 10-20 international system (American EEG Society Guidelines, 1994) (Figure 2-2 A) and connected to an amplifier device that is in turn connected to the acquisition computer synchronized with the stimulus presentation computer (Figure 2-2 B). The simultaneous recording using many electrodes helps to overcome spatial resolution limitations. Nevertheless, despite the use of a standard system for electrode positioning, the relative locations of the electrodes with respect to specific brain structures can only be estimated very roughly.

The EEG signal is recorded with respect to a reference (which can be a single electrode or the computed average of several electrodes) that may differ depending on
the purpose of the recording. EEG amplifiers providing sampling rates (SR) up to 20 kHz have enabled the study of high frequency components of the signal.

However, every recording is susceptible to artifacts (biological or electromagnetic noise) and signal correction prior to the analysis of the signal of interest has to be performed. During offline analysis these artifact related signals must be eliminated or corrected as they may mask and influence relevant results. Electromyography (EMG) is a high frequency signal resulting from muscle activity and may be confounded with gamma activity. Microsaccades may also distort the signal and characteristic oscillatory patterns around 10 Hz (the alpha rhythm) will appear if the subject closes his eyes. These artifacts (which may also be biological) can be corrected by low-pass filtering, by rejecting epochs that contain them, by visual inspection or by using advanced algorithms prepared to isolate and handle with artifact signals. Such a case is the Independent Component Analysis (ICA), which helps identifying artifact components based on their topography.

Figure 2.2. EEG channel locations and acquisition setup. A) 64 channel montage: Electrodes are placed according to the International 10-20 system and labeled according to their locations in the scalp. B) EEG acquisition setup: Data is recorded using an EEG quick-cap interfaced with the amplifier through the headbox. The SynAmps2 amplifier is connected to the acquisition computer that is synchronized with the stimulus presentation computer. Data are stored and further processed offline.
**EEG data analysis**

**Event-related potentials**

Among the main neurophysiological signals that can be recorded, two main types of activity can be listed: 1) spontaneous EEG activity or 2) the so-called event related potentials (ERPs) 1. ERPs are the electrophysiological measurements of neuronal activity that occur locked to a stimulus 5. The ERP components become visible by means of averaging the electric potential across many trials time-locked to a certain stimulus or response onset 4. They reflect the transient burst of neuronal activity of a large population of neurons in response to that event 12. By averaging, the brain response of interest is assumed to be the same or at least very similar from trial to trial and the random fluctuations will cancel each other out (they might be positive or negative in different segments) and brain activity time-locked to the stimulus presentation will add up and become visible in the average. ERP time-courses are classically labelled by polarity (negative or positive), amplitude, scalp distribution, and latency (e.g. P100 refers to a positive potential around 100ms and N170 is a negative peak with latency of 170 ms, a characteristic component widely believed to be selective for faces 13,14 (see Figure 2-3)).

< Figure 2-3. Example of an ERP. Evoked potential is shown for an average of 50 trials recorded from one subject during a Mooney face perception task. Note the N170 peak in the ERP waveform. Other components (P100 and P200) may also be identified.

The ERPs provide information on neurocognitive mechanisms with great temporal resolution (in the range of milliseconds) but have poor spatial resolution. Distortion of the electric potentials reaching the recording electrodes on the scalp occurs due to the resistive properties of the different tissues surrounding the brain, such as the cerebrospinal fluid, the skull and the scalp, which have different conductivities 15. Due to volume conduction, evoked potentials are significantly distorted by these compartments,
which act as low pass filters and they also may reflect more than one cognitive operation. Spatial resolution may be increased with high-density EEG, or, alternatively, Magnetoencephalography (MEG) or in special circumstances direct electrocorticographic invasive recordings. Since the magnetic fields penetrate the skull without impedance, the anatomical origins of the signals are easier to localize in MEG studies. Adding to the information yielded by ERP analysis, one can also explore the patterns of synchronization of neuronal activity as revealed by brain oscillations, which are typically classified into different frequency bands.

Neuronal brain oscillations

EEG data reflecting cognitive processes are composed of a combination of components that are usually analyzed in the time-domain, in particular, with the ERP methodology as described above. However, during the last decades efforts have been made to improve the analysis of these data in the frequency domain, since additional insights can emerge from decomposing the EEG signals into magnitude and phase information of different frequencies over time. The alpha (8-12 Hz), beta (around 20 Hz) and gamma (>30 Hz) bands have been widely studied and are related with many cognitive functions (for details see Chapter 1). Understanding oscillatory activity in these and other frequency ranges is crucial to understand communication in the brain as these are essential to integrate local information by coupling functional networks. For a detailed description of the neocortical circuits and mechanisms underlying the generation of oscillations and synchrony please refer to Uhlhaas et al., 2010.

Time-frequency analysis - Measuring evoked and induced oscillatory activity (amplitude and synchrony)

To measure the amplitude and synchrony of oscillations in EEG data the signal must be transformed into the frequency domain. New methods in the so-called ‘time-frequency analysis’ have allowed a better characterization of the spectral changes over time. In these signal decompositions, the amplitude and phase of the frequency bands of interest are estimated for different time windows along the trial, yielding both time and frequency descriptions. ERPs represent the average EEG signal over a number of experimental
trials and event-related brain oscillations (ERO) can also be assessed in a similar way \(^{24,25}\). An advantage of the time-frequency analysis of EEG, relative to ERPs, is the potential to reveal the brain’s parallel processing of information as conveyed by distinct frequency bands. The oscillations at distinct frequencies may act together to couple brain areas performing multiple neuronal processes and thus reveal co-occurring and interacting brain functions \(^{26}\).

A frequency transform of the EEG signal is made possible by using a fast Fourier transform (FFT) approach – the signal is decomposed into sinusoidal oscillations of different frequencies. Even though the obtained spectrum shows which frequencies are present in the EEG, the precise time information cannot be accessed. This can be obtained with time-frequency decomposition that combines amplitude and frequency information over time \(^{23}\). There are distinct approaches to this decomposition. The short-term Fourier transform (STFT) is based on a windowed analysis over the time series \(^{12}\). It uses a fixed duration time window irrespective of the analyzed frequency thus lacking temporal resolution for higher frequencies. A modified STFT that uses a time window adjusted across increasing frequencies may overcome this issue (this was implemented in EEGLAB software \(^{27}\)). Another possibility is to use wavelet analysis \(^{28}\). Wavelets also provide information on the time-frequency dynamics of the signal (reviewed in Herrmann et al., 1999, Samar et al., 1999, Bruns, 2004, Roach & Mathalon, 2008 or Wacker & Witte, 2013 \(^{12,28-31}\)). Time-frequency approaches can be optimized for time and frequency resolution but not for both simultaneously with the same quality because of the specificities used for transformation calculation. That is, at high frequencies the temporal resolution of a transform (e.g., wavelet, pseudo Wigner-Ville or Hilbert transformation) is better than at low frequencies and the inverse is also true – at low frequencies there is a good frequency resolution with worse temporal resolution \(^{31}\). Thus, frequency and time resolution are inversely related and a trade-off is needed to get the most information possible of the signal. The most commonly used wavelets are the Morlet wavelets \(^{12}\). A description of wavelet parameters and their influence on the resulting time-frequency can be read in Herrmann et al., 2005 and Roach & Mathalon, 2008 \(^{12,32}\). Figure 2-4 shows a time-frequency representation of an EEG signal. Every line of the plot is the result of a separate transform (each with one center frequency with a limited bandwidth around the respective frequency).
**Figure 2-4. Example of a Time-Frequency representation.** The plot illustrates the event-related spectral power of Mooney faces stimuli in a wide frequency range (7 to 100 Hz) obtained using EEGLAB. Colorscale is in decibel (dB). Note the increased activity for the Gamma-frequency range (30-80 Hz) around 200 ms.

As explained before, there is a distinction between the main types of oscillatory brain activity: stimulus-evoked oscillations or stimulus-induced oscillations as they can be phase-locked or non-phase locked to the stimulus onset, respectively.

To compute evoked oscillations, it is sufficient to average over trials and perform the time-frequency analysis of the resulting ERP. Since the activity has the same phase for every stimulus repetition it should therefore add up in the average.

By contrast, for the induced activity, averaging across trials would cancel out oscillations owing to random phase shifts. Therefore, the transform is applied at the single-trial level and the resulting absolute values are averaged together yielding the total brain oscillatory activity (see Figure 2-5 for a schematic representation of the analysis). Then, the induced oscillations are obtained by subtracting the evoked from the total activity. For that reason, evoked and induced oscillations do reflect distinct aspects of cortical network processes. Evoked activity is closely related to bottom-up sensory processing and induced activity represents the dynamics of networks with higher cognitive functions.

Furthermore, phase synchrony and coherence are distinct estimates of the synchrony of brain oscillations. Phase synchrony provides an estimate of synchrony to reveal interactions between two coupled systems (e.g., two different electrodes) and can be calculated as the phase-locking value (PLV) averaged over trials. On the other hand, linear correlations between two signals can be quantified in the frequency domain by means of the cross spectrum as a coherence measure. Phase synchronization has a better time resolution than coherence, and is only sensitive to the phases, irrespective of the amplitudes of the two signals of interest. Hence, synchronization measures have
found widespread use in neurophysiology since the analysis can be restricted to certain frequency bands reflecting specific brain rhythms, which allows relating the results to distinct cognitive processes. Care must be taken since spurious synchrony may arise from volume conduction effects \(^{42}\), i.e., different electrodes measuring the same neuronal populations despite their distinct localization (Figure 2-6; see Box2. in Tan et al., 2013 \(^{43}\) for a brief overview about other parameters that may influence the data).

**Figure 2-5. Evoked and induced oscillatory activity analysis.** The EEG signal is recorded across individual trials. Evoked activity is obtained by computing the time-frequency map of the ERP. Activity is locked (in phase) to the onset of the stimulus event. On the other hand, induced activity is calculated across single-trial time-frequency maps. This reveals two peaks of oscillatory activity, corresponding to the evoked and the induced oscillations. The average of the single-trial maps shows the evoked and the induced components together. Evoked activity is subtracted from the total activity to obtain induced oscillations. (Adapted from Tallon-Baudry & Bertrand, 1999 and Uhlhaas et al., 2010 \(^{21,35}\) )
Figure 2-6. **Phase Synchrony analysis.** Groups of neurons have an intrinsic property of oscillating. Increases in the Power of oscillations reflect increased synchrony of local neurons (i.e. measured by a single electrode). Synchrony between distant areas can be estimated through phase synchrony — quantifying the temporal relation between two oscillatory processes as recorded in channel A and B as a covariance of phase-values between them, irrespective of amplitude.

A recent work by Gross, 2014 \(^45\) reviewed the development of analytical methods in this field and is pivotal reading for additional information. This work provides a structured overview of experimental methodologies, spectral analysis techniques and methods to establish relationships between brain oscillatory activity and behavior.

**Independent component analysis (ICA)**

The EEG signal that is measured at the scalp electrodes is a mixture of various cortical source signals that add together to create the observed waveforms. ICA \(^46\) is a very useful tool to disentangle these waveforms and identify their original components. The *cocktail-party problem* is usually used to describe this methodology \(^47\): you have two people speaking simultaneously and two microphones recording the ‘conversation’ from two different locations in the room. Each microphone gives you a signal that is a sum of the speech signal emitted by the two speakers. The problem is then to estimate the two original speech signals from the recordings (Figure 2-7), a problem that ICA can solve given that the sensors are at least as many as the sources.

The idea of applying ICA to brain activity is similar to that of the “cocktail party” example: one would like to decompose the acquired signal into their original set of
components. These can be revealed using ICA assuming that the source signals are statistically independent \(^{25,47,48}\). Statistical independency denotes that the information carried by one component cannot be inferred from the others \(^{49}\). Independent component analysis was shown to be an effective blind data-driven method \(^{46,50}\) to statistically separate a linear mixture of independent source signals \(^{47}\) in brain imaging recordings \(^{51,52}\). It is now possible to decompose the EEG data into components and scalp maps contributing with as much distinct information to the data as possible \(^{25}\) without taking into account any prior information about head geometry or electrode locations.

![Figure 2-7. Illustration of the cocktail party problem. A mixture of the sounds from the people speaking is recorded by each microphone. If one has as many microphones as speakers, it is possible to separate the recorded signal on its original components using ICA.](image)

In cognitive neuroscience, ICA has been shown to be very useful as a preprocessing step to time/frequency analysis \(^{48,50}\), in order to minimize the influence of volume conduction \(^{25}\), or on feature extraction for subsequent signal classification \(^{47,49}\). Another application has been artifact correction procedures including blinks, eye movements \(^{10,51,53,54}\), muscle activity and preprocessing steps prior to source localization \(^{47,50}\). Hence, ICA provides a useful way to remove artifacts that are independently mixed with the signal of interest. This is particularly important for complex EEG setups such as simultaneous EEG/fMRI recordings since the EEG signal is degraded inside the scanner. In this context, it has been widely used for pulse (ballistocardiogram) related artifact reduction \(^{55–58}\).
EEG Source Localization

Over the past 15 years algorithms for the localization of EEG and MEG brain activity have been improved to provide more accurate spatial resolution for these scalp recordings. The problem of how to reconstruct the sources underlying the EEG electrical currents measured in the brain at specified scalp positions (electrode locations), called the inverse problem, is somewhat hard to solve, given that it is possible to find an infinite number of source distributions to account for those measurements.

The so-called EEG source imaging started with the equivalent dipoles approach and scalp topography mapping but has now expanded to allow the mapping of 3-dimensional distribution of current densities inside the head. The general components of the source reconstruction process are described in figure 2-8.

The accuracy of the inverse solution is affected by several factors such as EEG noise or unrealistic head modeling. On the other hand, it can be improved by using caps with more channels and is independent of the position of the reference electrode.

The resistive properties of the various tissues involved (brain, skull and scalp) are also an issue. The low-conductivity of the skull may spread the volume currents thus distorting the potentials originated in the surface of the brain. Nevertheless, introducing some a priori known mathematical, anatomical, functional, biophysical or statistical constrains (e.g., head model, number of possible sources or fMRI statistical parametric maps), the most probable sources can be accurately localized.

There are many approaches available to define the inverse solution for that problem (e.g., LAURA, MNE, LORETA, sLORETA, MUSIC, and BESA; for details see Grech et al., 2008) and the co-registration of EEG and Magnetic Resonance Imaging (MRI) or fMRI data may help improving the source localization results. The segmentation of MRI data into gray and white matter is used to better select the head model, fit the shape of the head and restrict the solution space to structures where EEG sources can arise. This should be as accurate and realistic as possible.
Figure 2-8. Schematic representation of the source localization process. EEG data recorded at the scalp and anatomical and/or functional MR data are acquired. Then, the location of the electrodes is coregistered with the individual anatomical data and the segmentation of different tissues is performed. A realistic head model of the head tissue properties is built. Finally, sLORETA is used to calculate the inverse solution and current density maps are interpolated on the cortical surface or anatomical data ⁶²,⁷²,⁷³.

The sLORETA (standardized low resolution brain electromagnetic tomography) ⁷³,⁷⁴, gives the best performance in terms of localization error. This method is a standardized discrete, three-dimensional (3D) distributed, linear, weighted Minimum Norm inverse solution that compensates the tendency of the Minimum Norm solution to favor superficial sources. Minimum Norm localization approaches estimate the current source density (CSD) over the entire 3 dimensional volume of the head and the regions of highest current density are taken to be the location of the sources ⁷⁵. However, without
realistic head models, the inverse problem is highly undetermined and these approaches results in very blurred images of the sources\textsuperscript{76} favoring the superficial sources. sLORETA takes several neurophysiologic and anatomical constraints into account and has been shown to yield depth-compensated inverse solutions with exact localization even in the presence of measurement and biological noise\textsuperscript{74,75}.

**EEG source localization in the frequency domain**

The majority of the studies investigate the sources for the ERPs and few, mainly in psychiatry, focus in source estimation of frequency domain data\textsuperscript{77}. The issues of source localization are similar for ERP and frequency based localization, with only the pre-processing differing. The source analysis applied to FFT-based\textsuperscript{78-80} and time-frequency results have been used successfully\textsuperscript{81,82}. The latter has advantages because it allows tracing frequency changes with relatively high temporal resolution (subsecond range). Indeed, the topographical mapping of the power distribution over time can provide important information on the pathological and normal brain.

In sum, EEG has become a true neuroimaging technique. Using proper post-processing techniques, algorithms can provide ERP, time-frequency data and source estimation results with high accuracy. The use of ICA, also increase the reliability in the results. Hence, EEG is a very useful technique to study brain dynamics given its high temporal resolution, in contrast to the spatial limitations that, in any case, new advanced methods have proven to improve. Furthermore, the incorporation of information from other complementary imaging modalities can make EEG even more powerful.
**Electrocorticography (ECoG)**

Invasive recordings of electrical activity have paved the way to overcome some limitations of scalp EEG. ECoG is an invasive imaging technique that allows recording electric potentials directly from the cortex. The activity measured from ECoG is not distorted due to the ‘smoothing’ low pass filter of the different tissues in the head or muscle artifacts. It provides a unique opportunity to study the electrophysiological correlates of functional brain activation in detail. This imaging technique is applied mainly for pre-operative evaluation of patients with refractory epilepsy to locate the epileptic foci but it has been useful to study brain responses to cognitive tasks. The electrode strips or grids are surgically implanted and computed tomography (CT) and MRI anatomical data are acquired in order to precisely locate the electrodes (Figure 2-9).

![Figure 2-9. Example of ECoG electrodes placement](image)

By implementing high sampling rate invasive recordings it is possible to record extremely high frequency responses without contamination by artifacts. This approach has also been used in a multimodal perspective. For this reason, the use of ECoG is a great opportunity to study high frequency (particularly in the gamma-band range up to 200 Hz) oscillations in the brain without the pitfalls of EEG scalp recordings. In fact, many studies have already taken advantage of intracerebral recordings to study task-related brain responses across a wide frequency range in particular the holistic perception of ambiguous stimuli.
Magnetic Resonance Imaging

MRI is a non-invasive technique that provides structural and functional images of the tissues. An important variation of MRI for brain imaging is blood oxygenation level-dependent (BOLD) functional MRI, which indirectly provides information about brain function by measuring differences in the hemodynamic activity that seem to be related to the metabolic requirements of neuronal activity.

Basic Principles

The signal measured in MRI arises from the electromagnetic properties of hydrogen nuclei (i.e. protons). Hydrogen nuclei consist of one unpaired proton and therefore its spin (an intrinsic quantum mechanical property of elementary particles) is non-zero. These nuclei have a small magnetic dipole. In the presence of a strong magnetic field, these dipole moments are distributed amongst two energy states: aligned parallel (i.e. lowest energy level) or anti-parallel (i.e. highest energy level) to the main magnetic field. Applying a time varying magnetic field on resonance i.e. oscillating at the Larmor frequency (radiofrequency (RF) pulse), the net magnetization vector is tilted away from the magnetic field and the total flip angle is proportional to the duration and amplitude of the pulse. After the RF pulse is switched off, the tilted magnetization gradually returns to its original state with distinct time constants. These are relevant to produce image contrast. The longitudinal relaxation time is used to produce images of the brain anatomy (Figure 2-10 A). Furthermore, the inhomogeneities in the static magnetic field result in a large shortening of the transversal T2 relaxation time due to an accelerated signal decay with an effective transverse relaxation time constant T2*. In T2*-weighted sequences, contrast is maximized because spins have enough time to dephase and relax when acquisition starts thus increasing the signal-to-noise ratio (SNR). These concepts of contrast optimization are of major importance for functional imaging studies (Figure 2-10 B) providing the possibility to image the metabolic consequences of neuronal activity patterns in the brain.
**Figure 2-10.** Examples of structural (A) and functional (B) imaging of the brain using MRI. The colormap in B) represents the general linear model statistical parametric maps for one subject performing a visual perception task using Mooney stimuli (our results). It shows regions with significant hemodynamic effects related to the stimuli.

**Functional Imaging – BOLD**

The BOLD effect was first described by Ogawa et al.,\(^{102,103}\) and is attributed to a magnetic susceptibility change in the blood, which depends on its level of oxygenation. Hemoglobin, a substance that is present in red blood cells for oxygen transportation has different magnetic properties depending on its level of oxygenation: when oxygenated (oxyhemoglobin; HbO2) it is diamagnetic, and it is paramagnetic when deoxygenated (deoxyhemoglobin; Hb). This subtle difference in the magnetic properties of oxy- and deoxyhemoglobin is very important because while oxyhemoglobin does not perturb the surrounding magnetic environment, the same does not hold for deoxyhemoglobin which acts as an endogenous contrast agent causing local magnetic field inhomogeneities that lead to spin dephasing and signal decay\(^{100}\).

With cortical activation in response to sensory input there is an increase in energy and oxygen consumption in the eloquent cortex\(^{100}\). Hence, in order to overcome the energy requirements of the neurons, the oxygen levels in these areas have to be reestablished. Cerebral blood volume and cerebral blood flow increase, thus increasing the delivery of oxyhemoglobin\(^{98,101}\). This exceeds the oxygen consumption demands thus increasing the local concentration of oxyhemoglobin in the capillaries that feed the activated brain regions. The concentration of (paramagnetic) deoxyhemoglobin decreases while that of diamagnetic oxyhemoglobin increases, thus causing a decrease of magnetic
susceptibility differences in the surrounding environment. As a consequence, $T_2^*$ temporarily increases and this results in an increase of the measured BOLD signal $^{100,104}$.

These changes in MR signal are delayed with respect to the fast occurring neuronal activity. The slow and delayed coupling between neuronal and vascular activity is known as the hemodynamic response. The typical hemodynamic response function (HRF) follows a time-course with three main phases as described next (Figure 2-11) $^{101}$.

![Figure 2-11. Schematic representation of BOLD – contrast fMRI. The neuronal activity changes the local level of oxygen consumption leading to increased oxygen levels in the vessels after stimulus onset, and therefore larger MR signals due to the increase in oxygenated hemoglobin (diamagnetic) $^{101,135}$.](image)

Immediatly after stimulus onset, there is a momentary increase in the amount of deoxyhemoglobin due to an increased oxygen consumption which generates a decrease in the signal - initial dip $^{105,106}$. This is followed by a period where the cerebral blood flow and volume increase and overcompensates for the amount of oxygen being extracted $^{69,100}$. It is usually seen as a plateau in the cases where the neuronal activity is extended in time and is named as Positive BOLD response. For a long stimulation event an overshoot around 4-6 seconds after onset of stimulus presentation occurs due to the local oversupply of oxyhemoglobin. After stimulus ending and before the BOLD signal returns to the pre-stimulus baseline levels, an undershoot occurs most probably due to an accumulation of
deoxyhemoglobin in the vessels. At this time, the cerebral blood flow and the oxygen extraction rate have already returned to baseline while the cerebral blood volume takes more time to return to baseline. This mismatch causes an increase in the total amount of deoxyhemoglobin in the capillaries, which in turn leads to BOLD signal decrease. It is important to note that in practice this model of HRF may have different shapes depending on stimulus conditions and subjects.

The underlying physiological mechanisms of BOLD signal still remains unclear and an active area of research. Nevertheless, BOLD-fMRI is able to non-invasively image complex brain networks, such as those activated during cognitive tasks, and enables the characterization of the response profile in numerous regions-of-interest (ROIs).

**Experimental task design and data analysis**

During fMRI data acquisition, subjects are enrolled in a task (e.g. sensory, motor, cognitive) to study a specific brain function. The paradigms that are used for stimulus presentation in these tasks are typically divided in two main categories: *blocked designs* or *event-related designs*. In a block-design paradigm, trials of the same condition are repeatedly presented during a block that lasts on average 16 seconds to 1 minute. Blocks for different conditions are sequentially presented with an inter-block time interval of rest. In general, blocks where the task is performed are contrasted against the rest periods. The underlying assumption is that the rest state represents the baseline of brain activity against which activated areas related to the task can be identified with a high SNR.

On the other hand, event-related designs are based in a single trial presentation paradigm instead of blocks of trials. The trial-events of each condition are typically presented randomly, to reduce cognitive adaptation effects on the subjects, with an interstimulus interval time (ISI) separating the events. If the ISI is long enough, then the BOLD responses to each of the events do not overlap. This type of paradigm is particularly useful for experiments where the timing of the events is unpredictable or when trials have to be sorted or adjusted according to the subjects’ response.

A functional run, which consists of a four-dimensional (4D) volume spanning space and time information, is acquired during the experimental task as a time series of volumes. The volume time (T_v), i.e. the time it takes to acquire an entire brain volume consisting of multiple two-dimensional (2D) slices of the brain, specifies the temporal resolution of the functional images (similar to the time of repetition, TR).
There is often a trade-off between the temporal resolution of the 4D image series and other acquisition parameters such as spatial resolution. Shorter values of T₁ imply a decrease in the number of slices acquired per volume and/or, a reduction in the in-plane spatial resolution. The hemodynamic response to the activation of neuronal tissue is much slower than that of the neuroelectrophysiologic signaling that is the origin of scalp EEG. The consequence is that fMRI-BOLD has a temporal resolution in the order of a few seconds, whereas for the EEG, signal variations can be sampled with a time resolution in the milliseconds range. However, spatial resolution of fMRI can be as small as 1 mm. This spatial resolution is much better than that of EEG.

Prior to data analysis, several pre-processing steps are applied to reduce noise or subject’s movements during fMRI acquisition. Image pre-processing typically includes motion correction, linear detrending and spatial and temporal filtering.

Statistical tests are then performed to identify the brain regions activated or deactivated in response to the experimental conditions and statistical parametric maps (SPMs) are created for visualization of the results. A general linear model (GLM) framework is typically used to this end by searching for linear correlations between the fMRI time course and a reference model. It is a model-driven approach that is useful to calculate the differences in BOLD signal between activation and baseline conditions. As a result, SPMs show the brain regions significantly activated in response to a presented task or stimulus.

Simultaneous EEG/fMRI

The co-registration of EEG/fMRI measurements allies the superior spatial resolution of fMRI with the better temporal resolution of EEG and has become a popular method in the field of cognitive neuroscience. Moreover, performing simultaneous acquisition of EEG/fMRI may facilitate the elucidation of the mechanisms mediating the coupling between neuronal activity and blood flow.

EEG/fMRI is motivated by the idea that one can combine the best properties of each modality, taking advantage of the different perspectives on brain functioning that can be attained from each one separately. From a brain imaging perspective, EEG and fMRI are complementary in the sense that they detect different aspects of neuronal activity. Given the described characteristics, advantages and pitfalls of the MR
technique and EEG, there was an obvious interest in their simultaneous acquisition which drove some developments to combine fMRI and EEG setups.\textsuperscript{115,118,120}

After fast advances and the solving of the main technical problems, it is now possible to perform multimodal non-invasive brain imaging with good data quality, combining both high spatial and temporal resolution\textsuperscript{119,120}. In order to better understand this added benefit, see figure 2-12 to compare the spatial and temporal resolution of different brain imaging techniques, e.g. MEG, Positron emission tomography (PET), Transcranial magnetic stimulation (TMS) or ECoG.

![Figure 2-12: Comparison of the spatial and temporal resolution of different brain imaging techniques.](image)

**Figure 2-12. Comparison of the spatial and temporal resolution of different brain imaging techniques.** Electrophysiology approaches have higher temporal resolution and MR based approaches increased spatial resolution. Multimodal acquisition take advantage of both. (adapted from Lachaux et al., 2004 and He & Liu, 2008\textsuperscript{84,135})

Concurrent EEG/fMRI was first acquired with the aim of achieving higher spatial resolution when mapping epileptogenic EEG activity for pre-surgical planning\textsuperscript{121,122}. However, acquiring EEG data inside the magnet is a challenging task since the EEG equipment affects the quality of the MR image\textsuperscript{123} and poses several problems related to patient safety. Furthermore, magnetic field variations inside the MR scanner during the MR
pulse sequence, induces electric currents in the electric conductors of the EEG system (e.g. wires in the electrode cap) that cause (gradient) artifacts in the EEG signal \(^57,124,125\). In addition, variations in the cardiovascular activity of the subject within a varying magnetic field generate the ballistocardiogram (BCG) artifact.

In order to acquire EEG data inside the magnet, the components of the recording setup (electrodes, cables, conducting materials, cap) have to be MR compatible, non-ferromagnetic \(^122\). Furthermore, the electrodes have to be better bundled together and the EEG amplifier requires a large dynamic range to avoid signal saturation thus fully sampling the EEG gradient artifacts. Additionally, the sampling rate (SR) has to be larger than in standard EEG (>10 kHz), in order to sample the gradient artifact with high temporal resolution (Figure 2-13 shows a typical setup used for EEG/fMRI recordings).

**Figure 2-13. Schematic representation of experimental setup for simultaneous EEG-fMRI recordings.** EEG is acquired with an MR-compatible EEG cap connected to the amplifier by a headbox located in the control room. MR, EEG and stimulus systems are synchronized by means of a synchronization box. Stimuli are projected onto a projection screen inside the magnet and the behavior of the subject is reported with a compatible response box (not seen in the picture). This setup is similar to the one from Laufs et al., 2008 \(^114\).
Even though commercially available EEG MR-compliant systems have solved the problems related to hardware compliance \(^{126}\), the need for the correction of gradient and BCG artifacts remains \(^{127}\). Appropriate hardware and as well as artifact reduction algorithms are now available for routine use in co-registered EEG-fMRI \(^{58,120,125–134}\). After correction, it is possible to integrate EEG with fMRI analysis (the information from one modality is used to guide the analysis of the other) \(^{135}\).

There are two main approaches to the integration of simultaneously acquired EEG-fMRI data. The EEG-correlated fMRI analysis is an approach in which EEG features are extracted and used to inform fMRI analysis and generate EEG driven activation maps. In the fMRI-informed EEG, the fMRI data is used to constrain the solution of source localization algorithms \(^{111,119,136,137}\). For details about artifact correction and EEG/fMRI integration refer to Allen et al., 2000, Srivastava et al., 2005, Gonçalves et al., 2007, Ertl et al., 2010; \(^{55,127,129,134}\) and chapter 4 of this thesis.

The brain imaging techniques described in this chapter are all potentially useful in the understanding and clarification of brain information processing. Here we took advantage of the distinct modalities (EEG, fMRI, EEG/fMRI and ECoG) to study the neural correlates of perceptual integration, namely brain oscillatory activity with high temporal and spatial resolution. In the next 4 chapters, an overview of our experiments and the respective results are detailed.
References


RESULTS
To Perceive or Not Perceive:
The Role of Gamma-band Activity in Signaling Object Percepts

Abstract

The relation of gamma-band synchrony to holistic perception in which concerns the effects of sensory processing, high level perceptual gestalt formation, motor planning and response is still controversial. To provide a more direct link to emergent perceptual states we have used holistic EEG/ERP paradigms where the moment of perceptual “discovery” of a global pattern was variable.

Using a rapid visual presentation of short lived Mooney objects we found an increase of gamma-band activity locked to perceptual events. Additional experiments using dynamic Mooney stimuli showed that gamma activity increases well before the report of an emergent holistic percept.

To confirm these findings in a data driven manner we have further used a support vector machine classification approach to distinguish between perceptual vs. non-perceptual states, based on time-frequency features. Sensitivity, specificity and accuracy were all above 95%. Modulations in the 30-75 Hz range were larger for perception states. Interestingly, phase synchrony was larger for perception states for high frequency bands. By focusing on global gestalt mechanisms instead of local processing, we conclude that gamma-band activity and synchrony provide a signature of holistic perceptual states of variable onset which are separable from sensory and motor processing.
Introduction

Oscillatory processes in the gamma frequency range have been proposed to play a role in percept formation and object representation. Studies using EEG and MEG have suggested that gamma-band oscillations are related to integration of information and the ability to form coherent gestalts as well as attention and working memory processes. Gamma-band synchrony does indeed seem to reflect binding of information across different brain regions that leads to the emergence of a coherent percept. Studies of oscillatory patterning may be important to understand normal and abnormal cognitive function related to perceptual functions. Although some studies reported that gamma-activity may be influenced by artifacts of muscle activity and eye movements, valuable methods to attenuate this problem have been developed.

Here we examined the oscillatory large-scale neural correlates of gestalt-like perceptual recognition moments. As previous studies have relied on simple contrasts across inverted vs. upright static stimuli, we aimed to study the moment a coherent visual percept is formed. Paradigms where object recognition is variable in time would be helpful in elucidating this issue by isolating a neural correlate of coherent perception. Neurochronometric paradigms that allow the emergence of variable moments of perception would also help in further clarifying the role of gamma-band synchrony in gestalt based perception.

To help identify the processes underlying the emergence of a coherent object/face percept under ambiguous stimulus conditions, and the role of gamma-band activity in these processes, we have recorded EEG/ERP signals while performing two different tasks that used Mooney objects (two-tone black and white degraded images). The first task tried to achieve that goal with rapid serial visual presentation paradigms, where short lived target Mooney stimuli do appear randomly with low (1/30) probability. In a second experiment, based on time variable percepts, we designed a new face paradigm that takes advantage of the well-known role of face inversion in holistic processing. A configural/holistic-based processing mode operates for upright faces and a part-based processing mechanism is activated when faces are inverted. We used such a paradigm that delayed the time of recognition from stimulus onset. We were able to observe a delayed transition from non-perception to perceptual states reflecting a gradual transition from local to holistic processing. This leads to the simple prediction that gamma should increase during the transition moment.
We aimed to investigate the link between gamma-band activity and the aforementioned moment whereby a coherent visual percept is formed, which helped dissociating low level visual analysis from high level categorical perception. We used the time-frequency and phase synchrony analysis described elsewhere\textsuperscript{17,35-37}. Finally, we performed an additional independent validation by a data driven (non-hypothesis constrained) approach using support vector machine (SVM) classification tools\textsuperscript{38}. We were able to differentiate between perceptual states based on temporal activity patterns and thereby support their likely functional relation with object recognition.

Given that previous studies have shown that the brain codes different information in multiple oscillatory bands\textsuperscript{39-41}, we did analyze a broad range of frequency bands, beyond the usual focus on high-frequency (60–75 Hz) as well as lower frequency (30–45 Hz) EEG gamma bands and high beta (18-30 Hz)\textsuperscript{42}. We tested whether induced (non-phase locked) oscillatory activity might be differently modulated depending on the particular frequency band as a function of perceptual state, as tested using statistical classification tools.

**Materials and Methods**

**Ethics Statement**

This study and all the procedures were reviewed and approved by the Ethics Commission of the Faculty of Medicine of the University of Coimbra and were conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

**Participants**

All participants (n=20, mean age 27.78±4.88 years; 9 males and 11 females; 2 left-handed) had normal or corrected-to normal vision and no history of neurological disorders. All participants were naive regarding the purpose of the study. Participants took part in EEG recordings, distributed over 2 experiments (14 in experiment 2, 8 in experiment 1). Two of these subjects underwent both experiments.
Experimental paradigms

Experiment 1 – Perceptual reports on briefly presented ambiguous stimuli – perceptual task with delayed response

EEG/ERP data were recorded along with a rapid visual presentation task with target probability of 1/30 (see below). Subjects (n=8) performed a three way forced choice task where they had to report the presence of a target (Mooney Face or Mooney Guitar or none of these objects) among frequent stimuli at the end of the trial (delayed report moment; see Figure 3-1 A). The choice of guitars was due to the fact that they have a highly prototypical shape, like faces. Using these stimuli it is possible to modify local features without changing global configuration. Moreover there is little correspondence between face-selective areas and regions correlated with guitar identification. Frequent stimuli were scramble versions of the target. Each picture was presented briefly for 150 ms in trials of 4500 ms. Each trial contained a target and 29 random versions of it (frequent standard stimuli). 103 different target Mooney faces and 103 target Mooney guitars were shown (subjects did not know when the target stimulus would appear). The timing of target presentation was variable between 1500 and 3000 ms in the trial. Subjects performed 7 runs (6 runs with 30 trials and 1 run with 26 trials) with trials randomized across subjects. Stimuli were generated in Psychophysics Toolbox (running in Matlab®) to enable calculation on the fly of different random pictures (standard distractors – frequent stimuli). Stimuli were presented in a black background in a CRT monitor with a resolution of 1024x768 pixels and refresh rate of 85 Hz. They spanned a visual angle of 4.5°x4.5°.

EEG Recording – experiment 1

Subjects sat in a comfortable chair in a darkened room at a viewing distance of 120 cm from the stimulus presentation monitor. We have used a 128 channel EEG system (Compumedics Quick cap, NeuroScan, USA) for recording. Caps were interfaced through SynAmps2 (NeuroScan, USA) signal amplifier, which fed the signal through the Acquire Data Acquisition software (version 4.3.1, Compumedics NeuroScan, USA) at a sampling rate (SR) of 2000 Hz. No notch filters were used during recording and impedances were kept under 10 kΩ (electrodes with higher impedances were marked as bad). All electrodes were referenced during recording to one reference electrode located close of CZ.
Figure 3.1. Summary of the tasks. A) EEG Mooney rapid visual presentation task, with delayed response. Meaningful (perceived as faces or guitars) objects appear among noise images. Mooney faces and Mooney guitars are shown randomly with a likelihood of 1/30 (between the 10th and 20th presented images) at each trial masked backward and forward by a randomization of itself (each picture 150 ms). Subjects had to report the presence of a target (Mooney Face or Mooney Guitar or none of these objects) at the end of the trial (inter-stimulus interval is 2000 ms). B) Mooney dynamic stimuli - Time-line of one run; for clarity, representative snapshots are represented in separated boxes (in the experiment, movies run continuously and smoothly). Accordingly, only 4 snapshots are shown for each movie – faces rotate from inverted to upright in 12 s movies separated by a 3 s black screen. Subjects were instructed to provide a motor report, when they perceived the face, as quickly as possible.

EEG analysis – experiment I

We used Edit EEG/ERP analysis software (version 4.5, NeuroScan, USA) for data pre-processing and extraction of event related responses (target stimulus locked event related potentials (ERP)). The channels that did not fulfill the impedance criteria during acquisition were rejected. Offline re-referencing, using average reference was then performed. Data were digitally high-pass filtered at 1 Hz using a finite impulse response filter and amplitude based (-75, 75 μV) artifact rejection routines were then applied. Blinks were removed by rejecting epochs in which the electrooculogram bipolar channels exceeded ±100 μV. Stimulus presentation time (150 ms) was below the known latency of eye movements. Miniature saccades usually show an average peak around 250 ms. Data were filtered with a low-pass filter of 100 Hz and then segmented into epochs (-200 to 800 ms) locked to the onset of the target stimuli (Mooney faces and guitars). Only trials with correct categorization of the stimuli (>90%) were considered for analysis. We used Matlab®
(v.R2010a, The MathWorks, USA) and EEGLAB Matlab® toolbox (version 10.2.5.6b 45) for time-frequency, phase synchrony and additional statistical analysis (see below for details).

We performed source localization for the grand average ERPs of the target stimuli. Source analysis was performed in Curry 5.0 software (NeuroScan, USA) on a realistic head model. Group average ERP data was co-registered with anatomical magnetic resonance (MR) data using landmarks and applying standard xyz coordinates of the channel locations. A boundary element model (BEM) was created from standard anatomical MR data and current source density was estimated for the ERP peaks with no assumption regarding the number or location of active sources. The sLORETA algorithm (standardized low resolution brain electromagnetic tomography) was used 46,47. This method is a standardized discrete, three-dimensional (3D) distributed, linear, minimum norm inverse solution. It takes several neurophysiologic and anatomical constraints into account and has been shown to yield images of standardized current density with exact localization in the presence of measurement and biological noise 47.

Experiment 2—Perceptual reports on dynamic ambiguous two-tone (Mooney) stimuli - emergent percepts and concomitant responses

In this experiment (Figure 3-1 B), using dynamic stimuli, EEG data were recorded in 14 right-handed subjects. Rotating Mooney face movies — starting from inverted to upright — were presented. The starting inverted position ensured minimal likelihood of initial face perception and induced late recognition in a substantial proportion of trials (see behavioral results). This is a new paradigm (see Rebola et al., 2012 29) where the 2-tone images started in the inverted position (180°), where local processing prevails, and slowly rotate for 9 s until they reach the upright position (0°), favoring holistic processing, staying then fixed for 3 s. This design enabled the presence of different perceptual states for the same physical stimulus (starting from absent perception). This way one can compare between distinct perceptual states induced by the same stimulus and not only a simple contrast between different pictures. Each movie contained a single embedded face and every stimulus appeared only once during the experiments to prevent repetition effects. We choose only one category (faces) because the initial inverted configuration has only been proven to be non-holistic for faces 27,48. For this stimulus category there is a transition from non-holistic/non-perceptual to holistic/perceptual states. Stimuli were presented with Presentation (version 12.1, Neurobehavioral Systems, USA) in a setup similar to
experiment 1. The experiment was divided in 5 runs (total of 103 trials; 4 runs with 20 trials each and the last run with 23 trials). All runs started with a fixation period of 10 s followed by a Mooney dynamic face stimulus which was presented for 12 seconds. Stimuli were separated (inter stimulus interval) by a black screen during 3 s. Subjects were instructed to search for a face and to press a button (concomitant report moment) as quickly as possible, only when they were confident of its presence. Stimuli remained visible until the end (12 seconds) for the perceived trials (even after the response) and for the trials that never came to a full percept.

**EEG Recording – experiment 2**

Subjects sat in a comfortable reclining chair in a darkened, acoustically and electrically shielded room at a viewing distance of 120 cm from the stimulus presentation monitor. We have used a 64 channel system (Compumedics Quick cap; NeuroScan, USA) for recording. Electrodes were displayed as the 10-20 system in caps that were interfaced through SynAmps2 signal amplifier, which fed the signal through the Acquire Data Acquisition software (version 4.3.1, Compumedics NeuroScan, USA) at a SR of 2000 Hz. There were no filters applied during recording and impedances were kept under 10 kΩ (electrodes with higher impedances were marked as bad). Reference was set to one reference electrode located close of CZ.

**EEG analysis – experiment 2**

We used Edit EEG/ERP analysis software (version 4.5, Compumedics NeuroScan, USA) for data pre-processing and extraction of ‘decision’ event related responses. The data were inspected by eye for artifacts and bad channels were rejected. Offline re-referencing, using average reference was then performed.

Filtering and artifact rejection criteria were set as in experiment 1. We corrected for eye-blinks (and other artifacts) segment-wise by rejecting data trials where the maximum exceeded 100 µV in any of the EEG or electrooculogram (EOG) channels. Moreover, because of the dynamic stimuli used in this experiment, we used independent component analysis (ICA) for signal “correction”, in particular saccade potentials attenuation, based on all electrodes (including 4 EOG channels). We identified the ocular component based on the scalp topography (higher activity around the orbits) and its relation to EOG channel peaks, as described by Keren et al., 2010.
Data were segmented into epochs (~1500 to 500 ms) locked to the response or to the middle of the trial, for the perceived trials (perceptual “discovery” moment) and non-perceived trials respectively. Epochs were separated accordingly to the response (perceived, non-perceived). To guarantee that the baseline was equal between different trials, only trials with responses between 1 to 11 s were considered for analysis.

Topography, time-frequency, phase synchrony and additional statistical analysis were implemented in *Matlab*® (v.R2010a, The MathWorks, USA).

**Time-frequency and phase synchrony analysis**

Time-frequency analysis was performed as in Lachaux et al., 1999 and Uhlhaas et al., 2006 and is also described elsewhere. Signals were time-frequency-analyzed using the pseudo Wigner-Ville transformation. For every time window and frequency bin (frequency resolution of 1 Hz/frequency bin) the amplitude and phase were computed, using *Matlab*®, in the high-beta/gamma frequency range (15 to 90 Hz in steps of 1 Hz) and in the time period of the epochs described above with EOG correction performed as described in Keren et al., 2010. From these phase values we calculated the phase-locking value (PLV), that measures the inter-trial variability of the phase difference. PLV looks for latencies at which the phase difference between the signals varies little across trials at the target frequency. Because we were interested in long-range coordination of neural activity, for a given time window, the phase difference was calculated between all electrode pairs, and the stability of phase difference evaluated through all the trials across a large frequency range. Coherency is an indicator of neural synchrony but, this phase calculation can be affected by volume conduction since activity of a single source is measurable in many electrodes. To avoid spurious synchrony, we computed PLV between sources, using the imaginary component part of coherence (ImCoh) as a measure for functional connectivity at the sensor level. As described by Nolte et al., 2004, ImCoh more directly reflects true interaction. We performed this analysis as it is implemented in *Source Information Flow* toolbox for *EEGLAB* (SIFT version 0.9.7-alpha). To further examine the ImCoh we plotted the head-in-head plots to visualize interactions between conditions over all channels (pairs of channels). The topographies are plotted at each electrode position and represent the connectivity strength (ImCoh) between that given channel and all other channels for each frequency band (15-30 Hz, 30-45 Hz, 45-60 Hz and 60-75 Hz).
The time-frequency results are usually scaled in relation to a pre or post-stimulus baseline. Care must be taken when choosing the window for the baseline because the frequency components are smeared out due to time-frequency windowed transformation function analysis and due to the filter algorithms that use time points of the past and the future to compute each point of the filtered signal. To avoid distortions and edge effects the convolution should start and end one-half of the wavelet length before the baseline and after the end of the time interval of interest.\textsuperscript{54}

Time-frequency (induced) and phase were then normalized to the baseline. The normalization involves subtracting the baseline average and dividing by the baseline standard deviation on a frequency by frequency basis. Baseline was chosen accordingly to the task. In the first experiment the baseline was set to pre-stimulus interval (avoiding inclusion of post-stimulus oscillations in the baseline segment). For the second experiment, using dynamic stimuli, baseline time-window spanned the no perception time window (the time before perceptual decision). After normalization, individual time-frequency and phase synchrony data were averaged through subjects to obtain grand-averages.

**Statistical analysis**

The alpha level was set at 0.05 for all tests. After verifying for normality (Kolmogorov-Smirnov), statistical tests were performed. After a visual inspection of the results, we focus and tested four frequency bands of 15 Hz length each (15-30 Hz, 30-45 Hz, 45-60 Hz and 60-75 Hz) to assess the significance of the oscillatory patterns with paired t-test and Wilcoxon signed rank test. The Bonferroni-Holms correction for multiple comparisons at alpha level was applied when appropriate.\textsuperscript{55}

**Support Vector Machines (SVMs)**

A classifier is a set of rules that may be used to determine the class of different objects based on their discriminative characteristics, also known as features (the problem can be binary or multiclass).\textsuperscript{56,57} Moreover, these methods may clarify the importance (or weight) of each feature for the decision function and consequently to the classification task.

Let us consider an object as an array of features that describes a certain event. The idea of the SVM algorithm is to define a decision boundary that separates the training data from different classes based on some of those features. In this sense, the generalization
error is minimized by maximizing the margin, i.e. the distance between the decision hyperplane/boundary and the closest data points of each class (also known as support vectors) \(^{38-41}\). Then, after the learning phase, new test objects without label can be compared with the decision function (defined in the training phase) and classified \(^{62}\). Real life problems are usually highly non-linear and therefore the definition of a decision boundary in the original input space is not an easy task. To overcome this issue, the decision hyperplanes are determined in a high-dimensional feature space \(^{56,63-65}\) and a unique global optimal separation solution can be found either for linear or non-linear boundaries without much computational costs (using the so called kernel trick) \(^{64,66}\). Hence, its main advantage is the ability to deal with a large number of features allowing the linear characterization at the level of the individual \(^{67}\).

Currently in brain imaging, SVM is the most frequently implemented classifier (see Figure 3-2) \(^{56}\). The SVMs have been early on used in computational learning theory \(^{68,69}\) and are now used with many applications in biomedical statistical pattern classification \(^{60,62,70-74}\) in the context of diagnosis or treatment predictions \(^{57,67,75}\). For a review on other classification algorithms and comparison with SVMs see Lotte et al., 2007 \(^{65}\). In EEG data classification, time-frequency features from different electrodes already proved to be informative to separate groups of subjects \(^{60,63,72,76,77}\) or to improve brain computer interfaces applications \(^{66,78}\). The Library for Support Vector Machines (LIBSVM) software is used to classify the data \(^{38}\). The size of the available dataset is typically limited. To improve the size of our test set and avoid over training (reducing the generalization ability of the models), cross-validation is used \(^{57,61}\), in particular the leave-one-out cross-validation. The idea is to divide the original data into N subsets and then train the classifier in N-1 subset and test in the remaining set. This is repeated N times and the results can be averaged to obtain performance measures. To evaluate the performance of the method, the accuracy, sensitivity and specificity of the SVM solution is estimated \(^{59,64}\) and permutation tests are used to estimate the statistical significance of the solution.

As a further statistical analysis we performed SVM classification applied to matched perceived/unperceived time window. We have performed classification of perceptual “discovery” responses of experiment 2 for the subjects (n=6) that had at least 10 non-perceived trials. In 8 subjects the number of unperceived trials was too low for classification. However, single subject statistics were highly consistent across all 6 eligible subjects, permitting proof of concept validation.
Figure 3.2. SVM analysis – Basic Design. A) Two groups of subjects can be separated based in distinct features of each group. The support vectors mark the largest margin with the optimal separation between groups. If more features are available a higher dimensional space is used. B) Schematic representation of the SVM pipeline. Input data is recorded. These data or characteristics computed from the data are used as features to find the support vectors. Then, during the training of the classifier a training dataset with known labels is used and a trade-off between computational cost, biological interpretability and performance is set. Finally, classification is performed on unseen data and performance is evaluated and features, model or training/testing sample size are adjusted if necessary. (Adapted from Duarte et al., IBIL meeting 2012 oral presentation 91)

We were conservative in subject selection for SVM but we guaranteed that we had enough number of trials for both states. The algorithm classified between perceived/unperceived trials. We used linear SVM implemented in the LIBSVM library based on time-frequency data. Data were divided into training and test sets. The classifier used 3 seconds at the end of the movies (for the perceived movies only trials with responses not overlapping this period were considered; in this way we guaranteed no contamination of overlaid motor responses). It used time-frequency data features based on the average over time in occipito-parietal channels (C1/Z/2, CP1/3/Z/2/4, P1/Z/4,
PO9/7/5/3/Z/4/6/8/10, O1/Z/2) for each averaged time-point per frequency band. To determine the best regularization parameter, we used a 3-fold cross-validation scheme. Thirty repetitions were computed with random distribution of data among folds. We used a permutation test approach 56-79 to evaluate the statistical significance of the classification's results. In this procedure, labels are assigned randomly to the example trials, then the classifier is trained on the task with the permuted condition labels and finally the generalization performance is tested with a “leave-one-out” cross-validation strategy.

Results

Behavioral data
Hit rates for Mooney tasks were as follows, task 1: mean 89.75 ± 8.46 % for faces and 90.36 ± 5.96 % for guitar stimuli; task 2: mean 91.87 ± 7.28%. As an additional measure of reliable perception we computed the overall group % of wrong categorization. 9.22% of stimuli were incorrectly categorized as faces or guitars.

In task 2 (Mooney dynamic stimuli) participants required a mean detection time of 4.30 ± 2.95 s. When converting time into an angular rotation required for detection, a mean value of 86° from the inverted position was obtained, suggesting that the object had to be at least close to the orthogonal position for recognition. These results are consistent with the face inversion effect 27,80.

Neurophysiological Results
Emergent perception of faces elicited by both short lived or dynamic ambiguous two-tone (Mooney) stimuli is related to increases in gamma-band activity in visual posterior brain regions.

An ERP that peaks negative at 220 ms after face/guitar onset was found conspicuously for the short-lived stimuli in experiment 1 (see Figure 3-3). This peak preceded a positive peak at 300 ms that is a known mark of target rare events 81. We performed source localization that revealed increased activity from occipito-parietal regions for the N220 component and more inferior temporal sources of activation for the P300 to the N400 components both for Mooney faces or guitars (see source mapping in Figure 3-3).
Figure 3-3. Group average ERP for experiment 1 - Mooney Rapid Visual Presentation. Baseline is set to -250 to 0 ms. ERP peaks negatively around 220 ms and is consistent across subjects (line points means standard deviation). Source distribution maps show sLORETA standardized current density for the three different peaks (hot colors signals the highest current density reconstruction values).

Regarding time-frequency analysis we found increased gamma activity specifically related to the perception moments amongst noise pictures in experiment 1 (Figure 3-4). These patterns were indeed phase-locked to the detection of the target faces or objects and appeared in both low and high gamma frequency bands (30-45 Hz and 60-75 Hz respectively). In order to confirm these findings we performed statistical comparisons between target and frequent conditions with Wilcoxon rank-sum tests for each time point and frequency band. Significant differences revealed gamma activity was increased for face trials at low frequency (30-45 Hz, 373-685 ms; z=4.14; P corrected <0.001) as well as for guitar trials both for low (30-45 Hz, 370-600 ms; z=5.06; P corrected <0.0001) and high frequencies (60-75 Hz; 166-641 ms; z=10.33; P corrected <0.0001).
<Figure 3-4. Time-frequency plot for the posterior channels – Experiment 1. Data are
locked to the onset of the oddball salient frame (A: Face; B: Guitar), baseline corrected for the
pre-stimulus interval and normalized for the baseline interval. Significant differences revealed
prominent gamma activity for face trials at low frequency (30-45 Hz; 373-685 ms; z=4.14; P
corrected <0.001) as well as for guitar trials both for low (30-45 Hz, 370-600 ms; z=5.06; P
corrected <0.0001) and high frequencies (60-75 Hz; 166-641 ms; z=10.33; P corrected <0.0001)
when comparing with the baseline. Blue line marks the beginning of the target stimuli; Color scale:
normalized units.

In experiment 2, we replicated these findings. We analyzed temporal patterns of
activity prior and after the perceptual identification of faces using dynamic Mooney stimuli.
Our difficult stimulus conditions whereby stimuli start from an inverted position often
lead to a delayed recognition moment, as expressed by the angle of stimulus rotation that
is present at the moment of perceptual report (see behavioral results). We have found
increases in gamma activity that starts prior to the perceptual report (see time-frequency
plots in Figure 3-5 A and B for perception and no perception results respectively). We
found gamma-band peaks of activity patterns at two frequencies (group averaged gamma
peaks: 32.69±12.59 Hz and 70.33±7.75 Hz) in response to moments of perception. In
Figure 3-5, for each time-frequency plot, we depict the topography maps of the gamma
response. We found increased central activity for the perception condition (60-75 Hz
gamma response) and a decrease over the parietal regions for the no perception condition
(30-45 Hz gamma response).

Comparison of no perception vs. perception during a time interval corresponding
to the second before perceptual report revealed significantly higher gamma-band activity
for Perception in the frequency bands 30-45 Hz, 45-60 Hz and 60-75 Hz (see Table 3-1
for statistics).

We did also observe a peri-stimulus response reduction in the lower frequency
(15-30 Hz) beta band. This band in time-frequency plots was only observed when a motor
response was required.
Figure 3-5. Time-frequency representations and topographies in the perception (A) and no perception (B) conditions – Experiment 2. A) Group average responses to Mooney dynamic stimuli locked to response onset (blue line), baseline corrected and normalized for the baseline interval (fixation and stimulus); B) Group average activity for the non-perceived trials. Topography plots for high and low gamma response are shown for perception and no perception conditions respectively. The topographies are averaged across the time window (-800 to 0ms) for the higher gamma-band (60-75 Hz) and lower gamma-band (30-45 Hz; top right and bottom right, respectively). The gamma-band signal is expressed as relative power change during perception compared to baseline, averaged across all channels. Note that the frequency band where the effect size is highest is the higher gamma-band. Boxes highlight low (30-45 Hz) and high (60-75 Hz) gamma bands, here and in subsequent figures.

Table 3-1. No perception vs. Perception statistics for experiment 2. Results of statistical t-tests when comparing perception and no perception during the second before button press. We tested four frequency bands. P and t values are shown and differences were considered significant for P <0.0025 (corrected for multiple comparisons). Perception show increased activity for higher gamma frequencies. n.s. stands for non-significant.

<table>
<thead>
<tr>
<th>Frequency range (Hz)</th>
<th>p</th>
<th>t</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>15-30</td>
<td>0.011187</td>
<td>-2.7584</td>
<td>n.s.</td>
</tr>
<tr>
<td>30-45</td>
<td>6.7571E-06</td>
<td>-5.7881</td>
<td>Perception higher than no perception</td>
</tr>
<tr>
<td>45-60</td>
<td>4.3518E-07</td>
<td>-6.9535</td>
<td>Perception higher than no perception</td>
</tr>
<tr>
<td>60-75</td>
<td>4.7501E-14</td>
<td>-16.1611</td>
<td>Perception higher than no perception</td>
</tr>
</tbody>
</table>
Frontal gamma-band activity is decision related

We observed significant gamma-band activity in frontal electrodes subsequent to occipital gamma related activation (see Figure 3-6) suggesting the occurrence of these temporal patterns in anterior locations until the moment of perceptual decision.

Accordingly, for the higher frequencies, activity is increased not only in occipital electrodes but seems to change its ‘centre of gravity’ during the time to more parieto-frontal areas. The deactivation at lower (beta) frequencies seems to have its source in central sensorimotor related regions.

Figure 3-6. Normalized time-frequency plots in 2D scalp maps (experiment 2). These maps are plotted for the channels marked as a black point in the 2D topographies in five consecutive time windows of 200 ms. The red-dashed row is associated with higher gamma-frequencies: activity is increased not only in occipital electrodes but seems to change its ‘centre of gravity’ during the time to more parieto-frontal areas. The blue-dashed row shows topographic maps for low-gamma. Gray row show the deactivation at the lowest frequencies (beta) that seems to have their source in central regions usually reported as motor areas.
Phase synchrony increases before the recognition moment

After normalizing to periods corresponding to the no perception states, group averages showed a burst of synchronization that appears 400 ms prior to detection. In experiment 1 (suddenly recognizable objects) we observed higher synchronization at both low (30-45 Hz; 200-373 ms; z=4.69, P corrected <0.0001) and high (60-75 Hz; 178-629 ms; z=5.46, P corrected <0.001) gamma frequency bands (Figure 3-7 A). In experiment 2 increased synchronization (30-45 Hz) indexes were detected just prior (-324 to -166 ms; z=3.0516, P corrected <0.003) to the perceptual report (Figure 3-7 B).

![Phase synchrony - experiment 1](image1)

![Phase synchrony - experiment 2](image2)

**Figure 3-7. Phase synchrony results for the two experiments.** A) Phase synchrony for task 1, synchrony is locked to the “target” face. Boxes highlight the higher synchronization at both low (30-45 Hz; 200-373 ms; z=4.69, P corrected<0.0001) and high (60-75 Hz; 178-629 ms; z=5.46, P corrected <0.001) gamma frequency bands. B) Increased synchronization for the lower gamma-band (30-45 Hz) appears during task 2 (-324 to -166 ms; z=3.0516, P corrected <0.003) before the perceptual report (blue line).

We have observed a consistent pattern of increased synchronization in the both experiments for the lower gamma frequency band. Moreover we have analyzed the imaginary component part of coherence (ImCoh) as a measure of synchrony that controls for volume conduction spurious activity and we found different connectivity patterns for
the different conditions suggesting that distinct neurophysiological mechanisms were involved associated with the upcoming object recognition. Figure 3-8 shows ImCoh plots as function of frequency.

![Imaginary Coherence - Experiment 1](image1)

![Imaginary Coherence - Experiment 2](image2)

**Figure 3-8. Representation of imaginary coherence (ImCoh) over all channels (pairs of channels) as function of frequency.** For experiment 1 (top panel) baseline was set to the interval before stimulus presentation. For experiment 2 (bottom panel) a baseline was subtracted consisting of the coherence time-averaged in the interval -1200 to -1000 ms. Colorbar codes imaginary coherence. Increased connectivity for the high beta/low gamma and high gamma-band is present thus replicating the results of phase-synchrony.

We found increased connectivity for the same frequency bands and time-intervals thus replicating the results of phase-synchrony. Figure 3-9 shows the ImCoh head-in-head plots difference between perception and no perception conditions. The connectivity is stronger for the topographic distributions that contain frontal and right occipito-parietal regions although one cannot make inferences about the directionality of information flow.
Figure 3-9. Head-in-head plots for the imaginary part of coherency at each frequency band. ImCoh is represented between all channel pairs time-averaged for the second before the button press. The difference between perception and no perception conditions is shown. Each small black dot corresponds to the position of the reference electrode in terms of connectivity. Note the link between occipital and frontal sites in the gamma range. Colorbar codes connectivity.

SVM data driven analysis shows that temporal patterns of gamma activity are informative in the classification of perception vs. no perception states.

The SVM classifier was able to classify without an a priori hypothesis the perceived/non-perceived trials of the Mooney dynamic task with high accuracy (>95%), above chance for all subjects where a significant number of no perception trials were available. A group average result for accuracy, sensitivity, specificity and balanced accuracy is shown for each frequency band in Table 3-2. The classifier performed with balanced accuracy above 95% for all these bands (that are matching the time-frequency results). The permutation results yielded P values bellow 0.001 in all of the cases, for the classification of perceptual states (which is remarkable even if the set of subjects with a sufficient number of trials for
classification was low). The likelihood that this would happen by chance even at a group level is very low. The contribution of each sub-band to the classification was based in an increase in perception related activity.

**Table 3-2. SVM classification results.** Frequency data from occipito-parietal electrodes were used as classification features to separate between perceptual states. Only subjects with >10 non-perceived trials were used. A group average of accuracy, sensitivity, specificity and balanced accuracy is shown for each frequency band (results in %). These bands are matching the time-frequency results (Table 3-1). We performed a permutation test for each subject and all p values were below threshold (P <0.001). Classification was successful for all tested subjects.

<table>
<thead>
<tr>
<th>Frequency Bands (Hz)</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Balanced Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-30</td>
<td>98.04 ± 1.29</td>
<td>99.07 ± 0.93</td>
<td>91.24 ± 6.59</td>
<td>95.15 ± 4.00</td>
</tr>
<tr>
<td>30-45</td>
<td>98.11 ± 1.31</td>
<td>99.34 ± 1.06</td>
<td>91.78 ± 5.25</td>
<td>95.56 ± 2.96</td>
</tr>
<tr>
<td>45-60</td>
<td>97.87 ± 1.43</td>
<td>98.67 ± 0.99</td>
<td>92.93 ± 6.52</td>
<td>95.80 ± 3.91</td>
</tr>
<tr>
<td>60-75</td>
<td>97.83 ± 1.51</td>
<td>98.96 ± 0.94</td>
<td>93.34 ± 6.88</td>
<td>96.15 ± 4.20</td>
</tr>
</tbody>
</table>

**Discussion**

This study provides a direct link between gamma-band temporal patterns and the presence versus absence of emerging holistic perception of variable onset. We investigated visual perceptual recognition moments based on EEG/ERP analysis with two different experiments. The novelty of our study lies in the fact that we departed from classical paradigms which are based on contrasts between stimuli conditions that are fixed in time. Our approach improves conventional designs by using short lived rapid visual presentation of many events with 1/30 likelihood of target presence or novel dynamic approaches whereby percepts are variable in which concerns the moment of recognition. In the latter case, this ensured that the moment of perception of an emergent global pattern was variable. This way we could directly compare perception vs. no perception states for the same stimuli.

In the first task, the short lived presentations yielded a characteristic ERP with a negative peak at 220 ms. Previous studies have shown a similar component for decision
related paradigms. The emergence of this negative peak is very common and can be related with the decision making demands of our paradigm as reported previously by others even in other sensory modalities. Moreover, our ERP data shows a clear P300 peak that appears in response to the rare target stimuli. In this experiment, we have found that increased gamma-band patterns appear in response to brief moments of object percepts.

This result was replicated in the second experiment, using dynamic stimuli. The topography plots for the perception condition at each frequency band show a broad distribution of the activity over the scalp. In this case, higher gamma-band activity appears in more anterior areas, possibly corresponding to decision related central regions. Interestingly, for the lower band of no perception states activity appears reduced over the occipito-parietal areas.

Both high beta/low gamma (30-45 Hz) and high gamma (60-75 Hz) frequencies showed higher synchrony but with an expected decrease in amplitude for the lower band, for perceived objects (faces or guitars, which are both very prototypical objects). Although the brief presentation paradigm show good evidence for a perceptual role of distinct gamma-band patterns in the emergence of percepts, our dynamic paradigm extended this notion by taking advantage of the well-known role of face inversion in holistic processing. The face-inversion effect, has been replicated by behavioral studies but has also been reported by other brain imaging studies (e.g., Rossion et al., 2000 and Yovel & Kanwisher 2005). Our manipulation uses the bias of holistic processing in Mooney stimuli. It delayed the time of perceptual discovery from stimulus onset through a gradual rotation from inverted to upright position because objects are mostly perceived far from the inverted position. This way, sensory processing was separated from perception. We would like to emphasize (as reported in Rebola et al., 2012) that one departs from a configuration not favoring holistic perception to one favoring a holistic perceptual interpretation, as also discussed by Jemel and colleagues, 2009. By delaying the moment of global integration or rendering it unpredictable this paradigm focused on global gestalt mechanisms instead of local sensory to noise levels. The moment a coherent visual stimulus is perceived was therefore variable in its time of occurrence as well as in the Rapid Visual Presentation paradigm. Nevertheless, as reported by Melloni et al., 2011 the expectation for the stimulus at a short time scales may affect peak signal latency and amplitudes.
The analysis of phase synchrony patterns showed that object perception was associated with a burst of synchronous activity in low frequency gamma-band components in both tasks. This synchrony pattern for the lower frequency band (most specific in task 2 but also present in task 1) irrespective of amplitude of gamma-band changes suggests that, at lower gamma-band frequencies, a different neurophysiological process associated with the upcoming object recognition, was involved. Interareal synchrony between areas has been reported as a mechanism for binding of information across different brain regions. To validate this increased interaction we looked to the ImCoh as a reliable measure for neuronal interactions that is insensitive to volume conduction artifacts and we found a similar pattern of activity with increased connectivity for the same frequency bands and time-intervals (see supplementary figure S1). It is accepted that ImCoh represents brain connectivity at the sensor level. According to this view, the connectivity patterns we found can be related with the interaction between dorso-ventral stream regions involved in object perception and anterior areas usually reported as decision related. Remarkably, we found a decrease in gamma amplitude during an increase in synchrony. These patterns are evidence that the synchrony increase was not caused spuriously by neither a change in power of a common source or a volume conduction artifact.

These results shed light on the mechanisms underlying perceptual object processing and decision making and provide support for the role of gamma-band frequency patterning and synchrony in the well-known binding problem. Our data supports a functional role for distributed spatiotemporal patterns of gamma-band activity and synchronization in perceptual decision. Together, these findings provide support for the claim that gamma-band activity is a signature of emergent holistic perceptual states.

An additional contribution of this study was the independent validation by data driven (non-hypothesis constrained) approaches. Support vector machine classification approach enabled us to directly distinguish between non-perceptual vs. perceptual states, based on time-frequency features in the gamma frequency band. This SVM approach is a proof of concept that worked in all subjects that were eligible for classification in terms of number of trials. Classification balanced accuracy, sensitivity and specificity were higher than 95% thereby emphasizing perception related neurophysiological signatures. This analysis should be viewed in light of our evidence that sustained activity was dominant in the gamma-band, particularly in perception states. These results show that a broad range
of frequencies is informative, corroborating the tenet that the brain uses different oscillatory bands to code different information \(^{17,39,41}\). This statistical classification of perceptual states using an SVM approach suggests an important functional role for gamma activity patterns that can be generalized to ambiguous percepts. This analysis provides evidence that time-frequency patterns at gamma-band frequencies provide sufficient information to infer about perceptual states in a data driven manner.

**Conclusions**

In sum we have found evidence that gamma-band features can differentiate perceptual versus non-perceptual states, as confirmed by SVM classification. We conclude that a functional role for distinct distributed spatiotemporal patterns of gamma-band activity can be identified for the moment a holistic object percept is formed.
References


The dual facet of gamma oscillations:

Separate visual and decision making circuits as revealed by simultaneous EEG/fMRI
Abstract

It remains an outstanding question whether gamma-band oscillations reflect unitary cognitive processes within the same task. EEG/MEG studies do lack the resolution or coverage to address the highly debated question whether single gamma activity patterns are linked with multiple cognitive modules or alternatively each pattern associates with a specific cognitive module, within the same coherent perceptual task. One way to disentangle these issues would be to provide direct identification of their sources, by combining different techniques. Here, we directly examined these questions by performing simultaneous EEG/fMRI using an ambiguous perception paradigm requiring holistic integration. We found that distinct gamma frequency sub-bands reflect different neural substrates and cognitive mechanisms when comparing object perception states vs. no categorical perception. A low gamma sub-band (near 40 Hz) activity was tightly related to the decision making network, and in particular the anterior insula. A high gamma sub-band (~60 Hz) could be linked to early visual processing regions. The demonstration of a clear functional topography for distinct gamma sub-bands within the same task shows that distinct gamma-band modulations underlie sensory processing and perceptual decision mechanisms.
Introduction

It has been proposed that gamma-band activity signals emerging object percepts and decision-making\(^1\). Accordingly, gamma-band modulation is increased for ambiguous states prior to a perceptual decision\(^3\). Animal studies\(^6,7\) further support the ability to form coherent Gestalts\(^8\) as a function of gamma, in addition to the earlier mentioned signaling of emerging object percepts.

Furthermore, gamma oscillations have been related to many cognitive processes\(^1,2,9^-12\). Hence, electrophysiological studies showed a wide variety of gamma-band patterns and sources for different tasks\(^13^-21\) but not within the same type of cognitive task. Accordingly, it remains unclear whether distinct patterns related to different cognitive modules coexist in the same task. Unimodal studies do not have enough resolution to test for non-unitary sources and to establish their spatial distribution\(^10,22\). It is known that gamma patterns occur in a distributed manner across cortical regions\(^15,23,24\) and even at different frequency ranges\(^4,10,22,25\). A major question is however still open: is there a single gamma activity pattern of a broad frequency band that reflects perception\(^1,2\), decision mechanisms\(^24\) or both or, on the other hand, are there separable gamma narrow sub-bands with distinct spatial sources in the brain?

In line with this notion, earlier experiments (in particular in animals) suggested that low- and high-frequency oscillatory sub-bands may indeed reflect different neural processes and may be originated in distinct brain regions\(^14,24,26\). In this line, a recent MEG study also showed a dissociation of attentional processes influencing higher gamma oscillations in early visual cortex, but not higher visual cortex, with clear distinct source localization for distinct frequency sub-bands, but within the same region (lateral and medial visual cortex\(^27\). Interestingly, we have also identified two distinct patterns of gamma-band activity for the moment a holistic object percept is formed\(^5,28\). These were observed in a perceptual decision paradigm requiring object recognition from 3D structure-from-motion in normal subjects and in a clinical model of impaired perceptual coherence\(^28\). A low range gamma sub-band (near 40 Hz) was increased in patients while a higher gamma narrow band (\(\sim 60 \text{ Hz}\)) was increased in controls, suggesting the use of distinct cognitive modules. This finding inspired us to test whether a critical dissociation exists between low and high gamma-band oscillations\(^4\). If they reflect non-unitary processes, that would be relevant to understand normal cognition and neuropsychiatric disorders such as autistic
spectrum disorder and schizophrenia\textsuperscript{28,29}, which are clinical models of fragmented perception and decision.

Despite the technical challenges, simultaneous recordings of electrophysiological and hemodynamic activity can be successfully measured\textsuperscript{30–36} and potentially clarify the contribution and sources of relatively narrow low- and high- frequency neuronal dynamics\textsuperscript{14,24,26} given their complementary advantages\textsuperscript{31,37} and coverage\textsuperscript{30,38,39}. Besides, despite the known limitation of EEG/fMRI, gamma-band activity can be assessed using this technique at least in the 25-75 Hz range\textsuperscript{32,34,40} and linked to the Blood oxygen level dependent (BOLD) fMRI response\textsuperscript{41–44}, as assessed by general linear models\textsuperscript{45,46} in humans performing a cognitive task\textsuperscript{26}.

To investigate the neuronal sources of gamma-band responses to ambiguous perceptual states\textsuperscript{15,39} with high spatial resolution, we performed simultaneous EEG/fMRI recordings. Our hypothesis was that independent gamma processes underlie visual perception and decision. We used a face/object detection and categorization task that was previously shown to increase gamma-band activity patterns\textsuperscript{15,47}. Increased activity was found to perception vs. no-perception states. We predicted that separable sources of gamma-band activity should be identified in relation to different cognitive processes by means of an EEG-informed fMRI approach. This strategy implements EEG pre-processing to obtain a specific EEG feature (time-frequency activity within a specific frequency band\textsuperscript{48}) that can be used as predictor for the general linear model (GLM) analysis of simultaneous fMRI data\textsuperscript{49}. We found that low gamma (30-48 Hz) response is related to general perceptual decision-making networks and high gamma activity (52-70 Hz) is associated with low level visual processing. These non-unitary patterns are relevant for the understanding of normal and clinical impaired holistic integration and show that current neural models of gamma activity generation need to consider their duality.

**Materials and Methods**

**Subjects**

We performed a simultaneous EEG/fMRI experiment in ten healthy subjects (age=26.33±4.17 years; 6 males; two left handed). All participants had normal or corrected-to-normal vision and no history of neurological disorders and were naive regarding the purpose of the study. All subjects performed the simultaneous EEG/fMRI task (two runs) and one
prior run of EEG recording outside the scanner. This study was approved by the Ethics Commission of the Faculty of Medicine of the University of Coimbra and was conducted in accordance with the declaration of Helsinki. All subjects gave written informed consent to participate in the study.

Stimuli

Visual stimuli were Mooney pictures (black and white incomplete stimuli) of faces (upright and inverted), guitars (prototypical stimuli) and their scrambled versions (see Figure 4-1). Stimuli were presented using Presentation software (Neurobehavioral Systems). Subjects viewed the projected (LCD Projector, Avotec, USA) stimuli in a white screen located at the back of the scanner (stimulus size: 6.56°x6.60°) through a mirror placed on the head coil.

**Figure 4-1. Task timeline.** A) Examples of Mooney object categories and scrambled controls. B) Timeline details; each run started with a black screen for 10000 ms and contained 100 trials (25 trials of each category). Three runs were performed during the experiment (one outside scanner and two inside scanner). Stimuli were presented for 150 ms in a trial that lasted for 2000 ms. The average inter-stimulus-interval (fixation cross on a black screen) was 7850 ms (slow event-related design). Stimulus presentation was randomized across subjects and runs, and there were no repetitions. Subjects had to discriminate between categories (face, guitar or other) and to press the respective button in the response box only after stimulus disappearance. EEG and fMRI recordings were done concurrent with stimulus presentation.
Procedure

The experiment consisted of three runs of EEG recordings: two runs were collected during fMRI acquisition and one outside the scanner. fMRI was acquired concurrently with the EEG recordings. Each run included 25 Mooney face stimuli, 25 inverted Mooney faces, 25 Mooney guitars and 25 scrambled Mooney events. In total we had 50 trials of each condition for the recordings obtained inside the scanner. The number of trials for perceived faces vs. non faces stimuli was approximately identical thus precluding possible response-related attentional effects. Recognition of Mooney objects was previously linked to increased gamma-band activity\textsuperscript{1,47}. Stimulus presentation was randomized across runs and subjects, and no stimulus was repeated to prevent repetition induced effects. The trial duration was 2 s, during which one stimulus was presented for 150 ms. Stimulus presentation was triggered automatically by the fMRI pulse. The stimuli were randomly presented with an average inter-stimulus interval of 7850 ms (Figure 4-1 B). A fixation cross on a dark screen was shown during the baseline periods. Subjects performed a forced choice discrimination task between the Mooney categories and were asked to press one of three keys after the image presentation to discriminate between faces, guitars and scrambled stimuli. Note that responses were required only after the stimulus offset (for details on reaction times see results section), to prevent early contamination from motor responses.

EEG recording

For EEG recording we used an MR compatible EEG system (MagLink\textsuperscript{TM}, NeuroScan, USA) with a cap providing 64 Ag/AgCl nonmagnetic electrodes positioned according to the extended 10/20 system. The recording reference was set to an electrode close to CZ and EEG and fMRI data were acquired in a continuous way. The EEG signal was amplified and recorded at a sampling rate (SR) of 10 kHz. This high SR is necessary to sample accurately the gradient artifact. The amplifier was located and connected to a PC in the scanner control room. To improve the effectiveness of the offline artifact correction algorithms, EEG and fMRI recordings were synchronized by means of a Syncbox (NordicNeuroLab, USA)\textsuperscript{32,51}. The exact timing of stimulus onset and MRI scanner gradient switching were recorded together with the EEG signal as well as with the stimuli logfiles. Vertical and horizontal electrooculogram (VEOG and HEOG) bipolar channels were used to record EOG and two more channels were placed in the standard electrocardiogram (EKG)
electrode positions for EKG recordings. These data were required to detect the ballistocardiogram (BCG) artifacts in the EEG recordings. All artifacts (gradient artifact and BCG) were removed offline (see below). Channels that did not fulfill the impedance criteria (<15 Ω) or had problems during acquisition were marked as ‘bad’ and excluded (on average >55 channels were available for final analysis). To overcome this issue for group average results, the spherical interpolation of missing channels for each subject was used as it is implemented in EEGLAB (Matlab® toolbox v10_2_5_6a).

Functional MRI recording

MR imaging data were acquired on a 3T whole-body MR scanner (Siemens Trio) using a 12 channel head coil. Anatomical images were collected using a T₁ weighted MPRAGE (magnetization-prepared rapid-acquisition gradient echo). T₂*-weighted gradient-echo echo planar imaging (EPI) was used to collect fMRI data with TR=2 s, TE=39 ms, 27 slices per TR, 2.5×2.5 in-plane resolution, 3.5 mm slice thickness with 4.2 mm gap, flip angle=90° and matrix size=102×102. The slices were oriented to obtain whole brain coverage. 410 volumes were acquired for each run with a total scan time of 13'40 min. All runs were acquired in the same EEG/fMRI session. The first 3 volumes were discarded from the analysis. Data analysis was performed using BrainVoyager QX v2.4 (Brain Innovation, Maastricht, The Netherlands).

EEG Data analysis

Analysis of EEG data acquired concurrently with fMRI

Concurrent EEG/fMRI is challenging due to gradient artifacts (which are related to gradient switching) and ballistocardiogram signals (which represent physiological cardiac-related artifacts) 53. Several methods have been developed for offline correction of the data 33,53–56.

Artifact reduction

- Gradient Artifact

The gradient artifact is due to the magnetic field variations that are induced by gradient switching during the MR pulse sequence. As a direct consequence of Faraday’s law of induction, spurious electric potentials are thus superimposed to the EEG electric potentials that result from neuronal activity. Gradient artifacts are approximately three
orders of magnitude larger than the normal EEG activity and they are particularly strong during the application of the radio-frequency (RF) pulse. This artifact dominates the EEG signal (see Figure 4-2 A) but its temporal profile is consistent across channels and time. This reproducibility is exploited by the correction algorithms during offline analysis. Computing a template from the average artifact over a number of TRs and subtracting it from the observed signal yields the uncontaminated EEG. This methodology is based on the assumption that the artifact is not correlated with the physiological signal. It is improved by synchronizing the EEG and MR acquisitions hence obtaining a precise sampling of the artifact.

EEG artifacts related to MR gradient switch were corrected offline using average subtraction gradient correction implemented in Maglink RT Edit software (v4.5, NeuroScan, USA). In brief, this creates an average template of the artifact that is subtracted from the recorded EEG. The correction algorithm includes a low-pass filter of 75 Hz.

- Ballistocardiogram (BCG)

The BCG artifact (see Figure 4-2 B) has a much lower amplitude than that of the gradient artifact and is induced by variations of the cardiovascular activity of the subject within the magnetic field of the MR scanner. It occurs in synchrony with the cardiac rhythm (with a delay of about 200 ms) and has a complex topographical and temporal profile that differs from subject to subject as well as between channels (due to their orientation in the magnetic field) or MR scanners. The size of the artifact is proportional to the magnetic field strength and it is very variable over time. Nevertheless, its origin remains largely unknown.

The main approach that is used to correct for this artifact is analogous to the average-subtraction method that was explained before for gradient artifact correction. However, the BCG artifact is more difficult to correct with simple average-subtraction than the gradient artifact because its shape tends to change over time. This makes it more difficult to build a reliable artifact template. In order to overcome this difficulty, several approaches have been proposed. New algorithms based on independent component analysis (ICA) are becoming popular to correct MR (BCG) related artifacts since they do not require temporal stability for template subtraction. This approach divides the signal
into different statistically independent components (see ICA section in chapter 2 for details) and the ones that best represent the artifacts are extracted from the data $^{62-64}$.

**Figure 4-2.** Example of a time-window of EEG recorded inside the MR scanner during image acquisition. **A)** Raw data including the gradient artifact. **B)** Signal corrected for the gradient artifact with average subtraction template algorithm. BCG artifact indicated with the arrows. **C)** EEG data free of the gradient and BCG artifacts. BCG artifact corrected with Independent component analysis.

BCG artifacts were corrected using ICA $^{62}$ implemented in EEGLAB (Matlab$^\text{®}$ toolbox v10.2.5.6a $^{52}$). The independent components were inspected and the ones with higher correlation with the EKG signal recorded during the acquisition were removed from the signal. ICA, based on all electrodes (including 4 EOG channels), was also applied to perform correction of eye movement related artifacts as performed in Keren et al., 2010 $^{65}$. We used the scalp topography of the ICA components to identify the ocular component for ocular artifact attenuation. All channels were re-referenced to average reference. Epochs (-500 to 1000 ms) were obtained locked to the beginning of the correctly reported stimuli and automatic epoch rejection as implemented in the EEGLAB toolbox was then applied with an amplitude criteria of $\pm 75 \mu$V followed by visual inspection. After epoch rejection $84.5\pm 7.23\%$ (mean $\pm$ standard deviation) epochs of faces, $83.75\pm 12.07$ epochs of inverted faces, $82.5\pm 8.26\%$ epochs of guitars and $83.75\pm 13.48\%$ epochs of the scrambled condition per subject remained for further analysis.
Time Frequency analysis

Time–frequency analysis was implemented as in Uhlhaas et al., 2006 and is described elsewhere. This analysis was carried out in Matlab concerning induced (non-phase locked) activity for frequencies ranging from 10 Hz to 70 Hz (feasible range within the limitations of EEG/fMRI) in steps of 1 Hz for all channels. Time-frequency data were normalized (in the z-score sense) to the baseline defined to pre-stimulus time-window (-500 to -50 ms) by subtracting the mean of the baseline per frequency bin and dividing by their standard deviation. Results are shown for a cluster of parieto-occipital and temporal electrodes (PO7, PO5, PO3, POZ, PO4, PO6, PO8, CP5, TP7, P5, P7, CP6, TP8, P6 and PB).

For the statistical comparison between conditions, for each time-frequency point, nonparametric statistics (Mann–Whitney U tests, Wilcoxon rank sum test) were carried out and Bonferroni–Holms correction for multiple comparisons (P values divided by the number of comparisons) were applied when appropriate. Furthermore, Pearson correlation was computed to assess if reaction time covariate did explain the observed pattern of results.

ERP measures were also obtained. Data were filtered between 1-30 Hz and segmented into epochs (-100 to 500 ms) locked to stimulus onset. After baseline correction (pre-stimulus interval) ERPs were obtained averaging the remaining trials per condition. One-Way ANOVA with Bonferroni correction was computed to assess statistical significance of the resulting ERPs.

ERP Analysis of EEG data acquired outside the scanner

EEG data recorded outside the scanner were processed offline using the Scan 4.5 Edit Software (NeuroScan, USA) and EEGLAB (Matlab). ICA-based ballistocardiogram correction was performed to ensure that processing was exactly identical to the one performed inside the scanner. Data were filtered between 1-30 Hz and segmented into epochs (-100 to 500 ms) locked to stimulus onset. After baseline correction based on the interval before stimulus onset the automatic epoch rejection (followed by visual inspection) was applied with a criteria set to ±75 µV (with >88% of the trials accepted for further processing). The epochs were organized by stimulus type and event related potentials were obtained. One-way ANOVA statistical test was performed to assess
differences between conditions and Bonferroni correction was used to correct for multiple comparisons.

**ERP Source localization**

We performed source localization of the EEG data as it is implemented in *Curry 7.0* software (NeuroScan, USA) on a realistic head model. For each condition, group average ERP data were co-registered with anatomical MR data using landmarks and standard electrode positions. Standard anatomical MR data were used to create the boundary element model (BEM) and current source density (CSD) analysis was performed. CSD computes a local current pattern that explains the EEG data at a certain time-point. The CSD source localization was based in sLORETA (standardized low resolution brain electromagnetic tomography) method with no assumption regarding the number or location of active sources. This method is a standardized discrete, three-dimensional (3D) distributed, linear weighted Minimum Norm Inverse solution that takes several neurophysiologic and anatomical constraints into account and has been shown to yield depth-compensated zero localization error inverse solutions. sLORETA employs the current density estimate given by the minimum norm solution, and localization inference is based on standardized values of the current density estimates. It was computed in *Curry 7.0* yielding an estimate of activity measures, namely (for each location) the current strength divided by its error bar, from which a F-distribution of the current densities were extracted. This is not a statistical estimate in a strict sense and therefore it has only localizing value and not in the comparison of activities across sources, which justifies that some caution should be taken regarding interpretations of such activated sources. Source localization plots were computed for a 200 ms time-window including the N170 peak.

**fMRI data analysis**

Functional MRI data were analyzed (pre-processing and statistical analysis) using *BrainVoyager QX 2.4* (Brain Innovation, Maastricht, The Netherlands). The first 3 volumes were discarded to avoid saturation effects. Pre-processing was performed with the default parameters including 3D motion correction, spatial smoothing and temporal high-pass filtering of 0.00980 Hz (3 cycles in time course). The images were transformed into Talairach space. This is a limitation of the study since this atlas template is based in only
one subject. fMRI data were co-registered with anatomical data and statistical fMRI analysis was performed using the general linear model (GLM), as implemented in BrainVoyager QX.

**Gamma-band source localization**

First, to localize the sources of gamma activity in two distinct frequency ranges, Low (30-48 Hz; LF) and High Frequency (52-70 Hz; HF) per stimulus condition we performed time-frequency decomposition using Curry 7.0 software. The 48-52 Hz range was excluded to avoid line noise. We performed this frequency analysis using the Short Time Fast Fourier Transform (STFFT) as implemented in Curry 7.0 software. It provides time and frequency representation of the signal with high temporal resolution for the faster frequencies. Source localization was performed for all accepted channels combined. The resulting source analysis time points for each condition are based on the latencies of the time-frequency peaks extracted from the group average data over all parieto-occipital and temporal electrodes (see above) and locked to the beginning of the stimulus. This was performed for the low and high frequency ranges (data filtered between 30 and 48 Hz and between 52-70 Hz, respectively). Using the same procedure as in the ERP source localization we calculated the current source density maps from the group data, as revealed by sLORETA for these gamma-frequency ranges.

Furthermore, to precisely localize the active regions we performed an EEG-informed fMRI approach in which an EEG time-frequency feature is extracted from EEG data and used to inform fMRI GLM analysis (Figure 4-3 schematically shows how this approach is performed).
Figure 4-3. Schematic representation of EEG-informed fMRI analysis. After artifact correction, EEG data (acquired concurrently with fMRI) were split into 2 s epochs and time-frequency (TF) decomposition applied. For each epoch that contains an event of interest (perceptual conditions) the latency of the maximum of gamma activity was extracted and used to build the fMRI protocol. Separated low and high gamma frequency events for each condition were used as predictors. After convolution with the hemodynamic response function (HRF), a general linear model (GLM) analysis was performed in fMRI data and the resulting statistical source maps show the sources of gamma activity peaks.\(^2\)
Consecutive time-frequency analysis epochs of 2 s were applied to detect oscillations induced but not necessarily phase locked neither to the stimuli nor to the response. Concerning EEG-informed fMRI, as we found similar time-frequency results and current density source patterns for all readily perceived object categories, we used all Mooney object (faces + guitars) stimuli together vs. scrambled + inverted faces (difficult perceptual decision conditions). This analysis using balanced number of trials focused more on the issue of difficult perceptual decision. Separate EEG regressors for each condition (object and scrambled) from low (30-48 Hz  $^{25}$) and high (52-70 Hz  $^{18}$) frequency bands were constructed. The peak latencies were obtained on a trial by trial basis per subject, in the two gamma sub-bands of interest and calculated by averaging time-frequency results over the same parieto-occipital and temporal electrodes as before. The resulting predictors were introduced in the GLM design matrix  $^{39,46,72}$ as in event-related fMRI designs. Contrasts were computed against the baseline (with epochs without any peak neither in LF nor HF sub-bands). Statistical maps show the results for LF and HF based contrasts for a whole-brain analysis when computing the above mentioned contrasts. Corrections for multiple comparisons were made through the Cluster Threshold plugin (BrainVoyager QX) with 1000 iterations. The minimum cluster size was set to a significant voxel threshold of  $P<0.05$ for each contrast. T-tests were applied between conditions in each frequency band and the coordinates and statistical results of the peak analysis for all clusters (of increased or decreased BOLD signal) were obtained.

**Results**

**Behavioral data**

To study the neural underpinnings of EEG oscillations, we used a categorical perception task in which subjects had to discriminate between Mooney (two-tone incomplete patterns) object categories (Figure 4-1). Behavioral analysis showed that participants were well above chance in terms of categorization performance levels (96.7%±3.13% for “guitar-like” stereotypical objects; 81.4±13.3% and 81.06±13.46% for upright and inverted Mooney faces respectively; 75.79±18.90% for scrambled control stimuli; values indicate mean± standard deviation, except when otherwise indicated). An ANOVA revealed a main effect for condition (F=18.156,  $P<0.00001$) and the posthoc Bonferroni show that these differences in discrimination rates were significant between faces and
guitars (Mean difference: 20.51, \( P < 0.0001 \)), inverted faces and guitars (Mean difference: 12.08, \( P = 0.027 \)), guitars versus scrambled (Mean difference: 29.00 \( P < 0.0001 \)) and inverted faces vs. scrambled (Mean difference: 16.93, \( P = 0.001 \)). A bar graph summarizing these results is shown in Figure 4-4 (left panel). This shows that subjects were best at guitar discrimination. Regarding reaction times, a main effect of condition was also present (\( F=19.502, P < 0.000001 \)).

The Bonferroni posthoc analysis revealed that subjects performed slower for the scrambled condition in comparison with all the others (Mean difference: 241.09 ms, \( P < 0.000001 \), for scrambled vs. faces; 285.94 ms, \( P < 0.000001 \) for scrambled vs. guitars; 217.96ms, \( P = 0.000004 \) for scrambled vs. inverted faces). On average, subjects’ reaction times were 725.7±88.20 ms for Mooney faces, 751.20±98.02 ms for upside down faces, 682.69±77.81 ms for guitars and 968.22±136.13 ms for scrambled conditions, well after stimulus offset (Figure 4-4 right panel contains a plot summarizing these results).

**Figure 4-4. Behavioral results. Left panel:** bar plot showing the behavioral results per condition in terms of the discrimination rate. Significantly different comparisons are highlighted in the graph. Note that subject’s best rate level is for guitars. **Right panel:** a bar plot representing the reaction time per condition and the statistically significant differences between them. Scrambled conditions are slower than all the other stimulus.
Event-related potentials

Figure 4-5 A and B shows ERP measures for each category outside (single EEG/ERP recordings) and inside (simultaneous EEG/fMRI measurements) the scanner, respectively. After artifact correction, we were able to observe similar waveform patterns outside and inside the scanner, across conditions. We observed in both cases the expected positive P100 early visual component and a similar categorical dependent pattern around the typical face selective N170 \(^{74}\). Our results are in line with the report of Sadeh et al., 2008 \(^{75}\). An one-way ANOVA with Bonferroni correction for multiple comparisons (P <0.05) revealed the time points where the difference between conditions were significant (see gray bars in Figure 4-5 C) for detailed representation of significantly different time points). Differences were found close to the N170 and latter around 300 and 400 ms. Furthermore, note that the Mooney face stimuli are evoking the larger amplitudes of the N170 (150-200 ms, P <0.05).

![Figure 4-5. Group average ERPs for EEG data acquired outside (A) and inside the scanner (B). Separate plots are shown for each condition for the PO8 electrode. Bold lines represent the group average and thicker lines the respective standard deviations. C) Superimposed group average ERPs. Different colors represent ERPs for the PO8 channel for the different stimulus categories. Gray bars at the bottom show the time-points where differences are statistically significant as revealed by ANOVA with Bonferroni correction (P <0.05).](image-url)
**Time-frequency data**

We computed the time-frequency (TF) transforms of single epochs and averaged them across all trials and the posterior cluster of electrodes. In this way, we focused on the induced gamma activity patterns which are not phase-locked to the stimulus onset. The time-frequency results are summarized in Figure 4-6. Time-frequency representations of gamma activity patterns for objects minus control (non-perceived) scrambled conditions are shown in Figure 4-6 A, B and C for the faces, guitar and inverted faces categories respectively. We found increased activity at the gamma-band frequency range for all categories vs. the scrambled condition after the stimulus onset period (the TF result for the scrambled condition is shown in Figure 4-6 D).

**Figure 4-6. Normalized Time-Frequency** analysis reveals a similar pattern for all object categories conditions minus the scrambled (non-categorical) condition. TF results are shown for a group average of all parieto-occipital and temporal cluster of channels, for faces, guitars and inverted faces (A, B and C, respectively). Resulting time-frequency data from the scrambled condition (also shown in D) was subtracted in all conditions. Color scale means normalized units (in relation to baseline pre-stimuli) and the blue line marks stimulus onset. Note that subject decision was only reported after stimulus offset (see reaction time results in Figure 4-4). E) Plot showing the statistical p-values for the comparison between Object categories and the Scrambled conditions with the Wilcoxon ranksum test (P <0.025). For additional information on normalized time-frequency results obtained over different clusters of channels see Figure 4-7.
The Figure 4-6 E shows time frequency corrected P value maps (Wilcoxon rank sum test) represented in a plot showing the time-frequency points where the difference is significant (P < 0.025). It is noteworthy to point out that all objects vs. scrambled condition generate similar spectral signatures, with two bands, one at the low gamma range (~40 Hz) and the other at high gamma frequency range (~63 Hz). Moreover, scrambled conditions induced modulations dominating at lower frequency bands (high beta and low gamma) whereas object categories dominate for the higher frequency band. The time-frequency activity per electrode’s cluster for both (scrambled and object) conditions are shown in Figure 4-7.

![TF maps](image)

**Figure 4-7.** Time-frequency results obtained over different clusters of channels (occipital, parietal, temporal, frontal, and those Left and Right). **A)** Time-frequency results for the faces + guitars condition; **B)** Time-frequency results for the scrambled + inverted faces condition. Colorscale means normalized units (data normalized for the time preceding stimuli presentation) and blue line mark the stimuli onset.

We found a peak (increased gamma activity) with similar average frequency of 39.44±2.46 Hz and 40.28±3.70 (lower gamma-band, LF) for the readily perceived objects (faces + guitars) and difficult conditions (scrambled + inverted faces) respectively. For the high gamma frequency range (HF), the peaks were found for an average frequency of 63.11±3.75 Hz for objects and 62.95±3.10 Hz for scrambled conditions. Mean peak
amplitudes per frequency band were 9.49±6.99 and 7.97±3.78 (power) for objects and scrambled LF respectively, and 14.09±3.13 for objects HF and 7.75±3.72 for scrambled HF. Differences between peak frequencies of objects vs. scrambled were not significant neither for frequencies (t=−0.71, P =0.5 for LF; t=0.10, P =0.920 for HF) nor for amplitudes (t=1.009, P =0.347 for LF and t=1.228, P =0.259 for HF). Moreover, no correlation was found between differences in amplitude and differences in reaction times (RT; Pearson =0.017, P =0.967 for LF and Pearson correlation =0.294, P =0.479 for HF) or between differences in discrimination rates and differences in peak amplitudes (Pearson =0.5, P =0.253).

Source localization based on the EEG data

We performed source localization of group average ERPs using Curry 7.0 (NeuroScan, USA) for each stimulus category and found the expected ERP sources of activity at visual ventral regions 170 ms after stimulus onset. All object perception conditions showed indeed similar bilateral infero-temporal activations (Figure 4-8 A, B and C) that are distinct from the one evoked by the scrambled control (no object perception) condition (see Figure 4-8 D). Right hemisphere dominates for the faces, inverted faces and scrambled conditions while left temporal regions appear for the guitars. We only found dominant occipital and frontal regions for the scrambled (absent visual category) condition. Results shown in Figure 4-8 are displayed for the hemisphere revealing activity over a threshold of 75% of the CSD (see figure legend for F-distribution values per condition).

In addition, we performed time-frequency decomposition using Curry 7.0 software and calculated the current source density maps as revealed by sLORETA. We separated the analysis by conditions and low and high frequency ranges. Figure 4-9 summarizes the results for these frequency bands. The activity for the low frequency (LF) band (Figure 4-9 A to D) emerges mainly at infero-frontal regions (insula plus frontal operculum) known to be related to decision processes. This pattern is similar for all stimulus conditions.

On the other hand, high frequency patterns (HF; Figure 4-9 E to H) show distributed activity sources that differ depending on the condition. The faces are localized to parietal regions, inverted faces sources are localized to right temporal regions, while guitars show a pattern with conspicuously localized sources at frontal areas. In contrast, the scrambled (non-recognizable object) condition shows a different pattern reminiscent to the one observed with ERP source localization with dominant occipital and frontal regions.
Figure 4-8. Categorical perception leads to a pattern that is distinct from perception of random (scrambled) patterns. Source localization of group average ERPs reveals sources of activity at visual ventral regions. Results are shown for the N170 latency point. Color codes correspond to the range of min and max CDR distribution values based in sLORETA. Right hemisphere dominates for the faces (F-distributed (min-max): 5.2-6.7), inverted faces (F-distributed 3.9-5.6) and scrambled condition (F-distributed 5.0-7.0) while left temporal regions appear for the guitars (F-distributed 3.3-4.2). Note that all object conditions (A, B and C) have a similar infero-temporal pattern that differed from the one evoked by scrambled conditions (D). A display threshold of 75% of the CSD was applied and the sources are shown for the hemisphere that revealed higher activity.
Source localization with EEG-informed fMRI approach

Using a general linear model analysis of the fMRI data, to model the neural origins of the different gamma activity patterns, we were able to identify two different neural generators for these distinct frequency bands. To precisely localize these patterns of gamma-band activity we used the latencies (on a trial by trial basis, per subject) of the gamma activity peaks as event predictors of a GLM modeling the EEG oscillations. Separate predictors for the low (30-48 Hz) and high (52-70 Hz) frequencies were constructed. Differences in latency peaks between conditions were not significant (LF, object vs. scrambled: F=0.231, P =0.634; HF, object vs. scrambled: F=0.075, P =0.786). Results show that posterior sources mainly located at the parieto-occipital and temporal lobes were strongly linked to the higher gamma frequency band but also for the LF band, in particular for object category perception (Figure 4-10 A and C).

We found that different gamma sources are related to perception processes in decision related regions centered in the anterior insula (Figure 4-10 B and E) and in the visual processing areas (Figure 4-10 C and D).
**Figure 4-10. EEG-informed fMRI evidence for different neural generators for distinct frequency bands**, one related to visual processing (early visual areas) and the other to high level decision mechanisms (e.g., the insula). **A)** and **B)** show GLM maps based on gamma-band regressors - Low frequency (LF) for face + guitar objects (easily perceived) and inverted faces + scrambled conditions (not readily perceived), respectively. High frequency (HF) GLM maps are shown in **C)** and **D)**. Note that group analysis showed different significantly activated regions as a function of frequency band. We found increased activity for decision related areas (anterior insula) mainly for the LF predictor (~40 Hz) and visual areas activated in particular in response to higher Gamma oscillation predictors which are related to object perception and scrambled conditions. **E)** Brain regions activated for the contrast HF>LF. **F)** Areas with increased modulation for the LF>HF contrast. Map threshold was set to P corrected <0.05. See Table 4-1 for details regarding significant clusters sizes, locations and statistics.

Low gamma frequency related activity in the anterior insula was particularly strong, which is interesting given the role of this region in perceptual decision 47 (Figure 4-10 B; see Table 4-1 for details regarding regions’ coordinates, cluster sizes and statistics).

Furthermore, a dissociation was visible for the LF sources between perceived objects and scrambled conditions. Interestingly, activity for object percepts was lateralized to the right insula while for scrambled conditions it was higher at the left anterior insula. Negative BOLD activity, as measured by EEG-informed fMRI was visible for different contrasts. These activations were found to the scrambled LF predictors localized in areas
spanning from occipital regions to anterior areas including the sub-lobular insula and the anterior cingulate. Regarding the HF predictors, negative activity was found for parietal and frontal regions (see Table 4-1 for details). Figure 4-10 E and F show the GLM results for the two direct contrasts: HF higher than LF and vice-versa, respectively. Note that posterior (visual) activation is evident for the HF>LF and bilateral anterior activation on the insula is present for the LF>HF contrast.

Table 4-1. Location and statistics of significantly activated brain regions (clusters) for the different contrasts of GLM analysis (distinct conditions and frequency ranges). LF and HF stands for low frequency and high frequency respectively. R, L and BA stands for right and left hemispheres and Broadmann Area, respectively. Clusters were obtained correcting for multiple comparisons by applying the cluster-level statistical threshold estimator from BrainVoyagerQX Plugins with 1000 iterations and alpha <0.05.

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Discussion

In this simultaneous EEG/fMRI study we found that different gamma oscillatory patterns corresponding to different brain regions/networks can be separated within the same perceptual decision-making task. These regions were identified as visual and insular networks. High gamma-band patterning (~60 Hz) dominated in visual regions and a lower band (~40 Hz) dominated in the anterior insula, which is a region known to be involved in high level decision processes 16. Both visual and insular sources are known to be important for perceptual decision making and gestalt formation 16.47. We observed that under difficult decision conditions, when an object category was harder to detect, the insula showed a higher modulation. This is precisely the region most closely associated with higher beta and low gamma frequency modulation (together with the anterior cingulate 77, which is involved in conflict monitoring 78). Larger activity modulation at the higher frequency gamma-band around decision time was present for holistic categorical (object) vs. non categorical (scrambled) perception. Such a dissection of the cognitive and
neural components of perceptual decision suggests that different gamma sub-bands can be related to separable circuits within the same cognitive task.

Different Gamma sub-bands had been previously identified in different cortical regions and tasks \(^4,10,13-21,23,25,27\) but the focus of this study was whether distinct patterns could be found within the same task.

Current source density approaches in the above mentioned studies had found evidence for gamma patterns characterized by different frequencies, for different tasks. However, these did not have enough resolution (as here provided by combination of EEG and fMRI) to test for non-unitary sources and establish their spatial topography within the same task.

Separability of different sources of gamma activity \(^27\) had been suggested to occur within the same visual region (lateral and medial visual cortex). Koelewijn and colleagues have indeed found that a rotating grating induced gamma synchronization in medial visual cortex at 30-70 Hz, and in lateral visual cortex at 60-90 Hz \(^27\). Directing spatial attention to the grating increased gamma synchronization in medial visual cortex, but only at 60-90 Hz \(^27\), which is in the range identified in our study for visual regions. These results are also consistent with MEG study of Gruber et al., 2008 \(^21\) on gamma-band generators in focal visual areas. We argue that these generators reflect local processing \(^79\).

Our previous EEG study in Williams syndrome \(^28\) suggested that sub-bands related to either visual or high level processing can be differentially modulated as a function of the cognitive strategy, suggesting the recruitment of multiple cognitive modules, and also inspired the current study.

Following those findings we found that predictors for increased activity at higher gamma-band have their origin in low-level visual areas in particular during object perception, during which top-down perceptual effects dominate. On the other hand, predictors for the low frequency range have their source at anterior decision related areas, namely the insula. In spite of the fact that the dissociation between two frequency bands within the same experiment has already been reported from auditory cortex recordings \(^20\), the duality we found and its relation to distinct cognitive mechanisms within the same task was not identified before. These gamma sub-bands show different frequencies, different brain generators and different functional meaning, one related to visual processing and the other to perceptual decision-making. This was rendered possible by our simultaneous EEG/fMRI \(^43\) and the EEG source localization approach which enabled
to pinpoint the above described unexpected dichotomy of gamma activity during perceptual decision. EEG/fMRI yields better localization resolution than MEG, which has nevertheless been shown to have considerably better accuracy in localization than EEG.

Gamma oscillations are known to be stimulus-induced and are closely colocalized to BOLD response. Our finding that a clear functional and topographic distinction is present for separable anatomo-physiological substrates of gamma sub-bands may shed light on the controversy between studies that reported the differential patterns of correlation between BOLD and gamma-band oscillations. Therefore our results provide support to the idea that low- and high-frequency oscillations reflect different phenomena and may be caused by different mechanisms within the same task. Although we were limited by the artifact correction algorithms that restrict the frequency range one can analyze (maximum ~70 Hz), our results do indeed support the notion that gamma-band activity patterns (Low and High gamma sub-bands) are not related to holistic perception in a simple unified manner, since aspects related to visual processing and decision can clearly be separated in terms of their neural origins. Based on the information provided by time-frequency EEG analysis, we were able to determine their spatio-temporal profile. An analogous distinction between sub-bands was also discussed recently in a review by Sedley & Cunnigham differentiating gamma activity in terms of narrow versus broad-band patterns, with putatively distinct physiological meaning. Accordingly, Ray & Maunsell showed that in monkey multi-unit recordings, higher frequency oscillations only were associated with spiking rate, which was not the case for lower gamma oscillations. Interestingly the lower frequency oscillations that we observed were mostly related to the insula and not the visual cortex.

Concerning the negative BOLD response which is also known to reflect stimulus related and/or neurophysiological response we also identified a network that deactivated mainly in response to scrambled object conditions, in particular in anterior regions.

In sum, we could identify spatial substrates underlying the temporal dynamics of brain activity during perceptual decision and categorization. Our results indicate the existence of distinct low and high level functional modules in the same perceptual decision task that can be tagged by two distinct frequency bands. These findings are relevant for the understanding of normal and clinical impaired holistic integration and show that
current neuronal models of gamma-band spatial distribution need to consider their duality by separating low and high sub-bands.

**Conclusions**

We conclude that gamma-band activity patterns do not represent a unitary phenomenon within the same decision task, but rather distinct neurocognitive components. Accordingly, at least two separate neural modules are involved in holistic perceptual decision, one in the visual cortex and the other in the anterior insula. This provides a step forward in understanding the functional specialization of decision-making networks and the role of gamma frequency range sub-bands in signaling their different neural and cognitive components. The finding that gestalt formation elicits non unitary gamma-band patterns, underlying independent sensory processing and perceptual decision mechanisms is novel and may shed new light on the role of gamma-band response in normal cognition and in neuropsychiatric disorders such as autism and schizophrenia, where both visual and decision making circuits may be impaired. Further studies including electrocorticography and non-simultaneous MEG and fMRI studies may help elucidating their separability with even higher spatial resolution.
References


Oscillations or synchrony?

Disruption of central coherence and neural synchrony despite enhanced gamma oscillations

This chapter was based on: Castelhano, J., Bernardino, L., Rebola, J., Rodriguez, E. & Castelo-Branco, M. Oscillations or synchrony? Disruption of central coherence and neural synchrony despite enhanced gamma oscillations. (Under review).
Abstract

It has been hypothesized that neural synchrony underlies central coherence. The hypothesis of loss of central coherence has been proposed to be at the origin of abnormal cognition in autistic spectrum disorders (ASD) and Williams Syndrome (WS), a neurodevelopmental disorder linked with autism, and the most clearcut model for impaired central coherence. We took advantage of this model of impaired holistic processing to test a functional role for neural synchrony using EEG and a set of experimental tasks requiring visual integration. A profound reorganization of brain activity was identified. Neural synchrony was reduced across stimulus conditions and this was associated with increased amplitude modulation at 25-45 Hz. This combination of a dramatic loss of synchrony despite increased oscillatory activity is strong evidence that synchrony underlies central coherence. This is the first time, to our knowledge, that dissociation between amplitude and synchrony is reported in a human model of impaired coherence.
Introduction

The role of temporal coding in perceptual coherence, defined as hierarchical grouping of local elements, remains controversial.\(^1\,2\) It is unclear whether oscillation amplitude is relevant for encoding global stimulus properties or if neural synchrony plays instead a pivotal role in gestalt formation.\(^3\) The fact that synchrony and amplitude modulation do often co-vary, has jeopardized their separation in terms of mechanistic explanation.\(^4\,5\) Gamma-band activity has been related to holistic integration and proposed to represent a neural correlate of perceptual coherence, object representation and binding.\(^6\) Accordingly, phase synchronization was shown to correlate with object perception, feature integration\(^3\,7\,8\) and regional brain coupling.\(^9\) Neural coherence, defined as increased spatiotemporal phase-locking, would therefore reflect perceptual coherence.

Additional evidence for a role of oscillatory patterning in perceptual organization comes from studies in clinical populations\(^10\) with fragmented perception such as autism\(^11\,12\) and schizophrenia, which have been associated with abnormal gamma-band patterning\(^13\,14\).

The central coherence hypothesis is a well-known model for cognitive dysfunction in autistic spectrum disorders (ASD)\(^11\). Williams syndrome (WS), a neurodevelopmental disorder that shares an intriguing link with autism\(^15\), is associated with larger deficits in central coherence, and in particular local-global grouping than ASD\(^16\). WS thereby provides a suitable model to address the hypothesis of dissociation between amplitude modulation and synchrony.\(^5\) WS is a well-established genetic neurodevelopmental\(^17\) model of impaired spatial perception associated with abnormal visual integration and coherence.\(^18\) 2D and 3D motion coherence thresholds are impaired\(^19\) as subjects fail to integrate local and global information for object perception to a much larger extent than ASD participants.\(^20\) Previous studies demonstrated impairments in 3D structure-from-motion (SFM) perception in WS\(^19\,21\) or in response to static faces both in autism and WS\(^22\). Perception of SFM stimuli requires dorsal-ventral stream integration of local features to achieve holistic perception.\(^23\) Moreover gamma oscillatory patterns are differentially distributed for different sub-bands in a task and/or disease dependent manner\(^21\,24\).

Here we tested whether synchronization and amplitude modulation of gamma patterns can be segregated in this model of impaired central coherence.\(^16\) We designed a set of experimental tasks requiring feature integration at different complexity levels (static and motion stimuli as well as 2D and 3D face stimuli) and compared gamma-band activity...
and synchrony between groups. We found that neural synchrony and the amplitude of oscillatory patterns dissociate under impaired coherence, thereby providing a novel clue for their functional significance in health and disease.

**Materials and Methods**

**Participants**

Participants were 9 individuals with WS (4 males and 5 females) aged between 15 to 37 years (mean±SE = 21.44±2.30). The WS participants were recruited from a database used in previous studies. WS diagnostic was confirmed based on clinical and genetic criteria. For details regarding WS group characterization see Bernardino et al., 2013.

The eight control participants (4 males and 4 females) were aged between 15 to 34 years (mean±SE = 21.89±2.40). Groups were matched on chronological age (Mann-Whitney U test, P > 0.05), gender and handedness. Two subjects who demonstrated left-hand dominance participated in each group. All participants had normal or corrected-to-normal vision and were naïve regarding the purpose of the study.

For details regarding psychological assessment and demographic characteristics of both groups refer to Bernardino et al., 2013. Written informed consent was obtained from all participants or their parents. The study was conducted according to the declaration of Helsinki and was approved by the local Ethics Committees of the Faculty of Medicine of the University of Coimbra.

**Task setup and procedure**

Participants were seated in a comfortable chair and were positioned at a distance of ≈ 120 cm from the computer screen in a darkened, acoustically and electrically shielded room. The stimuli (size: 13° horizontally and 10° vertically) were presented using the Presentation software (v14.9, Neurobehavioral systems) in the center of a CRT monitor screen (1024x768 with a refresh rate of 60 Hz). The experimental paradigm included four different stimulus conditions: Static Dots, Static Face, Structure-from-motion (SFM) defined face and Random Dots (Figure 5-1). The emphasis on dynamic stimuli was based on the fact that motion integration requires spatiotemporal correlation in the sense of Reichardt coincidence detectors. The static dot stimulus consisted of one frame of the SFM video (Figure 5-1 A). In the static face condition, participants viewed a mask
composed by a large set of dots to be similar to the SFM stimulus appearance (Figure 5-1 B). In the SFM condition, the stimuli consisted of videos of SFM defined faces with 980 ms of duration (for further details, see Graewe et al., 2012 and Bernardino et al., 2013 21,23). In this type of stimulus, a large set of coherent dots is moving, rotating in a cycle from -22.5 degrees to 22.5 degrees centered at the frontal plane. This enables the formation of 3D shape percepts (3D face). Importantly, shape extraction can only be achieved when dots are moving because otherwise the shape remains invisible (Figure 5-1 C). In the random dot condition, a set of white dots was randomly moving on a black background (Figure 5-1 D).

![Image](image.png)

**Figure 5-1. Examples of stimulus conditions and task timeline. A) to D) Stimuli used in the task. A and B represent static and C & D motion stimuli. Note that integration of dots is required for face perception. E) Timeline of the task. Stimuli (100 trials per condition divided in four runs) were randomly presented for 980 ms and subjects had to report at the end of the trial if a face was present.**

The four experimental conditions were randomly presented during 980 ms and were followed by a fixation period in which participants were required to press a button if the presented stimulus was a face and another button if the presented stimulus was not a face (Figure 5-1 E). The response was reported using a Cedrus response box (RB-530 model) and was required only after the stimulus offset to avoid contamination by motor activity. Response time was unlimited and the next trial was only presented after the
participants’ response. A 300 ms fixation period separated the response time from the test trial presentation. The experimental task was composed of 100 test trials of each task condition resulting in a total of 400 test trials which were divided in four blocks. The behavioral task was conducted to ensure that participants were able to maintain attention on the visual stimuli throughout the acquisition session. Twelve learning trials were administered and the practice phase was repeated whenever necessary to ensure full understanding of the task and motor coordination.

**Electrophysiological Recording**

The electroencephalogram (EEG) was recorded using a SynAmps 2 (64 channels Quick-Cap, NeuroScan Compumedics, USA). The recording was digitized at a 2 kHz sampling rate and processed offline. The electrooculogram (EOG) was monitored via electrodes positioned at the standard positions (vertical, VEOG and horizontal, HEOG) in order to correct artifacts due to blinking and eye movements. No notch filters were used during EEG recording and impedances of each electrode were kept below 10 kΩ (electrodes that did not fulfill this criterion were marked as ‘bad’). Reference was set to one electrode located close of CZ.

**ERP Data processing and analysis**

The data were processed offline using the Scan 4.5 Edit software (Neuroscan, Compumedics, USA). A digitally low- and high-pass filter (at 30 Hz, 24 dB/oct and 1 Hz, 12 dB/oct, respectively) was applied. Data were then segmented into epochs starting 100 ms before stimulus onset to 500 ms after stimulus onset. Baseline time interval was set to the time before stimulus onset (-100 to 0 ms). An automatic artifact rejection was applied and epochs with amplitudes exceeding +/-75 μV were rejected (>80% of the trials were accepted for further processing). The epochs for each stimulus type were averaged together and Event Related Potentials (ERP) were obtained. We defined four different clusters of electrodes for further analysis, namely: Occipital, Temporal, Parieto-occipital and Parietal. The Occipital cluster included the O1, OZ and O2 electrodes, the Temporal cluster was constituted by the TP7, CP5, P7, P5, PO7, TP8, CP6, P6, P8 and PO8 electrodes, the Posterior-occipital (POs) cluster comprised the PO5, PO3, POZ, PO4 and PO6 electrodes, and, finally, the Parietal cluster included the CP3, CP1, P3, P1,
CP2, CP4, P2 and P4 electrodes. We segmented the timeline (from 100 to 280 ms) in 20 ms windows. For each time window, the mean amplitude value was extracted. This procedure was performed for each subject (WS and Controls) and experimental condition and statistical Wilcoxon tests with false discovery rate (FDR) correction for multiple comparisons were performed to assess significant differences.

**Time-frequency and Phase Synchrony Analysis**

After filtering the data (1-100 Hz) we performed signal correction of eye movement related artifacts as described previously 27, using independent component analysis (ICA) based on all electrodes (including 4 EOG channels) as implemented in EEGLAB (version 10.2.5.6). Artifact components were identified based on their correlation with the EOG electrodes and on the scalp topography (increased activity distribution around the orbits) and removed from the data. Data were segmented into epochs locked to the stimulus onset (from -0.5 to 1.5 s) for all conditions. Epochs with artifacts exceeding ±75 μV were rejected. On average 72.3 ± 12.17% for controls and 79.48 ± 7.65% for WS of the epochs remained for further analysis.

Time-frequency and phase synchrony analysis were performed as in Uhlhaas et al., 2006 28 and are described elsewhere 7,8,29. Time-frequency analysis was carried out in Matlab® across distinct defined electrode clusters (Parietal, Temporal, Parieto-Occipital and Occipital; see above) for frequencies ranging from 10 to 90 Hz in steps of 1 Hz. This way we focus on the modulations of activity in the gamma-band range. We analysed the so-called ‘induced’ gamma activity which is not phase-locked to stimulus onset (compute time–frequency transforms of single epochs and average them across all trials). Regarding the SFM condition we considered also the data acquired in a previous experiment 21. For every time window and frequency bin (frequency resolution of 1 Hz/frequency bin) the phase was also computed, using Matlab®, in the same high-beta/gamma frequency range. The phase-locking value (PLV), that measures the inter-trial variability of the phase difference was calculated as described in Lachaux et al., 1999 7. PLV looks for latencies at which the phase difference between the signals varies little across trials at the target frequency. To evaluate long-range coordination, these values were computed between all possible pairs of electrodes, and the stability of phase differences was evaluated through all the trials across a wide frequency range 7,8,28. Time-frequency and phase were then normalized by subtracting the baseline average (pre-stimuli) and dividing by the baseline
standard deviation on a frequency by frequency basis \(^7\). The spatio-temporal distributions of coherence (higher phase locking across channels are drawn in a 2D head map) were calculated for distinct frequency bands and 5 consecutive time-windows of 200 ms length locked to the stimulus onset. Graphs of synchrony lines, representing enhanced phase locking, are shown per group and stimulus condition for two gamma frequency sub-bands (LF 25-45 Hz; HF 60-90 Hz) across time. Synchrony lines are drawn only if the synchrony value is above a two tailed probability value of \(P = 0.01\) \(^8\).

**Statistical Analysis**

Statistical comparisons were performed to compare ERPs and time-frequency results between groups and conditions with the alpha level set to 0.05. For the statistical analysis comparison between groups, Mann-Whitney U tests and Wilcoxon rank sum test were carried out. The FDR method was used for correction for multiple comparisons when appropriate. Bootstrap statistics with 1000 bootstrap samples was used to assess significance comparing the number of synchrony lines between groups. All statistical analysis were performed with the *IBM SPSS Statistics 19.0* software package and Matlab\textsuperscript{®}.

**Results**

**Behavioral and Event-Related Potential (ERP) Data Analysis**

In the present task, participants were required to discriminate between face stimuli and non-face stimuli. Both WS and control participants were performance matched when discriminating face stimuli from non-face stimuli (Mann–Whitney tests, \(P > 0.05\); error rates per condition: Static dots 11.63±4.93\%; 3.29±1.52\%; Static faces 9.63±4.42\%, 1.43±0.98\%; SFM 17.12±12.90\%, 1.93±1.20\%; Random dots 11.75±5.56\%, 7.43±6.44\%; mean ± SE for WS and controls, respectively). Accordingly, results indicated that all the participants were successfully performing the task.

ERP data (grand average waveforms for WS and typical controls), are shown in figure 5-2. For control participants, we found the expected early positive P100 visual component, followed by the expected face related negative N170 component. In accordance with our previous study \(^21\), we confirmed that WS subjects showed lower
P100 amplitude (particularly in the moving task conditions) than control participants, an earlier N150 component as well as a novel positive peak - P200.

![ERP Plots](image)

**Figure 5-2. ERP Plots are represented for each condition** (columns) for the parieto-occipital cluster of electrodes. Controls (red) and WS (green) differed mostly around N170 and P200 peaks except for the static dots (see text for details). Gray bars represent the time-windows of significant differences (FDR corrected).

We performed statistical analysis (Mann-Whitney tests with False Discovery Rate (FDR) correction to control for the family-wise error rate) for all clusters (Occipital, Occipito-Temporal, Occipito-Parietal and Parietal regions) and task conditions (Static Dots, Static Face, SFM and Random Dots). Significant differences between the two groups (Mann-Whitney tests, P <0.05) were mostly identified just around the emergence of the N170 standard face component (120-180 ms time window) and the P200 component (220-280 ms time window). These differences were observed for all task conditions (Static faces, P <0.038; SFM, P <0.048; Random Dots, P <0.043) except for the Static Dots condition (see gray bars in Figure 5-2).

**Time-Frequency analysis: amplitude and synchrony differences across distinct gamma-band ranges for the high vs. low coherence groups**

We focused on induced gamma oscillations known to be important for holistic processing, in particular in the low 25-45 Hz frequency band. We found a dual gamma activity pattern at low- and high- gamma frequency ranges in both groups and dependent on the type of stimulus, for the different stimulus conditions in the parieto-occipital cluster (Figure 5-3). Details on statistical analysis (including P values) concerning the group comparisons for all conditions are reported in the respective figures.
Reorganization of gamma-band activity patterns was found in the impaired central coherence groups, both concerning the 25-45 Hz (believed to be involved in gestalt formation) and 60-90 Hz bands. Regarding static face stimuli, time-frequency results confirmed that in the 60-90 Hz gamma-frequency range WS patients show decreased activity in comparison to controls (see Figure 5-3 C for detailed FDR corrected P values).

Regarding the conditions containing moving stimuli (SFM and Random dots), between group comparisons revealed differences in both conditions for both the 60-90 and 25-45 Hz ranges. SFM results replicate our previous finding that control participants show increased activity for the 60-90 Hz band while WS participants show significantly stronger activation at the lower gamma-band range (25-45 Hz). A similar pattern of results was also observed for the random dot condition for two similar sub-bands.

**Figure 5-3.** Time-frequency analysis results per experimental condition shows distinct oscillatory patterns across groups. **A)** Normalized (to pre-stimulus baseline) gamma-band activity patterns for the four conditions for the control group; **B)** Time-frequency analysis for the WS group. Color codes indicate normalized scores. **C)** Statistical maps for the between group comparison (Ctr vs. WS, Wilcoxon test with FDR corrected p values shown per time and frequency points). The blue line represents the stimulus onset and the gray background highlight the low-frequency range. The analysis depicted here corresponds to the parieto-occipital cluster of electrodes. Stimulus driven ~ 60 Hz gamma oscillations dominate in the control group, while in WS modulations have larger amplitudes near 40 Hz.
Loss of synchrony in the low perceptual coherence group

We evaluated the phase synchrony, a putative measure of binding between distinct brain regions. We computed synchronization between all electrode pairs for the different stimulus conditions (see Figure 5-4 A and B). Phase synchrony was significantly different between groups for all conditions, except for static faces, in distinct frequency sub-bands.

Synchrony plots show a decrease of synchronization in the lower frequency range in the WS group (Figure 5-4 C) in spite of the larger amplitude modulation. This finding is further detailed in Figure 5-4 D showing the synchrony spectrum average over time in the interval after stimulus onset for individual stimuli, for both groups. Most importantly, spectral comparisons between groups show that controls have higher synchrony for the low frequency range (25-45 Hz) while WS participants have slightly increased synchrony for the higher gamma-band (60-90 Hz).

Regarding the spectral analysis, controls have also increased synchrony in the low range for the static dot condition ($2.1<z<2.9; \ 0.003<P<0.036$), for the SFM condition ($1.73<z<2.02; \ 0.043<P<0.046$) as well as for the random dot condition ($1.92<z<2.50; \ 0.011<P<0.027$). WS subjects showed increased synchrony for the higher band only for the static dot condition ($2.1<z<2.52; \ 0.012<P<0.036$), as well as for the random dot condition ($2.02<z<2.41; \ 0.016<P<0.043$). Moreover this finding was spatially restricted (see analysis of synchrony lines below) and differences did not reach statistical significance for the static face condition, in line with the idea that lower band (25-45 Hz) oscillations that have been classically related to feature binding were more relevant.

We also performed a pooled analysis across conditions and found that control participants have higher synchronization at 10-22 Hz ($2.52<z<3.09, \ P<0.017$ FDR corrected,) and 30-45 Hz ($4.50<z<6.36, \ P<0.02$ FDR corrected) when compared to WS (Figure 5-4 E). This analysis further confirmed that WS patients exhibit increased synchronization at the higher frequency band (62-79 Hz; $2.59<z<3.31, \ P<0.015$ FDR corrected) in comparison to controls.
Figure 5-4. Phase synchrony results for the four conditions. Synchrony was calculated for all possible combination of pairs of electrodes and is locked to the beginning of the stimuli (blue line) for the control (A) and WS (B) groups. Color scale depicts normalized units. Baseline normalization was performed for the time-window preceding the stimulus onset. C) Representation of the time-frequency points with significant differences between groups. Statistics were obtained using Wilcoxon test and P values were corrected for multiple comparison using an FDR approach (color scale shows the range of P values and the gray background highlight the LF range). D) Synchrony spectrum (within group averages over time of the results in A and B) separated by stimuli conditions. For sake of clarity, SD were omitted in these plots. Note that controls (red line) show higher synchronization for the lower frequencies except for the static faces condition. E) Group average synchrony spectrum pooled over conditions. Red and green bars at the bottom mark the frequencies where groups differed (P < 0.02 FDR corrected).

Since synchronization near the 40 Hz frequency is believed to reflect coherent perception, we analysed topography maps of synchrony across multiple gamma sub-bands. The spatio-temporal distribution of these sub-bands revealed distinct patterns of synchronization for different frequencies and time intervals. Figure 5-5 shows the synchrony patterns between electrodes pairs (thicker lines indicating stronger synchrony). Only pairs are considered whose synchrony value reaches a two tailed probability value of P = 0.01 (SFM condition; similar results were obtained for the other conditions, data
not shown). These topographic maps show that synchronization pairs for the low gamma frequency band (LF; 25-45 Hz) cover a large brain network over an extended time period in the control group. This is in contrast with the pattern exhibited by the WS group that shows substantially less synchrony in accordance with the reduced ability to form coherent percepts.

![Image of topographic maps showing phase synchrony changes in the LF (25-45 Hz) and HF (60-90 Hz) bands plotted across space and time.](image)

**Figure 5-5. Phase synchrony changes in the LF (25-45 Hz) and HF (60-90 Hz) bands plotted across space and time.** Circles indicate electrode positions, with anterior sites at top and posterior sites at bottom. Results are shown for the SFM condition over 6 time-windows spanning the entire duration of the stimuli (-200 to 0 ms was defined as baseline). Red lines mark increased synchrony between electrode sites for the control group, and green lines mark higher synchrony for the WS group (lines are plotted with a significance threshold of $P < 0.01$). Note the increased number of lines (representing increased synchrony) for the LF band in the control group.

Bootstrap statistical analysis including all stimuli combined revealed that this reduction of synchrony is significant for the first 2 time-windows after stimulus onset for the LF (25-45 Hz) band (0-200 ms: mean difference = 16.82, $P < 0.027$, bias = 0.32, SE = 7.53; 200-400 ms: mean difference = 27.32, $P < 0.020$, bias = 0.34, SE = 10.04). Interestingly, in the control group synchronization is highly attenuated for higher frequencies (HF; 60-90 Hz) for all stimulus conditions. However, between group differences in the degree of synchrony for this band were not significant ($P > 0.11$), further supporting the notion that it is the 25-45 Hz band that critically differentiates the two groups. The topography of synchronization patterns shows the electrodes that were consistently participating in
these coherent patterns. These were located at the parieto-occipital and temporal clusters which lie over posterior dorsal stream areas and temporal ventral regions, respectively. These electrodes synchronized mainly with the fronto-central electrodes over decision-related brain regions \(^{29,32}\).

These results demonstrate that synchronization is altered in WS particularly in the lower frequency band (25-45 Hz) in particular for dynamic stimuli, which inherently require more spatiotemporal integration and phase/coincidence detection. Imaginary coherence analysis \(^{33}\) further confirmed our findings. This helps ruling out volume conduction biases. A particularly important observation was that synchrony was remarkably increased for the frequency range in which the amplitude was reduced \(^{8}\).

**Discussion**

In the present study, we took advantage of a well characterized model of impaired central coherence to examine the functional role of neural synchrony \(^{11,34}\). WS patients show markedly disrupted visual coherence and holistic visual perception \(^{16,35}\). If synchrony is supporting holistic perception we predict that it should be diminished \(^{31}\) in this model. In previous studies gamma-band activity and synchrony co-varied in association with perceptual integration \(^{30,36-39}\) rendering it difficult to separate amplitude modulations from changes in synchrony.

We found, for the first time, that a model of fragmented coherence in perception is associated with abnormal phase-synchrony even when gamma-band activity increases in the same time-frequency interval. This allowed us to avoid confound of synchrony with amplitude covariance. Interestingly, the task condition requiring the highest level of integration (SFM, structure-from-motion coherence) was the one associated with the most dramatic loss of synchrony. This suggests a dependence on visual complexity and integration requirements and is in line with the proposal that perception requires mechanisms for integration and feature binding that rely on the neuronal synchrony \(^{19}\). Moreover, effects were more prominent for all dynamic conditions, in line with the notion that motion perception, requiring spatiotemporal correlation, is often impaired in syndromes associated with a reduction of perceptual coherence. This is in line with theoretical models that postulate correlation-based motion detection (Reichardt correlation models \(^{26}\)).
Previous studies in WS did not address synchrony. It is known that coherent visual motion induces high frequency gamma oscillatory activity in parieto-occipital regions of the visual dorsal stream. We now show that low frequency gamma activity reflects integration of dynamic information based on gamma synchrony irrespective of changes of amplitude. This provides an intriguing link to dorsal stream functions which have been implicated in the analysis of motion and spatial relationships between objects. Our findings extend the notion that gamma-band activity serves an important role in a variety of processes (in particular feature integration) reflecting distinct mechanisms and that different activity patterns for particular gamma sub-bands may have different roles and sources within the gamma frequency range.

The finding that controls and WS have distinct synchrony patterns is relevant to akin conditions such as autism spectrum disorder, as well as schizophrenia and other neuropsychiatric conditions with fragmented perception. The identified signature of a dramatic loss of synchrony as opposed to concomitantly increased gamma activity, provides evidence that it is a marker for central coherence, irrespective of performance levels.

Given that WS has a genetic basis which is implicated in cortical circuit specification with a direct impact on the phenotype, it is relevant to consider the molecular mechanisms underlying abnormal oscillatory patterning. It is known that high-frequency oscillatory activity in the cortex results from interactions between GABAergic inhibitory interneurons and is impaired in some neuropsychiatric disorders such as schizophrenia, epilepsy and Attention Deficit and Hyperactivity Disorder (ADHD). Moreover, it has been shown that GABA (Gamma-Aminobutyric Acid) concentration is positively correlated with stimulus-induced gamma-oscillations during a visual task. Hence, abnormalities in synchronous neural activity could be disrupted in WS due to a dysfunction of inhibitory interneuron networks. Thus, future studies should explore mechanisms underlying abnormal patterning of gamma-band activity in WS, in particular with respect to neural mechanisms of synchronization. Given the evidence that GABA levels are altered in other pathologies with visuospatial impairment associated with dorsal stream dysfunction, such as Neurofibromatosis Type 1 and autism, this theoretical thread is worth pursuing. Further studies focusing on the connectivity of distributed cortical networks, inter-hemispheric interactions and neurochemical phenotypes may also help elucidate this issue.
Conclusions

Our experimental results support the notion that gamma-band synchronization deficits are associated with loss of central coherence and suggest a new neural correlate for impaired cognition in neuropsychiatric disorders. This abnormality may be at the basis of some of the manifestations of disorders known to have central coherence deficits. In sum, our data indicate that neural synchrony in the lower gamma frequency range is important in the generation of perceptual coherence.
References


Perception’s shadow:

Distinct gamma activity sources and sub-bands as revealed by ECoG

This chapter was based on: Castelhano, J., Duarte, I.C., Sales, F. & Castelo-Branco, M. Perception’s shadow: distinct gamma activity sources and sub-bands as revealed by ECoG. (Poster #4148 presented at XX OHBM meeting, Hamburg) (2014).
Introduction

Gamma-band oscillations are thought to play a crucial role in brain function but, as explained before, the functional role of specific sub-bands within the Gamma range \(^{1,2}\) remains unclear. Therefore, the direct identification of their sources may help clarifying their roles. This may be achieved with Electroencephalography (EEG) with the added value of greater spatial resolution \(^{3}\). Here we performed presurgical mapping in a patient with refractory epilepsy and explored the spatio-temporal dynamics of cortical oscillatory gamma-band activity. We used a task similar to the reported by Rodriguez et al., 1999 \(^{4}\), who found increased activity at the gamma frequency range (30 to 70 Hz) and claimed the presence of a neural “perception’s shadow” (reflecting object perception) during holistic visual perception of Mooney pictures \(^{5}\).

Methods

We acquired task-related ECoG data from one epileptic patient (age 31 years) with surgically implanted electrodes during a Mooney perception task (Figure 6-1). Stimuli were delivered using Presentation software (Neurobehavioral Systems). Stimulus duration and inter-stimulus-interval were 150 ms and 2 s, respectively (slow event related design).

Computed Tomography and Magnetic Resonance anatomical data were acquired and co-registered (using Curry 7.0 Neuromaging software) to precisely localize the positions of the intracranial electrodes (see Figure 6-2 for electrode locations). Briefly, the subject was presented with Mooney pictures of faces and non-faces stimuli (upright or inverted), guitars and scrambled versions, for 250 ms and had to report if a face, object or scrambled stimulus was present, at the end of the trial (see Figure 6-1). ECoG data were acquired from platinum grid electrodes (n=50) at a high sampling rate (5 kHz). No filters were applied during the recording. Data were analyzed offline. After artifact correction, data were split into epochs locked to the beginning of the stimuli (-500 to 1500 ms). Event-related potential (ERP) and time-frequency (TF) analysis focused on the frequency range from 5 to 250 Hz \(^{4,6,7}\) were performed using EEGLAB toolbox \(^{8}\). Statistical tests were performed to compare between two conditions (Mooney objects vs. Mooney scrambled) using Wilcoxon rank sum test from Matlab \(^{9}\).
**Figure 6-1. Task Timeline:** Subjects have to discriminate between three Mooney categories (faces, guitars and scrambled) randomly presented for 250 ms each and respond at the end of the trial. For the analysis, Faces and Guitars were considered together, as an ‘Object’ category.
Results

All stimuli were reported with an accuracy above 92%. The ERP results reveal the typical negative potential around 200 ms (Figure 6-2).

Figure 6-2. electrode locations and ERPs. A left view of the brain is showing representative ERP results for the Mooney Objects condition. Peak amplitude varied between -20 and 20 μV. A and P stands for anterior and posterior, respectively.

Regarding the TF results (Figure 6-3), we were able to identify cortical locations with responses to the “Perception’s shadow” ⁴. We found a burst (~ 200 to 400 ms) of activity in anterior electrodes (Broadman Area BA21) below <100 Hz for both stimuli. Higher frequency responses (up to 150 Hz) to Mooney Object perception were found in BA37. For these stimuli, activity in the primary visual regions (BA18) spanned even higher frequencies (<200 Hz). Note that for the scrambled stimuli, the posterior vs. anterior difference is more evident below 100 Hz and for Mooney object perception a specific pattern emerged for higher frequencies (up to 200 Hz; for statistical P values see Figure 6-3 C).
Figure 6-3. Time-Frequency results. A) TF results are shown for representative electrodes for the Mooney Objects condition. B) TF showing 4 electrodes spanning anterior to posterior regions for the object and scrambled conditions. C) Statistical P values are shown for the comparison Posterior (BA18 and BA37) vs. Anterior (BA21) clusters. Note that for scrambled stimuli, activity is significantly increased at lower frequencies (<100 Hz) and higher frequencies (up to 200 Hz) are specifically found for the Mooney objects condition.

Discussion and Conclusions

We identified activity related to Mooney object perception which modulated at lower frequencies (<100 Hz) in sources located in the anterior ventro-temporal areas. Object perception specific higher frequency modulations (up to 200 Hz) were found posteriorly in the occipital regions. This is in line with previous reports suggesting a dissociation between low and high gamma sub-bands \(^{9,11}\) with distinct sources and cognitive functions. Our previous work, from single EEG, simultaneous EEG/fMRI in normal subjects and from the clinical WS study provided evidence along these lines. The present study extends the
ranges of potentially relevant biological rhythms even to higher frequency bands (> 100 Hz). We identified a graded postero-anterior sharpening of frequency responses (the ones below 100 Hz remaining). Although the conclusions are limited by the fact that we studied only one subject, it also suggests (with the added value of very high spatiotemporal resolution) that a distinct spatial source map is present for different gamma sub-bands activity during visual perception of Mooney faces.
References


Concluding remarks
General Discussion and Conclusion
General Discussion and Conclusion

An exciting area in Cognitive Neuroscience concerns the investigation of neural mechanisms underlying the emergence of visual perceptual representations and the neuronal mechanisms underlying decision-making. Studying the brain oscillations have gained tremendous importance in neuroscience during recent decades as they were shown a role to many of these and other cognitive processes. To understand the neural mechanisms underlying brain oscillations and their role in cognition is useful to clarify normal and pathological brain function. This chapter summarizes the findings of our work and their relevance for further studies.

Brain oscillations as mediators of the perceptual decision process

Perceptual decision represents one of the most fundamental cognitive functions, and is defined by the choice between ambiguous alternative interpretations of incoming sensory evidence. By reflecting the choice between interpretations of the sensory world it may crucially affect subsequent action selection. The main purpose of this work was to provide insight into the functional role of brain oscillations observed in response to perceptual decision making processes and clarify the role of distinct frequency sub-bands and their sources regarding visual stimuli integration.

Perception paradigms using ambiguous stimuli have been widely used in research to address decision-making mechanisms. In particular, Mooney faces (black and white degraded pictures of faces) stimuli were used to study visual and gestalt perception and percept formation. In the more recent decades there was an increasing interest in studying a functional role for oscillatory activity and we are beginning to understand the sub-processes related to brain oscillations that are likely involved in perceptual decision making. Neuronal gamma-band oscillations appear to have a fundamental role concerning brain function. However, no single theory can explain all of its functional implications within the framework of a unified account.

Such an account could be achieved by taking into consideration that multiple levels of decision can be analysed to unravel the functional architecture of core and extended decision-making networks using EEG, fMRI, MEG and EEG/fMRI. Such approaches may help defining the neural underpinnings of multiple levels of decision making, from the perceptual level to the cognitive control and motivational level.
Brain oscillations in response to unpredictable holistic percepts

Here we used Mooney stimulus paradigms in order to investigate visual binding and perceptual decision processes. We used EEG/ERP to investigate perception-related oscillatory responses and assess the neural correlates of perception vs. no perception decision moments using both model and data driven approaches. We designed original new experiments that use state-of-the-art stimulus arrays and data analysis techniques in the fields of neurophysiology and cognitive neuroscience. Experiments were set to disentangle the various components of perception (e.g., sensory inputs, perceptual grouping, motor response) using a paradigm that makes possible the assessment of local vs. global integration and holistic processing.

The novel dynamic approaches whereby the emergence of percepts was variable in which concerns the moment of recognition ensured that the moment of perception of an emergent global pattern was unpredictable. With these paradigms we addressed the question of whether gamma-band EEG activity is related to holistic perception.

In a series of experiments examining the oscillatory large-scale neural correlates of gestalt-like perception, we provided a direct link between gamma-band temporal patterns and the presence versus absence of emerging holistic perception and directly compared perception vs. no perception states for the same stimuli. Analysis demonstrate that gamma-band activity is modulated at low and high frequencies and is associated with the perception of an object (face or guitar) for which the appearance could not be predicted. Data showed a delayed gamma peak when the Mooney face was both backward and forward masked relative to only forward or only backward masked. Finally, subjects were required to indicate when they detected a face in a rotating image of a Mooney face starting from an upside-down position. Such a procedure delays the onset of object recognition. We found that gamma-band activity predicted the emergence of such perceptual moments. The detection of a face in the rotating image was associated with an increase of gamma activity before the time point of detection. This increase consisted mostly of high frequency components in the gamma-band (above 60 Hz) and it was associated with a concomitant decrease of the low components (40 Hz). In addition, phase synchrony analysis revealed an increase of synchrony across occipital and temporal areas for low frequency gamma-band components. These results are interesting since modulation in power was shown to be concentrated in the high frequency components at the gamma-band.
The contrast between amplitude and synchrony was further more evident in the third study and it is a proof that synchrony under the conditions of our experiments is not just spurious activity/synchrony. Moreover, data driven statistical classification of the last 3 seconds based on the gamma-band activity patterns yielded a near perfect classification of trials in which a face was detected to those in which no face was detected.

Our data contribute to the long-lasting debate on the role of gamma-band activity in gestalt perception. Emergent holistic percepts are linked to increased gamma activity thus confirming a functional role for oscillatory activity in the Gamma frequency band (>30Hz) in perceptual decision. This study confirmed that oscillatory patterning at low and high gamma-band is informative in relation to object categorization and holistic perception, thereby providing a signature of unpredictable holistic perceptual states (confirmed with a data-driven approach). Moreover, our data supports a functional role for distributed spatiotemporal patterns of gamma-band activity and synchronization in perceptual decision which may shed light on the mechanisms underlying perceptual object processing.

Functional specialization of distinct gamma sub-bands in relation to distinct cognitive modules

Gamma oscillations seem to index neural self-organization and have been shown to be functionally associated with a large variety of cognitive processes ⁴ such as attention, memory processes and other cognitive functions. Gamma-band activity is suggested to reflect not only binding of stimulus features but also activity of brain areas involved in other cognitive functions such as decision networks ⁵. The big question however remains: is there a functional specialization for each gamma sub-band?

Previous studies do lack the resolution or coverage to directly address this highly debated question, which impacts on the binding hypothesis. Moreover, electrophysiological studies often reveal a wide variety of gamma-band patterns and sources for different tasks ⁶⁻⁸ rendering interpretation of their cognitive significance rather difficult.

In order to address the role of gamma oscillations in holistic perceptual decision-making and to provide a better characterization of the perceptual decision components
with high spatio-temporal resolution, we used simultaneous EEG/fMRI imaging \(^1\). This way it was possible to measure and separate activity and sources of oscillatory activity, by comparing/combining activity measured simultaneously using distinct modalities in response to the same paradigm. An EEG-informed fMRI approach was used to reveal functional networks involved in that process \(^9\). This analysis supports the notion of a link between BOLD and gamma-band activity \(^10\) and make it possible to clarify the role and sources of distinct frequency sub-bands within the gamma-band. We investigated whether different aspects of gamma oscillations associate with different cognitive processes underlying decision-making, or whether different processes associate with the same gamma-band activity.

**Sources of independent gamma oscillation bands**

Using a novel EEG-guided fMRI analysis based on peak gamma sources from time-frequency analysis identified at the single trial level, we were able to identify independent gamma-band sources supporting distinct cognitive operations. We showed that within the same visual gestalt perception task (categorization of Mooney stimuli) independent gamma oscillation bands appear, with different frequencies, brain generators and distinct functional meaning. A low sub-band of gamma (40 Hz) was activated with decision making (arising in particular within the insula; \(^1\)) and a higher band (60 Hz) in visual regions (reflecting sensory processing). These findings of a functional and topographical differentiation between low and high gamma activity within the same cognitive task, are a step forward in understanding the functional specialization of decision-making networks.

Future studies regarding time-frequency analysis of electrophysiological data should have into account this proposed parcellation of distinct gamma-band components. Such a dissection of the cognitive and neural components of perceptual decision associated with different gamma sub-bands sheds new light on the role of gamma-band patterning in cognition and neuropsychiatry disorders such as autism and schizophrenia, where both visual and decision making circuits may be impaired \(^11\). Further studies including higher spatio-temporal resolution techniques and these neuropsychiatric disorders, may help elucidating this and to clarify if it is possible to use patterns of oscillatory activity as disease biomarkers \(^12\).
Phase-synchrony in pathological conditions

As previously shown, many cognitive functions do not solely rely on specialized areas but involve a distributed network of regions. Our EEG/fMRI study showed that one can identify clearly distinct sources of gamma-band patterns with different putative functional roles and distinct sources in the brain. We argue that effective parsing of the cognitive components of holistic decision making can best be made by combining EEG and fMRI studies.

Furthermore, network synchronization (in particular cortico-cortical connectivity) can also be explored as an extension of conventional EEG time-frequency analysis – using phase synchrony measures \(^5\,^13\). These were shown to be increased during visual tasks requiring integration. Besides, gamma-band synchronization was shown to bind activity across separated local networks, supporting rapid feature binding \(^3\,^14\). In contrast to the fixed anatomical connections, oscillatory patterning of neuronal activity offers a wide range of coding options in terms of the role of cortico-cortical interactions in feature binding, attention, sensory-motor coordination or memory \(^3\).

Previous research showed that oscillatory activity across distinct frequency bands are altered in pathological conditions \(^15\,^16\). Deficits in gestalt perception seem to be clinically relevant in autistic spectrum disorders \(^17\,^19\). Here we addressed how neuroimaging and related multimodal approaches \(^20\) may contribute to the understanding of perceptual decision making by elucidating the core and extended neural architecture of percept formation networks and their functional relevance.

Neurodevelopmental disease models are particularly useful for testing hypotheses not only about pathophysiological mechanisms but also about physiological mechanisms and biophysical properties underlying neural function and the interrelationship of distinct imaging techniques \(^9\). We have studied a neurodevelopmental disorder with disrupted visual coherence and holistic visual perception \(^18\,^21\). The central coherence hypothesis might explain abnormal cognition in ASD and WS. Additionally, it has been hypothesized that neural synchrony underlies central coherence. If this is true, we predicted that it should be diminished in WS \(^22\). We took advantage of this model of impaired holistic processing to test a functional role for neural synchrony \(^23\) using EEG and a set of experimental tasks requiring visual integration.

We found evidence for disrupted synchrony despite enhanced amplitude of measured gamma-band patterns. This was in line with previous studies of neural
synchronization but it was the first time to our knowledge that a clear separation of amplitude modulations from changes in synchrony associated to perceptual integration was found in a human model of loss of coherence. This was rendered possible by using this unique model of fragmented coherence in perception.

Hence, gamma-band synchronization impairment may be at the basis of some of the symptoms and signs of disorders known to have central coherence deficits. This may help further improving models of impaired decision making in diseases with both impaired/fragmented perception and/or behavioral control/motivation such as autism, schizophrenia and Parkinson’s disease. In sum, impaired phase synchrony may be a new neural correlate for cognitive deficits in neurodevelopmental/ neuropsychiatric disorders.

**Implications for the search for neurophysiological biomarkers of disease**

From these results and work from others regarding cognitive impairment it stays clear that analysis of oscillations across distinct frequency sub-bands may be of tremendous potential importance in the search for neurophysiological biomarkers of disease. These should also include brain imaging techniques able to measure activity from multiple locations in the brain as well as connectivity analysis between distributed neural networks. In this line, diagnostic strategies may comprise multiple methods and multiple frequency bands. Furthermore, analysis of the clinical effects of drugs (e.g., in clinical trials) can also benefit from time-frequency analysis of whole brain electrophysiological data.

Emerging research on the molecular mechanisms concerning generation of high frequency oscillatory activity can also add to this framework. Previous results have revealed the neural mechanisms responsible for generating this activity. Several neurotransmitter systems that are abnormal in neuropsychiatric/neurodevelopmental disorders are also crucially involved in the generation and synchronization of cortical beta and gamma oscillations. A network of mutually connected inhibitory (GABAergic) interneurons is important in gamma-band generation that is important for information processing in the brain. Moreover, various types of interneurons fire at different frequencies and phases (for review see Bartos et al., 2007) and N-methyl-D-aspartate receptor (NMDA) antagonists influence fundamental frequency bands in cortical and
subcortical brain regions. This is in line with results showing that oscillatory activity is impaired in some psychiatric disorders in distinct frequency sub-bands. These includes alterations of GABA levels, and abnormal synchronization of neuronal activity that is key for generation of oscillations. Hence, it is important to consider the biological mechanisms that underlie impaired neuronal oscillations in future studies. These (and in particular studies in animals) can help link data from electrophysiological recordings with clinically impaired populations to changes in neurotransmitter systems or anatomical deficits (for a review regarding neurobiology of abnormal oscillations and mechanisms underlying the generation of gamma oscillations and synchrony please refer to Uhlhaas et al., 2010). Research into the underlying mechanisms of neural oscillations (including also PET and TMS) may therefore help mapping genetic and biological causes and mechanisms of some diseases such as schizophrenia, WS, autism or neurofibromatosis type I.

**Gamma oscillations (synchronization) patterns are essential for cortical computation**

The research field on oscillatory pattern generators is still lacking a standardization of applied paradigms, acquisition and analysis methods. This precludes the comparison of results between different studies because tasks are distinct or methodology may still largely differ (filtering, frequency bands, evoked power, induced analysis, phase synchrony). In future there is a strong need to address these points in particular in what concerns the definition of gamma sub-bands classes. In spite of the technical questions and controversies, gamma oscillation (synchronization) patterns are likely essential for cortical computation and will continue helping clarifying complex dynamics of brain circuits in health and disease. Model-driven approaches will also constitute an important step to address research questions in this field and help identify links between modalities with distinct spatio-temporal resolution with increasing computational power. The use of ECoG allowed a unique opportunity to extend and validate this approach by providing direct access to the cortical surface at high temporal resolution. This allowed to further confirm the role of high frequency oscillations in encoding changes in perceptual states in particular in the ventral stream. Multimodal approaches including invasive recordings and other brain imaging techniques may also help improving these knowledge.
CONCLUSION

Using neuroimaging and electrophysiological techniques we were able to provide new insights into mechanisms generating perceptual decision phenomena. By combining advanced neuroimaging techniques with high spatial and temporal resolution, and new experimental paradigms, we have found that gamma-band activity and synchrony play a functional role in holistic object perception. Moreover, we clarified the sources and neural correlates of the spatio-temporal patterns of distinct gamma sub-bands patterns of activity found to be related with information processing of global perceptual features. Using simultaneous EEG/fMRI it was possible to identify a clear functional and topographic distinction for distinct sub-bands within gamma-band in a perceptual decision task. In this line, we showed that separate gamma-band patterns represent distinct neurocognitive components underlying either sensory processing or perceptual decision mechanisms.

These gamma sub-bands components could also be identified in a clinical population with impaired coherence (WS). Moreover, we took advantage of this model of loss of central coherence and found that these patients have decreased stimulus induced synchrony patterns despite increased gamma amplitude for similar frequency sub-bands (40 Hz). With this study we add new insights into the binding by synchrony hypothesis and show that quantitative analysis of oscillatory activity in the gamma-band may represent potential disease biomarkers providing new evidence about neuronal population dynamics in health and disease. Our case-study with ECoG that yields a high spatio-temporal resolution revealed an anterior-posterior pattern of increased oscillations thus corroborating our previous findings on the emergence of perceptual representations.

Outstanding questions remain such as the ongoing debate on the role of low level (sensory) and high level (top down) mechanisms in explaining perceptual alternations, and the interaction between categorization and value attribution in high level difficult choice. Future studies should elucidate how perceptual decision can illuminate outstanding issues in decision-making neuroscience. They should include high temporal and spatial resolution brain imaging techniques to take into account the identified distinctions within the gamma frequency band and hopefully elucidate how our results can be generalized to general principles of cognitive and clinical neuroscience.
References


List of Publications


**Castelhano, J.,** Bernardino, I., Rebola, J., Rodriguez, E. & Castelo-Branco, M. Oscillations or synchrony? Disruption of central coherence and neural synchrony despite enhanced gamma oscillations. *(Under review).*


**Castelhano, J.,** Rebola, J., Rodriguez, E. & Castelo-Branco, M. Neural synchronization in the gamma frequency range is tightly associated with detection of ambiguous face stimuli. *Neurolmage* 47, Suppl 1: S155 - S155 (2009)
Agradecimentos

A caminhada começou a todo o gás, os objectivos estavam traçados e o tempo ajudava à prática desta modalidade de PhD. Quando dei o primeiro passo nesta caminhada não sabia quantos km ainda tinha pela frente. E ainda não sei... Pelo caminho tenho encontrado pessoas que me ajudam a carregar a mochila, que me chamam atrás quando não vou no caminho certo, que me dão a mão para atravessar mais uma dificuldade, que me indicam o próximo destino no mapa, me oferecem um pouco de água quando o cansaço aperta, me motivam a continuar mesmo quando a chuva cai forte ou simplesmente me acompanham em cada passo com um sorriso motivador. Cheguei a um ponto do percurso em que quero agradecer a quem esteve sempre, e a quem vai estando, a fazer caminho conigo.

'Olá meninos!' Tornou-se invariavelmente esta, a saudação com que o Prof Miguel nos cumprimenta. E não estou a brincar quando digo que esta expressão simboliza a proximidade que demonstrou sempre ao longo deste caminho. Obrigado Prof, pela paixão que pôe em tudo o que faz e por tudo o que me permite aprender! Pela motivação para o querer saber sempre mais, pela curiosidade nos resultados que aos seus olhos são sempre promissores e que tão bem sabe descobrir e interpretar, com base na imensa experiência adquirida. Experiência essa que vem do contacto com tantos outros que foram também presença importante nesta senda: Dr. Eugénio Rodrigues que facultou as funções para análise de Time-Frequency, o Dr. Michael Wirbal que providenciou as Mooney para a tarefa de EEG/fMRI, o Dr. Francisco Sales que possibilitou a colaboração com a unidade de Epilepsia dos CHUC e a Professora Catarina Oliveira minha tutora do PhDHS. A todos um obrigado especial pela colaboração e ajuda neste e noutros projectos.

Mas se o caminho se faz com quem nos indica o rumo a seguir, mais ainda com quem vai conosco. 'Então artista?', 'Então rapaz!' responde o Rebola. Obrigado pelos momentos científicos de percepção ambigua na tua visão de engenheiro artista, e por tantos momentos entre amigos dignos de registo fotográfico. E como o caminho ‘dá pro que for’, obrigado Inês porque no teu sorriso vejo também uma razão para sorrir (ne que seja num passe para golo quando somos da mesma equipa!). Este trilho foi recheado por ‘Hellos’, de uma pessoa adorável, que me ensinou que a vida faz mais sentido se formos transparentes. Agradeço-te Susinha por sermos amigos para sempre. Um sempre que, para a Filipa, começou ainda na Sala de cálculo, num troço de caminho que mudaria muitas coisas do ‘jhony’. Obrigado Filipa! Graças a Deus, este é um caminho que não temos de percorrer sozinhos, por isso obrigado Inês pelos ‘Hi5 Casti’ muitas vezes sem motivo aparente mas que alegremente nos fazem andar mais um passo, porque "se tu procuras, só tu podes encontrar...". Passo esse que todos os dias começava cedo, quase sempre com um ‘café’, contigo Inês, e que terminava numa ‘Bjoka’ ao fim do dia. Agradeço-te (vizinha por pouco tempo) pela tamanha disponibilidade e ajuda científica que culminava em massagens de escadas, que suavizavam as dores de mais um dia de caminho. Uma etapa que encontraria num escuteiro, um engenheiro que se tornou também um amigo. Mark Simons, para ti uma canhota forte! Esta etapa começou com o alto patrocínio de uma simpática coleguinha a quem ainda ‘não disse uma coisa hoje’! ‘Agora já disseste’ diz ela!
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Muitas vezes arriscamos um novo caminho e o percurso leva-nos então a pessoas que diariamente deixavam um ‘fary tale’ animado na SCA (Malhão, Mingas, Meneses, Manuel, Brás, Fábio). E esse trajecto apresenta-nos pessoas desportistas como os João, o Carlos ou o Gonçalo; práticas como a Maria; organizadas como a Paula e a Fátima; disponíveis como o Gabriel; empenhadas como a Britta e a Marta; cheias de talento como a Otilia (já tens o meu desenho?); que só comem sandes de queijo a qualquer refeição como o Francisco; ou simplesmente amigas gozonas, como a pessoa que me ajudou em muita coisa desta tese, e caminha agora (todos os dias) costas com costas comigo – obrigado Catdu! Nesta caminhada, por entre paisagens deslumbrantes, aparecem ainda a Joana (que achava que o meu sorriso era de gozo); a Catarina que queria por tudo que arranjasse namorada; a Marieke pronta a ajudar; o pessoal da ressonância que me aturou tantas experiências; as colegas de gabinete com música e conhecimentos sempre actuais; os amigos do VE, em particular as minhas amigas gêmeas que encontro sempre com os seus simpáticos olás sincronizados, ou aqueles com que me cruzo p.e. num jogo de futsal; a malta do Justica nos encontros com o nosso Amigo (alguns smilés praticamente diários); os amigos do Seixo (tantos!); o pessoal da bala; os colegas do PhDHS (em particular o Ricardo, os Joões, a Márcia e as minhas queridas colegas de grupo); todo o pessoal com que sempre me cruzei nos corredores como dizia a Aldina num célebre poema, ou os inúmeros ‘vizinhos’ do IBILI e do ICNAS, sem esquecer os ‘bom dia jovem!’ das senhoras da limpeza, ou as continências daqueles senhores da estatística (xará!) que andam sempre ao telefone. A todos um obrigado sincero por me sorrirem por aí, em particular todos aqueles que passaram pelos Calções&Tshirt.

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João Castelhano 😊  
Julho de 2014

“…The Camino begins when the Camino ends…”  
Unknown author
Curriculum Vitae

João Castelhano, was born in December 13, 1985 in Coimbra. His primary education was done in Seixo-Mira, Portugal and his basic and secondary education were completed in Mira, Portugal. In 2003, he went to the University of Coimbra (UC) to study Biomedical Engineering. He completed the Integrated Master Degree in Biomedical Engineering in September 2008 from the University of Coimbra. The work conducting to his master thesis have been done at Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, UC, under the supervision of Professor Miguel Castelo-Branco.

From June 2008 to May 2010, he pursued his research work at IBILI with a grant from the project “Neural correlates of object recognition: structure-function correlations within the visual ventral stream, striatal and limbic circuits in health and disease”.

Since October 2010, he is a PhD student in the PhD Health Science Doctoral Program from the Faculty of Medicine, UC. His PhD research is being held at the Visual Neuroscience Lab, IBILI and focuses on the “Neural substrates of 2D/3D object perception applying combined EEG/fMRI approaches”.

Since September 2013, João is working at Institute of Nuclear Sciences Applied to Health (ICNAS). Here he gives technical support to data acquisition and analysis of multimodal research and clinical projects (including human and animal studies; EEG, fMRI, MRS, TMS, EyeTracking, ECoG, and PET).